VIETNAM NATIONAL UNIVERSITY
OF AGRICULTURE

ACADÉMIE DE RECHERCHE
ET D'ENSEIGNEMENT SUPÉRIEUR

PROCEEDINGS

INTERNATIONAL CONFERENCE

AGRICULTURE DEVELOPMENT IN THE CONTEXT OF INTERNATIONAL INTEGRATION: OPPORTUNITIES AND CHALLENGES

December 7 - 8, 2016
Vietnam National University of Agriculture, Hanoi, Vietnam

AGRICULTURAL UNIVERSITY PRESS - 2016

PREFACE

The International Conference on *Agriculture Development in the Context of International Integration: Opportunities and Challenges* 2016 (ICOAD 2016) took place at Vietnam National University of Agriculture, Hanoi, Vietnam on December 7 to 8, 2016. This year Vietnam National University of Agriculture is celebrating its 60th Anniversary that will be held on 10th December. This conference is certainly a wonderful event which contribute to 60th anniversary celebration activities.

The Conference aims at creating a forum to exchange scientific ideas and debate research issues relating to agricultural development in the context of international integration. The topics that are covered in the conference include Animal Science, Veterinary Medicine, Aquaculture, Biological Technology, Food Technology, Economics, and Rural Development.

More than 50 papers have been submitted from research institutes and universities of different countries in order to be considered for presentation at ICOAD 2016. The selection of papers included in this Proceedings was based on international and national peer review procedure.

The program consisted of invited talks and contributed presentations, either in the form of oral presentations or posters.

Our sincere thanks go to Académie de Recherche et d'Enseignement Supérieur (ARES-CCD) and Vietnam National University of Agriculture for their support; the invited speakers for their acceptance to give keynote lectures on their respective fields of expertise; the participants, especially those of you coming from abroad, for joining us and sharing your valuable experience and ideas.

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CONFERENCE PROGRAM OF ICOAD 2016

Hanoi, December 7 and 8, 2016

Day 1: Wednesday, 7 December 2016

08.30 - 09.00 Registration

09.00 - 09.45 Opening ceremony

09.45 - 10.30 Keynote Speaker

Role of ruminant production on food security and global warming: feed resources and feeding interventions

Prof. Metha Wanapat (Khon Kaen University, Thailand)

10.30 - 11.00 Morning break and Poster session

11.00 - 12.00 Plenary speakers

11.00 - 11.30 Towards a Sustainable Aquaculture in Vietnam: Current Status and Selected Researches

Prof. Nguyen Thanh Phuong (Can Tho University, Vietnam)

11.30 - 12.00 Livestock production in Vietnam and Research in Perspective of Sustainable Development

Assoc. Prof. Vu Dinh Ton (Vietnam National University of Agriculture, Vietnam)

12.00 - 13.30 Lunch break

13.30 - 17.20 Session 1: Animal Science and Veterinary Medicine

13.30 - 14.00 Sustainable conservation and approach for utilization of native chicken

Invited speaker Assoc. Prof. Amonrat Molee, Suranaree University of Technology, Thailand

14.00 - 14.20 Induction of ovulation synchronization for pured BBB embryo transfer in Vietnam

Su Thanh Long, Trinh Dinh Thau, Nguyen Van Thanh and Vuong Tuan Phong

14.20 - 14.40 Genetic structure of candidate genes for litter size in Landrace and Yorkshire sows

Nguyen Thi Vinh, Do Duc Luc, Nguyen Hoang Thinh, Ha Xuan Bo, Hoang Ngoc Mai, Vu Dinh Ton

14.40 - 15.00 Prevalence of Antibodies to Porcine Parvovirus in swine in Ha Noi and its Vicinity

Le Van Truong, Nguyen Van Giap, Vu Thi Ngoc, Cao Thi Bich Phuong, Huynh Thi My Le

15.00 - 15.30 Afternoon break and Poster session

15.30 - 16.00 The use of Belgian breeds in the improvement of animal production in the tropics

Invited speaker Prof. Jean-Luc Hornick (University of Liege, Belgium)

16.00 - 16.20 Productive performance and yolk fatty acid composition of laying hens fed different dietary n-6 to n-3 fatty acid ratios

Molee W., Yaemphet T. and Khempaka S.

16.20 - 16.40 Monoclonal antibodies specific to water buffalo (Bubalus bubalis) myxovirus resistance protein 1

Dam Van Phai, Bui Tran Anh Dao, Desmecht Danie

16.40 - 17.00 Utilisation of Rice Distiller's By-product for Swine Production in Northern Vietnam

Oanh Nguyen Cong, Dang Pham Kim, Luc Do Duc, Jérôme Bindelle, Ton Vu Dinh and Jean-Luc Hornick

17.00 - 17.20 Dong tao chicken breed in Hung Yen province (Vietnam): Characteristics of an indigenous chicken breed with big legs

Nguyen Van Duy, Moyse Evelyne, Nassim Moula, Do Duc Luc, Nguyen Thi Xuan3 Vu Dinh Ton and Frederic Farnir

Day 2: Thursday, 8 December 2016

09.00 - 12.00 Session 2: Economic and Rural development

09.00 - 09.30 Urgency of farmer participation in livestock value chain development in Indonesia

Invited speaker Assoc. Prof. Budi Guntoro (Universitas Gadjah Mada, Indonesia)

09.30 - 10.00 Biosecurity and diseases control practices and perceptions of smallholder pig farmers in Vietnam

Max Barot, Fred Unger, Nguyen Thi Thu Huyen, Ninh Xuan Trung, Tran Van Long

10.00 - 10.20 Performance of the chicken contract farming and its affecting factors in Vietnam: A case study in Hoa Thach commune, Quoc Oai district, Hanoi

Bui Thi Nga, Philippe Lebailly

10.20 - 10.40 Morning break and Poster session

10.40 - 11.00 The Local State in the Northwest Highlands: Learning from collective field research in Yen Chau (Son La Province)

Pierre Pettit (Université libre de Bruxelles, Belgium)

11.00 - 11.20 Rice and shrimp farming in the Xuan Thuy National Park: sustainable and unsustainable practices

Nguyen Thi Trang Nhung, Philippe Lebailly, Tran Huu Cuong

11.20 - 11.40 The dynamic pathways of agrarian change in the Red River delta of Vietnam

Nguyen Thi Minh Khue, Nguyen Thi Dien and Philippe Lebailly

11.40 - 12.00 Status of phosphorus solubilizing microorganizism in some kind of alluvial soils cultivating wet rice

Nguyen Tu Diep, Cao Ky Son, Dinh Hong Duyen

12.00 - 13.30 Lunch break

13.30 - 16.40 Session 3: Aquaculture and Food Science

13.30 - 13.50 Physiologycal and immune pathway responses of rainbow trout juveniles to dietary supplementation with bovine lactoferrin

Trinh Dinh Khuyen, S.N.M Mandiki, Jessica Douxfils, Valérie Cornet, Stéphane Betoulle, Felipe E. Reyes López, Lluis Tort and Patrick Kestemont

13.50 - 14.10 Effect of fish meal replaced by soybean meal on growth performance and feed utilization of black carp (mylopharyngodon piceus)

Tran Thi Nang Thu, Tran Anh Tuyet, Nguuyen Tu Tuan Anh, Tran Thi Thap Hieu, Tran Quang Hung

14.10 - 14.30 In vitro investigation of antioxidant capacity of herbal extracts and commercial products used to improve aquaculture products quality

Nguyen Le Anh Dao, Guy Degand, François Brose, Tran Minh Phu1, Joëlle Quetin-Leclercq, Bui Thi Buu Hue, Le Thi Bach, Truong Quynh Nhu, Bui Thi Bich Hang, Do Thi Thanh Huong, Nguyen Thanh Phuong, Patrick Kestemont, Marie-Louise Scippo

14.30 - 14.50 Isolation and evaluation of antibiotic activity of endophytic actinobacteria on may chang tree (Litsea cubeba) against pathogenic bacteria causing diseases on common carp and tilapia

Trinh Thi Trang, Kim Van Van, Truong Dinh Hoai, Nguyen Thanh Hai, Nguyen Van Giang, Nguyen Ngoc Tuan

14.50 - 15.10 A pathway to climate change adaptation for agriculture production: Shrimp case in Mekong delta

Ngo Tien Chuong

15.10 - 15.40 Afternoon break and Poster session

15.40 - 16.00 Bacteriocins as food preservatives

Nannan Catherine, Vu Quynh Huong, Nguyen Thi Thanh Thuy and Mahillon Jacques

16.00 - 16.20 Ultrasound-assisted extraction and anticancer activity of piceatannol from sim (Rhodomyrtus tomentosa) seed

Lai Thi Ngoc Ha, Bui Van Ngoc, Tran Thi Hoai, Hoang Hai Ha

16.20 - 16.40 Screening and characterization of β -Glucanase produced by Bacillus spp. isolated from Muong Khuong chili sauce

Nguyen Thi Thanh Thuy, Nguyen Hoang Anh, Nguyen Vinh Hoang

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18.00 - 21.00 Farewell party

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KEYNOTE, PLENARY AND INVITED SPEAKERS

THE USE OF BELGIAN BREEDS IN THE IMPROVEMENT OF ANIMAL PRODUCTION IN THE TROPICS

Pascal Leroy[1*], Jean-Luc Hornick[1], Frédéric Farnir[1], Alain Huart[1], Emile Leroy[1], Renaud Cassart[1], Patrick Ruppol[1], Moussa El Fadili[2], Philippe Lebailly[3], Dang Vu Binh[4], Nguyen Van Thang[4], Johann Detilleux[1], Luc Do Duc[4], Désiré NFundiko[5], Benoît Nienhaus[5], Nassim Moula[1], Nicolas Antoine-Moussiaux[1]

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1. INTRODUCTION

The livestock sector accounts for 40 percent of global agricultural production and contributes to livelihoods and food security of almost a billion people (FAO, 2009). Rising incomes, urbanization, as well as population growth, contribute to an increasing demand, especially for meat, milk and eggs and their derivatives. Meat consumption is more particularly linked to GDP (figure 1) (FAO, 2005). Facing this revolution by demand, an intensification of animal production is observed, either through an increase in scale and numbers of intensive production units or through a gradual mutation of extensive systems. These mutations are more pronounced in suburban areas, i.e. close to the consumption centers. This massive development of demand, often termed "livestock revolution", thus the response in intensive production, is mostly borne by milk and even more by monogastric production, i.e. poultry and swine meat, and eggs. The latter species are indeed characterized by short cycles, low investment and low risk, meaning a rapid entry in production of new comers and a rapid increase in production of established producers. Dairy production, on its side, generates steady revenue and is also technically prone to rapid intensification. Facing the challenge posed to production in a wide array of socio-economical and ecological contexts, several disciplines must be integrated to ensure successful breeding activities. Animal nutrition, veterinary care and prevention, housing, as well as the genetics at the basis of the system must be managed as a comprehensive interdisciplinary approach for productivity including sustainability and animal welfare concerns.

In this particular context, genetics have a central role to play, as a fundament of the technical and social system, allowing for the gradual adaptation of breeds to more intensive practices. This effort, through a strategy of sustainable crossbreeding, is illustrated through a strong history of action-oriented research

mobilizing the unique animal genetic resources of Belgium led by the University of Liège in the Democratic Republic of Congo, Vietnam, Morocco and Brazil.

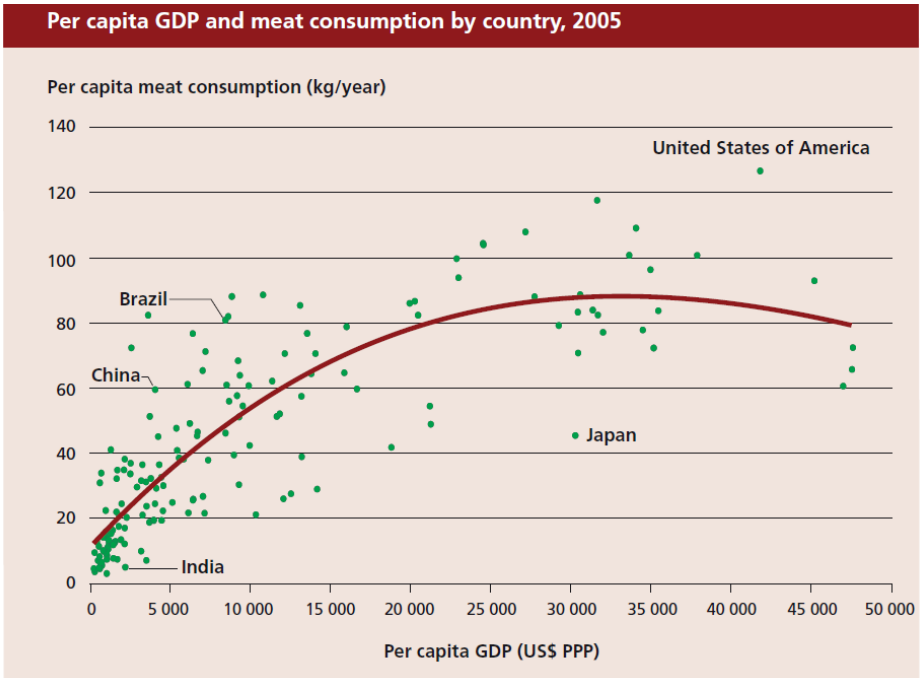
2. SELECTION AND CROSSBREEDING

Selection, generally called purebred selection, exploiting additive effects of genes, on the one hand and crossbreeding on the other hand, exploiting dominance and interaction, are the methods of choice used by animal breeders.

Purebred selection, as practiced in the developed world, based on own performance, progeny testing and molecular tests, requires expensive infrastructures and important human resources. In addition, genetic progress is slow.

For all these reasons, especially in least developed areas where purebred selection is also operational, stakeholders seek the most appropriate technical instruments to achieve rapid results, building on various crossbreeding schemes. Some schemes lead to spoilage of indigenous genetic resources that are needed for long-term development and therefore may be incriminated for their lack of sustainability. On the contrary, properly controlled, the so-called terminal crossbreeding contributes to the economically viable use of local breeds and therefore to their conservation.

The dairy sector of many countries has used the Holstein, the Brown Swiss, the Montbeliarde and the Normande with varying success. In the meat industry, in cattle, swine, sheep, as well as poultry, the crossbreeding of indigenous breeds with imported exotic breeds leads to interesting results. Moreover, the nature of the production lends itself well to the terminal cross scheme, hence its feasible implementation in well-framed sectors.



Note: GDP per capita is measured at purchasing power parity (PPP) in constant 2005 international US dollars.
Source: Based on data from FAOSTAT (FAO, 2009b) for per capita meat consumption and the World Bank for per capita GDP.

Figure 1. Relation of meat consumption and GDP in the World

In Belgium, following the hard work of breeders, remarkable breeds have emerged in cattle, swine and sheep, all showing exceptional performances in intensive meat production. Belgian Blue bulls, bred in natural conditions, show average daily gains as high as 2,000 g/d with slaughter yields close to 70%, the Piétrain pigs reach killing-out percentages around 83% and extreme dressing-out percentage values as high as 59% have been observed in the Texel sheep selected in Belgium. All these animals are very efficient, presenting carcasses with more muscle, less fat and less bone.

While the know-how required for their rearing as purebred is not to underestimate, their use in crossbreeding alleviates this need while delivering the rapid results sought by the typical suburban producer of countries and regions undergoing the economic transition described in introduction. We give several examples of the use of Belgian Blue cattle, Piétrain pig and Belgian Texel sheep in the context of the improvement of animal production.

3. THE USE OF BELGIAN BLUE CATTLE IN BRAZIL

In order to improve the efficiency of beef production, crossbreeding is a widely accepted means of incorporating desirable traits from various breeds, exploiting effects of complementarity and heterosis (Cundiff et al., 2000; Lunstra and Cundiff, 2003). The benefit of the Belgian Blue Breed (BBB) (Photo 1) in crossbreeding was studied in an experiment involving the BBB, the Brazilian Zebu Nelore and secondly the Braford, an American synthetic breed obtained from Zebu Brahman and Hereford.

Double muscling in cattle is recognized as an autosomal recessive trait (locus mh) widespread in the BBB) (Charlier et al., 1995). Braford is a synthetic breed, approximately 3/8 Brahman and 5/8 Hereford, developed in Florida since 1947 and currently used in USA and South America. Nelore is a Brazilian cattle breed (Photo 2) originated from the Ongole breed (India) first introduced in Salvador, Bahia, in 1868.

A total of 90 animals (36 BBB x Nelore and 54 Braford) of the Fazenda Lagoa do Morro, Agribahia (Photo 3a and 3b), were studied from 2002 to 2005 by the Center of Excellence in Animal Productions of the State of Bahia (CEPAB), a joint project of the Faculty of veterinary Medicine (University of Liege), Gembloux Agro-Bio Tech (ULg) and SEAGRI (Ministry of Agriculture of Bahia).

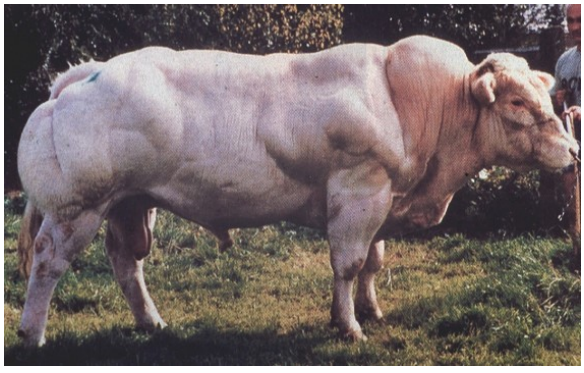


Photo 1. Bull Opticien, Belgian Blue Breed

Photo 2. Bull of the Zebu Nelore breed - Brazil

Table 1. Results of the dissection of the 7th rib of Braford and Belgian Blue x Nelore bulls

	Breeds			
Traits of the rib	Braford	BBB x Nelore	Difference BBB x N - Braford	
Age at slaughter (d)	755.9	750.4	-5.5	NS
Weight at slaughter (Kg)	553.5	539.7	-13.8	NS
Carcass weight(Kg)	286.6	292.0	+5.4	NS
Killing out (%)	51.78	54.10	+2.32	NS

	Breeds			
Traits of the rib	Braford	BBB x Nelore	Difference BBB x N - Braford	
Fat (g)	771.2	661.5	- 109.7 g	P < 0.25
Bone (g)	1203.5	939.5	- 264.0 g	P < 0.001
Muscle (g)	2015.5	2401.5	+ 386.0 g	P < 0.01
Total Rib (g)	3990.2	4002.5		NS
Fat % in the rib	19.11	16.56	-2.54%	P < 0.20
Bone % in the rib	30.40	23.50	-6.90%	P < 0.001
Muscle % on the rib	50.50	59.94	+9.44%	P < 0.001

	Breeds			
Traits of the rib	Braford	BBB x Nelore	Difference BBB x N - Braford	
Longissimus dorsi (g)	216.5	264	+47.5 g	P < 0.05
Longissimus dorsi (% of the rib)	5.49	6.62	+1.13%	P < 0.10
Periph. Muscles (g)	413	528	+114.6 g	P < 0.01
Periph. Muscles (%)	10.44	13.13	+2.68%	P < 0.005

Photo 3a. BBB x Nelore calf
Agribahia, Lagoa do Morro, Bahia, Brazil

BBB x Nelore crossbred bull

Photo 3b. Aladin BN Das Reunidas , BBB x Nelore crossbred (1240 kg at 39 month)
(Credits: Leroy ans coworkers, 1998 - 2001)

The Nelore cows were inseminated by two BBB bulls and the breeding season of the comparison animals (Braford) was the same. All calves were born without any assistance. 10 bulls of the two groups (BBB x Nelore and Braford) were slaughtered in Tecnocarne in Salavador (Bahia) at about 2 years of age (750 days on average) followed by the dissection of the 7th rib of the right carcass.

The results given in table 1 illustrate the potential of the BBB in Brazil and more specifically in crossbreeding with Zebu-type cattle. The BBB x Zebu crossbred, compared to Braford, showed a higher killing out percentage (+ 2.32%) and a higher muscle percentage in the 7th rib of the right carcass (+ 9.44%) on average, a situation that corresponds to a redistribution of muscle, fat (-6.9%) and bone (- 2.54%) in the 7th rib, and thus by extrapolation in the carcass.

Moreover, the forepart of the carcass is more developed in the BBB x Nelore crossbreds with new possibilities in terms of transformation of the carcasses.

4. THE BELGIAN BLUE CATTLE IN KATANGA - DRC

The Grelka farm (Grands Elevages de Katongola) is divided into two areas: the first on the Biano plateau (120,000 ha), including 8,000 head of cattle and the second near the city of Kamina, in the district of Haut Lomami (330,000 ha), with 24,000 heads.

The ranching operations started in 1914. The initial goal was to achieve a uniform red color, like that seen in Bonsmara cattle and called Grelka cattle (Photo 4).

In 2008 - 2009, BBB x Grelka crosses were produced. Moreover, the company also acquired pure Bonsmara animals. The animals in this study were born from September 2009 to October 2009.

Monthly weighings were carried out on 35 Bonsmara calves (17 females and 18 males) and 25 BBB x Grelka calves (10 females and 15 males).

Statistical analysis indicated a significant breed effect ($P < 0.01$) in favor of the BBB x Grelka crosses (on average +9.21 kg at 270 days). At the age of 3 years, the difference was in average + 152.24 in favour of the BBB x Grelka, (average weight of 481.91 kg) to be compared with Bonsmara purebred (329.67 kg) (NFundico *el al.*, 2012).

5. USING THE PIÉTRAIN IN DRC AND VIETNAM

The Pietrain pig is a native pig breed of Belgium appeared in the Southern part of Brussels. Easy to recognize by its small ears, black spots and the extreme muscular development, the Pietrain was also characterized by its stress susceptibly called Porcine Stress Syndrome (PSS).

Photo 4. Grelka cattle

The Piétrain has been selected from the 1980s at the Faculty of Veterinary Medicine of the University of Liège. The idea was to produce Pietrain pigs free of PSS.

Before the discovery of the locus Ryanodine (RyR) by Fuji et al. 1991, animals were first selected according to their resistance to malignant hypedhermia (response to 3% Halothane), later on, by the analysis of blood samples (presence/ absence of linked genes), and finally on the molecular analysis of the punctual mutation giving homozygotes stress negative (CC), heterozygote stress negative (CT) and stress positive (TT).

Following this selection work, stress negative Pietrain pigs were born with a muscle level comparable to the original Piétrain but without stress and its side effects (PSE meats) (Leroy and Verleyen, 1999).

In addition, it appeared that animals also exhibited a form of resistance to heat stress.

In Democratic Republic of Congo (DRC), especially in Kinshasa, after the last war, due to a lack of monitoring, leading to some degeneration of the species, a slower growth, carcasses of lesser value have been observed.

Given these observations, efforts have been made to correct the negative effects of the past through pig genetics.

The first Piétrain pigs of "stress negative Piétrain" line of the University of Liege, arrived in Kinshasa, in 2002, following a proposal of the Centre Agro-Vet Tropical Kinshasa (CAVTK), an association of the Faculty of veterinary Medicine (University of Liege), Gembloux Agro-Bio Tech (ULg) and the Walloon Region. 30 pigs including 5 females were airlifted from Liege Airport in Kinshasa. The breeding program, for the distribution and genetic improvement, actually began from 25 males and 3 females breed Piétrain stress negative (all born at the University of Liège), giving rise to more than 100 stress negative Piétrain. Subsequently, a total of 193 purebred boars have been shared in as many different farms and in 2005, more than 200 farms have benefited from the Pietrain breed. Very quickly, in Kinshasa, more than 4,000 pigs were born of this initiative which led to a high percentage of "Pietrain blood."

In this particular situation, the genetic improvement is obtained through the crossbreeding; it is practiced preserving local material (Photo 5) which is well adapted to the climate and to the pressure of infections. The effect of the crossbreeding, leading to pigs of the "commercial type" which could, in the long term, be marketed nationwide through multiplier breeders.

The effect of the introduction of the exotic breed (the stress negative Piétrain) on growth parameters was immediate on the weight and, in several farms comprising the different genetic types, favorable growth curves were observed.

The most favorable growth curve concerns half blood stress negative Piétrain (Photo 6); however, they have slightly more fatty carcasses. The animals reached 100 kg body weight between 7 and 9 months which corresponds to a two-month gain compared to the previous situation.

Thus, when the technical level and the nutrition level are high, with "good farming practices", the results of the use of a high level of genetic appear quickly. In other cases, it is appropriate to do it gradually by using progressively better genetics (in our case, the use of boars with increasing % of Pietrain) (Photo 7) requiring more care and inputs.

Photo 5. Local sow

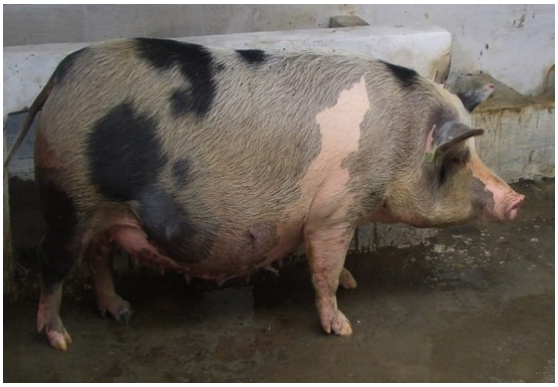


Photo 6. Pietrain x Local sow

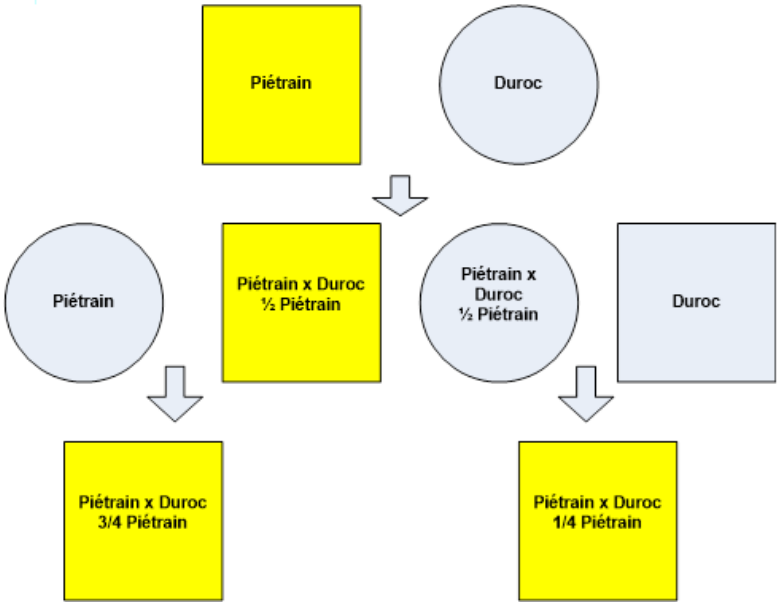
Photo 7. Pietrain x (Pietrain x Local)

The availability of a "variable genetic" particularly at the boar level is strategic because it takes into account virtually all situations encountered and especially allows breeders to make progress.

These notions of progress, improvement, goals to be achieved are many elements of success and enable progress by stages. This method has been proven elsewhere, particularly in Europe, where regional production averages are published and allow each farmer to compare himself to others.

In DRC, the so-called variable level of genetics in the pig sector is actually obtained from boars , , and 100% stress negative Pietrain, produced with Duroc or Large White sows well-known breed for their adaptability to the tropical climate.

The Pietrain crossbreds were produced according to the following cross breeding scheme.



6. GENETIC IMPROVEMENT OF PIG PRODUCTION IN VIETNAM

The university cooperation program involving the University of Liege (ULg), Gembloux Agro-Bio Tech (ULg) and the Hanoi Agricultural University was initiated in 1997. It is in the framework of this cooperation that stress negative Pietrain boars (Photo 8) were exported from Belgium to Vietnam.

Favorable results were first obtained from crosses (Photo 10) involving the Stress Negative Pietrain and Vietnamese breed Mong Cai (Photo 9).

These studies showed that crossbred animals exhibit a very good adaptation and weaning survival rate of 95% with significantly improved carcass quality in the crossbreds (Dang Vu Binh and Nguyen Van Thang, 2004; Do Duc *et al.,* 2011; Do Duc *et al.*, 2012).

Later, the 3-way cross Pietrain x[Large White x Mong Cai] (Photo 11) has shown that the maintenance of local genetics (Mong Cai) is compatible with the production of a commercial product.

Photo 8. Pietrain Boar

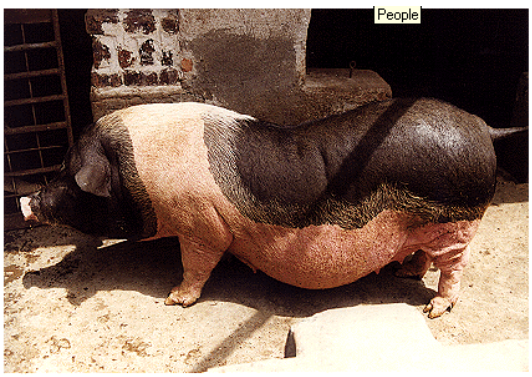


Photo 9. Mong Cai sow

Photo 10. Pietrain x Mong Cai

Photo 11. Pietrain x (Large White x Mong Cai)

First, the Large White boar (white dominant) is crossed with a Mong Cai sow (spotted recessive) giving rise to white crossbred animals whose conformation is already improved. The best F1 sows are then selected and crossed with a stress negative Pietrain boar leading to crossbred products (3/4 white and spotted) having a considerable improvement in the carcass which can compete with commercial lines.

The end result has an expected proportion of spotted piglets and considerable muscle development giving Vietnam a protection system (Economic) of the local breed Mong Cai.

7. GENETIC IMPROVEMENT IN THE SHEEP MEAT SECTOR IN MOROCCO

In Morocco, the low productivity of local sheep breeds can be explained by the moderate growth of the lambs and the tendency to be prematurely fat. Following the evolution of the trend of demand for Moroccan consumers, especially the urban consumers, the fat content of sheep meat has become an economic constraint.

Studies on crossbreeding using improved breeds: Ile de France, Lacaune, Merino a, conducted at the National Institute for Agricultural Research (INRA) in Morocco (El Fadili 1996, El Fadili and Leroy 1998, El Fadili and Leroy 2000, El Fadili et al.2000, 2001, El Fadili 2004, 2005, 2006), have showed the interest of the use of these breeds to improve growth and muscle development of the crossbred lambs.

In addition, in 2004, and in the same perspective of improving the carcass quality and meat content in lambs born from the terminal crossing with meat breeds from Europe, INRA has benefited from 3 Belgian Texel rams imported from Belgium.

The Belgian Texel breed is well known for its excellent meat traits: high efficiency, high surface area of the longissimus dorsi muscle and high meat/fat ratio (Leroy *et al.* 1995).

The study conducted to evaluate the performance terminal cross of the Belgian Texel breed with local breeds (T D'man (D) black head and Timahdite) brown-headed and their cross products "DT" showed that

in general the Belgian Texel rams showed no adaptation problems, including the first year of their arrival in July (summer) since they were mated immediately after their arrival. Fertility of the ewes mated to Belgian Texel rams can be considered high, and similar to other local sheep breeds.

The productivity estimated by live weight of lamb per ewe is higher in the cross involving the Belgian Texel breeds compared with D'man (D), Timahdite (T) and DT cross.

The Belgian Texel (BT) crossbred lambs showed pre-weaning and post-weaning growth performances superior to those of pure D'man and Timahdite. In addition, these lambs showed no difficulty during lambing or during their growth till weaning and till slaughter.

Moreover, the Belgian Texel crossbred lambs presented carcasses with good conformation and a good compactness with significantly less fat. The analysis of the composition of the various parts of the left half carcass has shown the superiority of Belgian Texel crossbred lambs for muscle development. The muscle percentage was significantly higher than in T, D and DT lambs. Similarly, the percentage of fat was lower in carcasses from crosses with the Belgian Texel breed. The percentage of bone was similar and no significant difference was observed in crossbred and purebred lambs.

The most important result with the 3-way cross (Figure 2) involving three breeds (BT x [DxT]) was the 7.61 kg increase (+30%) of lamb produced per ewe at 90 days (23.21 kg per ewe with Timahdite to be compared to 30.82 kg in BT x [DxT])

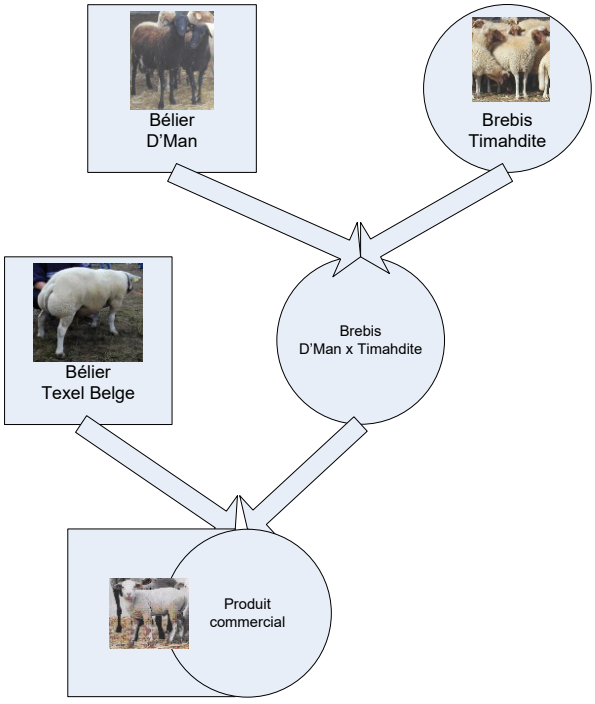


Figure 2. Three-way cross involving three breeds (BT x [DxT])

Source of variation	N	Fertility (%)	TPN (lambs)	PPN (kg)	TPS (lambs)	PPS (kg)
Genotype	388	*	***	***	***	***
D'man	27	85 ± 6	1.99 ± 0.10	4.88 ± 0.28	1.12 ± 0.11	22.02 ± 1.60
Timahdite	75	94 ± 3	1.15 ± 0.05	4.14 ± 0.15	1.06 ± 0.06	23.21 ± 0.78
D x T	138	99 ± 3	1.20 ± 0.04	4.42 ± 0.11	1.14 ± 0.04	24.68 ± 0.59
Texel x D	22	88 ± 6	1.88 ± 0.11	5.46 ± 0.30	1.46 ± 0.12	28.66 ± 1.61
Texel x T	67	81 ± 4	1.18 ± 0.06	5.00 ± 0.17	1.14 ± 0.07	28.19 ± 0.91
Texel x DT	59	85 ± 4	1.79 ± 0.07	5.86 ± 0.20	1.55 ± 0.08	30.82 ± 1.05
Age of the ewe		ns	**	***	**	***
age ≤ 2 ans	82	82 ± 4	1.41 ± 0.06	4.43 ± 0.17	1.12 ± 0.07	24.35 ± 0.94
2 < age ≤ 3	53	89 ± 4	1.38 ± 0.06	4.37 ± 0.18	1.11 ± 0.07	24.20 ± 0.99
3 < age ≤ 4	59	86 ± 4	1.46 ± 0.06	4.71 ± 0.17	1.09 ± 0.07	26.17 ± 0.96
4 < age ≤ 5	56	88 ± 4	1.50 ± 0.07	5.00 ± 0.18	1.28 ± 0.07	27.48 ± 0.98
5 < age ≤ 6	64	87 ± 4	1.72 ± 0.06	5.39 ± 0.17	1.39 ± 0.06	28.91 ± 0.91
6 < Age ≤ 7	49	84 ± 5	1.62 ± 0.07	5.51 ± 0.20	1.44 ± 0.08	28.12 ± 1.05
Age > 7 years	24	89 ± 6	1.61 ± 0.09	5.31 ± 0.26	1.38 ± 0.10	25.60 ± 1.36
Year		ns	ns	**	ns	***
2004	159	90 ± 3	1.51 ± 0.04	4.70 ± 0.11	1.17 ± 0.04	22.89 ± 0.64
2005	125	84 ± 3	1.56 ± 0.05	5.26 ± 0.13	1.26 ± 0.05	28.57 ± 0.70
2006	104	83 ± 3	1.52 ± 0.05	4.92 ± 0.15	1.30 ± 0.06	27.32 ± 0.79
R^2 (%)		10	32	27	14	28

Least Squares Means (± SE) weight. fertility. the litter size at birth (TPN) and 90 days (TPS). the litter weight at birth (PPN) and 90 days (PPS) by genotype. age of ewe per year. R ² of the linear model.
*ns = p > 0.05 ; * P < 0.05 ; ** p < 0.01 ; *** p < 0.001*

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ROLE OF RUMINANT PRODUCTION ON FOOD SECURITY AND GLOBAL WARMING: FEED RESOURCES AND FEEDING INTERVENTIONS

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ABSTRACT

Animal protein products including eggs, meat and milk will be in greater demand as a consequence of human population growth especially an increase from 6.7 billion now to an estimated population of 9.5 billion in the year 2025. Other driver demands are the impacts from improved livelihood and well-being, urbanization, income growth and consumption shifting behavior. Hence, these are urgent needs to improve livestock production efficiency as well as developing infra-structure, empowering research capability, enhancing research personal in order to obtain new information and innovations for further implementations. Furthermore, technical and research cooperations and collaborations are highly encouraged particularly the focus between South-to-South linkages. Besides, the improvement of suitable breeds to better adapt to environment and available resources, among the important factors feed resources and feeding interventions are essential. The establishment (E), development (D), utilization (U), of on farm feeds are highly encouraged for the sustainability(S)(EDU-S). Food-feed-system (FFS) has been recommended for smallholder farmers, while new technology and innovations are required for feed processing and feed formulation at industrial level. Importantly, increasing animal production efficiency required the attention and management on reducing greenhouse gas for global warming and to maintain a friendly-environment scenario. Among many methods to mitigate rumen methane production, dietary approach has been demonstrated promising and applicable. Potential feed resources containing plant secondary compounds have reported hiving impact on rumen microorganisms and mitigating methane production. With existing information, more effects should be further implemented on –farm engaging all stakeholders to participate to develop a more sustainable animal production.

1. INTRODUCTION

Wanapat *et al.* (2015) reported that animal agriculture has been an important component in the integrated farming systems in developing countries. It serves in a paramount diversified role in producing animal protein food, draft power, farm manure as well as ensuring social status-quo and enriching livelihood. Ruminants are importantly contributable to the well-being and the livelihood of the global population. Ruminant production systems can vary from subsistence to intensive type of farming depending on locality, resource availability, infrastructure accessibility, food demand and market potentials. The growing demand for sustainable animal production is compelling to researchers exploring the potential approaches to reduce greenhouse gases (GHG) emissions from livestock. Global warming has been an issue of concern and importance for all especially those engaged in animal agriculture. Methane (CH_4) is one of the major GHG

accounted for at least 14% of the total GHG with a global warming potential 25-fold of carbon dioxide and a 12-year atmospheric lifetime. Agricultural sector has a contribution of 50 to 60% methane emission and ruminants are the major source of methane contribution (15 to 33%). Methane emission by enteric fermentation of ruminants represents a loss of energy intake (5 to 15% of total) and is produced by methanogens (archae) as a result of fermentation end-products. Ruminants' digestive fermentation results in fermentation end-products of volatile fatty acids (VFA), microbial protein and methane production in the rumen. While, Wanapat et al. (2013) reported that the availability of local feed resources in various seasons can contribute as essential sources of carbohydrate and protein which significantly impact rumen fermentation and the subsequent productivity of the ruminant. Recent developments, based on enriching protein in cassava chips, have yielded yeast fermented cassava chip protein (YEFECAP) providing up to 47.5% crude protein (CP), which can be used to replace soybean meal. The use of fodder trees has been developed through the process of pelleting; *Leucaena leucocephala* leaf pellets (LLP), mulberry leaf pellets (MUP) and mangosteen peel and/or garlic pellets, can be used as good sources of protein to supplement ruminant feeding. Apart from producing volatile fatty acids and microbial proteins, greenhouse gases such as methane are also produced in the rumen. Several methods have been used to reduce rumen methane. However, among many approaches, nutritional manipulation using feed formulation and feeding management, especially the use of plant extracts or plants containing secondary compounds (condensed tannins and saponins) and plant oils, has been reported. This approach could help todecrease rumen protozoa and methanogens and thus mitigate the production of methane. At present, more research concerning this burning issue - the role of livestock in global warming - warrants undertaking further research with regard to economic viability and practical feasibility.

Thornton (2010) started the needs to develop breeds, nutrition and animal health to increase potential animal production efficiency especially in the developing countries. Rapid population growth will continue to be an important impediment to achieving improvements in food security. While urbanization and income growth rate (2.1% per capita) will further impact on the need of livestock products consumption. As Steinfeld et al. (2006) indicated that in the year 2050 the annual consumption per capita of meat and milk will be 44, 78 kg with total consumption of meat and milk 326, 585 Mt., for developing countries, while 94, 216 kg with total consumption of 126, 295 Mt., for developed countries, remarkable increase in developing countries.

Smith *et al.* (2013) have reiterated the importance the role livestock production beyond the supply of milk, meat and eggs. Livestock can enhance food and nutrition security and providing income to support the livelihood and well-being of the household formers. The challenges are those how to manage the trade-offs to enable livestock's positive impacts to be achievable while minimizing the negative issues and to maintain environmentally-friendly. Godber and Wall (2014) additionally stated the importance of livestock production as an important contributor to sustainable food security, as the animal products account for one-third of global human protein consumption. Livestock-based food security will be more vulnerable to impacts of

climate change in addition to prevailing lacks of technical support and economic, as well as other supporting infra-structure and available markets.

To achieve productive, profitable, sustainable and environmentally friendly in the topics the following recommendations are highly recommended;

E = Establishment of feed resources

D = Development of feeding system

U = Utilization of feeds including feeding method and processing

S = Sustainable of the animal production system.

The proposed feeding system for ruminants, food-feed system (FFS) to produce a year round feeding calendar, as well as to enrich the environment on-farm is illustrated in Figure I. Under this system, both type can be used to maximize the biomass by grazing and/or cut-and-carry. Root from cassava can be used as carbohydrate source while feed and whole top can be dried as hay as protein.

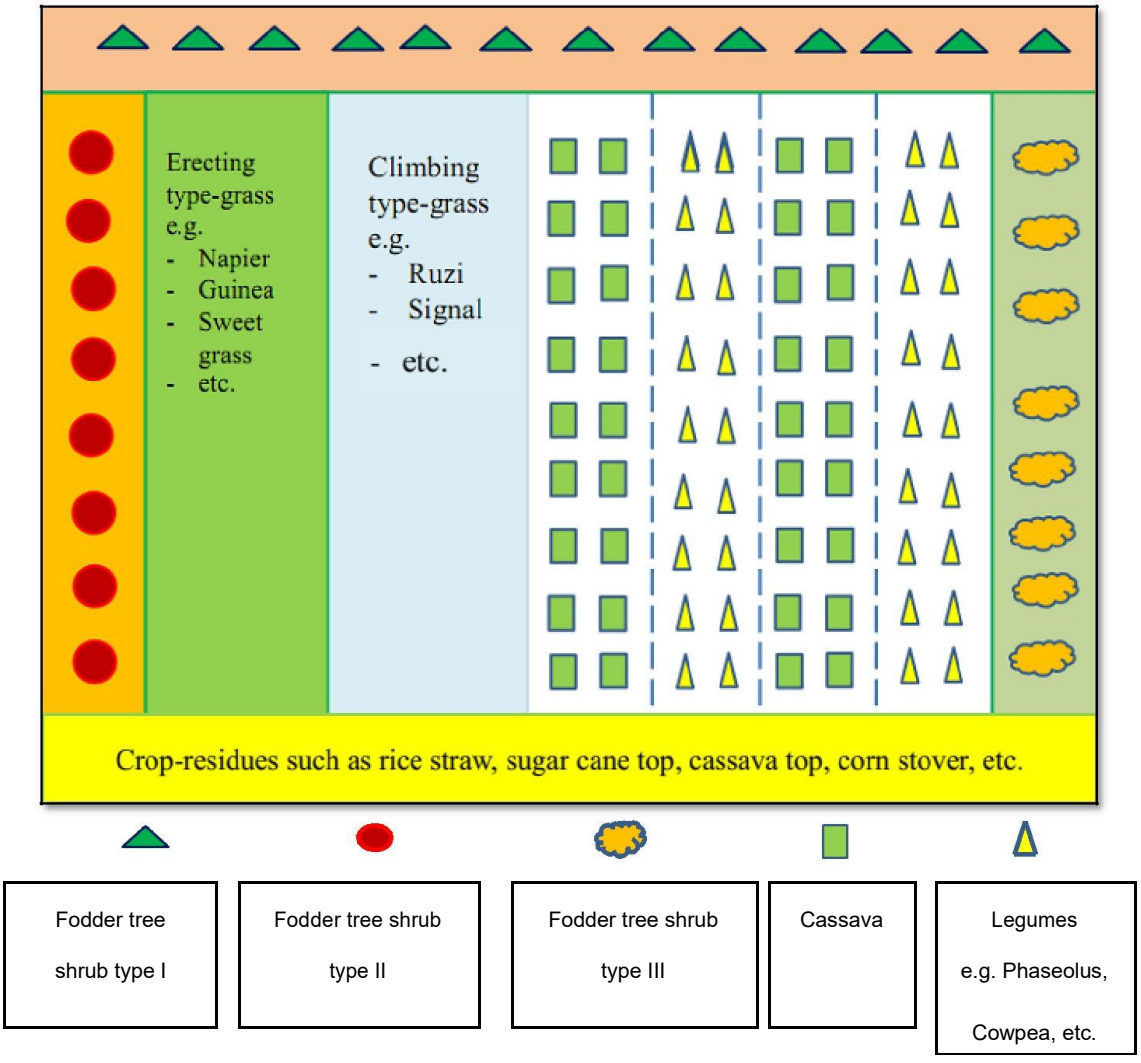

Figure 1. The proposed feeding system for ruminants, food-feed system (FFS) for ruminant feeding system for smallholder farmers in the tropics

2. CONCLUSIONS

Ruminant production will play a more important role to promote and support the livelihood and well-being of the rural population. EDU-S of local resources especially the feed resources with innovations will enhance the production efficiency, the profitability and sustainability of the systems. More efforts in linkages, sharing experiences among researchers are highly encouraged. Furthermore, on-farm feeding interventions are recommended for implementations.

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LIVESTOCK PRODUCTION IN VIETNAM AND RESEARCH IN PERSPECTIVE OF SUSTAINABLE DEVELOPMENT

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1. INTRODUCTION

In Vietnam, livestock production sector plays an important role in the socio-economic development. In the period of 2011 -2015, the livestock sector has a high growth rate, about 4.5-5% annually. In 2015, the total value of livestock production of the nation is about 205.44 thousand billion dongs. Pigs, poultry, and dairy cattle production have grown rapidly over some last years (annual growth rate of pork, poultry meat, egg, and milk production from 2011-2015 were 2.7%; 10%; 7.56%, and 22.1%, respectively). The animal-origin products meet basically the domestic consumption demand, some products even were exported to other countries (Hoang Thanh Van, 2016). In 2013, Vietnam had exported about 40,000 tones de pork (Statica, 2016). Livestock production not only provides enough food for domestic consumers demand (at least 10% of per capita calorie intake of consumers provided by livestock products), but also generates employment and income for a high number of farmers in rural areas (about 6.5 million households or 42% total households in rural areas engaged in livestock production, and shares about 14% in total household income) (Lucila Lapar, Ma., 2015). In the coming years, livestock production sector in Vietnam will be projected to rise due to the rapid increase of consumption requirement in the domestic market.

Research and technology transfer has contributed significantly to the development of livestock production in Vietnam over some last decades. The government always spends a considerable budget for the research and agricultural extension program. Thus, various research projects that have been taken at the agricultural universities and institutes have been applied by farm households and many issues of livestock production have been solved basically. Vietnam National University of Agriculture (VNUA) is one of the leading universities that plays important role in developing agriculture in general and animal production in Vietnam. This manuscript aims to introduce the livestock production in Vietnam and main axes of researches on animal sciences at VNUA towards sustainable development of livestock production.

2. OVERVIEW OF LIVESTOCK PRODUCTION DEVELOPMENT IN VIETNAM IN THE LAST DECADE AND ITS CURRENT CHALLENGES

2.1. General trends in the development of livestock production

The livestock production sector in Vietnam is much diversified with the development of different livestock species, including pigs, poultry, cattle and others. In recent years, due to the rapid growth of the economy, the livestock production has

been developed significantly to meet the increasing demand of domestic consumption. However, the development of livestock production sector is effected by many external and internal factors and changed slightly over some last years. The changes have seen in not only population of livestock herds, but also the production scale of animal farms. Figure 1 and 2 show the variation of population of pig, buffalo, cattle, and poultry over some last years. Figure 3 and 4 indicate the variation in meat production and consumption in the last decade.

According to figure 1 and 2, the population of livestock herds (pig, cattle, buffalo and poultry) has varied slightly over some last years. The pig population varies about 27,000 thousand heads, cattle population is about 5,000 thousand and buffalo one decreased from about 2,900 thousand to about 2,500 thousand from 2010 to 2015. However, dairy cattle and poultry populations have been increased considerably: from 300,000 thousand to more than 340,000 thousand heads in 2010 in 2015 for poultry population, and from 128.5 thousand heads to 275.3 thousand heads in the same period (more than 2 times in 5 years) (Figure. 3).

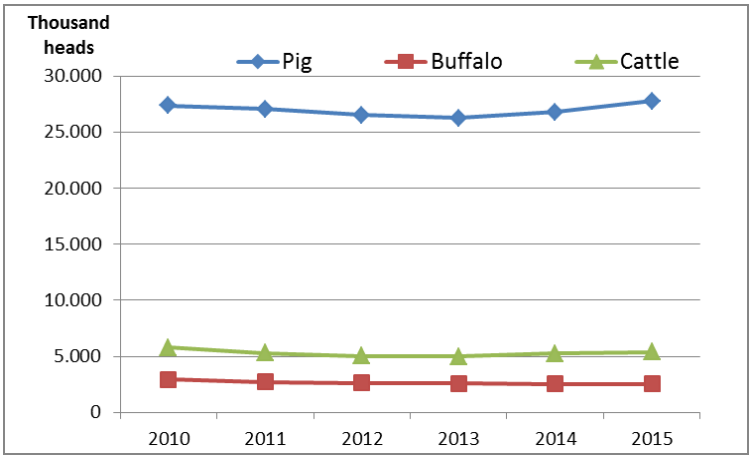


Figure 1. Changes in population of some livestock herds from 2010 to 2015

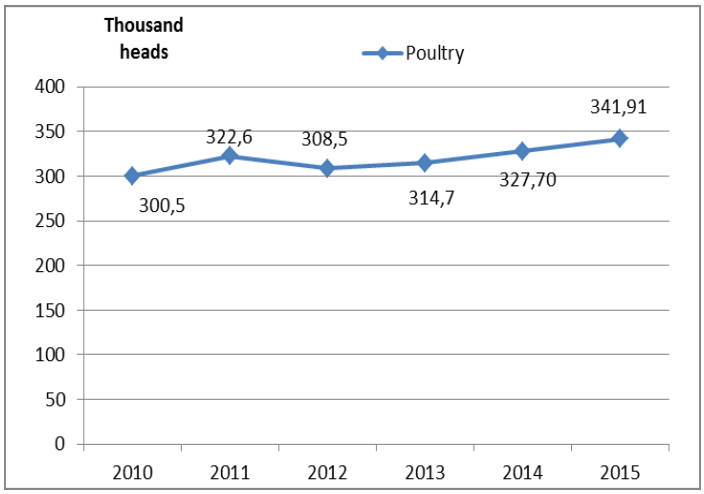
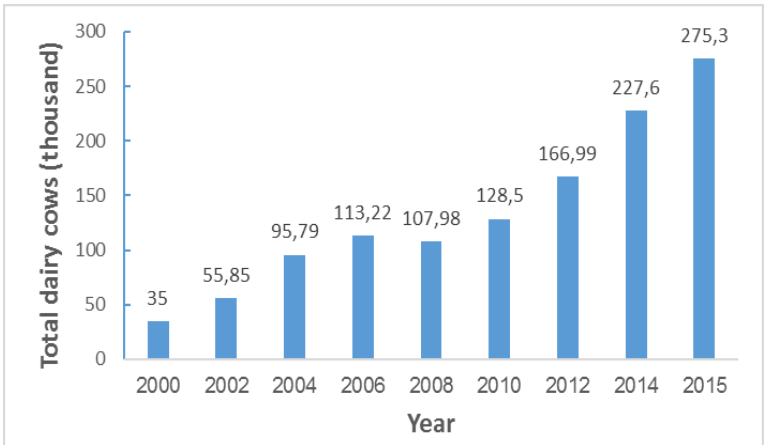
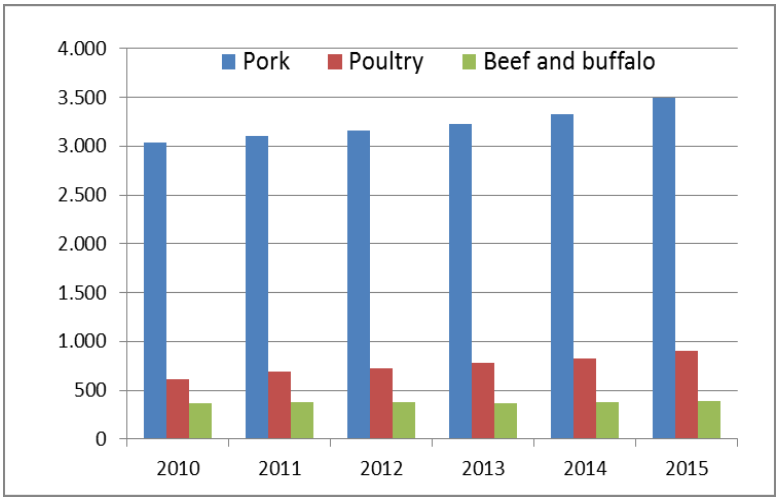


Figure 2. Changes in population of poultry herds from 2010 to 2015



Figue 3. Changes in popultation of dairy cattles from 2000 to 2015



Figue 4. Changes in meat products over the last years

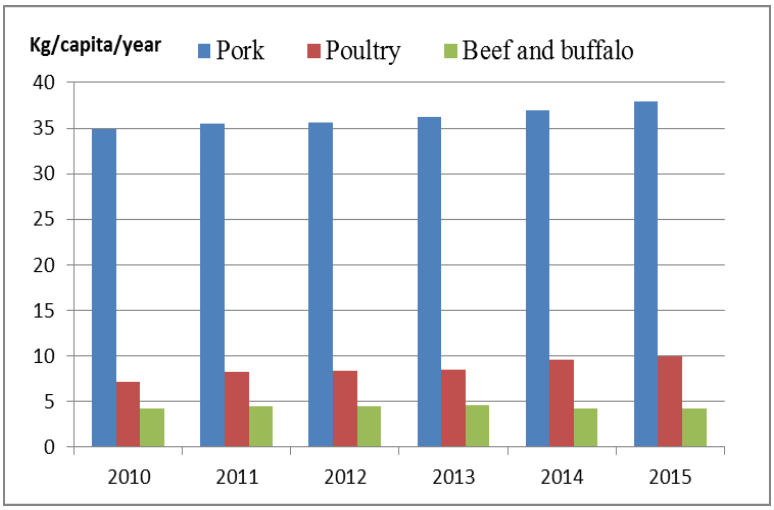


Figure 5. Changes in average meat comsum-ption per capita per year

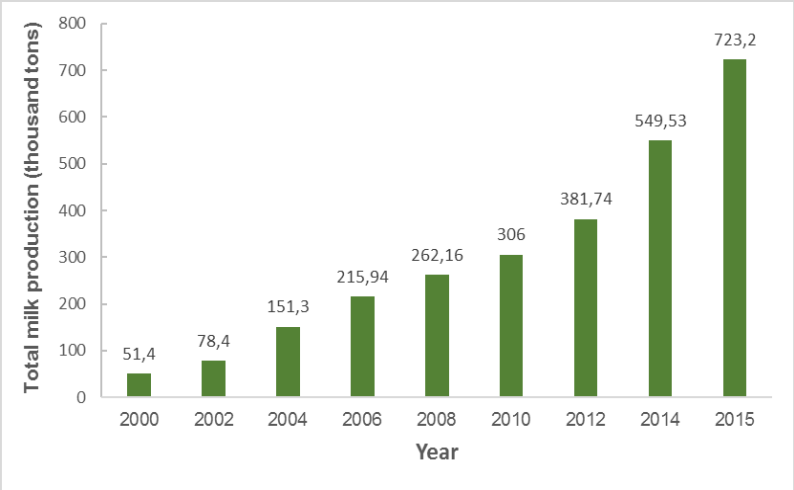


Figure 6. Changes in fresh milk products from 2000 to 2015

The pork production has risen from 2.012 thousand tons per year in 2010 to 3.491,6 thousand tons per year in 2015 (Figure 4). The statistical data indicates that pork is one of the most important types of meat in Vietnam with the average meat consumption per capita per year risen from 15.1kg live weigh in 2010 to 37.9kg in 2015 (Figure 5), sharing 72.67% of total consumed meat in the market.

The growth of dairy cattle population has created the very high increasing of fresh milk production. The milk quantity produced has been increased from 51.4 thousand tons in 2000 to 723.3 thousand tons in the year 2015 (near about 15 times).

One of the most remarkable changes in livestock production in Vietnam over some last years is the intensification and rapid development of large-scale farms. The statistical data indicated that over the last years, the number of pig farms with less than 10 heads of pigs has reduced 2.2 million farms (equal to a reduction of 38.5% in the total pig farms) from 2006 to 2011. On the other hand, the amount of medium-scale farms (with a pig herd size of 10 to 49 heads) and large-scale farms (with more than 50 heads of pigs per farm) has an increase of 3.4% and 80%, respectively (GSO 2012).

The trend in poultry production is also similar to that of pig production. The number of large-scale farm (with more than 1000 heads of chicken) in 2011 was 16.6 thousand farm, equal to 4.32 times higher than that in 2006 (GSO 2012). It is clear that the livestock production systems have changed remarkably to the intensive farming.

In dairy industry has the same situation, almost of dairy farms are small and very small ….. However, there are some very large farms (mega-farms) existing in Vietnam. The TH true milk company develop the farm with about 50,000 heads, VN milk future with more than 1000 heads…

2.2. Some difficulties and challenges

Although the livestock production sector has grown at a high rate recently, it still remains some difficulties and challenges.

The first and foremost challenge is threats and risks from animal diseases. Since 2003, many animal farms in nearly all provinces have been facing with the outbreak and repeat of several infectious

diseases such as avian influenza, PRRS (Porcine Reproductive & Respiratory Syndrome). In 2007, the PRRS had occurred at 13.355 farm households in 14 provinces, causing a loss of approximate 30.000 heads of pigs. In 2008, the disease had been broken in 28 provinces and the number of culling pigs was 10 times higher than that in 2007 (Binh *et al.*, 2010). In 2010, the PRRS has broken in 49 provinces and 812,947 heads of pigs were contaminated and 442,961 pigs have been destroyed (Department of Veterinary, 2010).

FMD (Feet and Mouth Disease) is one of important diseases in Vietnam. In 201, this disease has been broken in 13 provinces with about 3000 contaminated animal heads. Besides, there are some other diseases that have been occurred such as diarrhea symptoms, pasteurellosis… which caused an important loss in animal production.

The second is low productivity in animal production (such as the number of weaned pigs/sow/year in Vietnam is equal to 40 - 50% compare with developed countries). In the southern east area northern one sow produce annually 1212 kg live weight of fattening pigs, but in the mountainous area, one sow produce only 530 kg live weight. This is about 2800 to 3100 kg/sow/year in Canada and United States (Nguyen Thanh Son et al., 2016). This is caused by the important population of unimproved sows herd. The locale breed of sow represent about 12%, crossbred sows represent about 70%, the rest are exotic sows (Nguyen Thanh Son *et al.*, 2016).

The third is the big fluctuation of animal feed and product price in the market. On average, the price of animal feed in Vietnam is 10% to 20% higher than that in surrounding countries. One of the reasons is the excessive dependence on the imported raw materials for feed formulation. In which, about 45% of energy feed, more than 70% of protein and more than 85% of additives are imported. Thus, farmers have many difficulties in expanding their production scale.

The fourth is the challenges to threats of environmental pollution from intensive animal production. The expansion of animal herd size produces an extreme amount of wastes (including liquid and solid wastes) that need a comprehensive and effective management and treatment system. However, a high proportion of animal wastes have not been managed and treated well. In estimation, around 40 to 50% of total produced animal waste have been treated before discharging into the surrounding environment.

Last but not least, the food safety is now also an increasing concern of domestic consumers. The overuse of antibiotic and other chemical substances for both purposes of disease prevention and treatment and growth stimulation causes an alarm of food safety. By MARD (2015), there were 7 feed meals that used forbidden substances (Auramine O, Salbutamon, Clenbuterol) in order to increasing the lean meat, growth rate, creating better color of the products and preventing the diseases.

2.3. Strategies and policies towards sustainable development of livestock production sector

According to the development strategy of livestock production sector towards 2020 issued by the Ministry of Agriculture and Rural Development, livestock production will be fostered to achieve a rapid growth rate (5.5-6% per year in 2015-2020) and share a high proportion in total agricultural output value (about 42% in 2020). In order

to gain that goal, the livestock production will be oriented toward a concentrated intensive farming system with a high biosecurity, food safety, and environmental protection. In 2020, the share of livestock products provided by intensive large-scale farms will be 68-70% in total animal-original products. It is expected that 70-72% of animal feed will be formulated and produced from the factories. The environmental issues will be well-controlled by developing the waste treatment system at the livestock farms. There will be 78-80% of total animal farms having the waste treatment system in 2020 (Ministry of Agriculture and Rural Development 2008). Furthermore, the disease prevention and control program should be implemented effectively to limit its affects on livestock production of farm households.

Beside, the government also set up a program of restructuring the livestock production sector. The restructure program of livestock production sector will be focused on the small and medium-scale farms to enhance its value-added and sustainability. The main contents of this program is to support small and medium farms by providing partly the expenses of breeding (support half of expense of semen for artificial insemination) and expenses of waste treatment system construction (support by 50% of total expenses of waste treatment system construction, but not more than 5 million dongs per farm) (Prime Minister 2014). By Decision No. 984-QD-BNN, program of restructuring the livestock production sector will change the animal population: in period of 2013 to 2020, the pig population in Red River Delta will be decreases from 25.74% to 15% of total swine population of the country, from 10.51% to 5.0% in South-East region. Contrary, the mountainous area the number of pigs will be increased (northern mountainous area will be increased from 24.1 in 2013 to 30% in 2020). The redistribution of animal population will create more jobs for the remote and backward areas, decrease the pollution causing from livestock production.

3. MAIN AXES OF RESEARCH ON ANIMAL SCIENCE AT VIETNAM NATIONAL UNIVERSITY OF AGRICULTURE (VNUA)

Vietnam National University of Agriculture is one of the leading universities in agricultural research fields in Vietnam. Over the last decade, a number of research programs on animal science conducted by researchers at the university have been applied to the livestock production of many farms. These researches have been focused to the issues and requirements of livestock production of farm households towards more sustainable development. These researches covered all aspects of livestock production of farms as followings.

3.1. Animal genetic and breeding

- Over the last decade, the researches on the animal genetic and breeding have been focused on the improvement of animal productivity and product's quality by the importation of exotic breeds and the crossbred programs. Since 2008, a herd of pure stress negative Piétrain (*ReHal* Pietrain) pig breed was imported directly from Belgium to VNUA. Pietrain and PiDu boars (Pi had been crossed with Duroc breed) were supplied to the farms. Recently, a herd of other breeds with high performance (Landrace, Yorkshire, Duroc) have been imported from European countries to improve the genetic of pig herds. The pig breeding farm at VNUA is now one of the important farms to provide good breeds for farmers.

Using the gene technology in selecting pigs and chicken (selecting pigs with genes relating to litter size (RNF4; RPB4, IGF2) to stress resistance (halothane), to disease resistance (FUT1), in chicken with the genes relating to growth.

Research programs on the conservation and development of some native animal breeds. Some chicken and swine breeds have been studied in order to find out the ways of effective conservation such as Ho, Dong Tao and Mia chickens and Ban pigs. These native breeds were very famous in Vietnam and are in danger of genetic loss. Thus, our research uses the in situ and ex situ conservation of these breeds for biodiversity.

3.2. Animal nutrition

In the domain of animal nutrition, we have concentrated on:

- Treatment of by- agricultural products (rice straw, maize, etc.) and by-products of food processing (ethanol production from rice and manioc …) to produce animal feed.

- Looking for different protein resources at low price in animal feed (red worm, some kinds of bean…).

- Improving the diet quality by using the micro-organisms to fermentation.

- Trying some different feedstuffs in order to improving the product quality.

3.3. Food safety

Recently, the food safety is increasingly alarmed due to the overuse or abuse of antibiotics, minerals and other forbidden substances (hormone) in livestock production of several farms. Some researches regarding the antibiotics usage (in animal production, and in aquaculture) and detection method system of antibiotic residues in animal-origin products have been carried out.

3.4. Animal waste management

As mentioned above, environmental pollution from intensive livestock production is now increasingly concerned by not only the producers, but also the government and community. Thus, our researches have firstly assessed the polluted level of water and air at animal farms as the basic for the management and treatment. Some researches were carried out as follows:

- Treating the solid animal waste (fermentation, bio-digester, raising red worm)

- Treating the used water after biogas

- Decreasing the gas emission by applying the appropriate diets (using the tannin and some kinds of oils)

3.5. Animal welfare

Animal welfare is a new concept in Vietnam, but increasingly concerned in the country in the context of accelerating international integration. It is of great importance to study about animal welfare, especially in the intensive production system. In recent years, researchers at our university are the leading in Vietnam in doing research on animal welfare on farm. Several studies have been implemented to identify welfare issues of pigs and chickens kept in different production systems at farm households. Moreover, a model pig farm has been also developed at the university to study on efficiency an improved welfare housing for sows (group housing and outdoor access). These studies are the basic for the expansion of animal welfare research in Vietnam. It also helps to make some recommendations for the improvement of animal welfare quality while ensuring a high productive performance and economic efficiency of livestock production of farm holdings, towards a more sustainable development of livestock production sector in future.

3.6. Commodity chain and marketing system development

Beside the researches on animal sciences, we also have several studies on the commodity chain of animal origin products such as pork, milk, etc. By applying the systematic and interdisciplinary approaches, we have analysed the value chain of pork and milk products and also identified factors influencing on its efficiency. Thus, several recommendations on developing the chain of animal origin products have been made to foster the livestock production of farm holdings, towards sustainable development.

4. CONCLUSIONS

Over the last decade, the livestock production sector in Vietnam has changed significantly into an intensive production system. There is a decrease in the number of small-scale farms, while an increase trend in number of medium and large-scale farm holdings. Pig, poultry, and cattle production systems have been all developed at a high rate in order to meet the increasingly requirement of animal origin products of consumers.

As one of the leading research centers in animal science in Vietnam, researchers at Vietnam National University of Agriculture have conducted various studies on a wide range of animal science fields, covering the animal genetic and breeding, animal nutrition, food safety, animal waste management, animal welfare, and commodity chain and marketing system. These research topics are all oriented to the emerging issues facing by producers. Many of our research results have been applied effectively by animal keepers, contributing significantly to the sustainable development of livestock farming systems in Vietnam.

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TOWARDS A SUSTAINABLE AQUACULTURE IN VIETNAM: CURRENT STATUS AND SELECTED RESEARCHES

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ABSTRACT

Aquaculture is a fast growing food-producing sector in Vietnam; where its production reached 3.553 millions in 2015. The aquaculture production of Vietnam has developed differently depending on geographical locations. The Mekong river delta (MRD) located the Southern part of Vietnam is the main region, where accounts for over 70% of total production and farming area (*e.g.* the production of 2.516 mil. tons and the culture area of 1.37 mil. ha in 2015). The aquaculture of Vietnam has been a traditional practice for home-food since 1960s, and has become a business practice after 1980s. There are many species of fishes, crustaceans, mollusks are being farmed, but the commodities that have high production and play a vital role of export are marine shrimps (*e.g.* black tiger shrimp - *Peneaus monodon* and white-leg shrimp - *Penaeus vannamei*) with 0.596 mil. tons and striped catfish (*Pangasianodon hypophthalmus*) with 1.22 mil. tons in 2015. Aquaculture in Vietnam, in common with all other countries, is facing challenges for sustainable development. It has been realized that the sustainable development and responsible production of aquaculture, in the long run, require strong enforcement of regulation and better governance of the sector, the participation of the producers in the decision-making and regulation process, the involvement of researchers in generating new technologies*, etc.* In addition, the sustainable development of aquaculture in Vietnam, especially in the Mekong river delta has to take into account of climate change impacts. In terms of research, there are a number of issues required such as improvement of farming technologies (such as pond management, disease control, feed and feeding practice, alternative use of bio-products for drugs and chemicals), zonation of culture areas, reduction of environmental impacts, and increasing ratio of certified farms using national and international standards. In this presentation, the authors would like to review major issues of aquaculture development such as production growth, production systems, food safety and export and also to highlight selected current researches conducted by Can Tho University related to the alternation of drug and chemical uses in aquaculture and potential effects and adaptation of major aquaculture species to climate change, which hope to contribute to the sustainable development of aquaculture sector.

Keywords: Aquaculture, Mekong river delta, Sustainable.

SUSTAINABLE CONSERVATION AND APPROACH FOR UTILIZATION OF NATIVE CHICKEN AMONRAT MOLEE AND KANOK PHALARAKSH

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ABSTRACT

Southeast Asia is the region of plentiful variety of native chicken those are valuable natural resource. However, the situation of the chicken particularly in the aspect of diversity is sensible. They are confronting with high chance of declining diversity of the chicken since some factors such as the expansion of industrial commercial broiler, or the transition from local society to urban society, for example. The declining of diversity will impact particularly to mankind, they have no genetic stock for coping future need, no saving, insecurity of protein diet especially for poor people. These are the reasons why native chicken need to be existed.

Conservation and utilization are the approach for sustainable conservation. The objective of this presentation is for exchange direct and indirect experience of conservation and utilization approach those were implemented in Thailand.

Identification status of native chicken is needed since different statuses are different meaning, and different utilization. In Thailand, the native chicken was divided into 3 statuses, firstly, is upstream chicken, that means chickens which belong to villagers in remote area that disperse thorough the country. Secondly, is midstream chicken, they were drawn from upstream and collected in government stations. Thirdly, is downstream, they were developed their genetic for specific utilization by private companies, government units, or universities.

The upstream chicken is the pool of genetic variation that need to be secured. The aspect of In Situ conservation, the theory of population genetics, and system analysis need to be integrated, and collaboration between some government organizations need to be performed. Information about the effective number of the chicken which will be used to evaluate the conservation rate, and inbreeding coefficient, factors may effect on the existence of the chicken in the status, are necessary information. The information inform us the situation of the chicken and also help us to speculate the situation in the future. Policy and strategy plan of conservation and utilization can be performed by using this information. The work to gain this information now is financial supported by Biodiversity-Based Economy Development Office (BEDO).

The midstream chicken is buffer that was used to protect the upstream chicken from various interferences. To maintain genetic variation, therefore genetic selection is prohibited, random mating is applied and effective number of the chicken in each herd must be concerned. Nowadays, there are 4 herds (at least), under the responsibility of Department of Livestock Development (DLD). The chickens were collected from the upstream chickens which disperse across country since 2002, with collaboration between Thailand Research Fund (TRF), DLD, and some of universities, Ubon Ratchathani University, for example.

The downstream chicken is developed herd for specific purpose, alternative commercial broiler, or layer, for example. Their genetic will be changed by artificial selection. Theory of animal breeding, and molecular breeding were used in breeding program. Moreover, some techniques of modern biotechnology will be applied to identify groups of gene involved with interest traits, and gene markers will be identified to aid in selection. In Thailand, there are some herds of the chicken, Korat chicken which was established under the collaboration between Suranaree University of Technology (SUT), TRF, and DLD, is one of downstream chicken. They are used as the tool of small holder farmers' occupation.

This is the summarized picture of conservation and utilization of native chicken in Thailand. Since situation of native chicken and related factors of the existence are dynamic, monitoring the situation particularly in the upstream is important mission of government. Moreover, in the aspect of genetic diversity, it is boundless, platform of collaboration between organizations, either internal or external country particularly in ASEAN community should be established. Working together is an approach that can ensure that the sustainable conservation will be happen in the region.

Keywords: ASEAN, native chicken, sustainable conservation, utilization.

URGENCY OF FARMER PARTICIPATION IN LIVESTOCK VALUE CHAIN DEVELOPMENT IN INDONESIA

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ABSTRACT

Small scale livestock farmers may also be legitimate part of the supply chain. They may be seen as producers of live animals and animal products that will distribute quality products through many actors in supply chain to end consumers. The majority of small farmers in Indonesia raise animals for sale. Even in the most remote areas, many small farmers are connected to markets, selling small amounts of animals or products in a local village market or to a trader. The farmers may sell surplus that they cannot consume themselves. This paper discuses about current condition of livestock development. How farmer participated in development, market oriented, and value chain.

1. INTRODUCTION

The Government of Indonesia has set a general policy to be self-sufficient by 2010 in beef consumption, but there are not sufficient government resources to support this policy. In the past the government did not implement many policies for promoting the livestock – meat sub-sector. In the 1990s it did encourage with certain incentives to feedlots to develop nucleus and outreach programs with small farmers to feed imported cattle. After the crash in 1997, the partnership feeding initiative declined, Although it is no longer mandatory, a few feedlots have continued on social development grounds (Sullivan and Diwyanto, 2007). The new policy target set by the government is to be self-sufficeient in beef consumption by 2014. A large number of cattle are imported from Australia to support this program and at the same time the government has initiated a breeding program to focus on highly quality of breed. One major challenge in the breeding program is of course that the cattle production is spread out among so many smallholder farmers.

The smallholder agricultural (including livestock) sector plays an essential role in ensuring food security, economic growth and employment creation in Indonesia. The smallholder sector in the country is characterized by diversified farming of crops and livestock. Livestock may be categorized into three major areas of production: beef, dairy, and small livestock (e.g. poultry, goats, sheep, and pigs). More technical assistance, marketing competences, and investments are required in order to commercialize the smallholder agricultural sector. Such technical, financial, and commercial contributions may boost production and enhance food security at the household, national and regional levels.

Smallholder farmers in Indonesia operate small, mix crop-livestock farms, which rely on common property feed resources. These feed resources have been

heavily over-utilized. Therefore, many farmers are faced with declining livestock productivity because of lack of feed, so they have to spend more and more time herding and feeding their animals.

In rural areas of Indonesia, markets are often poorly developed. Smallholders are thus unable to take advantage of potential market opportunities and must pay high costs to overcome market imperfections. Farmers often have troubles in receiving credit, obtaining information on market opportunities or new technologies, purchasing certain inputs and in accessing product markets, and when markets are accessible, farmers may be subject to price fluctuations. Such difficulties are barriers to their development and represent a 'bottleneck' in the development process of smallholder farming (Patrick, 2004).

To be competitive in the future, smallholder livestock producers need to intensify and be able to provide higher value products. Major constraints faced by smallholders are the relative higher costs of quality inputs (e.g. improved animals and better feed stuffs) and increased knowledge to produce more efficiently in a sustainable manner. The greater risk associated with the loss of an animal due to higher intensification is a further constraint. Public investments has a central role in overcoming the constraints through knowledge and technologies that deliver quality feed, animal health, breeding, technical advice and other services (McDermott, 2010).

Smallholders should be supported to be competitive when vertically integrated livestock food chains are developed. In such developments economies of-scale are becoming more important for processing, distributing and marketing of livestock products, particularly when enhanced standards for quality and safety are demanded. However, it is important that any investments in chain and market developments is planned carefully with serious considerations given to the commercial viability and meaningful participation of poor people (McDermott, 2010).

Major constraints in livestock production faced by small farmers in Indonesia are:

- Fluctuations in weather. Droughts (in some areas), foods, and unpredictable weather patterns have direct effect on livestock feed availability and water supply and consequently the quality and quantity of production

- Marketing. Many farmers cannot acces markets due to poor infrastructure. Some roads are impassable during the rainy season, hence a lot of waste of livestock products

- Expensive breeding services such as artificial insemination as well as expensive quality breeding stock

- Unavailabity of suitable credit facilities to livestock farmers especially in the small-scale sector

- Lack of commercialization of the smallholder sector, i.e. high degree of subsistence farming

- Poor storage capacity for farm produce

- Unfavourable international trade policy and barriers

The adaptation of new livestock farming technologies and practices remains important for any livestock farmer as diseases, high mortality, low productivity and low income are general major constraints in livestock production. This leads to the major problem that many livestock farmers in Indonesia produce

below the potential capacity in comparison with livestock farmers who operate in the developed countries. This happens despite a large number of Indonesian organizations at national/state levels are generating and transferring information, knowledge and skills among livestock farmers.

The question is therefore whether the large numbers of smallholders in Indonesia will be able to meet the growing demand for livestock products in Indonesia, or these products will be provided by other producers (such as big private companies or oversea farmers)?

2. PARTICIPATION TOWARDS EMPOWERMENT

The term participation is used in many ways, meaning different things to different people and agencies. Most commonly, participation refers to people's voluntary contributions to projects in the form of labor, cash or kind. This may be one important aspect of participation (Muller-Glodde, 1991). Participation may also means co-determination and power sharing throughout the development program cycle. More specifically, in this sense participation is related to problem identification and ranking, decision-making, planning, implementation including mobilization of resources, benefit sharing, and monitoring and evaluation of a program. The programs should be understood as part of a wider development process. Thus, the participation in such programmes may be regarded as steps towards political and economical empowerment of hitherto inarticulated people living in poverty. Participation of the farmers in such processes is in a sense empowerment of their business activities.

In this regard, farmer paticipation in value chain is realized when the farmers are actively involved in the process of decion making, planning, implementation, sharing of benefits, and monitoring and evaluation of value chain development. Awareness of the farmer as a producer not only increase the chance of increasing standard of living but also the chance to meet the comsumers' expectation and satisfaction. Deschler and Sock (1985) in Selener (1997) mentioned that the last step of genuine participation type is empowerment where the power and authority to develop their work conditions is in the hand of local people.

Empowerment is vital for sustainability. Confronted by short project timeframes and limited funding, development organizations often make the mistake of trying to intervene too much – for example, by taking over management of the chain, rather than enabling the farmers' organization (or other players) to do it themselves. When the project finishes and the development organization withdraws, the value chain is left without the key resources, so it collapses. Intermediary organizations should aim instead to support farmer organizations to strengthen their capacity to develop and manage chains or chain activities. They should embrace the following principles before engaging smallholders in a value-chain development process. Principles that may help ensure that interventions target development objectives such as equity, gender, sustainable development, and poverty reduction. The ability of farmers to respond to development interventions depends on a number of factors. These factors include (KIT, Faida, IIRR, 2006):

- The farmers' access to capital assets: economic/financial capital, physical capital (such as infrastructure), natural resources (land, soil, water), human capital (skills, education, labour), and social capital (ability to organize, links with outsiders, etc.).

- The level of social integration of the community. Some groups are relatively isolated (e.g., forest dwellers, pastoralists and ethnic minorities), while other communities are in regular contact with urban centres and have strong social and economic ties with influential outsiders.

- The stability of the environment where the community lives. Has the community been exposed to security problems such as civil war or ethnic conflicts? Is it recovering from a disaster, or has it been exposed to economic shocks? Is it subject to chronic emergencies, such as repeated drought, disease or political discrimination?

3. PARTICIPATION AND MARKET-ORIENTED

The described types of participation focus on enabling smallholder farmers to increase their level of competitiveness, to produce marketable products rather than trying to sell what they have already produced and also seeking new market opportunities that offer higher levels of income. These goals can be achieved through better economic coordination and institutions. Farmers' participation in the development and governance of agricultural and food supply chains can improve their ability to make the right marketing decisions. Farmer organizations can play a key role of organizing economic activities beyond local boundaries. They can build up relationships with various chain actors (those involved in producing, processing, trading or consuming a particular agricultural product) and create commitments from various actors to cooperate on mutually beneficial actions and investments and thus create enhanced value chains.

Small scale livestock farmers may also be legitimate part of the supply chain. They may be seen as producers of live animals and animal products that will distribute quality products through many actors in supply chain to end consumers. The majority of small farmers in Indonesia raise animals for sale. Even in the most remote areas, many small farmers are connected to markets, selling small amounts of animals or products in a local village market or to a trader. The farmers may sell surplus that they cannot consume themselves, for example a farmer may sell eggs or milk to help cover household expenses. They may raise animals for cash such as native chickens and other native poultries such as duck and quails or native pigs. They may also process some of their produce and sell it to their neighbours, for example: salted-eggs from ducks. Selling their products make these farmers part of a value chain. The chain may be very short (they may sell directly to the consumers), and some take a long chain road to the final consumers. In this situation the question often is how these farmers can improve their performance as a chain actor. They may be able to increase the quality or volume of their output, or improve ther farm management in order to enhance their incomes and thus improve their livelihood. For many farmers, this is a necessary first step before any other type of chain development may take place.

Many small farmers have traditionally produced their own basic food needs, and sold surpluses to provide for additional needs of the household. Moreover, raising livestock is still becoming secondary activity of smallholder farmers to support integrated farming system. Examples for this commodities are native chickens, ducks, beef cattle, sheep, goats, and pigs. However, this livelihood pattern is increasingly seen as insufficient to raise rural incomes, provide the stimulus for rural development and alleviate poverty. Therefore, more efforts should be initiated

to increase rural economic activities by increasing the quality of animal production and diversify products to the markets. Efforts to link small farmers to markets face a number of constraints:

- The need to diversify into new markets for higher value products and/or add value to current products through improved quality, niche market, etc.

- The need to build economies of scale to compete with large farms and increase bargaining power with buyers that require certain quantities through collective action, farmer associations/groups, cooperatives, etc.

- The risks associated with major changes e.g. the change to new production systems and/or to new relationships to other chain actors

- The need to reorient research and extension from a "supply-driven" mode of generating and disseminating information led by researchers to a "demand-driven" or "client-oriented" mode of service delivery.

- The need to improve information flows and develop positive synergies between the different actors in the production and marketing system.

4. PARTCIPATION IN VALUE CHAIN PROJECT CYCLE

To know how the value chain development through farmer participary approach can be effective, let us look at the value chain project cycle. Figure 1. describes the project cycle of the value chain. Effective value chain development programs described by USAID (2008) are designed and carried out in a dynamic process referred to as the project cycle. The cycle comprises five phases: Value chain selection, Value chain analysis, Competitiveness strategy, Design and implementation, and Monitoring and evaluation.

Value chain selection is the process of prioritizing industries or value chains based on their potential for growth and competitiveness, impact, and other benefits targeted by a donor or implementer. Cross-cutting objectives may also be considered, such as conflict mitigation, food security or natural resource management. The selection process is inherently subjective, and there is always a danger of selecting a value chain for the wrong reasons.

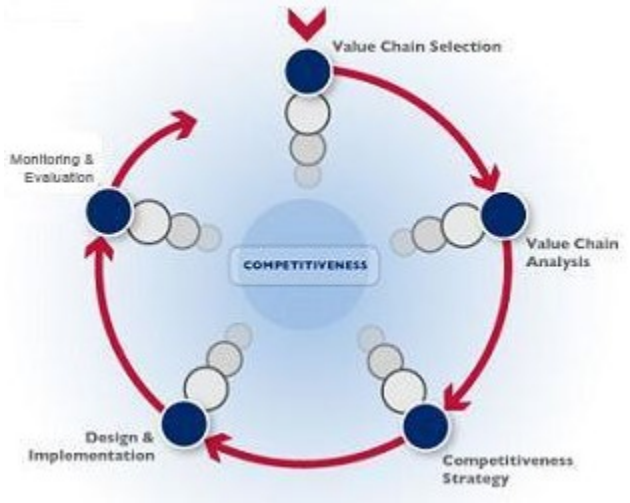

Figure 1. Value chain project cycle (adopted from USAID, 2008)

Value Chain Analysis to identify the greatest opportunities for improving value chain competitiveness, the constraints to exploiting these opportunities, the set of stakeholders who will benefit from investments in upgrading, and a subset of this group with the incentives, skills, resources and power to help drive or make these investments.Value chain analysts gather information about end markets and chain-level opportunities and constraints that, taken together, illuminate how the market/value chain system functions and - based on trends - how it might change over time.

Competitiveness strategy is a plan for moving the industry toward sustained growth. The list of key constraints to exploiting identified opportunities generated during the value chain analysis is used to develop a competitiveness strategy**.** It represents a vision for how firms might collaborate to achieve growth, rather than seeing one another solely as competitors.

Design and Implementation should include a causal model that articulates how project interventions will facilitate implementation of the competitiveness strategy. Facilitation is defined as an agent or action that stimulates a market system without becoming part of it. The goal of facilitation is to foster behavior that drives upgrading and that makes the value chain more competitive by capitalizing on firm-level incentives and by cultivating collective action.

Monitoring and Evaluation. A well-designed monitoring and evaluation process provides information to program managers and implementers that is critical to judging the effectiveness of particular interventions so that modifications can made to optimize project impact. The goal of a monitoring and evaluation system is to increase the density and quality of information flow to improve decision-making at all levels, from the field through managers to donors and other stakeholders. Since those changes will be most helpful during a project rather than after, the monitoring and evaluation are an ongoing feedback mechanism used throughout the project's implementation.

Monitoring and impact assessment are a continuous process. Feedback on the effectiveness of the project interventions is necessary throughout implementation in order to make changes before it is too late. Monitoring is critical to this process of continuous reflection and adjustment. Impact assessment benefits from a baseline survey at the beginning of the project and one or more follow-up assessments to track results over the course of the project.

5. LIVESTOCK MARKETING

Most livestock produced by smallholder pastoralists and farmers are marketed by private entrepreneurs who, operating as a marketing chain, collect, regroup and distribute the livestock and livestock products to terminal markets. Although the marketing chain is well known, the economic and institutional barriers to livestock marketing (transportation costs, quality standards, inadequate and uncoordinated livestock market information systems) limit livestock-sector development, with a consequent negative impact on the welfare of the large population of smallholder producers and others who depend on the sector for their livelihoods. The potential exists for an improved and well-functioning market that will enable smallholder producers to derive greater benefits from their production

activities. Constraints The following are some of the main constraints:

- External constraints: Adverse macroeconomic conditions (high taxes, high interest rates), lack of institutional support;

- Quality constraints: Little understanding of processors' requirements, lack of laboratories and instruments for quality control, price and quality of the veterinary services;

- Financial constraints: Lack of capital to invest in assets, equipment and inputs that would improve quality;

- Gender constraints: In comparison to men, women face higher disadvantages, in particular in terms of mobility, access to assets and to productive resources, and access to market information, with the result that they find it more difficult to access and maintain profitable market niches and capture a larger slice of incomes;

- Infrastructure constraints: Lack or inadequacy of, among others, roads, electricity, weighing stations, cattle dips, slaughtering and processing facilities (which raises transaction costs, exacerbates information asymmetries between producers and traders, and discourages investment in processing);

- Information constraints: Limited access to market-related information (e.g. on prices, value chains, competitors, consumer preferences);

- Skills and knowledge constraints: Lack of business management skills (e.g. production planning) and, in particular, inadequate access to the knowledge and technologies needed to meet rising sanitary standards, making it extremely difficult for smallholders to gain credible certification of compliance with marketing requirements; and

- Market constraints: Low demand, a multiplicity of intermediaries (which increases the charges and shades the transparency of the operation).

However some needs to be fulfiled to reduce the contraints:

- Secure and adequate access to basic production inputs together with riskcoping mechanisms for natural disasters and price shocks;

- Dissemination of livestock market information to livestock producers;

- Strong relationships among various chain actors (including commitments from these actors to cooperate on mutually beneficial actions/investments) and strengthened farmers' organizations;

- Policies and strategies to enhance the ability of smallholders and small-scale market agents to compete in livestock product markets;

- Standards and branding mechanisms to identify high-quality livestock products;

- Kick-starting of domestic markets to allow the poor to exploit market opportunities;

- Reduced fees on the sale or slaughter of livestock;

- New or adapted marketing strategies (for example, promotion of alliances with fairtrade chains);

- Adequate responses to volume demand and ability to expand to match increased demand;

- Product differentiation to create niche markets; and

- Linking of poor livestock keepers to expanding urban markets.

6. IMPLICATIONS FOR PRACTICE

The global demand for livestock products is predicted to double in the next 20 years due to world population increases, urbanization and economic growth

(Delgado, 2003). The rapid growth in this sector, will have a strong impact on world markets, creating opportunities for the rural poor but also posing significant threats. Trade chains are becoming more complex where the standardization and food safety and quality requirements imposed are making it increasingly difficult for poor livestock farmers to participate in livestock globalized markets.

Value chain approaches play an important role in characterizing and understanding the complex networks, relationships and incentives that exist in different livestock systems. They further provide a framework for mobilizing pro-poor development in the context of agri-food networks that facture livestock across a range of livelihood-improving roles for the rural poor.

Enhanced collaboration among e.g. government agencies, non-governmental agencies, farmers, cooperatives and private retailer and agribusinesses offer the greatest potential for applying value chain concepts for smallholder farmer, with the aim of increasing the income and employment opportunities among poor farmers. The supply chain apporaches can be applied to a wide range of situations either directed farmer groups and/or other beneficiary groups, including youths and women's groups. It can be used in relationship to specific sub-sectors, commodities or groups of products and/or in formulating e.g. a strategy for including smallholder farmers in livestock development projects.

With respect to future research priorities, value chain approaches in research, teaching and dissemination (for example in public-private partnership between universitiess and research institution) can improve the technologies available to small-scale producers and processors, while capacity building can help smallholder farmers to meet new quality and safety requirements, as well as learning how to manage cash flows. Value chain approaches may also facilitate and support the establishment of producer organisations, which allow economies of scale in buying inputs and selling products.

Improved business services to small-scale farmers and agribusinesses might help to improve quality and production efficiency by reducing costs and expanding operations. It is important that governments anticipate future vulnerabilities and build the capacities of chain participants to innovate, diversify or exit as structural changes occur in markets. Changes value chains can increase vulnerability if these changes favour products and services susceptible to disrupt and large shifts in demand and prices. The risks of value chain development to increase vulnerability underscore the importance of appraising comparative advantage investment requirements and an assessment of risks mitigation before any new interventions are implemented.

In the future, there is ample justification to consider a participatory strategy for value chain development as a key concept for defining and formulating agricultural development interventions in Indonesia. More specific, it is suggested that donors, regional organisations, researchers, and decision makers in government and aid agencies consider milk and beef as well as related products such as lamb, mutton, chicken etc. as the most promising production branches for agribusiness ventures in rural areas. All these braches have the potential to substitute for food imports and thus improve the national food security.

Higher emphasis must be placed on the establishment of agribusinesses and farm related enterprises in rural areas in order

to mitigate the pressures of migration to major urban areas by stimulating the growth of employment and income opportunities. Increased rural development may be an important step in providing fresh, locally grown food for growing urban populations.

In the livestock sector there could be support for creating domestically available feed resources and for the construction of clean and safe slaughter facilities close to major consumption areas. The livestock sector is an important sub-sector which has a considerable potential for growth. A sustainable growth in the livestock production may become a substitution for unhealthy and nutritionally poor imported products as well as a device to create new employment opportunities along the entire livestock value chain, i.e. from domestically grown feed to marketing, veterinary health and distribution of the final livestock products to the consumers.

A key area for future government agricultural support is improvements of the infrastructure, especially with respect to transportation and communication. Implementation of improved communication and information technologies can have an immediate positive impact since it provides farmers with better access to market and production information and thus enables them to optimise their production and realise new income opportunities.

It is now generally accepted that for any development intervention to be successful and sustainable, in the long run, some kind of a participatory approach is necessary to engender ownership of development projects by the beneficiaries.

Lessons learned from previous experiences with participatory approaches in the development of livestock systems in developing countries:

- It is more secure to produce for a well-known market rather than seek new market opportunities

- Livestock trade is less risky within a given developing country (domestic segment) than between different countries (cross border segment). This is mainly due to trade barriers as well as marketing risks lack of export credits and the increased risk of losing animals associated with cross-border trade

- Transportation and handling costs are the largest components of marketing costs. These can be reduced by lowering tariffs and taxes, including fuel tax. The elimination of corruption and e.g. illegal taxation along trade routes could also contribute to improved market performance

- Enhanced direct communication between buyers down and/or upstream in the value chain and the primary producers can be a powerful tool to boost the income of producers and increase their competitiveness

- Learning innovation and adaptation are essential competences for sustaining competitiveness

- Low market integration can best be overcomed by developing and effectively deploying livestock market information systems at both national and regional levels

- Market institutions such as livestock traders' associations and intermediaries can play a key role in reducing transaction costs, facilitating livestock trade and achieving better market integration at the national and regional level.

In a fast-changing business sector many other factors may potential limit smallholder participation including low market prices, an inadequate infrastructure, high quality standards, financial constraints, difficulties in accessing productive assets and lack of knowledge. Within this context,

development practitioners are called upon to support the integration of small-scale and poor farmers into formal livestock markets as an important step towards reducing rural poverty.

Effective, market-oriented livestock production can potentially increase output quantity, quality and prices; and improve the margins with the utilization of more efficient production and distribution technologies (FAO, 2007).

Attention should be focused not just on increasing productivity and improving animal health services, but also on increasing advocacy efforts through improved farmers' organizations, and building the capacity of local institutions to deal with the standards and regulations of regional and international markets.

7. CONCLUSIONS

It is better to produce for an identified market, rather than trying to sell what the farmers have already produced and then seeking new market opportunities. Livestock trade is more competitive and functions better within countries (domestic segment) than between countries (crossborder segment). This is mainly due to the high capital outlay, lack of credit and increased risk of losing animals associated with cross-border trade. Transportation and handling costs are the single largest component of marketing costs. These can be reduced by lowering tariffs and official taxes. The elimination of illegal taxation along trade routes could also contribute to improved market performance. Direct communication between end buyers and producers can be a powerful tool in helping producers to understand the implications of competitiveness. Learning and innovation are essential for sustaining competitiveness. Low market integration can be best overcome by developing and effectively deploying livestock market information systems at national and regional levels. Market institutions, such as livestock traders' associations, and intermediaries, both at local and national levels, can play a key role in reducing transaction costs, facilitating livestock trade and achieving market integration at the regional level.

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ANIMAL SCIENCE

PRODUCTIVE PERFORMANCE AND YOLK FATTY ACID COMPOSITION OF LAYING HENS FED DIFFERENT DIETARY N - 6 TO N - 3 FATTY ACID RATIOS

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ABSTRACT

The effect of different n - 6 to n - 3 fatty acid ratios in laying hen diets on productive performance and yolk fatty acid composition is investigated. A total of one hundred and eighty 42 - wk - old ISA brown laying hens were randomly assigned to 3 dietary treatments based on n - 6 to n - 3 fatty acid ratios in diets: 10:1, 5:1 and 1:1, respectively. Egg production, egg weight, feed intake, feed conversion ratio, and egg yolk fatty acid composition were determined at 12 wk of experimental period. The results showed that egg production, egg weight and feed intake were decreased in laying hens fed dietary 1:1 ratio of n - 6 to n - 3 fatty acid ($P < 0.01$), but feed conversion ratio was better in laying hens fed dietary 1:1 than in those fed 5:1 and 10:1 ratio of n - 6 to n - 3 fatty acid, respectively ($P < 0.01$). The proportion of n - 3 fatty acids in egg yolk was higher ($P < 0.01$) and the n - 6 to n - 3 fatty acid ratio was lower ($P < 0.01$) in laying hens fed dietary 1:1 than in those fed dietary 5:1 and 10:1 ratio of n - 6 to n - 3 fatty acid, respectively ($P < 0.01$). These data indicated that lowering the dietary n - 6 to n - 3 fatty acid ratio had negative effect on egg production and egg weight of laying hens, but enhanced beneficial n - 3 fatty acid and decreased n - 6 to n - 3 fatty acid in egg yolk.

Keywords: Egg production, laying hens, n - 6 to n - 3 fatty acid ratio, yolk fatty acid.

1. INTRODUCTION

The n - 6 and n - 3 fatty acids are polyunsaturated fatty acids that cannot be synthesized by human or animal body, and must be obtained from food or feed. Linoleic acid (LA, C18:2n - 6) and α - Linolenic acid (ALA, C18:3n - 3) are the precursors of the n - 6 and n - 3 family of fatty acids, respectively. The n - 6 and n - 3 fatty acids are both required for the body to function but have opposite effects when it comes to the inflammatory response and cardiovascular health. Too much n - 6 fatty acids and too little n - 3 fatty acids are among the causes for many diseases in modern society. Simopoulos (2008) reported that human beings evolved on a diet with a ratio of n - 6 to n - 3 fatty acid of about 1 whereas in modern diets the ratio is 15 to 16.7:1. This suggests that modern diets are deficient in n - 3 fatty acids. Daily optimum gain of n - 6 and n - 3 fatty acids can reduce risk to be cardiovascular disease, rheumatoid arthritis and neurological disease (Whelan and Rust, 2006; Patterson *et al.*, 2012). The optimum ratio of n - 6 to n - 3 fatty acids in human is not confirmed nor official announce from any organization. However, Haz *et al.*, (2004) and Wijendran and Hayes (2004) suggested that the optimal ratio of n - 6 to n - 3 fatty acid was about 5:1 or less.

The n - 3 fatty acids are mainly found in marine fish that may not be readily available everywhere, so other sources for n - 3 fatty acids are needs. It is known that fatty acid composition in eggs or meat can be modified by dietary fat. Recently, eggs have gained attention as alternative to fish oil and some oilseeds (such as linseed oil) as a source of n - 3 fatty acids (Gonzalez - Esquerra and Leeson, 2000; Garcia - Rebollar *et al*., 2008). These eggs are healthy, inexpensive and high essential nutrition for human especially for infant (Oliveira *et al*., 2010). Various kind of oils have used to feed laying hens for modify lipid composition in egg yolks, including linseed oil or marine fish oil for n - 3 source and soybean oil for n - 6 source. This present work was carried out to evaluate effect of different n - 6 to n - 3 fatty acid ratios in laying hen diets by using soybean oil for n - 6 fatty acid source and tuna oil for n - 3 fatty acid source on productive performance and egg yolk fatty acid composition.

2. MATERIALS AND METHODS

2.1. Birds and Experimental Diets

One hundred and eighty 42 - wk - old ISA brown laying hens were randomly assigned to 3 dietary treatments based on n - 6 to n - 3 fatty acid ratios in diets: 10:1, 5:1 and 1:1, respectively. Each treatment was represented by 4 replications with 15 birds each. Birds were fed ad libitum during the experimental period (12 weeks). The experimental diets were corn - soy based, formulated to meet the recommendations for major nutrients (NRC, 1994) and to be equivalent in crude protein and metabolizable energy. Soybean oil (n - 6 fatty acid source) and tuna oil (n - 3 fatty acid source) were analyzed fatty acid composition and used to adjust n - 6 and n - 3 fatty acid ratios in experimental diets. The nutrient composition of experimental diets are shown in Table 1.

Table 1. Nutrient composition of experimental diets

Nutrient composition (%)	n - 6:n - 3 fatty acid		
	10:1	5:1	1:1
Analyzed nutrient composition			
Dry matter	90.52	90.07	90.71
Crude protein	17.42	17.64	17.28
Crude fat	8.47	8.53	8.76
Crude fiber	3.92	4.11	4.07
Calcium	4.13	4.03	3.94
Total n - 6	59.05	50.47	26.96
Total n - 3	5.07	9.62	20.35
n - 6:n - 3	11.65	5.25	1.32
Calculated nutrient composition			
ME (kcal/kg)	2,907	2,903	2,900
Available phosphorus	0.38	0.38	0.38
Lysine	0.92	0.92	0.92
Methionine + cystine	0.66	0.66	0.66

2.2. Data collection

Eggs were collected daily, feed consumption was collected weekly for calculated hen - day egg production, feed intake (FI), feed conversion ratio (FCR, kg feed/kg egg) and average of egg weight.

2.2.1. Fatty acid composition Analysis

A total of 8 eggs per groups were collected randomly at wk 12 of experimental period and separated yolk from albumen then stored at -20°C for analyse fatty acid composition. Egg yolk and sample diets were analyzed following the method of Folch *et al.*, (1957) and Metcalfe *et al.*, (1966). Lipids were extracted in methyl ester form and run through gas chromatography (Hewlett Packard, HP 6890 series GC system).

2.2.2. Statistical Analysis

The results were statistically evaluated by analysis of variance (ANOVA), experimental design by completely randomized design (CRD) and Duncan's new multiple range test with P = 0.05 using SPSS 13.0 (2004).

3. RESULTS AND DISCUSSION

3.1. Egg production, Feed intake, FCR, and egg weight

The effect of different n - 6 to n - 3 fatty acid ratios in laying hen diets on egg production, feed intake, FCR and egg weight is shown in Table 2.

Feed intake in birds fed dietary 1:1 ratio of n - 6 to n - 3 fatty acid was lower than other groups ($P < 0.01$). This result was also found in previous study in broiler diets (Chashnidel *et al.*, 2010), due to unpleasant specific flavor compounds of tuna oil using in high level. However, FCR was better in birds fed dietary 1:1 than in those fed 5:1 and 10:1 ratio of n - 6 to n - 3 fatty acid, respectively ($P < 0.01$). Egg production and egg weight were decreased in birds fed dietary 1:1 ratio of n - 6 to n - 3 fatty acid than other groups ($P < 0.01$) due to the lower feed consumption.

3.2. Fatty acid composition of egg yolks

The effect of different n - 6 to n - 3 fatty acid ratios in laying hen diets on egg yolk fatty acid composition is shown in Table 3.

The proportion of SFA and MUFA were higher, but the proportion of PUFA was lower in birds fed dietary 1:1 ratio of n - 6 to n - 3 fatty acid than other groups ($P < 0.01$). The porportion of total n - 6 fatty acid was lower, on the other hand, the porportion of total n - 3 fatty acid was higher in birds fed dietary 1:1 than in those fed 5:1 and 10:1 ratio of n - 6 to n - 3 fatty acid, respectively ($P < 0.01$). These results can be explained by the rich in n - 3 fatty acid and poor in n - 6 fatty acid in tuna oil that used in high level in dietary 1:1 n - 6 to n - 3 fatty acid than other groups. It is widely known that the composition of dietary fat will affect the composition of fat deposited as body fat (Du et al., 2000). These results agree with the findings of Garcia - Rebollar et al., (2008) and Gonzalez - Esquerra and Leeson (2000). The ratio of n - 6 to n - 3 fatty acid in egg yolk was lower in birds fed dietary 1:1 than in those fed 5:1 and 10:1 ratio of n - 6 to n - 3 fatty acid, respectively ($P < 0.01$), according to the ratio of n - 6 to n - 3 fatty acid in experimental diets.

4. CONCLUSIONS

The low - dietary n - 6 to n - 3 fatty acid ratio had negative effect on feed intake, egg production and egg weight of laying hens, but enhanced beneficial n - 3 fatty acid and decreased n - 6 to n - 3 fatty acid in egg yolk.

Table 2. Effect of different n - 6 to n - 3 fatty acid ratios in laying hen diets on productive performance

Parameters	n - 6 : n - 3 fatty acid			SEM	P - value
	10:1	5:1	1:1		
Egg production (%)	92.22[a]	91.82[a]	89.02[b]	1.123	0.003
Egg weight (g)	59.79[a]	60.29[a]	57.48[b]	0.920	0.001
Feed intake (g/b/d)	104.79[a]	103.97[a]	93.33[b]	1.207	0.001
FCR (kg feed/kg egg)	1.80[a]	1.71[b]	1.63[c]	0.012	0.001

Note: [a,b,c]Means within rows with different superscript letters are significantly different ($P < 0.01$).

Table 3. Effect of different n - 6 to n - 3 fatty acid ratios in laying hen diets on egg yolk fatty acid composition

Fatty acid (% of total fatty acid)	n - 6 : n - 3 fatty acid			SEM	P - value
	10:1	5:1	1:1		
SFA	22.64b	22.70b	25.50a	0.141	0.004
MUFA	47.64b	48.54b	51.30a	0.182	0.002
PUFA	29.72a	28.76b	23.15c	0.119	0.001
Total n - 6	26.65a	23.76b	15.05c	0.107	< 0.001
Total n - 3	2.59c	4.58b	7.93a	0.055	< 0.001
n - 6:n - 3	10.29a	5.19b	1.90c	0.037	< 0.001

Note: SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, n - 6: omega - 6 fatty acids, n - 3: omega - 3 fatty acids; n = 8 per group; [a,b,c]Means within rows with different superscript letters are significantly different ($P < 0.01$).

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REPRODUCTIVE PERFORMANCE OF DONG TAO CHICKEN BREED

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Dong Tao chicken is a famous native chicken breed in Vietnam with a specific pattern of legs that is of great significance for conservation and development. This study aimed to investigate reproductive performance of Dong Tao chicken when using artificial insemination. A total of 8 cocks and 30 hens at experimental farm of Vietnam National University of Agriculture were used from February to July 2016. The cocks were trained to exploit semen by abdominal massage method. The semen parameters were ejaculate volume, sperm motility, sperm concentration and semen pH. The artificial insemination has been done by deposition 0.05 ml of semen into the oviduct of hens. The results showed that ejaculate volume, sperm motility, sperm concentration and semen pH were 0.49ml, 73.64%, $2.03x10^9$ sperms per ml and 7.46, respectively. The egg quality of Dong Tao chicken was relatively good for hatchery, with an average weight of 50.75g and a normal shape index of 1.30. The artificial insemination in Dong Tao chickens was highly effective, resulting in a higher rate of embryonated egg than that from natural mating (73.41% compared with 58.73%).

Keywords: Dong Tao chicken, native chicken, reproductive performance, semen quality.

CHARACTERIZATION OF THE KABYLE BREED OF HEN (THAYAZIT LEKVAYEL) AND ITS FARMING SYSTEM IN THE REGION OF CHEMINI AND BOUZEGUENE (ALGERIA)

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ABSTRACT

The rural poultry in Algeria is a supplier of popular products. The aims of this study are to characterize local chickens in Kabylie in terms of the livestock practices, management, flock structures, uses, performance and phenotypes. The present survey addresses this issue in the case of Kabylie, a mountainous coastal region of Algeria, and concerns 28 households raising poultry and a morpho - biometric description of 388 (290 females and 98 males) adult local chickens. The farming practices is characterized by a reduction in the time allocated for this activity and to deal with production costs which are aspects of an unproductive extensive livestock system considered as a secondary activity. The body weight is 2.03 ± 0.22 kg and 1.61 ± 0.19 kg respectively in the male and the female. The age at first egg, egg weight, egg production per hen per clutch, number of clutches per year, hatchability and number of chicks weaned are 28.00 ± 3.82 weeks, 49.03 ± 3.66 g, 13.57 ± 1.64, 3.82 ± 1.14, 89.29 ± 9.79% and 7.82 ±1.75 respectively. The comb type is mostly single (89.43%). Skin colours are principally white (39.18%) and yellow (39.43). The comb and wattles are mostly red (93.30). Shanks colours are principally yellow (49.74%) and white (24.23%). The most common plumage colours are salmon (14.43%), white (12.11%), black (17%), grey (9.54%) and gold (9.02%). The genetic improvement of local poultry breeds is also to consider, provided that the suitability of the animal with respect to the context of its breeding is preserved.

Keywordss: Algeria, biodiversity, indigenous chicken, Kabyle breed, livestock practices, morpho - biometric description.

1. INTRODUCTION

In recent years, the management of animal biodiversity has become an important issue in the international scientific community due to changes in large - scale production systems. Animal genetic resources are capital for sustainable development and production of poultry. However, a gradual and relentless depleting of available breeds is now rife at the scale of the planet. The appearance of diseases and epidemics, natural disasters and other conflicts also threaten these resources either through direct extinction or indirect effects such as the reduction in suitable habitat. Traditional poultry breeds contribute significantly to meat and egg production. Indigenous breeds represent in excess of 80% of the world poultry production (Besbes, 2009). However, most of these breeds have not been surveyed,

and not even known to the scientific community. According to the FAO, about 40 % of all avian breeds have an unknown risk status (Besbes, 2009). Important efforts are, therefore, necessary to evaluate these breeds.

The efficient management of animal genetic resources in general and avian, in particular, requires first the identification of the concerned breeds. But, it also requires their statistics such as their population sizes, their geographic distributions (their habitats) and, where financial means are available, their genetic diversity (Moula, 2012).

«The best-known native chicken breed of Algeria is called Kabyle. This breed was already described in Chasse et pêche 1927 » (Luuk Hans, 2014). Moula *et al.,* (2012a) reported a noticeable change in the poultry genetic resources in Kabylie. The period at which they situate the beginning of this change is the early 1990's. The nature of the change discussed is a widening of phenotypic variability, a decrease in flock size and a loss in « quality » and flavor of both meat and eggs. The original phenotypes are described as predominantly black with some white in the plumage (pure black, mottled or barred) and with blue or black shanks. The partridge plumage along with the black and white varieties as the «true Kabyle chickens».

The aims of this study was to characterize local chickens in Central Kabylie in terms of the livestocks practices, management, flock structures, uses, performance and phenotypes. The present survey addresses this issue in the case of Kabylie, a mountainous coastal region of Algeria, through a survey conducted in 84 households raising poultry and a morpho-biometric description of 388 adult local chickens.

2. MATERIALS AND METHODS

2.1. Survey of households keeping backyard poultry

This study was investigated in Kabylie (Algeria). The investigation concerned exclusively with the traditional aviculture. The study has been carried with 84 local chicken breeders from the districts of Chemini and Bouzeguene (Figure. 1). The interviews were semi-structured and covered household characteristics, poultry keeping practices as well as breed description, management and perceived evolution.

2.2. Morpho-biometric characterization

Adult males and females (290 and 98 respectively) were used for morpho - biometric characterization. The different body measurements were recorded by means of a digital balance, an electronic sliding caliper and a tape measure. The data collected were sex, body weight and reported age of animal, thoracic girth, feathers type and color, the comb's type, length, height and color, wattles height and color, tarsus length and diameter, wings length as well as the length and color of the beak.

2.3. Statistical analysis

All statistical analyses were performed with the R software (version 3.0.0). Descriptive statistics were calculated for both, quantitative and qualitative variables (mean, standard deviation, percentages). The Student test was used to compare the different body measurements between male and female. Finally, Pearson's correlation coefficients between the different body measurements were calculated.

3. RESULTS AND DISCUSSION

In Algeria few studies have concerned the characterization and identification of

poultry genetic resources (Moula *et al.,* 2009; 2012a; Halbouche *et al.,* 2009; Ait Kaki and Moula, 2013; Mahammi *et al.,* 2014; 2016; Dahloum *et al.,* 2016).

3.1. Survey of households keeping backyard poultry

The first backyard chickens are obtained by bought (39.29%), through inheritance (25%) and gifts (35.71%). Concerning feed and feeding systems for chickens, all poultry farmers (100%) provided supplementary feeding to their chickens as following: kitchen leftovers (91.67%), industrial feed (25%), crops and their residuals (14.29%). The animals live of what they find their environment (insects, worms, grasshoppers, larvae, grass, crops…). All farmers provide water to the birds. Drinking water sources cited are: the water tap (57.14%), the well (32.14%) and other sources such as streams, springs, fountains (10.71%). These farming practices are characterized by a reduction in the time allocated to them and to deal with high production costs which are the mark of an unproductive extensive livestock system considered as a secondary activity (Moula *et al.,* 2009; 2011; 2012a,b).

Use of eggs from the indigenous chickens include hatching chicks (100%) to get replacement stocks, eating at home (100%), sold for cash (14.29%) and a few (10.71%) are used for ceremonies. The chickens are kept for home consumption (100%), ceremonies (32.14%), gifts (28.57%) and a (21.43%) for cash. As reported in table 1, the indigenous chickens are valued mainly for their ability to scavenge (82.14%), good meat quality (85.71%), disease tolerance (78.57%) and general hardiness (78.57%). As reported in the studies of Moula *et al.* (2011) and Moula *et al.* (2012a,b), the purpose of keeping indigenous chickens at household level in the rural areas is for consumption and income.

The mainly cited constraints on the productivity of family - based poultry are: expensive chicken feed (46.43%), predators (32.14%), diseases (10.71%) and low production of local breeds (10.71%). The same constraints have been reported by Moula *et al.* (2011) and Moula *et al.* (2012a). These constraints are the expression of the extensive livestock with limited financial resources.

The mean egg performance traits of local chickens are shown in table 2. The mean age at first egg, egg weight, eggproduction per hen per clutch, number of clutches per year, hatchability and number of chicks weaned were 28.00 weeks, 49.03g, 13.57, 3.82, 89.29% and 7.82 respectively. These limited performances are the characteristics of local chicken breeds intended for mixed production (Moula *et al.,* 2009, 2011, 2012a, b; Duy *et al.*, 2015).

Table 1. Farmers responses (%) to main uses of eggs, chicken and special attributes

Use and attributes	n	%
Eggs		
Food	84	100
Cash	12	14,29
Ceremonies	9	10.71
Chicks	84	100
Chickens		
Food	84	100
Cash	18	21.43
Gifts	24	28.57
Ceremonies	27	32.14
Special attributes		
Disease tolerance	66	78.57
Meat quality	72	85.71
Ability to scavenge	69	82,14
General hardiness	66	78,57

Table 2. Performance characterization of indigenous chickens in local Kabyle chicken (28 households

Variable	Mean ± SD	Median	Max	Min
Age at first egg (weeks)	28.00 ± 3.82	28.00	36	24
Egg production / hen / clutch	13.57 ± 1.64	13.50	16	10
Egg weight (g)	49.03 ± 3.66	48.63	57.01	39.35
Number of clutches per year	3.82 ± 1.14	4.00	6	2
Hatchability (%)	89.29 ± 9.79	90.00	100	60
Average number of chicks weaned	7.82 ± 1.75	8.00	11	4

Table 3. Number (n) and percentages (%) of different feather colours in local chicken in Kabylie

Feather colour	Male		Female		Total	
	n	%	n	%	n	%
Black	11	11.22	35	12.07	46	11.86
White	10	10.20	37	12.76	47	12.11
Golden	5	5.10	30	10.34	35	9.02
Silver	9	9.18	11	3.79	20	5.15
Light brown	7	7.14	17	5.86	24	6.19
Dark red	4	4.08	8	2.76	12	3.09
Dark brown	0	0.00	19	6.55	19	4.90
Barred	7	7.14	24	8.28	31	7.99
Blue	4	4.08	5	1.72	9	2.32
White Columbian black	4	4.08	8	2.76	12	3.09
Grey	7	7.14	30	10.34	37	9.54
Mottled	9	9.18	8	2.76	17	4.38
Patridge	0	0.00	6	2.07	6	1.55
Salmon	15	15.31	41	14.14	56	14.43
Tan	6	6.12	11	3.79	17	4.38

3.2. Morpho-biometric characterization

Colour variation of plumage, shanks, comb, wattles and skin with comb type is shown in tables 3 and 4. As reported in Table 3, the most common colours were salmon (14.43%), white (12.11%), black (17%), grey (9.54%) and gold (9.02%). Other colours such as silver, blue, mottled, light brown, dark red, barred, partridge, dark brown, white columbian black and tan were less frequently encountered (between 1 and 8 percent). Globally, the population thus turned out to be highly heterogeneous. Male plumage colour in the largest group was salmon in 15.31% of the roosters.

Other principal groups shown were the black (11.22%) and white (10.20%) feathering. Like the cocks, hens were presented mainly salmon, white and black plumage colors with respectively 14.14%, 12.76% and 12.07%. The shanks colours varied from gray (7.73%), black (18.30%), white (24.23%) to yellow (49.74%). The predominant comb type was single (89.43%), followed by double (5.41%), pea (3.35%) and then walnut comb (1.80%). This extraordinary diversity reported in this study corresponds to those described by Moula *et al.* (2009) and Mahammi *et al.* (2014). The genetic diversity could be the result of uncontrolled crossings of the local populations with industrial strains of hens.

Sex had a significant effect ($P < 0.001$) on all the quantitative traits. Males had heavier body weight and larger body size compared to females (Table 5). Pearson's coefficients of correlation among various morphometric variables are shown in figure 2. All body measurements were positively ($r \geq +0.32$) and significantly ($P < 0.001$) inter - correlated.

Table 4. Number (n) and percentages (%) of different types and colours of the comb and the wattles and the skin and shanks coulour in local chicken in Kabylie

	Male		Female		Total	
	n	%	n	%	n	%
Skin colour						
White	27	27.55	125	43.10	152	39.18
rose	16	16.33	43	14.83	59	15.21
Yellow	50	51.02	103	35.52	153	39.43
pigmented	5	5.10	19	6.55	24	6.19
Comb type						
Single	84	85.71	263	90.69	347	89.43
Double	7	7.14	14	4.83	21	5.41
Pea	5	5.10	8	2.76	13	3.35
Walnut	2	2.04	5	1.72	7	1.80
Comb and Wattles colour						
Red	91	92.86	271	93.45	362	93.30
Pink	2	2.04	11	3.79	13	3.35
Black	5	5.10	8	2.76	13	3.35
Shanks colour						
Black	25	25.51	46	15.86	71	18.30
Yellow	30	30.61	163	56.21	193	49.74
Gray	11	11.22	19	6.55	30	7.73
White	32	32.65	62	21.38	94	24.23

Table 5. Body weight and measurements according to sex in local Kabyle chicken (mean ± SD and median in parentheses)

Variable	Male (n= 98)	Female (n= 290)	P - value
Body weight (kg)	2.03 ± 0.22 (2.00)	1.61 ± 0.19 (1.58)	***
Tarsus length (cm)	9.94 ± 2.80 (9.63)	7.67 ± 2.93 (7.30)	***
Tarsus Diameter (mm)	12.26 ± 1.58 (12.00)	11.02 ± 1.53 (11.00)	***
Comb length (cm)	6.95 ± 0.95 (7.10)	5.29 ± 1.86 (5.70)	***
Comb height (cm)	3.20 ± 0.41 (3.11)	2.46 ± 0.57 (2.60)	***
Ear lobe length (cm)	3.02 ± 0.55 (2.98)	2.21 ± 0.61 (2.16)	***
Ear lobe width (cm)	2.38 ± 0.70 (2.45)	1.50 ± 0.63 (1.40)	***
Wattles length (cm)	3.66 ± 0.38 (3.52)	2.98 ± 0.51 (3.08)	***
Wattles width (cm)	2.94 ± 0.60 (3.06)	2.30 ± 0.58 (2.28)	***

Figure 1. Location of the region of Chemini and Bouzeguene, Algeria

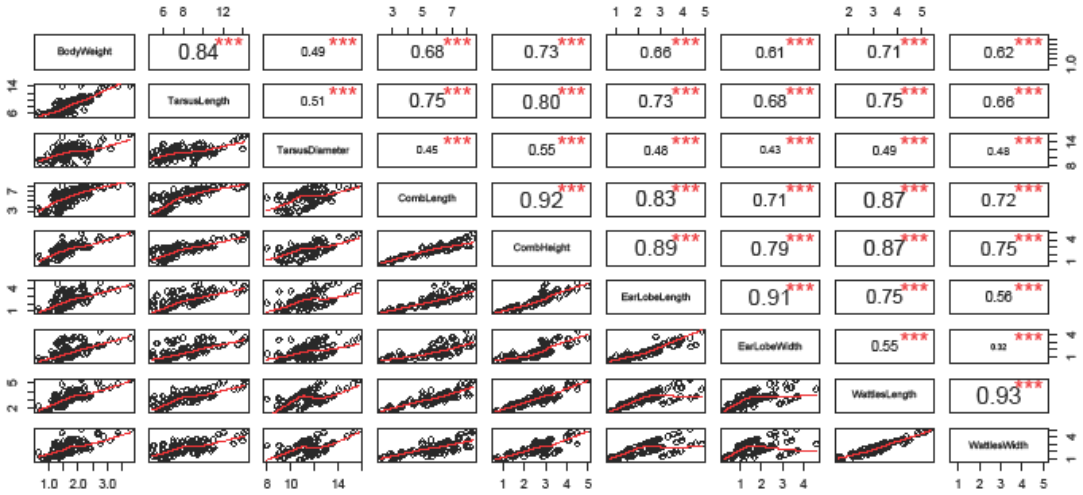

Figure 2. Correlation between all measurements

4. CONCLUSIONS

The rich genetic diversity of local chickens in Kabylie seems to evolve in an anarchic way. This wealth should nevertheless provide a useful gene pool of major interest for the establishment of a true local breed, based on the strong commitment of farmers to produce what corresponds to a collective ideal. Improved farming practices are needed to improve the productivity of village poultry. It's important to select the animals with good production, then afford them a balanced diet, proper housing and establish a prophylactic measures to fight diseases rampant in the study area.

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DONG TAO CHICKEN BREED IN HUNG YEN PROVINCE (VIETNAM): CHARACTERISTICS OF AN INDIGENOUS CHICKEN BREED WITH BIG LEGS

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ABSTRACT

This study aimed to determine morphological characteristics, reproductive performance and egg quality of Dong Tao chicken, an indigenous chicken breed in Vietnam. This study was conducted on 10 households raising purebred Dong Tao chicken. A sample of 240 adult individuals was morphologycally characterized. Reproductive performances were assessed on 10 backyard chicken (1 cock and 5 hens), and egg quality was assessed on 60 eggs. The results showed that Dong Tao chickens have a variety of phenotypes, as indicated in their feather colors, tarsus colors and comb types. The size of Dong Tao chickens was large, the body weight and all body measurements of the cocks were greater than those of hens ($P < 0.001$). Their reproductive performances were low but egg weight, yolk weight and the maximum breakage strength were higher compared to other chickens breeds. The Dong Tao breed, in the present state of things, is endangered. It should improve its reproductive performance before considering the *in-situ* conservation.

Keywords: Biodiversity, Dong Tao chicken, egg quality, indigenous chicken, productivity, Vietnam.

1. INTRODUCTION

In recent years, human population in the world is growing rapidly. The estimated growth rate of the human population is 1% annually over the next decade. The world's population will increase up to 9,1 billion people by 2050 (7.2 billion people currently) (FAO, 2014). Rapid population growth will lead to the increase in the demand for foods in general and demand for animal source foods in particular. According to FAO, in the next decade, the global meat consumption will increase by 1.6% per year (FAO, 2014). Agricultural production not only produces food but also generates income and supports for the livelihood of rural population (FAO, 2014). Livestock production is an important and promising agricultural sector in the current context of increasing global demand for livestock products (Delgado et al., 2001). The use of indigenous breeds in livestock production contributes to animal biodiversity conservation and *in-situ* protection. The adaptation of indigenous poultry breeds to the natural conditions and the fact that their products meet the expectations of local consumers are factors adding value to these products from local breeds. The

dramatic development of intensive livestock production systems is causing the decline of indigenous animal breeds. In particular, poultry production is also concerned in the general phenomenon of the reduction of animal genetic resources (Besbes, 2009).

In 2014, Vietnam's population is estimated at about 90728 900 people, with a density of 274 inhabitants per km^2. The rural population accounts for 66.9% of the total population (GSO, 2014). In Vietnam, poultry production plays an important role in rural economic development and in providing food for human consumption. According to General Statistics Office of Vietnam (GSO), in 2013, poultry meat production was 774.7 thousand tons and ranked at the second position, after pork production, which was 3228.7 thousand tons, in the national ranking of meat production level (GSO, 2014). Biodiversity in livestock production in Vietnam is very important. Vietnam is ranked as one of the countries with a high diversity of animal and plant breeds in the world. More than 10% of the world's animal species have been found in Vietnam (Ly, 1993). The local poultry accounts for 84% of the total poultry (Do Duc *et al.*, 2012). Moula *et al.*, (2012) reported that, in Vietnam, 15 local chicken breeds are identified, namely Ri, Te (or Lun, which means "short legs"), Tau Vang, Ac (black meat, white or black feather), Oke, H'môngTre, Choi (fighting-cock), Mong, Te, Dan Khao (six toes), Mia, Ho, Dong Tao (thick legs) and Van Phu.

Among local chicken breeds in Vietnam, Dong Tao chicken breed is famous for its massive body weight, thick legs and the good meat quality which is favored by consumers (Lan Phuong *et al.*, 2015, Thanh, 2008). According to Tieu (2009), the greater number of local chicken breeds in Vietnam are in danger of extinction. The conservation of local animal genetic resources is a major challenge in the future, especially for sustainable economic development. This study aimed at determining morphological characteristics, reproductive performances and egg quality of Dong Tao chicken. This study is a foundation for a rehabilitation of Vietnamese local chicken breeds, which is especially suitable with less intensive farming conditions and that plays a paramount role on socioeconomic and cultural aspects.

2. MATERIAL AND METHODS

This study was implemented from September 2014 till April 2016 on 10 households raising purebred Dong Tao chicken, in Dong Tao commune (located 30 km from Hanoi), Khoai Chau district, Hung Yen province in Northern Vietnam.

2.1. Morpho-biometric characterization

Morpho-biometric characterization was based on measurement of 240 adult individuals (40 males and 200 females over six months of age). The different body parameters were recorded in accordance with the FAO recommendations (FAO, 2012), a weighing scale (precision 10 g) was used to measure chicken body weight; a tape measure and an electronic sliding caliper (precision 0.01 mm) were used to measure body parameters. The collected data were thus the sex and body weight, body parameters including body length, neck length, back length, wing length, thoracic perimeter, breast length, thigh length, tarsus length, and tarsus diameter, beak length, comb length, comb height. The characteristics about feather color, tarsus color, beak color and comb style were determined based on visual observations.

2.2. Egg production performances

Egg production performances were recorded on 10 chicken family livestock (1 cock and 5 hens per family) on 10 households, adult chickens were reared in the traditional livestock farming system. Chicken were fed by agricultural products including paddy rice (49.05%), corn (9.25%), commercial feed (39.40%), vegetables (2.30%). The production traits were number of eggs per clutch, number of clutch per year, number of eggs/hen/year, number of embryonated eggs per clutch, rate of embryonated egg per clutch, number of chicks born per clutch, rate of chicks born per clutch, number of chicks born alive per clutch, survival rate of chicks per clutch and number of chicks per year. Egg production performances in each chicken family were recorded and then divided equally for the total amount of hens in each family.

2.3. Egg quality

A total of 60 eggs were collected from 10 chicken families at 20th laying week for physical quality analysis. Eggs weight, their length and width, the maximum breakage strength, Haugh unit, yolk and albumen weight and albumen/yolk ratio were measured. Egg weight was measured by means of an electronic balance (accuracy 0.01 g). Thereafter, their length and width were measured by means of an electronic sliding caliper (precision 0.01 mm), hence, an egg shape index could be calculated as the ratio between width and length multiplied by 100. The egg shells were broken and the yolks were carefully separated from the albumen. The shells including the membranes and yolks were weighed separately (electronic balance, accuracy 0.01 g). Albumen weight was determined by subtracting yolk and shell weights from the total egg weight. The yolks and the albumen were carefully separated for chemical analysis including protein, lipid and ash content. The egg quality analysis was carried out at the laboratory of Faculty of Animal Science, Vietnam National University of Agriculture.

2.4. Statistical analysis

The data were analyzed using the general linear model procedure of SAS software (Statistical Analysis System, 1989) to determine the effect of the sex (male and female) on each morpho-biometric parameter.

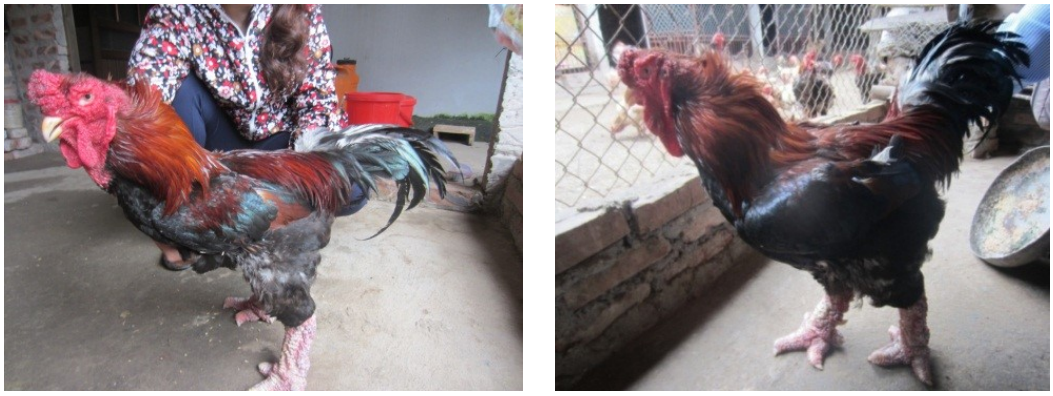
3. RESULTS AND DISCUSSION

3.1. Feather color and aspect

The feather colors and aspects of Dong Tao chickens are given in Table 1. Dong Tao chickens are characterized by 5 basic feather colors (2 for males and 3 for females). Two main types of feather colors were observed for the males: the black with gold hackle (Figure. 1a) predominant over the black copper (Figure.1b). For the females, the tan color (Figure. 2a) was the most frequently observed color, followed by the wheat (Figure. 2c) and the tri color (Figure. 2b). The skin color of all observed males was red, while for females, the color was red (97.5%) or yellow red (2.5%) (Table 1). Red toe (Figure. 3a) and yellow toe (Figure. 3b) are the two observed types of tarsus color, with a majority of the red ones. The red is thus the dominant color for skin and tarsus for both males and females. Beak colors of Dong Tao chickens were yellow and dark horn, with a dominant yellow color (Table 1). There were 3 observed comb types, for both males and females, where the most frequent one is the cushion (Figure. 4a), followed by the strawberry (Figure. 4b) and the pea (Fig 4b).

Table 1. Feather, skin, tarsus, beak couleur and comb types of Dong Tao chicken

Variable		Male		Female		Total	
		n	%	n	%	n	%
Feather color	Black copper	3	7.5	-	-	3	1.3
	Black with gold hackle	37	92.5	-	-	37	15.4
	Wheat color feather	-	-	151	75.5	151	62.9
	Tri color feather	-	-	31	15.5	31	12.9
	Tan color feather	-	-	18	9.0	18	7.5
Skin color	Red	40	100	195	97.5	235	97.9
	Red yellow	-	-	5	2.5	5	2.1
Tarsus color	Red	37	92.5	170	85.0	207	86.3
	Red yellow	3	7.5	30	15.0	33	13.8
Beak color	yellow	16	40.0	129	64.5	145	60.4
	Dark horn	24	60.0	71	35.5	95	39.6
Comb type	Cushion comb	17	42.5	86	43.0	103	42.9
	Strawberry comb	19	47.5	108	54.0	127	52.9
	Pea comb	4	10	6	3.0	10	4.2

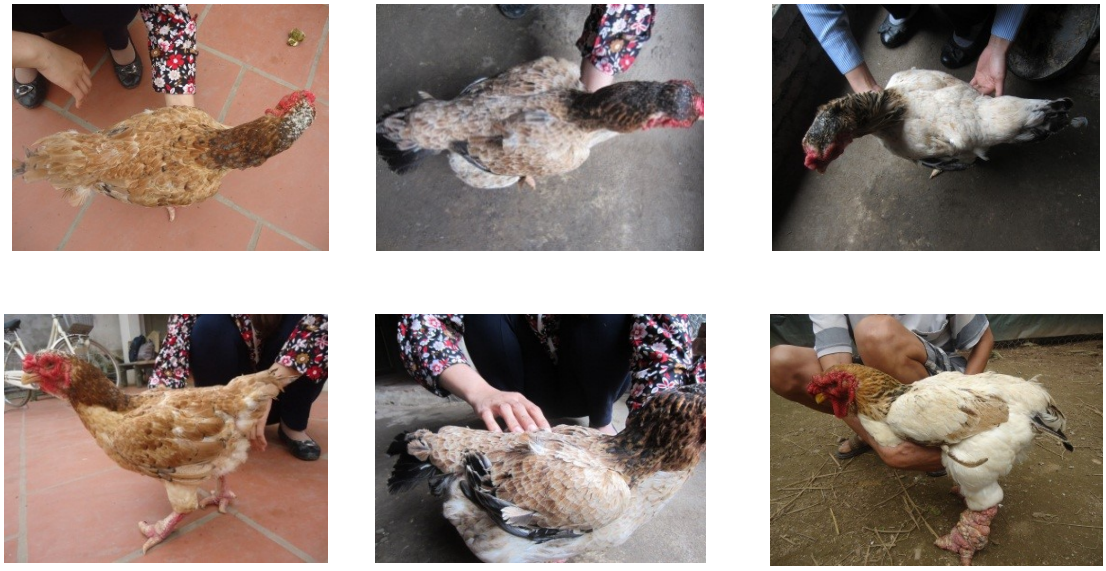


(a) (b)

Figure 1 (a-b). (a) Black with gold hackle feather; (b): Black copper feather

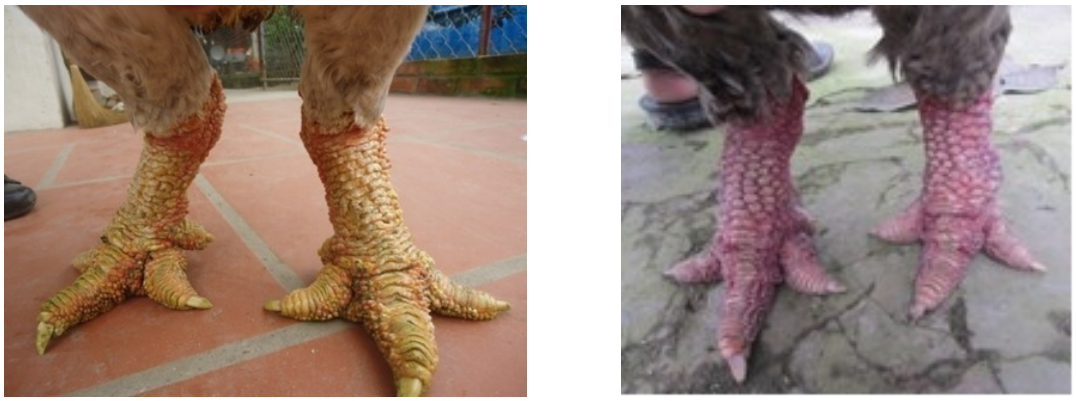
3.2. Morphometric traits

The live weight and body measurements of mature Dong Tao chickens are shown in Table 2. There are significant differences between the mean values of males and females. The body weight, body length, back length, thoratic perimeter, breast length and all other remaining measurements on males were larger than those on females ($P < 0.001$). Dong Tao chickens have high body weight, which are 3.97 kg per male and 2.85 kg per female. The body weight of mature Dong Tao chicken is much larger than that of adult Ri chicken, another Vietnamese local chicken breed, which is of 1.87 – 2.08 kg for cocks and 1.36 – 1.50 kg for hens (Moula *et al.*, 2012).



(a) (b) (c)

Figure 2 (a-c). (a): Wheat color feather, (b): Tan color feather, (c): Tri color feather



(a) (b)

Figure 3(a-b). (a): Yellow, (b): Red

(a) (b) (c)

Figure 4(a-c). (a): Cushion comb, (b): Strawberry comb, (c): Pea comb

3.3. Egg production performance

Table 3 presents the results of the egg production of Dong Tao hens. The age of laying the first egg was late (166.27 days). As compared with other local chicken breeds in Vietnam, the age laying the first egg of Dong Tao chicken breed is earlier than that of Ho breed (219.5 days) (Van Duy et al., 2015), but later than that of Ri breed (140 days) and of H'mong breed (133 days) (Thanh, 2008). Their egg production performance was low, which was shown in number of eggs per hen per year, number of embryonated per hen per year, embryonated egg rate and hatching rate per hen per year (Table 3). The high egg production is often observed at egg laying chicken breeds. For example, the egg production of Dominat breed is of more than 200 eggs/hen/year and the hatching rate egg rate of this breed is of 94.33% (Nguyen Duc Trong et al., 2011). The hatching rate egg on a local chicken breed in Bac Giang province is of 96.75% (Lam Thi Ha, 2011). The results of egg quality analysis are shown in Table 4 and Table 5. The weight of eggs, their length and width, maximum breakage strength and yolk weight were of high values (Table 4). Dong Tao eggs were larger than 50 gram/egg, which are at the same weight of Ho eggs (Van Duy et al., 2015). As compared to other local chicken eggs in Vietnam, such as Ri eggs (41.8 gram/egg) (Thanh, 2008), H'mong eggs (43.37 gram/egg) (Dao Le Hang, 2001); eggs of Dong Tao chicken breed are larger. Dong Tao eggs had high nutrition value with high protein content (Table 5). Results showed that dry matter content of the yolk was 51.89%; protein content was 17,01% and lipid content was 26.68%. The dry matter content of albumen was 12.18%; protein content was 10.57% and lipid content was 0.09%. The dry matter content of the yolk was thus 4.26 times higher than that of the albumen. According to Al-Obaidi et al., (2011), the albumen of chicken egg contains 11.43% protein and 0.02% lipid; the yolk of chicken egg contains 16.59% protein and 33.72% lipid.

Table 2. Morpho-biometric stature of Dong Tao chicken (Mean ± SD)

Variable	Males (n=40)	Females (n=200)	p-value
Body weigth (kg)	3.97 ± 0.06	2.85 ± 0.03	***
Body length (cm)	46.18 ± 0.23	41.75 ± 0.13	***
Neck length (cm)	21.41 ± 0.16	19.82 ± 0.08	***
Back length (cm)	24.77 ± 0.18	21.93 ± 0.09	***
Wing length (cm)	25.73 ± 0.22	22.67 ± 0.09	***
Thigh length (cm)	18.78 ± 0.16	15.91 ± 0.08	***
Breast length (cm)	21.07 ± 0.16	17.09 ± 0.10	***
Tarsus length (cm)	8.82 ± 0.10	6.98 ± 0.08	***
Tarsus diam 1 (mm)	36.20 ± 1.04	24.67 ± 0.35	***
Tarsus diam 2 (mm)	31.56 ± 0.45	23.30 ± 0.22	***
Beak length (mm)	40.44 ± 0.32	36.65 ± 0.19	***
Comb length (mm)	38.78 ± 0.65	25.02 ± 0.29	***
Comb height (mm)	31.05 ± 0.83	14.76 ± 0.34	***

*Note: Diam 1: Diameter at widest part of tarsus, Diam 2: Diameter at thinnest part of tarsus, ***: $p < 0.001$*

Table 3. Reproductive performance of Dong Tao chicken

Variable	n	Mean ± SD
Age laying the first egg (day)	10	166.27 ± 12.52
Body weigth in age laying the first egg	50	2 211.11 ± 13.82
Number of eggs per clutch	50	12.75 ± 2.71
Number of clutch per year	10	5.75 ± 5.90
Number of eggs layed per hen/year	50	73.31 ± 7.58
Number of hatched eggs per clutch	50	12.61 ± 10.04
Rate of hatched egg per clutch (%)	50	98.90 ± 1.82
Number of hatching eggs per clutch	50	9.35 ± 4.21
Hatching rate per clutch (%)	50	74.15 ± 2.04
Number of chicks born per clutch	50	7.40 ± 6.04
Number of chicks per year	50	42.55 ± 2.12

Table 4. Quantitative traits of Dong Tao chicken eggs (n = 60)

Variable	unit	Mean ± SD
Egg weight	g	50.62 ± 0.86
Egg length	mm	52.44 ± 0.46
Egg width	mm	41.93 ± 0.41
Shape index	%	1.25 ± 0.02
Maximum breakage force	N	40.08 ± 2.27
Haugh	Hu	81.29 ± 2.35
Egg shell weight	g	5.65 ± 0.20
Yolk weight	g	17.67 ± 0.75
Albumen weight	g	27.29 ± 1.07
Albumen/ Yolk ratio		1.60 ± 0.11

Table 5. Chemical composition of Dong Tao chicken eggs (n = 60)

Variable	Albumen			Yolk		
	Mean	±	SD	Mean	±	SD
Dry matter (%)	51.89	±	0.60	12.18	±	0.27
Ash (%)	3.54	±	0.18	1.01	±	0.09
Crude protein (%)	17.01	±	0.18	10.57	±	0.29
Lipit (%)	26.68	±	0.48	0.09	±	0.02

4. CONCLUSIONS

Dong Tao chicken is a Vietnamese indigenous chicken breed with high body weight and body measurements. Dong Tao chicken phenotypic characteristics are quite variable, potentially revealing genotypic heterogeneity. The egg production performances are low but the egg weight is large and the maximum breakage force is high.

Conservation objectives of this breed requires to have continued researches aiming to inprove the reproductive performance of

Dong Tao chicken and to increase its economic efficiency while conserving the genetic resources of the breed, more widelyand maintaining the biodiversity in Vietnamese local chicken breeds.

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A STUDY OF EGG QUALITY AND INCUBATION PARAMETERS IN SIX BREEDS OF TAIWANESS CHICKENS

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ABSTRACT

In this study, we investigated the differences of egg quality and incubation parameters traits in six breeds of Taiwanese chickens: Hua - Tung (HT), Hsin - Yi (HY), Ju - Chi (JC), Shek–Ki (SK), Nagoya (NG) and Quemoy (KM) breeds. The study eggs were collected when hens were 29 - 30 weeks of age. Two eggs per hen were collected to study egg quality traits. The incubation traits, including loss of egg weight during storage (storeloss), 18 days in the setter (inculoss), 21 days of incubation (hatchloss), time to hatch, and hatchability were tested for the effect of breed, egg weight and storage period. The results showed that egg qualities of Nagoya were better than other breeds with higher eggshell breaking strength and Haugh unit. Eggshell breaking strength of Quemoy and Shek - Ki were lower than other breeds. The highest inculoss (%) and hatchloss (%) were found in Quemoy, while the lowest was Shek - Ki. The longest hatchtime was found in Quemoy and the shortest was Ju - Chi. The highest hatchability was found in Hua - Tung breed, while the lowest was Shek - Ki breed. Furthermore, long egg storage time increases egg weight loss and incubation duration (hatch time), but decreased hatchability. When eggs stored for one more day, egg weight loss increased 0.034 % during storage period (storeloss %), 0.017 % during 18 days in the setter (inculoss %), and 0.037% for entire incubation period (hatchloss %), increased the hatch time longer by 0.723 hr, but decreased hatchability 0.026 %.

Keywords: Egg quality, egg weight loss, incubation, hatchability.

1. INTRODUCTION

Egg quality is an important parameter for embryo development as well as for chick quality at one day old and growth (Tona *et al.*, 2003). Egg quality is determined by egg shape, egg weight, Haugh unit and eggshell attributes (Robert, 2004). Production of eggs which are good egg shell quality and good internal quality not only critical factors affect reproduction but also the cost of economic viability of the industry. Therefore, it is importance to understand the factors affect egg quality and the differences of egg quality traits laid by six breeds of local chickens kept in the research farm of National Chung - Hsing University.

The fate of chicks largely depends on the quality of hatching eggs. Various breeding practices and handling of eggs from egg laying to hatching, particularly pre - incubation storage condition, and incubation parameters have affected hatchability and quality of day old chicks (Tona *et al.*, 2001). Hatching eggs are collected at breeding farm, stored for sometime there, or directly transferred to the hatchery. Here, these are stored for certain limit of time under specific environmental conditions. The main objective of holding period is to maintain the fertility of hatching eggs.

Hua - Tung, Hsin - Yi, Ju - Chi, Quemoy, Nagoya, and Shek - Ki are six

breeds of local Taiwan chickens (Chen *et al.*, 1994; Lee, 2006) kept at the research farm of National Chung Hsing University. During the annual reproduction period, their eggs were collected at farm and kept there for one to two weeks in storage before moved to the University for incubation. During this stage, there were many changes of egg's characteristics. Brake *et al.*, (1997) reviewed the changes in eggs components associated with egg handling, storage and concluded that hatchability and chick quality varied by age of flock, age of egg, ambient temperature, strain and handling procedures. Better understanding of these details, the aim of this study, we investigate the differences of egg quality and incubation parameters among six breeds of local chickens during the incubation period.

2. MATERIAL AND METHODS

2.1. Animals and samples collection

The eggs used in this study were obtained from six breeds of Taiwaness chickens in research farm of National Chung Hsing University, Taichung, Taiwan from September to November 2015: Hua - Tung (HT), Hsin - Yi (HY), Ju - Chi (JC), Shek–Ki (SK), Nagoya (NG) and Quemoy (KM) breeds when hens were 29 - 30 weeks of age.

2.2. Traits measured

2.2.1. Egg quality traits includes

Egg weight, shape index, egg shell color, eggshell breaking strength, internal egg quality, shell thickness and membrane thickness.

2.2.2. Incubation parameters

Egg weight, storeloss, inculoss, hatchloss, hatch time, chicks weight and hatchability.

2.3. Statistical analysis

Observation of egg quality and incubation parameters of eggs laid by hens of six local breeds chicken were analyzed together using General Linear Models procedures of SAS Institute (version 9.3.1).

3. RESULTS AND DISCUSSIONS

3.1. Egg quality traits

Analysis of variance and least - square means of egg quality traits in six breeds of Taiwaness chickens are showed in Table 1.

The effect of breeds were highly significant ($P < 0.01$) on egg weight, yolk weight %, albumen weight %, shell thickness, shell breaking strength, yolk height, shape index, shell whiteness and shell color; significant ($P < 0.05$) on shell weight % and Haugh unit, but not significant on shell membrane. The highest egg weight was found in Hua - Tung breed, and the lowest was Shek - Ki breed, these are the same as reported by Sukanya (2007). Yolk weight %, however, the highest was found in Shek - Ki breed, and the lowest was Ju - Chi breed. The highest albumen weight (%) was found in egg laid by Ju - Chi breed, and the lowest was Shek - Ki breed. The highest shell weight % and shell thickness were found in Nagoya and Shek - Ki breeds, while the lowest was Hsin - Yi breed. Egg shell breaking strength of egg laid by Nagoya breed higher than other breeds, whereas, egg shell breaking strength of egg laid by Quemoy breed was lowest. This result is the same as reported by Sukanya (2007), but different from reported by Chang (2002). Chang (2002) reported that egg laid by Shek - Ki breed had egg shell breaking strength higher than other breeds.

Haugh unit of egg laid by Nagoya and Hua - Tung breeds were higher than other breeds, and the lowest was found in Shek -

Ki breed. This result is the same as reported by Sukanya (2007) but Shek - Ki breed. As a report by Chen (1994) Haugh unit of egg laid by Ju - Chi breed was higher than other breed, and the lowest was Nagoya breed. Different from reported by Chen (1994), Sukanya (2007) reported that Haugh unit of eggs laid by Nagoya breed was higher than other breed, while the lowest was Hua - Tung breed. These differences among reports might be caused by hen's age. As reported by Chen (1994) eggs were collected when hens were 60 weeks of age, while Sukanya (2007) collected eggs when hens were 30 - 50 weeks of age. The highest shape index was found in Shek - Ki breed, whereas the lowest was Hsin - Yi breed. These results are the same as results reported by Chang (2002), Chen (1994), and Sukanya (2007).

There were very few studies the effect of egg shell color on egg quality. The white shell eggs were thicker and heavier than brown shell (Cutis *et al.*, 1985). Sukanya (2007) reported that blue eggshell eggs had higher shell weight percentage and thicker shell membrane. In this study, we found that Quemoy had the highest shell whiteness, whereas the lowest was Shek - Ki breed. In contrast to egg shell whiteness, shell color of eggs laid by Quemoy breed was lowest, and the highest was egg laid by Shek - ki breed.

Table 1. Analysis of variance (mean squares) and least - square means for egg quality traits in six breeds of Taiwaness chickens

Source of variation	df	Egg weight (g)	Yolk (%)	Albumen (%)	Shell (%)	Shell thickness (10^{-2}cm)	Shell membrane (10^{-2}cm)
Breed	5	138.27**	37.83**	51.87**	1.90*	0.017**	0.0013
Error	362	11.27	3.39	5.78	0.64	0.008	0.0005
Hua - Tung		47.60 ± 0.46^{a}	30.85 ± 0.25^{b}	53.86 ± 0.33^{ab}	9.26 ± 0.11^{bc}	39.6 ± 0.04^{ab}	0.29 ± 0.010
Hsin - Yi		44.61 ± 0.45^{b}	32.56 ± 0.25^{a}	52.55 ± 0.32^{cd}	9.11 ± 0.10^{c}	38.4 ± 0.04^{c}	0.28 ± 0.010
Ju - Chi		47.10 ± 0.44^{a}	30.82 ± 0.24^{b}	54.31 ± 0.31^{a}	9.13 ± 0.11^{c}	39.0 ± 0.04^{bc}	0.30 ± 0.009
Quemoy		44.99 ± 0.4 ^{b}	31.34 ± 0.24^{b}	53.99 ± 0.31^{ab}	9.22 ± 0.10^{bc}	39.0 ± 0.04^{bc}	0.26 ± 0.009
Nagoya		45.74 ± 0.48^{b}	32.19 ± 0.26^{a}	53.33 ± 0.34^{bc}	9.44 ± 0.11^{ab}	40.1 ± 0.04 ^{a}	0.30 ± 0.010
Shek - Ki		43.09 ± 0.51^{c}	32.77 ± 0.28^{a}	51.60 ± 0.37^{d}	9.63 ± 0.12^{a}	39.5 ± 0.04^{abc}	0.29 ± 0.010

Source of variation	df	Brk force (g)	Yolk height (mm)	Haugh unit	Shape index	Shell whiteness	Shell color
Breed	5	1.99**	2.99**	54.32**	218.70**	383.15**	151.71**
Error	362	1.21	0.60	33.08	4.29	27.18	19.62
Hua - Tung		2.26 ± 0.15^{ab}	16.80 ± 0.10^{b}	83.88 ± 0.79^{a}	76.82 ± 0.43^{b}	60.71 ± 1.07^{c}	24.70 ± 0.91^{ab}
Hsin - Yi		2.27 ± 0.15^{ab}	16.67 ± 0.10^{b}	81.42 ± 0.77^{ab}	69.58 ± 0.43^{e}	66.62 ± 1.07^{b}	24.00 ± 0.91^{ab}
Ju - Chi		2.54 ± 0.14^{a}	17.10 ± 0.10^{a}	82.19 ± 0.76^{abc}	72.81 ± 0.43^{d}	66.09 ± 1.08^{b}	22.32 ± 0.92^{b}
Quemoy		2.06 ± 0.14^{b}	16.56 ± 0.10^{b}	82.89 ± 0.74^{abc}	74.82 ± 0.37^{c}	70.86 ± 0.94^{a}	18.86 ± 0.80^{c}
Nagoya		2.55 ± 0.16^{a}	17.12 ± 0.11^{a}	83.33 ± 0.82^{ab}	76.05 ± 1.05^{bc}	68.04 ± 2.66^{ab}	20.39 ± 2.25^{bc}
Shek - Ki		2.25 ± 0.17^{ab}	16.71 ± 0.12^{b}	81.35 ± 0.88^{c}	78.80 ± 0.50^{a}	60.48 ± 1.26^{c}	25.62 ± 1.07^{a}

*Note:$^{a-e}$ For each measure, means of different breeds without the same superscript are significantly different ($P < 0.05$). $^{+}P < 0.1$; * $P < 0.05$; ** $P < 0.01$.*

3.2. Incubation parameters

3.2.1. Egg weight and Chick weight

The heaviest chick weight was Hua - Tung breed, whereas the lightest was Quemoy (Table 2). Overall, day - old chick weights increased with the egg weights (Tona *et al.*, 2003). The quality of newly hatched chick is a major factor in determining its livability, growth, and health. There was a highly significant correlation between egg weight and chick weight at hatched time (Reis., 1997). Thus, the heavier egg weight will produce the heavier chick weight.

3.2.2. Egg weight loss

There were no significant on egg weight loss (%) during storage period ($P < 0.05$) among six beeds (Table 2). The Ju - Chi and Quemoy breeds had the highest inculoss (%) while the lowest were found in Shek - Ki and Hua - Tung breeds ($P < 0.01$). In hatchloss (%), however, the highest were found in Quemoy and Hsin - Yi breeds while the lowest were still Shek - Ki and Hua - Tung breeds. Reis *et al.*, (1997) demonstrated that there is an inverse relationship between egg weight and hatchloss. Thus, egg had smallest egg weight has largest hatchloss. This is as the same with our results but egg laid by Shek - Ki hens. The present results agree well with the observations obtained by *Kirk et al.*, (1980), North and Bell (1990), and Roque and Soares (1994), who reported that proportional weight loss decreased slightly with flock age, probably because of the associated increase in egg weight. As larger eggs have less shell area per unit of interior egg weight than do smaller eggs. Another explanation of the breed's difference in inculoss might be caused by the shell difference.

3.2.3. Hatchtime

Among six breeds, we found that the Hsin - Yi breed hatched earlier than other breeds (478.68 h) (Table 2), whereas the longest hatchtime was found in Quemoy and Shek - Ki breeds (492.02 and 491.82 h, respectively).

3.2.4. Effect of storage time on hatching traits

The partial regression coefficients of storage time on hatching traits were shown in table 3. Long egg storage time increases storeloss (%), inculoss (%), hatchloss (%) and incubation duration (hatch time) but hatchability. When eggs stored for one more day, egg weight loss increased 0.034% during storage period, 0.017 % during 18 days in the setter, and 0.037% for entire incubation period, increased the hatch time longer by 0.723 hr, but decreased hatchability 0.026%. Reis *et al.* (1997) reported that eggs submitted to 1 day storage hatched about 3h earlier than eggs not stored. Yassin et al. (2008) showed that each day of storage up to 7 days reduced hatchability by 0.2%, whereas, further storage reduced hatchability by 0.5% daily. Theses suggest that the effect of pre - storage incubation on hatchability when storage time is prolonged depends on the developmental stage of the embryo after pre - storage incubation.

4. INCONCLUSIONS

Egg qualities of Nagoya were better than other breeds with higher eggshell breaking strength and Haugh unit. Eggshell breaking strength of Quemoy and Shek - Ki was lower than other breeds

The heavier egg weight will produce the heavier chick weight, thus the heaviest chick weight was Hua - Tung breed, whereas the lightest was Quemoy.

Table 2. Analysis of variance (mean square) and least - square means of hatching traits in six breeds of local chickens

Source of variation	df	Egg weight (g)	Storeloss[1] (%)	Inculoss[2] (%)	Hatchloss[3] (%)	Hatch time[4] (hr)	Chick weight (g)	Hatchability (%)
Breed	5	810.52**	0.21	212.83**	388.57**	12741.81**	589.91**	16.77*
Error	3087	12.79	0.18	4.03	4.29	74.63	8.00	2.41
Hua - Tung		47.56 ± 0.16^{a}	0.32 ± 0.02	12.05 ± 0.09^{c}	27.86 ± 0.10^{d}	484.96 ± 0.39^{b}	34.22 ± 0.13^{a}	88.84 ± 0.95^{a}
Hsin - Yi		45.12 ± 0.15^{d}	0.35 ± 0.02	13.02 ± 0.08^{b}	29.00 ± 0.09^{b}	478.68 ± 0.35^{c}	31.93 ± 0.12^{d}	87.62 ± 0.95^{a}
Ju - Chi		46.90 ± 0.15^{b}	0.34 ± 0.02	13.29 ± 0.08^{a}	28.56 ± 0.08^{c}	484.90 ± 0.35^{b}	33.39 ± 0.12^{b}	88.48 ± 0.95^{a}
Quemoy		44.37 ± 0.16^{e}	0.36 ± 0.02	13.25 ± 0.09^{ab}	29.57 ± 0.09^{a}	492.02 ± 0.38^{a}	31.15 ± 0.13^{e}	83.59 ± 0.95^{b}
Nagoya		46.12 ± 0.17^{c}	0.38 ± 0.02	13.04 ± 0.09^{ab}	28.96 ± 0.10^{b}	485.72 ± 0.40^{b}	32.64 ± 0.13^{c}	87.40 ± 0.95^{a}
Shek - Ki		44.61 ± 0.19^{e}	0.33 ± 0.02	11.63 ± 0.10^{d}	26.90 ± 0.11^{e}	491.82 ± 0.45^{a}	32.52 ± 0.15^{c}	83.09 ± 0.95^{b}

*Note: $^{a-e}$ For each measure, means of different breeds without the same superscript are significantly different ($P < 0.05$). $^{+}$ $P < 0.1$; * $P < 0.05$; ** $P < 0.01$.*

[1]Storeloss is loss of egg weight during storage period. [2]Inculoss is loss of egg weight during 18 days of incubation. [3]Hatchloss is loss of egg weight entire 21 days of incubation. [4]Hatch time is number of hours required to hatch.

Table 3. Estimates of partial regression coefficients of hatching traits

Source of variation	Storeloss[1] (%)	Inculoss[2] (%)	Hatchloss[3] (%)	Hatch time[4] (hr)	Hatchability(%)
Storage (d)	0.034**	0.017*	0.037**	0.723**	- 0.026*
Egg weight (g)	- 0.001^{ns}	- 0.091**	- 0.005^{ns}	- 0.162^{ns}	0.019^{ns}
Egg shape (%)	- 0.003^{ns}	0.015^{ns}	- 0.053*	- 0.088^{ns}	- 0.043^{ns}

*Note: $^{+}$ $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; ns: none significant;*

[1]Storeloss is loss of egg weight during storage, [2]Inculoss is loss of egg weight during18 days in the setter, [3]Hatchloss is loss of egg weight entire 21 days of incubation and [4]Hatch time is number of hours required to hatch.

The highest egg weight loss (%) was found in Quemoy breed, while the lowest was Shek - Ki breed. The highest hatchability was Hua - Tung breed, while the lowest was found in Shek - Ki breed.

Long egg storage time increases egg weight loss, incubation duration (hatch time), and decreases hatchability. When eggs stored for one more day, egg weight loss increased 0.034 % during storage period, 0.017 % during 18 days in the setter, and 0.037% for entire incubation period, increased the hatch time longer by 0.723 hr, but decreased hatchability 0.026%

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EFFECT OF CASSAVA DISTILLERS DRIED GRAINS FROM ETHANOL PRODUCTION ON PERFORMANCE OF GROWING PIGS

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ABSTRACT

The objective of this study was to evaluate the effects of levels of cassava distillers' dried grains (cassava DDG) in the diet on feed intake, body weight gain, feed conversion ratio (FCR) of pig (PiDu x LY) and on feed cost per 1 kg weight gain. A total of 120 pigs with 14 ± 0.5 kg were randomly assigned according to a completely randomized design (CRD) into 4 treatments with different levels of cassava DDG at 0; 5; 10 and 15%. The results indicated that the diet with 5% cassava DDG improved feed intake, weight gain and feed cost per 1 kg weight gain, while diets with 10 and 15% cassava DDG increased the total feed intake, FCR and feed cost per 1 kg weight gain, but reduced weight gain. In CONCLUSIONS, cassava DDG could be used in the diet at level of 5% to improve the performance of growing pigs.

Keywords: Body weight gain, cassava DDG, feed intake, feed cost per 1 kg weight gain.

1. INTRODUCTION

In pig production, feed cost can account approximately 65 - 75% of the production costs. In Vietnam, pig feed ingredients such as soybean, corn, fish oil are mostly imported from overseas. Therefore, in order to reduce the production cost as well as the dependence on importing materials, the use of local feed resource and agro - industrial by - product is essential.

Distillers dried grains (DDG) is the main co - product of ethanol production from cereal grains such as corn. Since only starch and sugars are converted by yeast cells into ethanol and CO_2, the concentration of other nutrient components increases in DDG (Cozannet *et al.*, 2011; Monceaux and Kuehner, 2009; Pahm *et al.*, 2008; Stein and Shurson, 2009). Cromwell *et al.* (1983) reported that corn DDG contains high level of protein, lipid, mineral, fiber content, so, DDG is potentially utilisized for animal feeding in the world However in Vietnam, the DDG from ethanol industry have either been underutilized or probably caused the environmental pollution.

In Vietnam, cassava is an attractive raw material for bio - ethanol production thanks to the following advantages: (i) easy of plantation in various soil types and climate conditions; (ii) very low input and investment for planting; (iii) "all year round" availability of feedstock in the form of fresh roots and dry chips; (iv) high starch raw materials and a lower proportion of fibers (Sriroth *et al.*, 2007). According to the Vietnamese Ministry of Industry and Trade declared that bio - fuel production

will achieve 1.8 million tons in 2025, which accounts for 5% of country's demand (Ministry - of - Industry, 2007a). Moreover, the government also adapted the policy to improve the beverage ethanol industry in Vietnam. By the Development strategy of beverage ethanol production in Vietnam from 2007 - 2025 (Ministry - of - Industry, 2007b), ethanol industry will produce 188 million liters of ethanol for food industry in 2025. Thus, a vast amount of cassava DDG is available and can be used as ingredient feed for pig production. However, few investigation has been conducted, and therefore, the objective of current study was to investigate the effect of different levels of cassava DDG on the performance of growing and finishing pig.

2. MATERIALS AND METHODS

2.1. Animals, diets, experimental design and animal management

This experiment was conducted at the farm of Duc Anh Company, Luong Son, Hoa Binh, from March to June 2016.

Total 120 F1 (PiDu x LY) pigs with initial body weight (BW) of 14.0 ± 0.5 kg were randomly assigned according to a completely randomized design (CRD) into four dietary treatments with 2 lots/treatment/period (15 pig/lot) and the experiment was timely replicated twice. Pigs were housed in groups of 15 with smilar weight, age and gender. All pigs were allowed for an adaptation period of 7 days and were vaccinated before the experiment period. The diets and water were fed *ad libitum* during the entire 135 days study.

The dietary treatments were formulated with 4 levels of cassava DDG: 0; 5; 10 and 15%. Diets were formulated to meet requirement of growing pigs for 3 periods (period 1: 15 - 30 kg; period 2: 30 - 60 kg and period 3: 60 kg - slaughter weight) according to NRC standard (1998) (NRC, 1998). The ingredients and chemical composition of experimental diets are presented in Table 1.

2.2. Chemical analysis

Wet distiller grain (WDG) from a bioethanol cassava - based plant (BSR - BF) located in Quang Ngai province (Center of Vietnam) were collected and transported immediately after production directly to Hanoi University of Science and Technology and were dried at 90°C for 30 min, then 80°C for 2.5 - 3 h and finally 70°C for 1 h in a circulating dryer to make dried distiller grains (DDG). The DDG were packed in a plastic bag and store at room temperature in dry and clean place for chemical analysis.

Cassava DDG and other feed ingredients were dried at 60°C in an oven and ground for analysis of crude protein, ether extract, crude fiber and ash (AOAC 1990). Calcium and phosphorous were analyzed according to TCVN 1526 - 1:2007 and TCVN 1525: 2001, respectively. For determination of amino acids, samples were hydrolyzed by concentrated HCl with phenol at 120°C for 24 h. Amino acids were derivertized with OPA and analyzed by HPLC (Agilent, USA) with GromSil OPA C18 column, DAD detector, λ absorbance 340 nm. Mobile phase were A: NaH_2PO_4 40 mM, pH 7.8, B: ACN/MeOH/H_2O: 45/45/10, Mobile phase flow rate was 0.05 ml/min, the column temperature was maintained at 30°C. Digestible and metabolizable energy (DE and ME) values were calculated according to NRC (1998).

2.3. Measurements

Feed intakes were recorded daily by weighing the offered and refused feeds.

Animals were weighed at the beginning and finishing time of each period to determine average dailly gain (ADG). Feed conversion ratio (FCR) was calculated by dividing feed intake and body weight gain. Based on raw ingredient prices and cost of feed production, feed costs/1 kg weight gain was calculated by the total feed consumption and weight gain of each stage.

Table 1. Ingredients and chemical composition of dietary treatments

Item	Period 1 (15 - 30 kg)				Period 2 (30 - 60 kg)				Period 3 (60 – finisher)			
	Control	DDG 5%	DDG 10%	DDG 15%	Control	DDG 5%	DDG 10%	DDG 15%	Control	DDG 5%	DDG 10%	DDG 15%
	Ingredient (%)											
Probiotic	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Lasasu enzymes	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Corn	60.00	55.00	52.09	30.88	61.70	55.25	51.95	50.00	63.52	58.96	53.91	50.00
DDG cassava	0.00	5.00	10.00	15.00	0.00	5.00	10.00	15.00	0.00	5.00	10.00	15.00
Wheat	12.00	12.68	10.66	30.00	12.92	14.84	13.70	10.00	11.42	11.34	11.43	10.24
Soybean meal	20.85	20.32	19.54	18.12	18.92	18.76	17.17	17.76	17.96	16.79	17.44	17.99
Fish meal	2.51	2.50	3.21	1.50	1.96	1.65	2.68	2.60	1.90	2.72	2.01	1.50
Oil	0.44	0.30	0.30	0.30	0.30	0.30	0.30	0.44	1.00	1.00	1.00	1.07
MIX 02	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
Price (VND/kg)	8400	8200	8154	7606	8248	7959	7943.5	7815.3	8228	8188	7918	7691
	Chemical composition, as feed offered.											
ME (kcal/kg)	3125.0	3125.0	3125.0	3125.0	3125.0	3125.0	3125.0	3125.0	3125.0	3125.0	3125.0	3125.0
CP (%)	17.26	17.25	17.33	17.33	16.30	16.35	16.35	16.42	15.75	15.87	15.90	15.90
Fat (%)	3.74	3.51	3.45	3.28	3.62	3.49	3.47	3.47	4.29	4.25	4.09	4.00
Fiber (%)	4.20	6.24	8.00	11.78	4.23	6.40	8.21	9.87	4.04	5.97	8.00	9.90
Ca (%)	0.33	0.35	0.40	0.32	0.30	0.30	0.37	0.39	0.30	0.36	0.34	0.33
P (%)	0.44	0.44	0.43	0.51	0.43	0.43	0.43	0.40	0.41	0.42	0.40	0.38
Ash (%)	3.45	3.93	4.45	5.17	3.24	3.73	4.30	4.69	3.11	3.69	4.09	4.48
Lys (%)	0.90	0.89	0.89	0.86	0.83	0.83	0.82	0.82	0.80	0.80	0.79	0.78
Met (%)	0.29	0.29	0.29	0.27	0.28	0.27	0.27	0.26	0.27	0.27	0.26	0.25
Tryp (%)	0.21	0.20	0.20	0.21	0.19	0.19	0.19	0.18	0.18	0.18	0.18	0.18

Note: MIX 2: Vitamine, mineral premix produced Bayer company; ME: Metabolism energy; CP: Crude protein; Lys: Lysine; Met: Methionine; Tryp: Tryptophan. Coltrol: the basal diet; DDG 5%: the diet with cassava DDG 5%; DDG 10%: the diet with cassava DDG 10%; DDG 15%: the diet with cassava DDG 15%.

2.4. Statistical analysis

All data from the experiment were statistically analyzed as completely randomized design (CRD) using the GLM procedure of Minatab 16. Differences among means with < 0.05 were accepted as representing statistically significant differences. Data were analyzed using the model:

$Y_{ij} = \mu + T_i + e_{ij}$

Where: Y_{ij} is dependent variable; μ is the overall mean; T_i is effect of the level of cassava DDG (i = 0, 5, 10, 15%); e_{ij} is the residual effect.

3. RESULTS AND DISCUSSION

3.1. Voluntary feed intake

Table 2 presents the voluntary feed intake of pigs fed various levels of cassava DDG. There was a change in feed intake when pigs fed diets with different levels of cassava DDG (P < 0.001). In the period of 15 - 30 kg, feed intake was higher for pig fed the diets containing 5 and 10% cassava DDG than for pigs fed the diets without or containing 15% cassava DDG. The diets added 10 and 15 % cassava DDG reduced feed intake in the period of 30 - 60kg. In general, during growing periods (15 - 60 kg), feed intake declined linearly by the increase in levels of cassava DDG in the diet. It can be explained by increasing dietary fiber when increase percentage of cassava DDG added in the diets. In this present study, crude fiber were greater when added 5, 10, 15% of cassava DDG (Table 1). Several studies reported that feed intake is inversely related to the amount of dietary fiber, and feed intake could be decreased when the diets contain high fiber content (Nyachoti *et al.,* 2005; Phoemchalard *et al*., 2014).

In contrast to growing period (15 - 60 kg), in finishing period (from 60 kg to slaughter), feed intake were increased when pigs fed diets with increasing level of cassava DDG (P < 0.001). Similar to studies by Ndindana *et al.* (2002) and Thacker (2006), the authors reported that feed intake were not affected by different levels of wheat DDGS (4.86; 9.71; 15.7; and 19.2%); or even feed intake were increased when pigs fed high fiber diets (Low, 1993). This result indicated that pigs in finishing phase could use the high fiber diets without negative effect on feed intake.

In overall (15 kg – finishing period), feed intake were increased for pigs fed diets added 10 and 15% cassava DDG; while it was unaffected by 5% of cassava DDG. It was due to the significantly increasing feed intake in the diets added 10 and 15% cassava DDG during finishing phase. Feed intake is one important indicator that affect to weight gain and animal health; however, this feed can be well digested or not is another crucial indicator.

Table 2. Voluntary feed intake of pigs fed various levels of cassava DDG (kg/head)

Period	Control	DDG 5%	DDG 10%	DDG 15%	SEM	P
Period 1	44.39^{b}	47.59^{a}	46.84^{a}	44.17^{b}	0.83	P < 0.001
Period 2	79.95^{a}	79.49^{a}	75.97^{c}	77.23^{b}	1.57	P < 0.001
Period 3	103.63^{c}	105.94^{b}	111.30^{a}	113.57^{a}	0.67	P < 0.001
Overall experiment	227.98^{b}	233.01^{ab}	234.12^{a}	234.97^{a}	3.67	P < 0.001

Note: ^{a,b}Means in the same row with different superscripts differ (P < 0.05). SEM: Standard error of the mean. Coltrol: the basal diet; DDG 5%: the diet with cassava DDG 5%; DDG 10%: the diet with cassava DDG 10%; DDG 15%: the diet with cassava DDG 15%. Period 1: 15 - 30 kg; period 2: 30 - 60 kg and period 3: 60 kg - slaughter weight.

Table 4. Effect of cassava DDG levels on growing preformance and feed utilization efficiency

Item	Control	DDG 5%	DDG 10%	DDG 15%	SEM	P
Initial Body Weight (kg)	14.55	13.31	13.23	13.23	0.18	ns
Weight gain (kg)						
Period 1	36.42[a]	36.64[a]	34.72[b]	32.27[c]	0.48	P < 0.001
Period 2	64.87[a]	65.23[a]	61.85[b]	58.72[c]	1.27	P < 0.001
Period 3	109.81[a]	110.71[a]	107.65[b]	104.33[c]	1.72	P < 0.001
Overall experiment(kg)	95.26[b]	97.40[a]	94.42[b]	91.10[c]	1.80	P < 0.001
Overall ADG (g/head/day)	705.63[b]	721.48[a]	699.42[b]	674.83[c]	11.08	P < 0.001
Overal FCR (kg/kg)	2.39[c]	2.39[c]	2.48[b]	2.58[a]	0.03	P < 0.001

Note:[a,b]Means in the same row with different superscripts differ (P < 0.05). SEM: Standard error of the mean. Coltrol: the basal diet; DDG 5%: the diet with cassava DDG 5%; DDG 10%: the diet with cassava DDG 10%; DDG 15%: the diet with cassava DDG 15%. Period 1: 15 - 30 kg; period 2: 30 - 60 kg and period 3: 60 kg - slaughter weight.

3.2. Body weight gain and feed conversion ratio

Body weight gain, average daily gain (ADG) and feed conversion ration (FCR) are presented in Table 4 and Figure 1. There are significant different body weight gain and FCR among treatments in all experimental periods (P < 0.001). Body weight gain, ADG, and FCR were decreased by 10 and 15% cassava DDG, while body weight and ADG were higher and FCR was lower for pigs fed 5% cassava DDG compare to pigs fed control diet. This is in agreement with results of other researchers *(Avelar et al., 2010; Emiola et al.*, 2009; Thacker, 2006, 2009), who reported that increasing the level of wheat DDGS in the diet resulted in a linear decrease in weight gain but increase in FCR. The result of feed intake indicated that diets formulated at 10 and 15% cassava DDG increased feed intake, resulting in reducing body weight gain and FCR. It could be explained by the increasing fiber content in the diets added 10 and 15% cassava DDG and thus decreased feed digestibility (Bell *et al.,* 1983; Kennelly and Aherne, 1980; Nortey *et al.*, 2007).

Overall, the diet formulated at 5% cassava DDG resulted in greater weight gain, ADG and lower FCR. According to this result, the additional cassava DDG can be used in the diet to replace (at 5 %) other expensive ingredients such as corn meal.

3.3. Feed cost

Feed cost are typically at least 2/3 the total cost of producing pig, so a little change in feed cost can affect significantly to the profitability. Therefore, the strategy in pig production is to find available feed resources that are cheap and can be used in the pig diet.

In present study, the use of cassava DDG in the diets reduced price of 1 kg feed. Particularly, in the period of 15 - 30kg, price for 1 kg feed of control diet was 8400 VND; for diets added 5; 10 and 15% were 8200; 8154; 7606 VND, respectively (Table 1). In the period of 30 - 60 kg and finishing period, price of feed were also decreased when cassava DDG were supplemented into the diets.

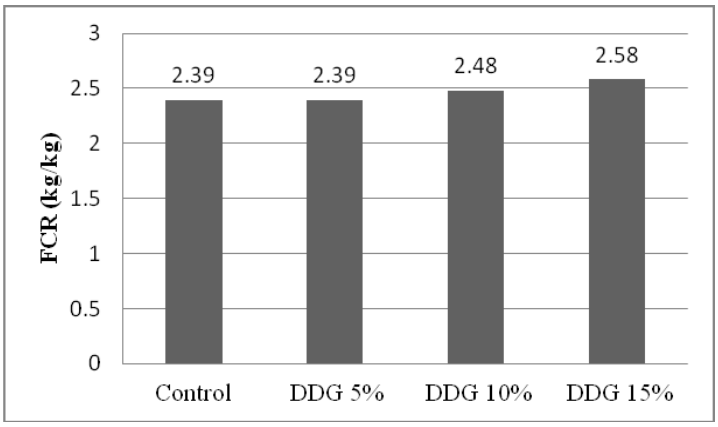

Figure 1. Effect of cassava DDG levels on on feed convertion ratio

Table 5. Effect of cassava DDG on feed cost (Unit: 1,000 VND/kg weight gain))

Feed cost	Control	DDG 5%	DDG 10%	DDG 15%	SEM	P - value
Period 1	372.89^{b}	390.23^{a}	381.97^{ab}	335.98^{c}	18.62	P < 0.001
Period 2	659.43^{a}	632.63^{ab}	603.46^{b}	603.57^{b}	34.25	P < 0.001
Period 3	852.70^{b}	867.42^{ab}	881.30^{b}	873.45^{a}	16.84	P < 0.001
Overall experiment	1885.02^{a}	1890.28^{a}	1866.74^{ab}	1812.99^{b}	77.40	P < 0.001
Feed cost/kg weight gain	19.79^{b}	19.41^{c}	19.77^{b}	19.90^{a}	0.03	P < 0.001

Note: ^{a,b}Means in the same row with different superscripts differ (P < 0.05). SEM: Standard error of the mean. Coltrol: the basal diet; DDG 5%: the diet with cassava DDG 5%; DDG 10%: the diet with cassava DDG 10%; DDG 15%: the diet with cassava DDG 15%. Period 1: 15 - 30 kg; period 2: 30 - 60 kg and period 3: 60 kg - slaughter weight.

Based on experimental feed ingredients price, feed cost, and feed cost per one kilogram of body weight were calculated (Table 5). The results show that, total feed cost for three phases and for overall experiment and feed cost per kg BW were significantly different among treatments (P < 0.001).

Overall experiment, the diets supplemented cassava DDG at the levels of 10 and 15% have lower feed cost than the control diet and the diets with 5% cassava DDG. However, the feed cost per kg BW gain for the diet containing 5% of cassava DDG was lowest with reducing of 380.20 VND compared to the control diet.

4. CONCLUSIONS

Based on the results of this study, it could be concluded that the diet containing 5% cassava DDG could improve growth rate by 2.3% (from 705.63 to 721.48 g/a/day) and reduced feed cost/ by 1.9% (from 17,79 to 19,41 thousand VND kg BW gain). Therefore, the cassava DDG can be used as an alternative ingredient at ratio of 5% in the diet of growing pigs to improve the efficiency of pig production. However, these finding should be studied further in production trials in order to investigate performance and carcass characteristics in growing pigs.

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UTILISATION OF RICE DISTILLER'S BY - PRODUCT FOR SWINE PRODUCTION IN NORTHERN VIETNAM

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ABSTRACT

The objective of this study was to investigate the production of rice distiller's by - product and its use as feed for pigs in three provinces (Hai Duong, Hung Yen and Bac Giang) of Northern Vietnam to identify annual supply resource of the by - product as pig feed. A total of 120 rice alcohol producers classified by production scales (large, medium and small) were interviewed from January to August 2015. Additionally, sixty - three rice distiller's by - product samples were collected from investigated areas to determine nutrition components and to evaluate the effect of storage time (from the first to the seventh day) on quality of rice distiller's by - product. Annual rice distiller's by - product production was 17.26, 6.26 and 3.59 tons per household for large, medium and small scales respectively ($P < 0.001$). The dominance of swine quantity in large - scale alcohol producing households in comparison with smaller number of pigs raised in medium and small ones ($P < 0.05$) proved a clear relation between number of pigs in household and alcohol production scales. The amount of by - product used in daily diet of sows in all three scales gradually reduced from pregnant to lactating sows. The utilisation of by - product for fattening pigs was significantly diversified among different scales (33.6%, 29.3% and 25.3% for large, medium and small scales respectively) ($P < 0.05$). The rice distiller's by - product was a rich source of crude protein (26.2%), neutral detergent fiber (33.7%), lactic acid (2.27 %) and gross energy (20.41 MJ/kg DM). Furthermore, its nutritive values were stable under ambient condition during a week ($P > 0.05$).

Keywords: Nothern Vietnam, nutrition value, rice distillers' by - product, pig production.

1. INTRODUCTION

In pig production, feed cost accounts for a weighty portion (70%) of total cost (Huynh *et al.*, 2007; Bergstrom *et al.*, 2014). In order to reduce this cost, a number of pig producers preferred to use by - products from alcohol, beer production or agricultural processing plants as animal feed (Manh *et al.*, 2009; Rosenfelder *et al.*, 2013; Woyengo *et al.*, 2014). In Vietnam, a large amount of rice distillers' by - product (RDP) could be obtained after rice fermentation for alcohol production in

traditional villages (Oanh *et al.*, 2016). In general, diet formula with RDP was often experience - based and fluctuated in each farm (Hong *et al.*, 2006; Hong *et al.*, 2013). Previous studies (Manh *et al.*, 2009; Hong *et al.*, 2013; Oanh *et al.*, 2016) showed RDP was used as sources of protein (17 - 32%) and energy (20 - 21 MJ/kg DM) in pig diets because of cheap cost and frequent availability in the whole year. Moreover, diets containing RDP were assumed to reduce a number of E.coli bacteria and total coliform in the gastrointestinal tract of piglets (Hong *et al.*, 2009), reduce feed cost and enhance economic benefits *(Manh et al.*, 2009). The preventive capability of digestive system against intestinal diseases can be improved by low pH value and high lactic acid ratio of RDP (Oanh *et al.*, 2016). However, researches on the utilisation of RDP as animal feed in alcohol villages in Northern Vietnam have been still limited. Therefore, the main objective of this research is to provide information about (1) the amount of RDP obtained in traditional alcohol trade villages, the proportion of this by - product in pig diets and the effects on organoleptic quality of pig meat, and (2) the nutritive values of RDP according to storage time. In both farming and scientific research respects, these results will provide more useful information in salvaging RDP as feed for pig production.

2. MATERIALS AND METHODS

2.1. Research area and samples

A cross - sectional study was conducted from January to August 2015 on 120 out of 825 rice alcohol producers based on various scale selection (large, medium and small) in three provinces (Hai Duong, Hung Yen and Bac Giang) in Northern Vietnam (Table 1).

2.2. Data collection

2.2.1. Interviews

Provided by local statistical officers, 40 households (hhs), who possessed both alcohol distillation and pig production, were selected randomly from alcohol producer list of each province. Based on the local criteria, alcohol production was classified into scale - based large, medium and small groups corresponding to alcohol daily out put per household achieved at more than 40 litres, from 20 to 40 litres, and lower than 20 litres, respectively.

Information was collected by interviews on study site by using a questionnaire. Before conducting official survey, a validation of the questionnaire was tested in 9 households, focused on (1) general information about material preparation, frequency of alcohol production, amount of alcohol, amount of RDP by a balance of 50 kg; (2) number of pigs raised, proportion of RDP in diet for sows and fattening pigs and its effects on pig performance.

Table 1. Sample distribution by scale and province in the study areas

Scale of alcohol production	Provinces			Total scales
	Hai Duong	Hung Yen	Bac Giang	
Large (> 40 l)	10	10	10	30
Medium (20 - 40 l)	15	15	15	45
Small (< 20 l)	15	15	15	45
Total by localities	40	40	40	120

Table 2. Amount of rice distiller's by - product per household from different alcohol production scales during a year (LSM ± SE)

Parameters	Large (n = 30)	Medium (n = 45)	Small (n = 45)
Frequency producing alcohol (time)	864a ± 60.5	682ab ± 49.4	552b ± 49.4
Rice for alcohol production (tonne)	20.3a ± 1.24	12.5b ± 1.01	8.83c ± 1.01
Alcohol production (litre)	15910a ± 729	9349b ± 595	5965c ± 595
Wet RDP (tonne)	156.3a ± 22.4	56.7b ± 18.3	32.6b ± 18.3
DM RDP *(tonne)	17.26a ± 2.47	6.26b ± 2.02	3.59b ± 2.02
DM RDP/1 tonne rice for alcohol production (kg)	241.78a ± 4.94	241.70a ± 4.04	243.18a ± 4.04

*Note: Value without the same letter in the same row differ significantly (P < 0.05); *DM of rice distiller's by - product estimated at 11,04% by Oanh et al., (2016).*

2.2.2. Sample collection

A total of 63 representative RDP samples were collected from 9 households typical for three different scales in three study provinces. Equally, 3 households of each provine by different scales (> 40 litre, 20 - 40 litre and < 20 litre) was chosen. According to Vietnamese standard No 4325 (TCVN, 2007), in each household, 7 RDP samples (approximately 500ml per sample) were collected from a single alcohol distillatory batch and stored in the plastic bottles. The bottles were numbered 1 to 7 corresponding to storage time (day 1 to day 7) and stored under ambient condition. Rice and local fermentative yeast were input materials in the process of alcohol distillation. Traditionally, firewood or electricity were used to supply energy for this artisanal distillation. The yeast used to produce the alcohol was produced locally at artisan level. Chemical composition and nutritive values of these samples were analysed daily from the first to the seventh day. Later, parameters of pH value, dry matter (DM), crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), gross energy (GE), lactic acid, acetic acid, butyric acid, total organic acids, calcium (Ca), phosphorus (P) were analysed according to AOAC method (1990) at the Center Laboratory, Faculty of Animal Science, Vietnam National University of Agriculture.

2.3. Statistical Analysis

The data were analysed by using the SAS Software, version 9.0. The General Linear Model (GLM) and chi - squared test (χ^2) was used for quantitative and qualitative data in order.

3. RESULTS AND DISCUSSIONS

3.1. Characteristics of survey households

The average age of householders was not significantly different (P > 0.05) among alcohol production scales (47.60, 49.00 and 50.73 years old in large, medium and small scale respectively). In each household, average number of family members was 4.07, 4.16 and 4.20 while as average number of employees was 3.00, 2.78 and 2.68 in large, medium and small scale respectively. These values were not seemingly scale - oriented (P > 0.05).

Education level of household owners in large scale was higher than those in medium and small scale (P < 0.05). The number of owners who finished their highest study level at primary school and secondary school was low for large scale (13.33% and 36.67%), moderate for medium scale (20% and 42.22%) and high for small

scale (28.89% and 44.44%). In contrary, owners who finished high school were most popular for large scale of alcohol production model (covered up to 50%). Based on these data, we assumed that education level of owners could influence on the scale of alcohol production in study areas.

The importance of alcohol business were significantly different for each scale of prodution. Alcohol production were the main occupation for 100% of large - scale households, compared to 95.56% and 73.33% of households at medium scale and small scale respectively ($P < 0.001$).

3.2. Rice distiller's by - product productivity for pig production by scales of traditional alcohol production

3.2.1. Rice distiller's by - product productivity

The productivity of RDP released from alcohol production in large, medium and small scales were presented in Table 2. Frequency of alcohol production were statistically different between large scale and the other two scales ($P < 0.001$). However, there were no sifnificant disparity between medium scale and small scale ($P > 0.05$). On the other hand, the quantity of rice used for fermentation was unequal among all three scales ($P < 0.001$).

The amount of RDP harvested was statistically different by alcohol production scale ($P < 0.001$). In large scale, this number was 2.8 times and 4.8 times greater than that those in medium scale and in small scale. There were no differences in DM RDP/tonne of rice calculated in all three scales ($P > 0.05$), which implied that the fermentation methods utilised in alcohol production households were similar. Obviously, the majority of this by - product was supplied from traditional alcohol producing villages. Besides, this by - product contributed as a potential feed source for pigs, which was important to develop effectively for the improvement of economic values.

3.2.2. Utilisation of rice distiller's by - product in pig diets

All producers mainly used RDP as pig feed (100%), 13.33% of hhs used for feeding chicken and only a small proportion of farmers used for fish production (2.5%). The daily output of this by - product was naturally enough for feeding pig herd in one day. Excessive amount could be stored in a jar for feeding animals within three days or sold to other farming villagers.

3.2.2.1. Relation between alcohol production scales and pig number

The model of fattening pig raising was popular in all three alcohol production scales (Table 3) while households raised both sows and fattening pigs occupied around 43.33, 46.67, 44.44% of all investigated households in large, medium and small scale respectively ($P > 0.05$).

Table 3. Number of pigs in a household by scales

Variable	Large	Medium	Small
Number of households	30	45	45
Number of households with sow	13	21	20
Number of households with fattening pigs	30	44	44
Number of sows	3.15^{a}	2.10^{ab}	1.70^{b}
Number of fattening pigs	66.6^{a}	39.4^{b}	30.1^{bc}

Note: Value without the same letter in the same row differ significantly ($P < 0.05$).

Table 4. Number of households and utilisation of raw materials (%) in diet for sow by scales (in DM)

Material	Large (n=13)		Medium (n=21)		Small (n=20)	
	Number Household	Raw material	Number Household	Raw material	Number Household	Raw material
Feed for sows in early stage of pregnancy (0 - 84 days)						
Maize meal	6	46.86	8	37.73	12	45.1
Rice bran	9	47.03	11	49.58	11	42.53
Wheat bran	3	41.16	9	58.31	8	51.32
Rice distiller's by - product	12	28.01	21	25.42	19	23.3
Concentrated feed	1	8	0	0	1	3.85
Completed feed mixtures	2	64	4	48.56	4	33.48
Feed for sows in late stage of pregnancy (85 - 114 days)						
Maize meal	6	45.36	8	31.84	11	42.57
Rice bran	8	47.11	12	42.05	11	40.05
Wheat bran	3	35.00^{b}	7	61.75^{a}	7	48.78^{ab}
Rice distiller's by - product	12	26.13	16	24.39	17	22.38
Concentrated feed	1	8.7	0	0	2	5.93
Completed feed mixtures	4	55.94	11	47.1	9	38.27
Feed for lactating sows						
Maize meal	5	33.23	9	23.65	10	37.02
Rice	0	0	2	26.32^{b}	1	29.41^{a}
Rice bran	9	32.98	10	40.36	9	39.39
Wheat bran	1	27.78	9	48.24	9	35.57
Rice distiller's by - product	10	20.19	11	19.24	12	17.91
Concentrated feed	1	5.71	0	0	2	5.64
Completed feed mixtures	11	54.7	15	52.34	15	45.77

Note: Values without the same letter in the same row differ significantly (P < 0.05).

The number of pigs in household related to the alcohol production scales was shown in table 3. There were statistically significant difference in number of sows and fattening pigs among large scale, medium and small scales (P < 0.05).

3.2.2.2. Utilisation of rice distiller's by - product in diet for the sows

The sow diet formulas were not the same for farmers in three different scales (Table 4).

Number of households salvaging rice distiller's by - product as sow feed was higher than that using other raw materials. However, this by - product was preferablly used at lower proportion for late stage of pregnancy and lactating sows because producers reported that malformation rate of new - born piglets was supposed to increase when sows were fed with this by - product in late stage of pregnancy. In deed, Manh *et al.* (2009) indicated sows fed with 33% RDP ended up with lower reproductive performance (9.7 piglets per litter and 1.20 kg per piglet) compared to those fed without RDP (10.10 piglets per litter and 1.50 kg per piglet) while live weight of weaning piglets was similar. This research - based supervision implied that decreasing

RDP ratio in diets for pregnant sows was reasonable but it was not nessessary to reduce RDP components in diets for lactating sows.

3.2.2.3. Utilisation of rice distiller's by - product in the diet for fattening pigs

The proportion of RDP used in fattening pig diets in each alcohol production scale was significantly different ($P < 0.05$) from the others (Table 5).

It was previously indicated that diet mixture containing 15 - 30 % rice distiller's by - product did not influence fattening pigs' growth performance (Hong *et al.*, 2013). In this study, experience - based diets for fattening pigs containing 25.33 - 33.46% RDP were prefered by farmers because of the stable growth performance in their swine herds. In comparison with the results published by Hong et al., this range of RDP is slightly higher.

Table 5. Number of households and utilisation of raw materials (%) in diet for fattening pigs by scales (in DM)

Material	Large (n = 30)		Medium (n = 44)		Small (n = 44)	
	Number Household	Raw material	Number Household	Raw material	Number Household	Raw material
Maize meal	6	35.65	21	38.05	22	38.21
Rice	0	0	0	0	2	56.00
Rice bran	9	41.75	16	30.46	17	32.08
Wheat bran	20	42.61	26	36.19	23	43.41
RDP	30	33.46^{a}	44	29.32^{ab}	44	25.33^{b}
Concentrated feed	6	8.20	14	10.50	14	11.78
Complete feed mixtures	21	24.07	32	22.98	29	21.53

Note: Values without the same letter in the same row differ significantly ($P < 0.05$).

Table 6. Effects of rice distiller's by - product on the sows

Parameters	Large (n = 13)	Medium (n = 21)	Small (n = 20)
Stimulating feed intake of sows	7	3	9
Piglet's skin and hair shiny and smooth	9	11	11
Reducing piglet diarrhoea	9	11	11
Improving pig health	9	12	12
Reducing feed costs	12	21	20

3.2.3. Effects of rice distiller's by - product on qualitative parameters of swine production

3.2.3.1. Effects of rice distiller's by - product on quantitative parameters of sows

The effect of RDP on the qualitative paramters of sows was classified by farmer opinions (Table 6). Investigational results showed that qualitative parameters were not significant difference ($P > 0.05$) among three different scales. By farmer opinion, the present of RDP in diet was not only effective in reducing feed cost, but also in improving pig health, reducing piglet diarrhoea and stimulating feed intake

of sows in all scales. Therefore, the utilisation of RDP in sow diets positively influenced both technical parameters and production costs.

3.2.3.2. Effects of rice distiller's by-product on qualitative parameters of fattening pigs

Qualitative parameters of fattening pig fed RDP were also assessed based on farmers's observation. Difference in qualitative parameters was not statistically significant between scales (P > 0.05). All farmers was optimistic about effective usage of RDP for fattening pigs to reduce feed costs, improve pig meat with higher organoleptic quality and satisfy customers' demand.

Moreover, RDP included in pig diet not only contributed to the improvement of feed intake and the appearance of skin and hair, but also enhanced swine general health. For this reason, it is believed that swine growth performance and quality of meat benefit from the combination of RDP and local raw materials in pig diets.

3.3. Chemical composition and nutritive value of rice distiller's by - product

Chemical composition and nutritive values of RDP were analysed daily from the first to the seventh day of preservation (Table 8). Chemical composition and nutritive values were not significantly different (P > 0.05) among storage times. The pH value and DM content of wet RDP samples were low and stable from day 1 to day 7 (pH value: 3.12 to 3.18; DM proportion: 10.97 to 11.37 %) (P > 0.05). The pH value in this study was similar with previous study done by Manh *et al.*, (2009), but DM content was slightly higher than that in other studies (Manh *et al.*, 2009, Hong *et al.*, 2013). This inconsistence could be explained by different distillative equipments, yeast generation and especially proportion of rice and water in alcohol production process.

Crude protein (CP) and GE values of RDP was considerably high (achieved at 26.22 % and 20.41 MJ/kg DM respectively) and relatively stable during 7 days of preservation (P > 0.05). CP content of RDP in this study was higher than that in a study conducted in Mekong River Delta (Manh et al., 2009), but was lower than that in another study in the Middle of Vietnam (Hong et al., 2013). Alternatively, GE value was similar to results published by Manh et al., (2009). The different results among different studies could be explained by various selections of rice breeds, fermentative yeast and distillative methods.

Table 7. Effects of rice distiller's by - product on fattening pigs

Parameters	Large (n=30)	Medium (n=44)	Small (n=44)
Stimulating feed intake of fattening pig	10	23	22
Pig's skin and hair shiny and smooth	17	30	26
Improving animal health	30	43	42
Reducing production costs	30	44	44
Pig meat with higher organoleptic quality	30	44	44
Consumer preferences and choice	30	44	44

Table 8. Chemical composition and gross energy of rice distiller's by - product from the first to the seventh day *(n = 9)*

Parameters	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Average	SEM
pH	3.14	3.14	3.12	3.15	3.13	3.18	3.15	3.14	0.05
Dry matter (DM), %	10.97	11.37	11.37	11.15	11.24	11.37	11.02	11.22	0.40
Composition based on the dry matter (%)									
Crude Protein (CP)	26.37	26.31	25.94	26.72	26.35	25.60	26.24	26.22	1.40
Ether Extract (EE)	3.69	3.96	3.47	3.05	3.95	3.89	3.76	3.68	1.14
Ash	5.14	5.09	5.18	5.14	5.20	4.97	5.33	5.15	0.27
NDF	34.06	34.67	34.37	33.25	35.32	32.95	31.07	33.67	4.10
ADF	16.66	16.95	16.79	16.01	17.13	15.56	14.86	16.28	4.26
Calcium (Ca)	0.14	0.15	0.14	0.16	0.18	0.16	0.20	0.16	0.02
Phosphorus (P)	0.64	0.63	0.62	0.66	0.61	0.63	0.66	0.64	0.12
Gross Energy (GE), MJ/kg DM	20.34	20.44	20.31	20.11	20.56	20.53	20.60	20.41	0.12
Composition in g/100g fresh sample									
Lactic Acid	2.38	2.30	2.27	2.31	2.28	2.20	2.25	2.27	0.14
Acetic Acid	0.07	0.05	0.06	0.07	0.08	0.06	0.06	0.06	0.02
Butyric Acid	0.12	0.14	0.13	0.18	0.19	0.17	0.16	0.16	0.04
Total organic acid*	16.89	16.86	16.57	17.17	17.15	16.34	16.44	16.77	0.95

*Note: *presented according to gram of sulfuric acid in 01 kilogram of rice distiller's by - product.*

The consistently high level of NDF in RDP samples (33.67 on average) confirmed the appreciable fibre value of this by - product during preservative period. This result was higher in comparison with publication (15.4%) by Manh *et al.,* (2009). This could be caused by type of rice and fermentation.

From day 1 to day 7, the components of calcium and phosphorus in DM were found to be rather low, ranging from 0.14 to 0.2% and 0.61 to 0.66% respectively. Meanwhile, Manh *et al.* (2009) reported that calcium and phosphorus of rice distller's by - product were achieved at 0.55 and 0.35%, in order. These differences suggested a vast array of choices during alcohol production process, especially distillative techniques and rice variety.

Current study advocated the consequences published in the remarkable study of Carpenter (1970) and reaffirmed the influence of initial materials, production methods and distillative equipments on chemical compositions and nutritive values of RDP.

There was not significant difference for single organic acid level by preservation time ($P > 0.05$). In this study, lactic acid level of RDP was meaningfully higher than levels of acetic acid and butyric acid, possibly caused by the usage of a typical yeast. Hong *et al.* (2009) reported that high lactic and acetic acid contents in pig diets from RDP and liquid fermented by - products played a role in decreasing the number of *E.coli* and total coliforms in

stomach and ileum of piglets (Hong *et al.*, 2013). Furthermore, fermented liquid feed was proved to increase lactic and acetic acid concentrations and reduce number of Salmonella in swine gastrointestinal tract (Van Winsen *et al.*, 2001). The quantity and frequency of diarrhea observed in piglets fed with wet wheat - distillers grain were lower than in those fed without (Pedersen *et al.*, 2005). Low pH environment and high level of organic acids (especially lactic acids) created a favorable condition for stimulating the development of useful bacteria and limiting activities of harmful bacteria. Moreover, the apprearance of RDP in pig diets contributed to decrease pH in intestine, leading to the reduction of swine diarhea disease.

In conclusions, the nutritive values of RDP were not statistically fluctuated during one week. For this reason, the rice distiller's by - product can be stored and preserved in jars for swine feeding up to 7 days without significant modification of chemical and nutritive components.

4. CONCLUSIONS

A large amount of rice distiller's by - product was produced by alcohol manufacturers in large, medium and small scale, contributing to meet demand of swine feed for the whole year.

Swine farming size of large - scale alcohol producers was bigger than that of medium - scale and small - scale ones. RDP ratio in diets was lower for lactating sows than that for pregnant sows in all three alcohol production scales. On the other hand, propotion of RDP in diets for fattening pigs was high in large - scale producers but increasingly lower in medium - scale producers and small - scale producers. Moreover, the contribution of this by - product in pig diets showed improvements in technical parameters and organoleptic quality of pig meat.

Rice distiller's by - product was a rich source of crude protein, neutral detergent fiber and crude energy but containing low dry matter. Especially, its low pH value and high lactic acid might influence swine gut health possitively and limit pathogens in swine gastrointestinal tract. Its nutritive values were stable under ambient condition of preservation during 7 days.

Further research is necessary to identify the energy metabolism and optimum proportion of rice distiller's by - product in pig production.

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WELFARE QUALITY OF GESTATION SOWS IN DIFFERENT PRODUCTION SYSTEMS IN THE RED RIVER DELTA OF VIETNAM

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ABSTRACT

Vietnam is one of the largest pork producers in the world, leading to the significance of pig welfare issues. This study was conducted at 65 pig farms in the Red River delta to assess welfare quality of gestation sows kept in four production systems using the Welfare Quality® protocol (2009). Sows in the small farms with a small herd size were often housed in individual pens (8.06 m^2 per pen) in open-side buildings and fed with an on-farm mixed ration between local feed resources and industrial feed. On the contrary, sows in the medium and large farms were confined in narrow crates in completely closed houses and provided with industrial concentrated feed. The main welfare issue of gestation sows in the small-scale production system were strongly related to housing conditions with an increasing risk of panting (52.59% of sows) in hot weather. Sows in the medium and large farms were at a higher risk of several injuries (wounds on body, skin condition, and local infections) and performed several stereotypies behaviours (sham chewing, tougue rolling, bar biting, and drinker biting) and feared of human with a higher prevelance than those housed in pens in small farms. The improvement of sow welfare quality should be focussed on environmental ventilation enhancement of sow housing in small farms and by redesigning sow houses to provide them with more space allowance and some beddings in medium and large farms.

Keywords: Gestation sows, pig production system, sow welfare.

1. INTRODUCTION

In Vietnam, the demand for animal source foods, especially pork has risen dramatically over the past decade. The contribution of animal agriculture to the total gross agricultural output has also increased significantly from 19.3 percent in 2000 to 26.5 percent in 2011 (General Statistics Office, 2012a). This has been accompanied by a decrease in the proportion of small-scale animal farms and a rapid increase in the proportion of larger farms. Between 2006 and 2011, the number of farms keeping less than 10 pigs decreased by 2.2 million farm holdings (35%), while the number of farms with more than 50 pigs increased by 80% in the same period. As of 2011, 4.13 million households were raising pigs, in which more than 87% had less than 10 pigs, and less than 1% kept more than 50 pigs (General Statistics Office 2012b). However, in the coming years, the government will continue to give priority to the development of larger, industrial animal farming systems, with the aim of having 37% of the total pig population in industrial systems by 2020 (Ministry of Agriculture and Rural Development, 2008).

The intensification of pig production systems has resulted in a great concern about animal welfare and the significance of conducting research projects not only in

developed countries, but also in developing countries involving Vietnam. Currently, animal welfare is still a new concept in Vietnam due to the lack of researches on this topic. In order to give implications of alternative improved-animal welfare practices for farmers and policy makers, the assessment of welfare quality of farm animals in different production systems plays as a basic role for setting up an appropriate code of practices for farmers as well as for administrative officers.

This study aimed to assess the impact of different pig production systems in the Red River delta on the welfare quality on gestation sows using Welfare Quality protocol (2009). Based on this assessment, recommendations were developed for farms of all sizes to improve animal welfare in their production practices.

2. MATERIALS AND METHODS

2.1. Selection of study sites and farm households

The study was conducted in the Red River delta of Vietnam. Specifically, Haiduong, Hanoi and Hungyen provinces were chosen as they have the highest population of pigs in the Delta (General Statistics Office 2012a).

Selection of farms was based on the farm size and housing systems. Firstly, the classification of pig farms were implemented by the participatory discussion with local government officials in study sites. Farms were classified into four categories according to size, as follows: 1) Small farm had less than 20 sows; 2) Medium farms had 20-100 sows; 3) Large farms had more than 100 sows. For small farms, they were divided into two sub-systems according to housing condition, including stall-confined house and pen-confined house. The number of sampled farms depended on the diversity or homogeneity in farm practices and management among farms within a category and also based on the sow population herd. In the small-scale pig production systems, due to the high diversity in farm management and practices and low herd size, the number of samples was higher than those in medium and large-scale farms. A total of 26 pen-confined house farms and 14 stall-confined house farms were chosen. On the contrary, because of high homogeneity in farm practice and management and big sow herd size, only 15 medium-scale farms and 10 large-scale farms were assessed. The selection of these farms was conducted by a random method according to the list of farms in each category provided by the local officials.

2.2. Welfare measurements

In each farm, gestation sows were selected randomly for the animal-based welfare quality assessment. The number of sampled sows varied from small farms to large farms, including 116 sows in pen-confined house of small-scale farm, 165 sows in stall-confined house of small-scale farm, 247 sows in medium-scale farm and 597 sows in large-scale farm.

A number of selected animal-based indicators was applied to assess welfare quality of sows according to the Welfare Quality protocol (2009). Sows were inspected individually and scored accordingly (two-scale or three-scale scoring system depended on crieria) according to the absence or evidence of the welfare signs as well as the quality levels of welfare (from 0: no evidence or good quality to 1: moderately affected and 2: evidence or severely affected). Sows were observed visually in the morning after feeding time.

2.3. Statistical analysis

The data was analyzed using GENMOD procedure of SAS software

version 9.4. Copyright 2014 SAS Institute Inc. In order to compare the indices in four farming systems, proportional odds model (ordered logit model) was used to fit data measured on an ordinal scale. Given the outcome indice has three levels: low, normal and high; their proportions are respectively p1, p2 and p3. Then the logarithms of the odds of answering in certain ways are:

Low: $\boldsymbol{log}\left(\frac{p_1}{p_2+p_3}\right) = \boldsymbol{log}\left(\frac{p_1}{1-p_1}\right)$

Low or Normal:

$$\boldsymbol{log}\left(\frac{\boldsymbol{p_1+p_2}}{\boldsymbol{p_3}}\right) = \boldsymbol{log}\left(\frac{\boldsymbol{p_1+p_2}}{\boldsymbol{1-(p_1+p_2)}}\right)$$

The binary logistic model was used when the outcome had only two levels.

Farming system was the only predictor variable in the model. However, it was adjusted by two variables: season (fix variable, two categories) and farm (random variable). We took in account of farm variable because the results in the same farm were more likely similar, in another word, there are correlations between observations in one farm.

These models could be written as: Logit(P(indice ≤ j)) = α + β1*system + β2*season.

3. RESULTS AND DISCUSSIONS

3.1. General characteristics of pig production systems in study sites

The characteristic of pig production systems in the study site differs from one type of production system to another, depending strongly on the farm size, housing and feeding system. According to farm size and pig housing system, it can be classified into four types of pig production systems, including the small pen-confined pig farm, small stall-confined pig farm, medium pig farm, and large pig farm. The general characteristics of these production systems are shown in table 1.

There was a great difference in housing and feeding system among pig production systems, that may have different impacts on welfare quality of sows. In the small farms, the sow herd size was about 5.42 to 9.07 heads per farm, much smaller than that in the medium-scale and large-scale farms (Table 1). These sows were totally confined in individual pens for each sow with an average space allowance of 8.06 m^2 per pen (Table 1). This type of housing system is very good for sow welfare because it provides enough space for sows to turn around and enable them to express their natural behaviours. However, sows in the small farms faced with a high risk of thermal conditions, especially in hot or cold weather because they were kept in the open animal buildings that are characterized by the open air conditions with a limited indoor control facilitities (such as electronic fans, window drapes, water pumping system over the roof). Sows in small-scale farms were often fed by on-farm mixed feed regime (76.92% of surveyed farms) that composed of both locally produced feed (such as corn, soybean, and rice bran) and industrial concentrated feed.

In the medium-scale farms and large-scale farms, with the high herd size, the gestation crates were used exclusively (100% of farms) with a very narrow space allowance (1.43 - 1.51 m^2/stall) (Table 1) which limit the movement of sows, causing a lot of welfare problems for animals. The advantages for sow welfare in these farms were the good thermal conditions because they were kept in a completely closed building with a good ventilation system (such as cooling pad, suction fans) to partly control the indoor environment according to the requirement of animals. Sows were also fed with completely concentrated feed regime (used by 86.67 - 90% of farms) which could be adjusted to meet the requirement of sows according to different production stages and body conditions.

Table 1. General characteristics of pig production systems (Mean ± SD)

	Small pen-confined pig farms (n = 26)	Small stall-confined pig farms (n = 14)	Medium pig farms (n = 15)	Large pig farms (n = 10)
Total number of sows (head/farm)	5.42 ± 3.42	9.07 ± 6.71	40.60 ±17.00	212.70 ± 130.39
Size of pen/stall (m^2/sow)	8.06 ± 3.11	1.48 ± 0.03	1.51 ± 0.02	1.43 ± 0.01
Completely closed building (% of surveyed farm)	0	0	93.33	100
Open-side buildings (% of surveyed farm)	100	100	6.67	0
Completely concentrated feed (% of surveyed farm)	23.08	92.86	86.67	90.0
On-farm mixed feed (% of surveyed farm)	76.92	7.14	13.33	10.0

Table 2. Prevelance and standard error of selected measures of good feeding and good housing of sow welfare quality

	Scores	Small pen-confined pig farms	Small stall-confined pig farms	Medium pig farms	Large pig farms	P-value
Body condition	0	50.86	52.12	21.05	18.76	< 0.001
	1	39.66	41.82	68.83	69.85	
	2	9.48	6.06	10.12	11.39	
Bursitis	0	94.83	87.88	94.33	93.80	0.12
	1	5.17	10.30	5.26	5.53	
	2	0.00	1.82	0.40	0.67	
Shoulder injury	0	91.38	91.52	87.04	86.26	0.26
	1	6.9	7.27	12.55	12.56	
	2	1.72	1.21	0.40	1.17	
Panting	0	47.41	69.70	68.42	75.38	< 0.001
	2	52.59	30.30	31.58	24.62	

Note: Body condition score: 0: good, 1: moderate thin, 2: thin; Bursitis score: 0: no evidence, 1: moderate bursitis, 2: severe bursitis; Shoulder score: 0: no evidence, 1: moderate injury, 2: severe injury; Panting: 0: no evidence, 2: evidence of panting.

Table 3. Logistic-regression model of selected measures of good feeding and good housing of sow welfare quality for production systems

		Small pen-confined pig farms	Small stall-confined pig farms	Medium pig farms	Large pig farms
Body condition	OR	1	0.8	3.17	4.06
	CI 95%	-	0.37 - 1.73	1.28 - 7.9	1.71 - 9.65
	P-value	-	0.56	0.013	0.0015
Panting	OR	1	0.64	0.15	0.04
	CI 95%	-	0.16 - 2.50	0.029 - 0.7	0.01 - 0.125
	P-value	-	0.52	0.02	< 0.001

Note: OR: odds ratio; CI: 95% confidence interval.

3.2. Feeding and housing systems and sow welfare

Good feeding and good housing system have a strong influence on sow welfare and are two out of four main principles of sow welfare assessment. Table 2 and table 3 presented several selected measures of sow welfare related to feeding and housing system in four production systems.

Body condition of sows is an important measure of good feeding principle. There was an increasing prevelence of thin sow in the medium and large farm compared with pigs in the small production systems ($P < 0.001$). Results in table 2 showed that the odd of having good body condition of sows (score 0) on small pen-confined farms was estimated as being 3.17 times greater than that on medium farms ($P = 0.013$; CI 95%: 1.28 - 7.9) and 4.06 times higher than that on large farms ($P = 0.0015$; CI 95%: 1.71 - 9.65). In the medium and large produciton systems, sows often had a very high producitivity (high number of born piglets per litter and high number of litters per year), leading to an increasing risk of thin sows. Moreover, because of feed restriction to prevent sows from being fat, a number of sows in medium and large farms were at risk of poor body condition. On the contrary, sows in small farms were often fed with high-energy feed (corn, maize, rice brain) that resulted in better body condition.

Generally, housing condition strongly impacted on the sow welfare, especially the "comfort around resting" such as bursitis and shoulder injury, and the "thermal comfort". The prevelance of bursitis, shoulder injury of sows was not affected by production systems ($P > 0.05$) (Table 2). A low proportion of sows in four production systems suffered from severe bursitis (0.4-1.82% of sows) or severe shoulder injury (0.4-1.72% of sows) (Table 2). However, a significant difference in panting of sows among production systems was observed. Sows kept in open buildings in small farms represented a higher prevelance of panting (52.59% of sows) than those in the medium and large ones (24.62% in large farm) ($P < 0.001$) (Table 2). Table 3 presented that the odd of no evidence of sow panting in small farms was 0.15 times less than that at medium farm (CI 95%: 0.029 - 0.7) and 0.04 times less than that at the large farm (CI 95%: 0.01 - 0.125) ($P < 0.001$). The assessment was conducted in the summer (July), and pigs at the small farm were not subjected to extremely hot environments as indoor temperatures ranged from 28°C to 35°C. Joseph M. Zulovich (2012) reported that the thermal comfort zone ranged from 10^0C to 21°C in gestation sows. Consequently, the temperature range in this study appeared to be insufficiently high to observe a high variability of panting of sows in small farms. This is a big challenge for small pig farms whrer needs more facilities to cool the indoor rooms in hot weather.

3.4. Health and sow welfare

Good health is an important principle of sow welfare because it affects directly to the productivity and economic outcome of the farm, consequently. Sows may suffer from injuries and diseases caused by the handling and herd management. Several representive injury and disease measures of gestation sows in four produciton systems are shown in table 4 and table 5.

Table 4 showed that the prevalence of moderate and severe lame sows were very low in all production systems (under 3% for moderate lame and 0% of severe lame). There was also no significant different in gait score of sow between systems.

Table 4. Prevelance and standard error of selected measures of good health principles of sow welfare quality

	Scores	Small pen-confined pig farms	Small stall-confined pig farms	Medium pig farms	Large pig farms	P-value
Lameness	0	97.41	99.39	100	98.32	0.10
	1	2.59	0.61	0	1.68	
	2	0	0	0	0	
Wounds on body	0	88.79	82.42	71.26	70.85	0.0003
	1	11.21	15.76	26.32	25.63	
	2	0	1.82	2.43	3.52	
Skin condition	0	78.45	90.91	80.16	90.45	< 0.001
	1	17.24	8.48	13.77	8.04	
	2	4.31	0.61	6.07	1.51	
Local infections	0	93.10	93.94	87.85	95.31	< 0.001
	1	6.90	2.42	9.72	4.52	
	2	0	3.64	2.43	0.17	

Note: Lameness score: 0: normal gait, 1: moderate lame, 2: severe lame; Wounds on body score: 0: good, 1: moderate wounds, 2: severe wounds; Skin condition score: 0: no inflammation, 1: less than 10% skin inflamed, 2: more than 10% skin inflamed; Local infections: 0: no evidence, 1: moderate infection, 2: severe infection.

Table 5. Logistic-regression model of selected measures of good health of sow welfare quality for production systems

		Small pen-confined pig farms	Small stall-confined pig farms	Medium pig farms	Large pig farms
Wounds on body	OR	1	1.67	3.17	3.36
	CI 95%	-	0.54 - 5.15	0.84 - 12	1 - 11.35
	P-value	-	0.37	0.09	0.051
Skin condition	OR	1	0.36	0.925	0.38
	CI 95%	-	0.13 - 1.01	0.37 - 2.34	0.11 - 1.41
	P-value	-	0.053	0.87	0.15
Local infections	OR	1	0.83	1.83	0.70
	CI 95%	-	0.27 - 2.56	0.50 - 6.65	0.26 - 1.9
	P-value	-	0.74	0.36	0.49

Note: OR: odds ratio; CI: 95% confidence interval

The housing system can be an important cause of wounds on the sow body, skin condition and local infections. When sows were kept in individual pen with a sufficient space allowance, there was a decreasing risk of wounds and local infections (0% of sows affected by severe wounds on body and severe local infections in small pen-confined pig farms). In the medium and large farms, sows were at increasing risk of wounds and local infections ($P < 0.001$) due to the insufficient space allowance and the inappropriate structure of mental crates (Table 4).

However, the confidence interval (CI) of these measure contained the unity ($P > 0.05$) (Table 5). The improvement of housing condition, especially the space allowance and bedding provision may prevent sows from risk of wounds on the body as well as local infections.

3.5. Fear of human and stereotypic behaviours of sows

Fear of human and stereotypic behaviours are the two main measures of sow welfare because they indicate the reduced animal welfare (Duncan, 1985). Table 6 and table 7 present the prevelance and odd ratio of these measures.

Fear of human is an indicator to express the human-animal relationship. Sow in stall systems at medium and large farms showed a significantly higher prevelance of extreme fearfullness (47.37% and 84.25% of sows with score 2, respectively) than than those in the small farms ($P < 0.05$) (Table 6). In the small farms, sows were confined in an open-side house having frequent contact with animal keepers, resulting in a good human-animal relationship. On the contrary, due to the high stock of animals and barren environments, sows were more inactive and fearful when being approached by assessors. In many large farms, sows were also forced to be oestrus after weaning by violent interventions such as forced movements or excessive excersises, causing the extreme fear of human in sows.

Stereotypies are abnormal behaviours when sows are maladaptive to changes of environment. These behaviours are occasionally performed by sows in gestation crates (Stolba *et al.,* 1983; Cronin and Wiepkema, 1984) cited from (Rushen and Anne Marie B' de Passille, 1992), including sham chewing, tougue rolling, bar biting, and drinker biting. There was a significant difference in the prevelance of these behaviours between sows housed in small pen system and those housed in stall system in medium and large farms ($P < 0.05$) (Table 6). The odd of performing sham chewing behaviour was estimated as being 2.47 times higher in sows housed in stall system than those housed in pen in small farm (CI 95%: 1.16 - 5.23, $P < 0.05$) (Table 7). Similarly, the estimated odd of these stereotypies were respectively 3.38 times and 2.92 times higher in sows housed in stalls in medium farms and large farms than those in small pen-housed farms (CI 95%: 1.58 - 7.24, and 1.85 - 4.59, respectively, $P < 0.001$) (Table 7). Arellano P.E et al. (1992) also reported that sows in crates developed significantly more stereotypies than sows in pens, especially vacuum chewing, and providing beddings and more space for exercise reduced the frequency of stereotypies.

4. CONCLUSIONS

Four types of pig production systems in the Red River delta were classified according to sow herd size and housing system. In the small farms (5.42 sows/farm), sows were provided with an enough space for movement (8.06 m^2 per pen) in individual pens of open-side houses and fed with an on-farm mixed ration between local feed resources and concentrated feed. In the medium and large farms (40.6 heads and 212.70 heads, respectively), sows were confined in conventional stalls (1.43 - 1.51 m^2/stall) in a completely closed house and mostly provided with an industrial concentrated ration.

Table 6. Prevelance of several selected measures of appropriate behaviour principles of sow welfare quality

	Scores	Small pen-confined pig farms	Small stall-confined pig farms	Medium pig farms	Large pig farms	P-value
Fear of human	0	68.97	50.30	41.70	15.41	< 0.001
	1	24.14	11.52	10.93	0.34	
	2	6.9	38.18	47.37	84.25	
Sham chewing	0	83.62	73.33	63.97	61.98	< 0.001
	2	16.38	26.67	36.03	38.02	
Tougue rolling	0	94.83	72.12	82.19	89.78	< 0.001
	2	5.17	27.88	17.81	10.22	
Bar biting	0	-	76.36	64.37	85.43	< 0.001
	2	-	23.64	35.63	14.57	
Drinker biting	0	-	76.97	65.99	86.60	< 0.001
	2	-	23.03	34.01	13.04	

Note: Fear of human score: 0: no fear, 1: moderate fear, 2: severe fear; Stereotypies behaviour (sham chewing, tougue rolling, bar biting, drinker biting) score: 0 : no evidence, 1: evidence of behaviour.

Table 7. OR and CI-95% of selected behaviour measures of gestation sows for production systems

		Small pen-confined pig farms	Small stall-confined pig farms	Medium pig farms	Large pig farms
Fear of human	OR	1	2.52	3.70	21.15
	CI 95%	-	1.13 - 5.61	1.34 - 10.23	4.49 - 99.54
	P-value	-	0.023	0.01	0.0001
Sham chewing	OR	1	2.47	3.38	2.92
	CI-95%	-	1.16-5.23	1.58 - 7.24	1.85 - 4.59
	P-value	-	0.018	0.0017	< 0.001
Tougue rolling	OR	1	5.71	3.68	2.63
	CI 95%	-	2.22 - 14.66	1.17 - 11.45	1.08 - 6.40
	P-value	-	0.0003	0.025	0.034
Bar biting	OR	-	1	1.84	0.59
	CI 95%	-	-	0.35 - 9.58	0.157 - 2.25
	P-value	-	-	0.47	0.44
Drinker biting	OR		1	1.87	0.63
	CI 95%		-	0.51 - 6.86	0.0526 - 1.50
	P-value		-	0.35	0.29

Note: OR: odds ratio; CI: confidence interval

Significant differences in sow welfare between production systems were indentified. Sows in the small pen-confined farms presented a higher prevelance of good body condition, but were at higher risk of panting in hot weather (52.59% of sows panting) than those in medium and large farms. There was also a trend for

increased wounds on body, skin condition, and local infections among sows on medium and large farms compared with sows on small farms; however, the probality was high ($P > 0.05$) and confidence interval (CI) contained the unity. Moreover, sows housed in stalls in medium and large farms performed several stereotypies behaviours (sham chewing, tougue rolling, bar biting, and drinker biting) and feared of human with a higher prevelance than those housed in pens in small farms. Improvement of sow welfare should focus on the adjustment of indoor thermal conditions by providing small open-side farms houses with more ventilation facilities. For medium and large farms, the priority should be given to the redesign of housing systems with more space allowance and supply of some beddings to prevent them from risk of injuries as well as to enable them to perform appropriate behaviours

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VETERINARY MEDICINE

PREVALENCE OF ANTIBODIES TO PORCINE PARVOVIRUS IN SWINE IN HANOI AND ITS VICINITY

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ABSTRACT

Disease caused by Porcine Parvovirus (PPV) is restricted to the pregnant sow or gilt with the virus capable of infecting and destroying both embryos and fetuses. It is the most common cause of what was traditionally called the Stillbirths Mummification Embryonic Death and Infertility (SEMI) syndrome. Our objective was to determine the serological prevalence of PPV of household porcine herds in some districts near Ha Noi with high (infection level) PPV antibodies. In this study, we collected 164 pig serum samples from 5 provinces including: Ha Noi, Hai Phong, Nam Dinh, Bac Ninh, Vinh Phuc. All 164 animals were sampled in 23 randomly chosen loosely housed pig herds, among 164 samples there were 130 samples in 16 loose - house herds from vaccinated pigs and 34 samples in 7 loose - house herds from unvaccinated pigs. Serum samples from pig were tested by using a hemagglutination inhibition (HI) test, a sample of herds was required to estimate prevalence at the 95% confidence level. Our results indicated that there were 94.51% (155/164) of all serum samples positive with PPV antibodies. However, among 155 positive samples there were 130 samples from vaccinated pigs. So, the real percentage of the pigs naturally exposed to PPV was only 29.68% (46/155) with high titre (> 1:512). There weren't difference about rate positive samples with antibodies PPV between Ha Noi, Nam Dinh, Hai Phong and Bac Ninh ($P > 0.05$) but rate positive samples with antibodies PPV between Ha Noi and Vinh Phuc had a significant ($P < 0.05$).

Keywords: Ha Noi and its vicinity, porcine parvovirus, prevalence of antibodies, unvaccinated pigs, vaccinated pigs.

1. INTRODUCTION

Porcine parvovirus (PPV) have been detected from normal and abnormal foetuses in sows and piglets (Mengeling *et al.*, 2000). This viruscaused reproductive failure in swine as fetal death and mummification, although infertility, abortion, stillbirth and neonatal death may also be consequence of in utero PPV infection (Joo *et al.,* 1976). PPV is a small, non - enveloped, single - stranded, negative - sense DNA virus. Capsids of PPV are assembled from three viral proteins (VP1, VP2, and VP3). The major structural protein, VP2 is the main target for neutralizing antibodies in PPV (Martinez *et al.,* 1992; Kamstrup *et al.,* 1998). When VP2 was expressed in large amounts using the baculovirus expression vector system, it assembled into virus - like particles (VLPs) similar in size and morphology to the original virions.VP2 protein caused hemagglutination when diluted up to 1:8.192 (Hongchao Zhou *et al.*, 2010). Virus were determined by antibodies in serum or antigen in body, but humoral immune, either as a consequence of natural exposure

or from vaccination and antibodies to PPV, as determined by thehemagglutination inhibition (HI) test. The haemagglutination inhibition (HI) test is based on the ability of PPV to agglutinate erythrocytes. This is the most frequently used test for detecting and quantifying humoral antibodies to PPV. Serum is pre - treated by heat activation and absorption with erythrocytes to remove naturally occurring haemagglutinins and kaolinto remove or reduce non - antibody inhibitors of haemagglutination (Mengeling *et al.*, 1999). These washed erythrocytes and an identified virus strain are added to thesample serum. If the sample has antibodies against the virus, haemagglutination does not occur. The HI test is usually performed on V - bottom microplates, where haemagglutination inhibition, i.e. a positive result, is seen as precipitation of the erythrocytes at the bottom. Diseases caused by PPV occur in a number of countries; in Vietnam, before 2007, Porcine parvovirus and Porcine reproductive and respiratory syndrome virus were determined caused reproductive failure in swine. Accordingly, determination prevalence of antibodies to Porcine Parvovirus in swine is very important to give prevention and treatment PPV diseases. Aim of this study is determining prevalence of Antibodies to Porcine Parvovirus in swine in Hanoi and its vicinity by using HI test.

2. MATERIALS AND METHODS

This study was carried out from January 2016 to June 2016 in 23 randomly chosen loosely housed sow herds on farms in Hanoi and its vicinity. We collected 164 pig serum samples from 5 provinces including: Ha Noi, Hai Phong, Nam Dinh, Bac Ninh, Vinh Phuc. All 164 animals were sampled in 23 randomly chosen loosely housed pig herds. However, among 164 samples there were 130 samples in 16 loose - house herds from vaccinated pigs and 34 samples in 7 loose - house herds from unvaccinated pigs. The results show in Table 1.

All serum samples from sows, gilts, porkers were tested by using a hemagglutination inhibition (HI) test. Blood samples were drawn from the vena coccyges orvena saphena. Samples were taken in a cool box within 4h to the laboratory for storage in the refrigerator untilcentrifugation the next day. The serum was stored at - 18oC until analysis in the virus diagnostic laboratoryof the Department of Microbiology and Infectious diseases.

Antibodies against parvovirus were detected with a HI test (Joo *et al.,* 1976), with slight modifications: only guinea pig erythrocytes and V - bottom microplates were used and no bovine serum albumin was used for a clearer end - point. Animals were considered to have low antibody levels when HI titres were ≤1: 512; titres > 1: 512 were considered high (Neuvonen *et al.*, 1979).

We used excel to calculate rate of prevalence PPV antibodies and used https://www.medcalc.org/calc/comparison_of_proportions.php to analyze statistics and Chi - square test was used for dichotomous variables and logistic regression for categorical variables in the univariate analysis with a P - value < 0.05.

3. RESULTS

Prevalence of positive antibodies PPV in places

All together 164 animals were sampled. The aim of results of HI test were

determined prevalence of positive antibodies PPV in places are presented in Figure 1; 2; 3 and Table 2.

The results showed that, 155/164 (94.51%) were positive for PPV antibodies. In that, the province with the highest rate PPV antibodies was Nam Dinh: 11/11 (100%) serum samples were positive for PPV antibodies. Contrast to Nam Dinh, Vinh Phuc had the smallest rate PPV antibodies about 26/32 (81.25%). The prevalence of antibodies did not differ between Ha Noi, Hai Phong, Nam Dinh, Bac Ninh ($P > 0.05$). But there were very significant differ about the prevalence of antibodies PPV between Ha Noi and Vinh Phuc ($P < 0.001$).

Prevalence of positive antibodies PPV belong to herd size

In this result, we only focus on samples in 7 loose - house herds from unvaccinated pigs The results of prevalence of positive antibodies PPV following herd size are presented in Table 3.

Table 1. Results of collecting samples

No	Places	Number of farms	Number of samples
1	Ha Noi	8	96
2	Hai Phong	2	8
3	Bac Ninh	3	11
4	Nam Dinh	4	17
5	Vinh Phuc	6	32
	Total	23	164

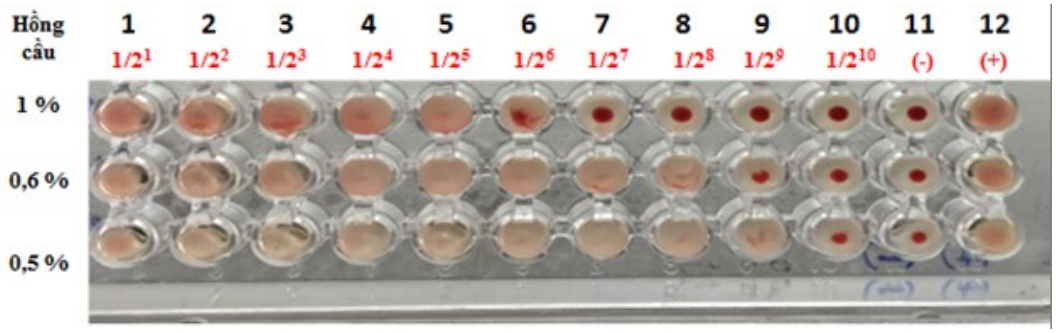

Figure 1. Results of HA test

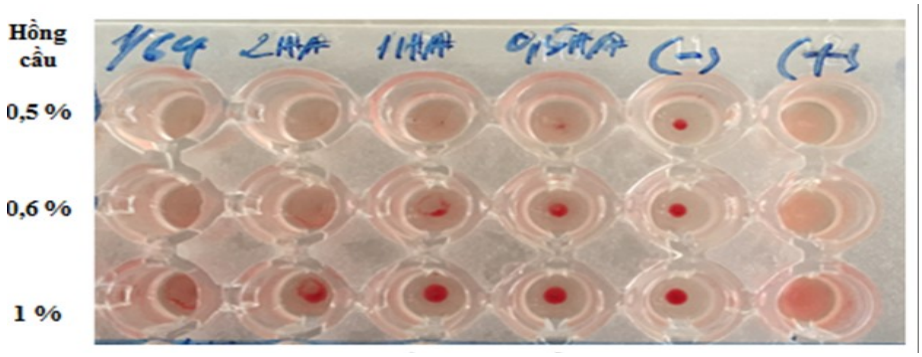

Figure 2. Results of back titration HA

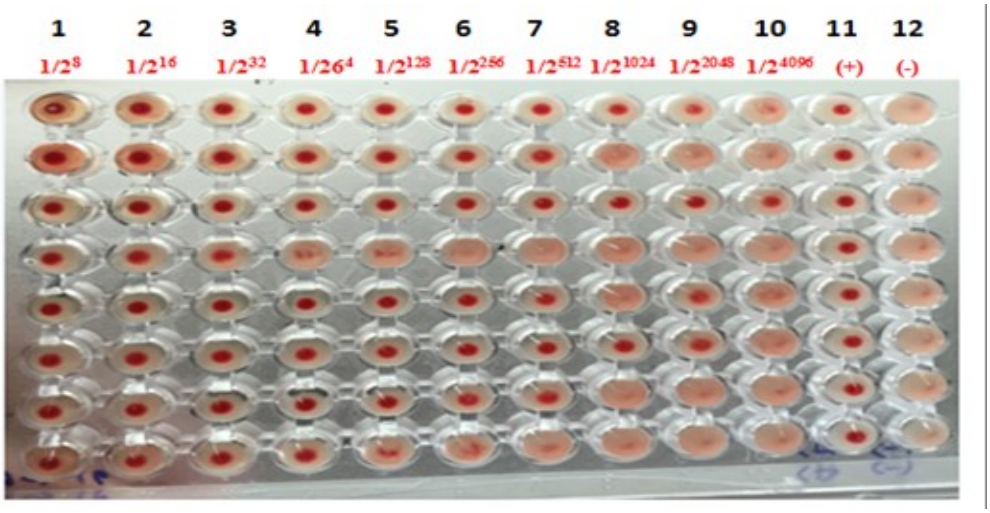

Figure 3. Results of HI test

Table 2. Prevalence of positive antibodies PPV in places

No	Places	Number of samples	Number of positive samples	Number of positive samples/ Number of samples
1	Ha Noi	96	95	95/96 (98.96%)
2	Hai Phong	8	7	7/8 (87.5%)
3	Nam Dinh	11	11	11/11 (100%)
4	Bac Ninh	17	16	16/17 (94.12%)
5	Vinh Phuc	32	26	26/32 *** (81.25%)
	General	164	155	155/164 (94.51%)

Note: (***) P < 0.001

Table 3. Prevalence of positive antibodies PPV belong to herd size

No	Herd size	Number of samples		Number of positive samples	Number of positive samples/ Number of samples
		Vaccinated	Unvaccinated	Unvaccinated	Unvaccinated
1	< 50	30	5	4	4/5 (80%)
2	50 - 100	18	7	5	5/7 (71.43%)
3	100 - 500	45	15	9	9/15 (60%)
4	500 - 1000	26	4	4	4/4 (100%)
5	> 1000	11	3	3	3/3 (100%)
General		130	34	25	25/34 (73.53%)

Table 4. Prevalence of positive antibodies PPV belong to ages

No	Ages	Number of samples		Number of positive samples	Number of positive samples/ Number of samples
		Vaccinated	Unvaccinated	Unvaccinated	Unvaccinated
1	Gilts (< 1 month)	46	14	11	11/14 (78.57%)
2	Porkers (2 – 5 months)	56	12	8	10/12 (66.67%)
3	Sows (> 12 months)	28	8	6	6/8 (75%)
	General	130	34	25	25/34 (73.53%)

The results showed that, with different herd sizes had different rate positive PPV antibodies, especially, in herd swine with large size (> 500) were high rate positive with PPV antibodies about 100%. However, with herd sizes popular from < 50 animals, 50 - 100 animals, 100 – 500 animals the rate positive PPV were in turn as 4/5 (80%); 5/7 (71.43%) and 9/15 (60%) respectively. The prevalence of antibodies didnot differ between herd sizes (P > 0.05).

Prevalence of positive antibodies PPV belong to ages

The results of prevalence of positive antibodies PPV following ages are presented in Table 4.

We tested antibodies titre belonging to ages swine and only focus on samples in 7 loose - house herds from unvaccinated pigs, so the results indicated that the smallest positive PPV rate were porkers with 66.67% respectively, this rate was less than rate of gilts and sows 78.57% and 75% respectively (P > 0.05). Although testing 8 serum samples from sows, but the rate antibodies detection were the highest about 75%. This antibodies can transfect passively to gilts, so that the rate positive antibodies in gilts were 78.57% and did not differ between gilts, porkers and sows about rate PPV antibodies (P > 0.05).

Prevalence of positive antibodies PPV belong to use vaccination or not

The vaccination programme was carried out according to official recommendations at farms. There were 7 farms without PPV vaccinated. The most common shortcoming was that the gilts were vaccinated only once before mating or farm didn't use vaccination, other farms used vaccination PPV for herd swine. We tested antibodies titre belonging to use vaccination or not, so the results indicated that the smallest positive PPV rate were herd not using vaccination with 73.53%. This rate was natural infection with PPV and less than herd using vaccination about 100% and had a significant difference between two groups (P < 0.001).

The results of rate positive PPV antibodies belong to HI titres

Antibodies against parvovirus were detected with a HI test (Joo *et al.*, 1976), with slight modifications: only guinea pig erythrocytes and V - bottom microplates were used and no bovine serum albumin was used for a clearer end - point. Animals were considered to have low antibody levels when HI titres were ≤1: 512; HI titres > 1: 512 were considered high (Neuvonen *et al.,* 1979).

Table 5. Prevalence of positive antibodies PPV belong to use vaccination or not

No	Status of herd	Number of samples	Number of positive samples	Number of positive samples/ Number of samples
1	Unvaccinated	34	25	25/34 (73.53%)
2	Vaccinated	130	130	130/130*** (100%)
	General	164	155	155/164 (94.51%)

*Note: ***: P < 0.0001*

Table 6. The results of positive PPV antibodies belong to HI titres

Titre HI	HaNoi		Hai Phong		Bac Ninh		Nam Dinh		Vinh Phuc		General		
	$P_{HI}/\sum P$ *	Tỷ lệ %	$P_{HI}/\sum P$	Tỷ lệ %	$P_{HI}/\sum P$	Tỷ lệ %	$P_{HI}/\sum P$	Tỷ lệ %	$P_{HI}/\sum P$	Tỷ lệ %	$P_{HI}/\sum P$	Tỷ lệ %	General ratio
1:8	0/95	0.00	0/7	0	2/16	12.5	0/11	0	2/26	7.69	4	2.58	109/155 (70.32%)
1:16	11/95	11.58	0/7	0	1/16	6.25	0/11	0	1/26	3.85	13	8.39	
1:32	10/95	10.53	0/7	0	2/16	12.5	0/11	0	0/26	0.00	12	7.74	
1:64	13/95	13.68	2/7	28.57	1/16	6.25	0/11	0	14/26	53.85	30	19.35	
1:128	12/95	12.63	4/7	57.14	1/16	6.25	1/11	9.1	2/26	7.69	20	12.90	
1:256	23/95	24.21	0/7	0	5/16	31.25	2/11	18.2	0/26	0.00	30	19.35	
1:512	10/95	10.53	0/7	0	0/16	0	3/11	27.27	1/26	3.85	14	9.03	46/155 (29.68%)
1:1024	10/95	10.53	0/7	0	0/16	0	3/11	27.27	2/26	7.69	15	9.68	
1:2048	5/95	5.26	0/7	0	0/16	0	2/11	18.2	0/26	0.00	7	4.52	
1:4096	1/95	1.05	1/7	14.29	4/16	25	0/11	0	4/26	15.38	10	6.45	

*Note: * P_{HI}: positive samples at once HI titre; P: general positive samples.*

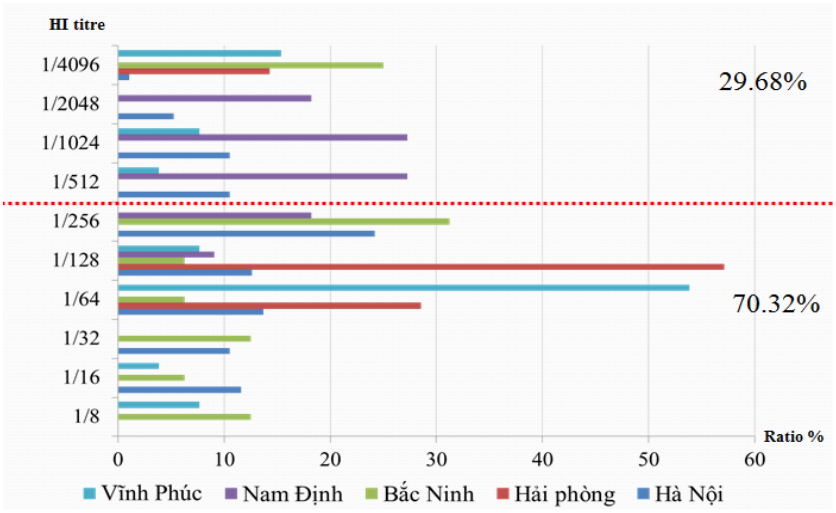

Figure 4. Ratio of positive samples with PPV antibodies at once HI titre

The results are presented in Table 6 and Figure 4, the results show that HI titre at swine groups were different and HI titre fluctuated between 1:8 and 1:4096. The percentage of low antibodylevels HI titres were 70.32% and high antibody levels HI titre were 29.68%.

4. DISCUSSION

Although the significance to the pig industry ofporcine parvovirus is still in doubt, the agent can cross the placenta and invade the foetuses (Van Leengoed *et al.,* 1983). Virus were determined by antibodies in serum or antigen in body, but humoral immune, either as a consequence of natural exposure or from vaccination and antibodies to PPV, as determined by the hemagglutination inhibition (HI) test. In this study, all 164 animals were sampled in 23 randomly chosen loosely housed pig herds to collect serum. All serum samples from sows, gilts, porkers were tested by using a hemagglutination inhibition (HI) test. The results showed that, 25/34 (73.53%) samples in 7 loose - house herds from unvaccinated pigs were positive for PPV antibodies. This antibodies prevalence was lower than that reported in Germany (77%) (Lutz et al.,1996), and the same Italy (ranging from 56.7% to 99%; Mignone et al., 1995). The prevalence of antibodies did not differ between Ha Noi, Hai Phong, Nam Dinh, Bac Ninh ($P > 0.05$). But there were very significant differ about the prevalence of antibodies PPV between Ha Noi and Vinh Phuc ($P < 0.001$). In herd swine with large size (> 500) were high rate positive with PPV antibodies. The prevalence of antibodies didnot differ between herd sizes ($P > 0.05$). The smallest positive PPV rate following ages were porkers with 66.67% respectively, this rate was less than rate of gilts and sows 78.57% and 75% respectively ($P > 0.05$). The rate antibodies from infection with PPV were less than herd using vaccination about 100% and had a significant difference between two groups ($P < 0.001$). Pointon et al, (1983) conducted a survey on four endemically infected PPV herds where sows and gilts were kept in the same groups and concluded that sows being 44–100%, so our results about rate positive antibodies PPV in sows and gilts are the same. The percentage of low antibody levels HI titres (< 1/512) were 70.32% and high antibody levels HI titre (≥1/512) were 29.68%. Based on evaluations of vaccinated animals and field cases, are as follows: antibody titres < 1:8 indicate that the animal has not seroconverted, 1:16 to 1:512 indicate intermediate seroconversion, and titres beyond this represent high level of antibodies from exposure PPV (J Oravainen et al., 2005) and HI titre can larger than 1/2000 (van Leengoed et al., 1983). So that, our results indicated that high antibody levels HI titre (≥1/512) were 29.68% (46/155) this mean that 29.68% swine were natural infection with PPV.

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INDUCTION OF OVULATION SYNCHRONIZATION FOR PURED BBB EMBRYO TRANSFER IN VIETNAM

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ABSTRACT

The study was conducted upon the crossbred dairy (75% Holstein Friesian) herd at Phudong farm (Hanoi Livestock Breeding Joint Stock Company), aiming to induce ovulation synchronization for serving BBB embryo transfer after 7 days of estrus (observation of early heat signs). A total 38 cows with BCS 2.75- 4.00 were suitable for being recepient cows. After treatment with GnRH, CIDR and $PGF_{2\alpha}$, 32 cows were detected estrus (84.21%). In addition, 9 cows presented natural estrus and met BCS requirements and were chosen for embryo transfer. At day 6 post-estrus, the 41 cows were collected blood samples to determine progesterone (P4) concentration by ELISA, and there were 22/41 (53.66%) ones having P4 ≥3 ng/ml. One day later (7 days post-estrus), frozen BBB embryos were nonsurgically transfered into the suitable recipients. All 22 transfered cows were palpated at 60 days old of embryo and 8 (36.36%) were in pregnancy. In CONCLUSIONS, a hormone therapy were used for ovulation synchronization and plasma P4 were quantified by ELISA resulted in the first successful transfer of BBB embryos in Vietnam.

Key words: BBB, embryo transfer, ovulation synchronization, progesterone.

1. INTRODUCTION

Nowadays, Vietnamese community is partly characterised by an increase in demand for food, especeially demand for beef. In 2013, more than 130,000 living beef cattle were imported to Vietnam, easily overwhelming the domestic beef cattle. So, the improvement of productivity and quantity of beef cattle is one necessary task.

Contributing to solve this task, high quality semen of famous beef cattle breeds have been more and more popularized. Remarkably, the success in improving productivity of local beef cattle herd in Hanoi by Blanc Bleu Belge (BBB) bull semen.

As the name implies, Blanc Bleu Belge or Blue Belgian Breed (BBB) originated in Belgium. Recently, Belgian Blue breed is spread all over Belgium and represents 50% of the national herd, which is made up of 1.083.408 cows. The breed attract enormous attention with their magnificient apperance and physique. The most distintive characteristic lies in the muscle structure, which is now known as “double- muscling”. The result of this so called “double-muscling” is much larger volumes of red meat and much reduced deposits of fat. The weigh of an adult bull ranges from between 1,100 – 1,250 kg. Indeed, it is by no means rare to see animals heavier than 1,300 kg. The average weigh of an adult cow is 700 – 750 kg. Cows can reach a weigh of 850 – 900kg. The cross and full blooding breed are perfectly suited to a great diversity of soils and climates encountered in its international expansion. They prosper in the bitter Canadian winters, as well as the hot Texas summer. An animal that is structurally correct and sound, docile in temperament, fertile in breeding, and above all the ultimate beef machine.

So far, Hanoi livestock breeding joint stock company has inseminated artificially the local beef cattle with over 10.000 straws of frozen Blue Belgian bull semen, to bring 3000 cross- bred calves into the world. Genral characteristics of the cross-bred calf as follow: birth weigh of calf is 28 – 30kg, 150 – 170kg in month 6 of age, 300 – 310kg in month 12 of age; carcass weight is 60%, carcass conformation is 51.03%, apart from feed efficiency, virtually no calving issues and docile temperaments (Nguyen Tan Anh, 2015).

In general, BB cross-bred calves is considered as the development way of Vietnamese beef cattle herd, aim to balance against the improtation of exotic cattle. Hanoi decided to import BBB frozen thawed embryo from Belgium to produce BBB bull by embryo transfer in Vietnam, thence gaining initiative in BB semen to improve Vietnamses local beef cattle herd. This study was conducted the induction of ovulation synchronization method to producce the first BBB bull in Vietnam via embryo transfer.

2. METHODS AND MATERIALS

2.1. Study target, study location and study time

2.1.1. Study target

Frozen thawed BBB embryos were imported from Belgium);

Cross blooding cows (75% HF).

2.1.2. Study location

Phudong dairy farm, Hanoi livestock breeding joint stock company;

Theriogenology – Surgery Department, Veterinary Medicine Faculty, Vietnam National University of Agriculture;

Biodiversity Conservation and Tropical Disease Research Institute.

2.1.3. Study time

From July, 2015 to March, 2016

2.2. Methods

2.2.1. Selection of recipients.

Cows were selected with following criterion: healthy, without apparent sign of pathology at clinical examnination, body condition scores varied between 2.5 – 4.00 (1 – 5 scaled)

2.2.2. Examination of the reproductive tract

Cows were clinically examined for the presence of purulent discharge, urovagina and any other abnormalities.

2.2.3. Measure the body score condition (BCS)

Body score condition (BCS) is determined by palpating over the back, ribs, and over the horizontal processes of the backbone (edge of loin). BCS ranges from 1 – 5, with a score of 1 being extremly thin and 5 being very obese.

2.2.4. Induction of ovulation synchronization method

Selected cows were synchronized with CIDR (Controlled Internal Drug Release) and GnRH (Gonadotropin-releasing hormone). Before CIDR insertion, the vulva was washed with warm water containing disinfectant, cleaned with towel papers, sprayed with 2% PVP idodine. (Figure 1).

The protocol included CIDR insertion for 7 days starting at first GnRH injection. The insert CIDR was removed 7 days after at least 1 – 2 h before the $PGF_{2\alpha}$ injection. Cows received the second GnRH injection in the next day of removing CIDR. Blood samples were collected 6 days after the fisrt sign of estrus to determine plasma progesterone concentration. Embryo were only transferred in cows with the concentration ≥3 ng/ml. Pregnancy diagnosis was carried out 53 days after.

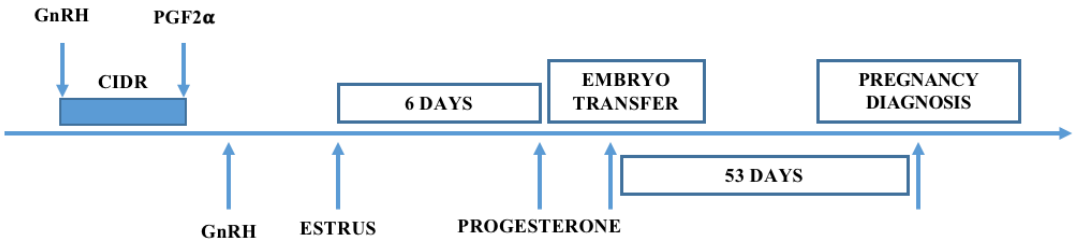

Figure 1. Induction of ovulation synchronization protocol

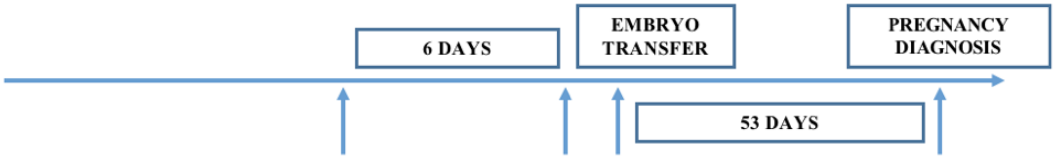

Figure 2. Protocol for natural estrous cows

2.3.5. Natural estrus

6 days after sign of estrus, all experiment cows were collected blood for determination of progesterone profile in plasma. (Figure 2).

2.3.6. Determination of plasma progesterone concentration

Blood samples were collected into heparinized vaccum tubes via the tail venepuncture and kept in an ice box during the path to the laboratory.

Plasma progesterone concentration were determined by enzyme immunoassay (ELISA)

2.3.7. Embryo transfer method

Embryo transfer were conducted by experienced veterinarians, follow non-surgical method established by Geogre and Sarah (1991)

2.3.8. Pregnancy diagnosis

Pregnancy diagnosis were made by trans- rectal palpation at day 60 (53 days after transfer).

2.4. Statistical analyses

Estrous rate and conception rate were analyses by Excel (Microsoft).

3. RESULTS AND DISCUSSION

3.1. Selection of recipients

Before conducting the experiment, we collected profile of dairy cattle at Phudong farm (table 1).

Table 1. Profile of Phudong dairy cattle

Nutrition	Hay, rice straw, TMR, fermented straw, beer waste
Calving interval	15,2 months
Vaccination	Twice/year
Mean milk yielding	15 l/d
Fat content in milk	3,8%
Milking	Portable milking machine

Table 2. Result of estrus synchronized and plasma progesterone concentration

Synchronize estrus	Estrus	Rate (%)	Progesterone (P4 ≥3 ng/ml)	Rate (%)
38	32	84.21	17	53.13%

After 4 times of assenment, we selected 38 cows that meet the criterion for embryo transfer.

3.2. Induction of ovulation synchronization and plasma progesterone concentration

A total of 38 selected cows were received treatment with the protocol based on GnRD injection cocurrent with CIDR insertion. Then, blood samples were collected to determine plasma progesterone concentration, results are presented in the table 2.

After treatment with the protocol, 32/38 (84.21%) cows showed estrus, lower than that in Japanese black cattle (Kawate et al. 2006) with 100% estrus cows after treatment with GnRH plus CIDR insertion protocol, 66.7% in group without CIDR insertion protocol. Looney et al (2005) revealed higher estrous rate with 94% in Bos indicus cattle herd. Maybe, the hot, humid climate and the poor nutrition in Phudong dairy farm negatively affect results of this experiment, according to studies of Gwazdauskas et al (1973; 1975; 1981), Wiltbank et al. (1977), McDowell (1972); Holness et al. (1978) that revealed the effect of ambient enviroment, nutrition to estrus rate, conception rate of cattle.

6 days after showed estrus, experimental cows were collected blood to determine plasma progesterone concentration. Of 32 oestrus cows, 17 (53.13%) cows had progesterone concentration ≥3 ng/ml. Moreover, 6/9 (55.56%) natural estrus cows with plasma progesterone concentration ≥3 ng/ml were selected being recipients.

Mann and Lamming (2001), Mann et al (2006) recorded the relationship between plsama progesterone concentration in post-estrus period, concentration of uterus interferon-tau and size of the fetus. According to Herrier et al (1990), František Novotný et al (2005), Callesen et al (1986; 1988), progesterone concentration in milk < 8 ng/ml and blood < 3 ng/ml decreased the conception rate of embryo transfer, so only cows with the concentration ≥3 ng/ml were slected to be recipients. Starbuck et al. (2004) recorded 50.0% miscarriage in cows with plasma progesterone concentration in 9 week of pregnancy lower than 2.8 ng/ml. In addition, Kenyon et al (2013) also revealed that cows with plasma progesterone concentration < 5.0 ng/ml at day 14 after pregnancy tended to be miscarriage in 28 - 42 days (P=0.01) and 28 - 63 days (P=0.07).

In general, a total of 22/41 (53.66%) cows were selected to be recipients plasma progesterone concentration ≥3 ng/ml. This seem to be the most important process of this study, due to saving 19 BBB embryo that were transferred into cows not enough platma progesterone in blood.

3.3. Result of embryo transfer

After transferring 7 day old frozen thawed embryo, recipients were examined for pregnancy in day 53. Results shown in the table 3 with 8/22 (36.36%) pregnant cows, lower than that in study of Van Soom et al. (1994), Kawate et al. (2004) and El-Zarkouny (2004).

Table 3. Result of embryo transfer

Recipients BCS=2.75–4.00	Estrus		P4 ≥3 ng/ml		Embryo transfer		
Estrus	No.	No.	%	No.	%	No.	Pregnancy	%
- Synchronize	38	32	84.21	17	53.13	-	-	-
- Natural	9	9	100.00	5	55.56	-	-	-
Total	47	41	87.23	22	53.66	22	8	36.36

Although, the results is not high as expected, the study will be conducted in larger cow herd with the proper nutrition to get better result.

4. CONCLUSIONS

By induction of ovulation synchronization based on GnRH, $PGF_{2\alpha}$ plus CIDR insertion, 84.21% treated cows showed estrus. Determination of plasma progesterone concentration of experimental cows at day 6 after estrus, 46.81% of estrous cows meet the criteria of plasma progesterone concentration. Conception rate was 36.36%.

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STATUS OF CATTLE TICKS INFECTION IN YELLOW AND DAIRY COWS IN BA VI DISTRICT

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ABSTRACT

A total of 160 cattle ticks were collected from 273 yellow and dairy cows at the grass farm in Ba Vi district, Ha Noi, Vietnam. Cattle ticks were identified as *Boophilus annulatus* at rate of 16.25% (26/160), followed by *Boophilus microplus* as 83.75% (134/160). The average prevalence of cattle ticks in yellow and dairy cows was 24.18% (66/273). The prevalence of cattle ticks was higher in yellow cows (29.86%) than in dairy cow (17.83%). There was no difference of prevalence of tick infection in cow host among its age groups or collected sites.

Keywords: Ba Vi, *Boophilus* spp., cattle ticks.

1. INTRODUCTION

Cattle ticks cause tick-borne diseases in cattle are major significance as factors reduce meat and milk production by transmitting of hemoparasites of *Babesia* spp and *Anaplasma* spp. Cattle ticks of *Boophilus annulatus*, *Boophilus microplus* causes a huge economic loss for cattle farms worldwide, estimated at between 13.9 and 18.7 billion US$ per year(De Castro, 1997). The cattle tick is well established in Latin America (Evans *et al.,* 2000) and Australasia (Estrada-Pen˜a and Venzal, 2006) and is currently invading the West African region where it was first reported in 2007 (Madder *et al.,* 2007).

There are few reports on cattle ticks in Southeastern Asian including Vietnam and neighbor countries, Lao PDR and China (Petney, 1993; Jiang *et al.,* 2011; Khamsing *et al.,* 2016). Those supposed a threatening status of cattle herds with infection of ticks and hemoparasitic diseases transmitted by the tick vector. The grass farm in Ba Vi District is one of the biggest grass farms for cattle industry in northern Vietnam. The present study was conducted in small scale farms in the grass farms of Ba Vi District to clarify cattle tick component and their prevalence in yellow and dairy cows. This better knowledge is needed for further studies and control program of hemoparasites in cattle herds in the area.

2. MATERIALS AND METHODS

2.1. Study area

Bavi district (21°04′0″N, 105°20′05″E) (Figure 1), a haft mountain haft plain area, locates in Northern-east of Hanoi Capital contains a biggest grass farm for cattle industry in Northern Vietnam. Two mountainous communes of Tan Linh and

Phu Son of the district, gathering many small scale farms raising both yellow and dairy cows were selected for study.

2.2. Tick collection

From September to November (2016), total of 274 yellow and dairy cows from 100 small farms in two communes of Tan Linh and Phu Son were randomly selected for tick sampling.

Ticks were collected as description of De Clercq et al., (2012), in brief: the full body of cow host was sampled using forceps. Collected ticks from each cow were fixed in falcon tubes containing ethanol 70 % which were labeled with a unique ID number which also appears on the extra information sheet, following by the sampling date and the village name.

2.3. Tick identification

Ticks were identified base on morphological characteristics by using a stereoscope (a magnification of 80x) and a light microscope (a magnification of 100x – 200x). Only adult specimens were identified up to species level following the morphological identification key of Walker et al. (2003) and Madder (2012a, b). The information of cattle breeding, cattle age, etc, was recorded during tick sampling.

Data were inserted in the excel sheet (Microsoft Office, version 2007) for analysis.

3. RESULTS

A total of 160 ticks were identified and belong to *Boophilus* genus. There were two *Boophilus* species, including *Boophilus microplus* at rate of 83.75% (134/160) and *Boophilus annulatus* at rate of 16.25% (26/160).

For *Boophilus microplus*: Both male and female ticks have the hypostome dentition of 2x4 rows; and seta on the palpal segment 1 is absent. In the male tick, the caudal process is present; the cornua is available; and the spurs on coxa 1 is long internal and short external spur; whereas, in the female tick (Figure 2), the external spur is present on coxa 2 and 3.

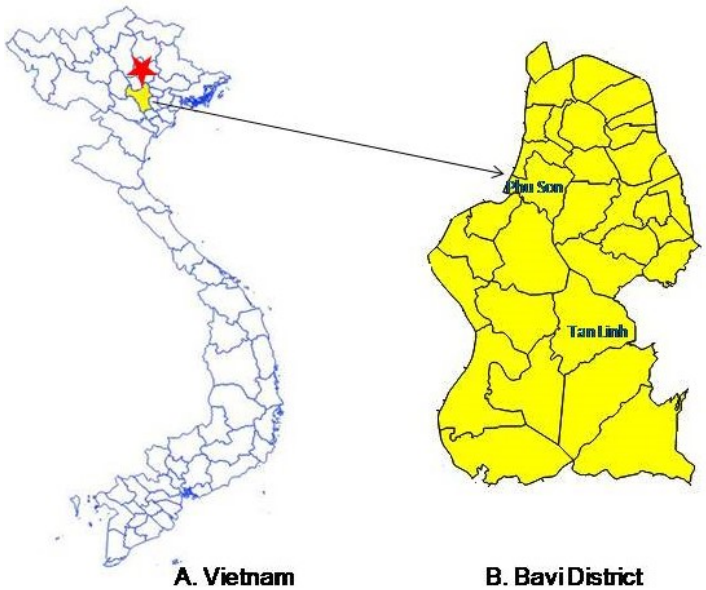

Figure 1. Study area

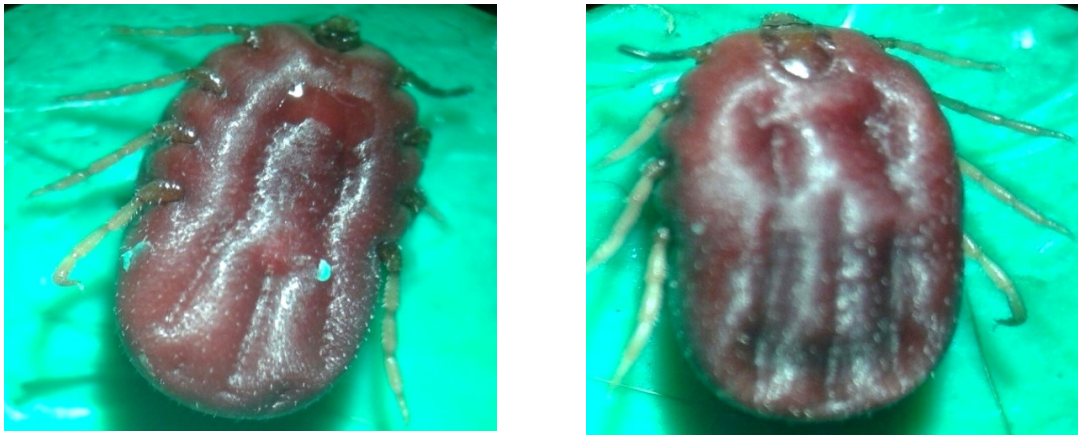

Figure 2. *Boophilus microplus* (female) in dairy cow in Ba Vi, Ha Noi

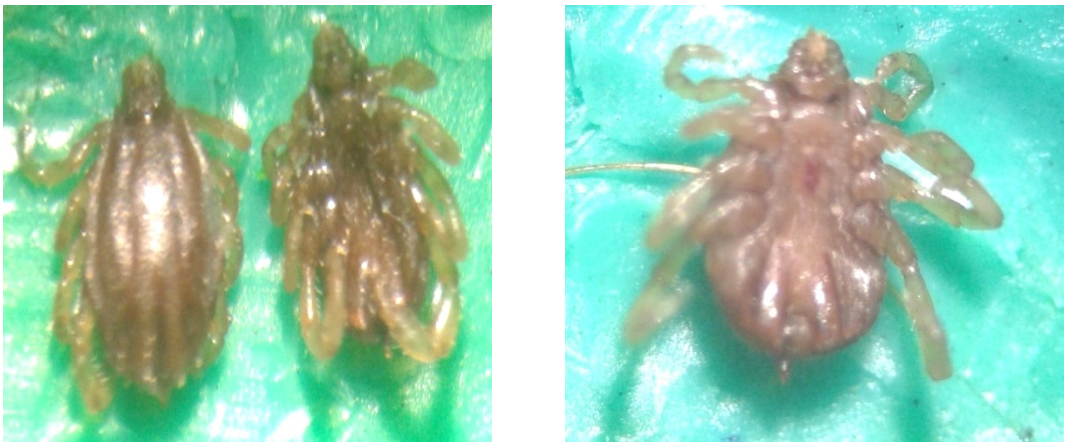

Figure 3. *Boophilus microplus* (male) in dairy cow in Ba Vi, Ha Noi

Figure 4. *Boophilus annulatus* (male) in dairy cow in Ba Vi, Ha Noi

Table 1. *Boophilus* spp. infection in yellow and dairy cows in Ba Vi, Ha Noi

Factor		No. examined cows	No. infected cows	Prevalence (%)
Total		273	66	24.18
Breeding				
	Yellow cow	144	43	29.86
	Dairy cow	129	23	17.83
Age (years old)				
	< 1	61	16	26.23
	1-2	100	25	25.00
	> 2	112	25	22.32
Commune				
	Tan Linh	200	49	24.50
	Phu Son	73	17	23.29

For *Boophilus annulatus:* The hypostome dentition is 2x4 rows, and no seta on the palpal segment. In the male tick (Figure 3), the caudal is absent; the cornua is present; the internal spur of adanal plates long is absent; and on the spurs on coxa 1, both internal and external spur short are present. The female (Figure 4), external spur on coxa 2 and 3 is absent, and the palpal segment 1 is not present with seta.

The prevalence of *Boophilus spp.* in yellow and dairy cows in Ba Vi were 24.18% (Table 1). This value in yellow cows (29.86%) was higher than in dairy cows (17.83%). Neither age nor commune was affected to the *Boophilus* infection rate in yellow and dairy cows in Ba Vi District (Table 1).

4. DISCUSSION

Two cattle ticks *Boophilus microplus* and *Boophilus annulatus* were found in Ba Vi grass farm at moderate prevalence (24.18 %). *B. microplus* was known as Asian ticks and the most abundant (Meaza et al., 2014). This hard tick also was known as major pest species of cattle tick transmitting hemoparasites of both *Anaplasma* spp and *Babesia* spp (De Castro, 1997). The mid-rate of infection of cow with ticks in the study was due to time of sampling. Ticks were collected around dry season, from September to November, when ticks need to hide itself into deep soil to avoid progress of dehydration of its body (Madder, 2011). The presenting of two cattle ticks in cows indicates a potential present of either anaplasmosis or babesiosis in cattle population in Ba Vi grass farm in Northern Vietnam.

The prevalence of ticks in yellow cows was higher than in dairy cow, indicating that free grazing on grass farm of yellow cows created more chance for tick attached cow host; whereas dairy cows commonly confined in cage with higher hygiene conditions were less infection with ticks. The small scale farms in the Ba Vi grass farm usually have both yellow and dairy cows. This is risk for dairy cows to get more infection with tick from yellow cow.

5.CONCLUSIONS

Further studies on the pathway of tick transmission and the disease can be transmitted by these *Boophilus* cattle ticks

in grass farm of Ba Vi District should be done. More understanding on epidemiology of these cattle ticks and its veterinary importance is needed for a sustainable strategy of prevention and control programs of ticks and tick borne diseases in the cattle herds.

ACKNOWLEDGEMENT

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PREVALENCE OF CEPHALOSPORIN RESISTANT AND EXTENDED-SPECTRUM - β-LACTAMASE PRODUCING *ESCHERICHIA COLI* ISOLATED FROM PIG MANURE IN VIETNAM

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ABSTRACT

Using antimicrobials for livestock is not well managed in Vietnam as well as in other developing countries. The study was implemented in Soc Son and Thai Binh provinces between May and September 2015. Household owners of 100 pig farms which contain 15-50 pigs each were selected to answer questionnaire form on current situation of antimicrobial used for pig on farms. Prevalence of cephalosporin resistant *E.coli* and potential ESBLs producing *Escherichia coli* was also detected in pig manure samples by a cross-sectional study. The results show that 73/100 household owners bought antimicrobials for pig disease treatment based on local veterinarian's advice and 24/100 bought antimicrobials based on their personal experience. Antimicrobials were added in feed as growth promotion on 12 farms (12%), including 8 farms using semi-commercial feed and 4 farms using traditional feed. Antimicrobials were used for pig disease treatment not following description on 15/50 (30%) farms in Thai Binh and 36/44 (81.8%) farms in Soc Son. Particularly, antimicrobial dosage was 0.5-2 times higher than description on 13 over those 15 farms in Thai Binh and on all those 36 farms in Soc Son. The lab results showed that cefotaxime resistant *E. coli* was detected in pig feces samples at 82/100 farms (82%). 220 *E. coli* isolated strains (maximum 5 colonies/sample) were test for cephalosporin resistant. Prevalence resistant to cephamaldole, ceftriaxone, cefuroxime, cefuroxime, and ceftazidime of cefotaxime resistant *E.coli* isolated strains were 87.7%, 75.5%, 67.7%, 52.7%, and 34.5% respectively. The number of multi-resistant strains (resistant to at least three kinds of antimicrobials) was 163/220 (74.1%) strains. Prevalence of ESBL producing *E. coli* in pigmanure was 83.8% in Thai Binh and 88.6% in Soc Son.The result of the present study is an evidence for the prevalence of cephalosporin-resistant *E.coli* and ESBL producing *E. coli* in pig manure and will contribute to an antimicrobial resistant bacteria monitor program in Vietnam. Further studies on molecular ESBL producing *E. coli* reservoirs should be implemented for elucidating the dynamics of antimicrobial resistant genes transmission.

Keywords: Cephalosporin resistant, *E. coli*, ESBL producing *E.coli,* pig manure.

THE IMMUNE RESPONSE OF RED JUNGLEFOWL VACCINATED BY LASOTA AND MUKTESWAR STRAINS OF NEWCASTLE DISEASE VIRUS

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ABSTRACT

The red junglefowl (Gallus gallus spadiceus) has been raised and multiplied in Cuc Phuong National Park since 2007. And the prevention measures against infectious diseases are demanded. This study provided data of the immune response of red junglefowl post vaccination by Newcastle disease vaccines. It was also aimed at designing a suitable vaccination schedule. The immune response after the primary immunization by LaSota strain showed that: at 7 day post vaccination (DPV), there were 86.70% of vaccinated chickens having the antibody titer ≥ 3 log2. The titer raised gradually, was above the protective level (3 log2) at 21 DPV, then it dropped at 28 DPV. After the first booster by LaSota strain, a higher level of immune response was invoked. As such, the antibodies titer of chickens was above the protective titer, and maintained at least to 28 DPV. A solid immunity against Newcastle disease virus was obtained by a booster with Mukteswar strain. Of which, the titer was always higher than 3 log2 for at least 35 DPV, and got peak (6.1 – 6.3 log 2) on 28 DPV. Overall, this study showed that the red junglefowl well responded with the Newcastle disease vaccines. It was suggested that the optimal immunization protocol should be a combination of LaSota and Mukteswar strains.

Keywords: Immune response, Newcastle disease, Red junglefowl.

BIOSECURITY AND DISEASES CONTROL PRACTICES AND PERCEPTIONS OF SMALLHOLDER PIG FARMERS IN VIETNAM

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ABSTRACT

Pork is the most widely consumed meat in Vietnam and plays a key role in meeting the growing public demand for protein rich foods. Despite the recent introduction of large scale commercial farming systems, it is estimated that 80% of pork consumed in Vietnam is sourced from smallholder farmers. These farmers can often have their animal production and livelihood significantly impacted by introduction and spread of infectious diseases. Implementation of sound disease control and bio-security policies can play a crucial role in negating these impacts. This study sampled 420 smallholder farmers in two provinces of Vietnam to identity current farmer practices and perceptions relating to disease prevention, control and farm bio-security. The study found that an overwhelming majority (82%) of farmers reported experiencing one or more instances of disease in 2012, with self-treatment as the first response to disease for 70% of farmers. Disinfection mattress and visitor control were used by 67% and 54% of the farmers respectively. However other bio security measures such as rodent control and quarantine of new animals were poorly adopted, with respective adoption rates of 9% and 4%. Farmer perceptions also revealed a strong desire to improve their knowledge and understand of production. Findings from this study will form a key part of a participatory approach to improving farm production and livelihoods through a better understanding of current disease control and biosecurity practices and perceptions.

FUNCTIONAL AND TOPOLOGICAL ANALYSIS OF TMS 11 OF THE STAPHYLOCOCCAL MULTIDRUG EFFLUX PROTEIN QacA

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ABSTRACT

Staphylococcus aureus is a major problem in both the clinical setting and within the community. It is an opportunistic pathogen that can cause a range of diseases with high mortality rates including septicaemia, pneumonia and toxic shock syndrome. *S. aureus* has the ability to quickly acquire and spread antibiotic resistance genes through horizontal gene transfer on mobile genetic elements such as plasmids. This has resulted in many strains having high levels of resistance, limiting treatment of infections to just a few options. One of the mechanisms important in antimicrobial resistance is the action of transport proteins located in the *S. aureus* cell membrane. One such transporter is the QacA efflux protein, encoded by the *qacA* determinant, which is a 514 amino acid protein with 14 transmembrane segments (TMS). QacA mediates resistance to a broad range of antiseptic and disinfectant agents by extruding them out of the cell using a proton motive force generated from the transmembrane electrochemical proton gradient. A number of amino acids within QacA have been found to be important for the function and structure of QacA. The aim of this research was to determine the functional importance of amino acids within the TMS 11 of QacA. Fifteen amino acids within TMS 11 of QacA were successfully substituted with cysteine using site-directed mutagenesis of the plasmid harbouring the *qacA* gene. Western blot analysis revealed that only the cysteine substitution at position 353 affected QacA expression levels. Functional analysis, using minimum inhibitory concentration assays with six different compounds and fluorimetric transport analysis using ethidium bromide, revealed that L343C, G346C and G353C were important for QacA function and G353 may be part of the chlorhexidine dihychloride binding site. In conclusion, this study reveals that TMS 11 may have a general role in QacA function rather than specific interaction with the investigated substrates as only cysteine substitution for three amino acid residues (L343, G346 and G353) caused reduction in QacA function to some tested substrates. The knowledge gained in this study contributes to our overall understanding of QacA structure and function, which may assist in the development of new chemotherapeutic agent designed to inhibit the activity of multidrug efflux systems.

Keywords: *Staphylococcus aureus*, QacA effux pump, *qacA* gene, TMS 11, site-directed mutagenesis

AQUACULTURE

PHYSIOLOGYCAL AND IMMUNE PATHWAY RESPONSES OF RAINBOW TROUT JUVENILES TO DIETARY SUPPLEMENTATION WITH BOVINE LACTOFERRIN

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ABSTRACT

The objective of the present study was to determine the ability of bovine lactoferrin (BLf) of the low dose (Lac 0.1%) diet versus the high dose (Lac 1%) diet to improve the immune status of rainbow trout juveniles as well as their resistance after bacterial challenge. We intended to characterize the physiological and immune pathways by analysing different biological endpoints, including immune gene expressions, humoral immune parameters and blood leukocyte populations at different time points on day 35 (D35), day 51 (D51) and day 54 (D54) (D54 - after challenged test with Aeromonas salmonicida achromogenes). After the short term nutrient test (D35), feeding to trout juveniles with BLf diets, interleukin (pro-inflammatory) genes releasing (il-1, il-6,il-8) in spleen and kidney samples did not display any significant difference compared to those of the control (accepted for the il-8 gene of the low dose of BLf, Lac 0.1%, in spleen). Meanwhile, pro-inflammatory genes release (il-1,il-6,il-8) were recorded for up regulation in spleen and kidney samples on D51 (accepted for the il-1and il-6 genes of the low dose of BLf, Lac 0.1%, in spleen). On the other hand, pro-inflammatory genes release (il-1,il-6,il-8) in kidney samples of the low dose of BLf (Lac 0.1%) after bacteria injection (D54) was observed for a significantly up regulation compared to the control, but those of the high dose of BLf (Lac 1%) in kidney samples, low and high doses of BLf (Lac 0.1%, Lac 1%) in spleen samples on D35, D51, D54 did not show any difference compared to those of the control.

Expression level of the gene coding for the antibacterial enzyme (lysozyme) in spleen and kidney did not differ after short and long term nutrients test (D35, D51), regardless of the dose. However, on D54 there was a significantly higher expression of lysozyme in both spleen and kidneys from fish belonging to Lac 1% than those of the control and low dose of BLf (Lac 0.1%). Regarding macrophage colony stimulating factor (rabbit) gene (MCSFRa), only MCSFRa releasing gene of the low dose of BLf on D51 and high dose of BLf on D54 presented significant up regulation, while those of other BLf diets in spleen and kidney samples at different time points did not show any differ. Relevant to MCSFRa analysis, respiratory burst activity (RBA) analyzed (by flow cytometer) in raw spleen samples on D35 and D51 of BLf diets were not illustrate any difference compared to those of the control too. Moreover, On day 54, Interleukin 10 (il-10, an anti-inflammatory) gene in spleen was significantly more expressed in low dose of BLf diet (Lac 0.1%) than those of the control while, other BLf diets did not show any significant difference after short and long term nutrient tests and bacterial injection. Moreover, il-10 transcript level of BLf diets were significantly different from control in the kidney samples on D35, D51 and D54. Anti-inflammatory (TGFβ1) gene expression of the high BLf dose (Lac 1%) in spleen and both low and high doses of BLf diets (Lac 0.1%; Lac 1%) in the kidney samples exhibited significantly high levels on day 54. Meanwhile, other BLf diets did not show any difference compared to those of control on D35, D51 and D54.

In this present study, there was a significantly higher of cytotoxic T cell (CD8) genes of BLf diets on D51 than those of the control and BLf diets on D35 in the kidney samples but, no difference of CD8 gene in spleen samples was recorded on D35, D51 and D54. However, there was a significantly higher of CD8 in spleen samples of BLf diets compared to those of the control on D54. Beside, high doses of BLf (Lac 1%) induced a significantly higher transcript expression of T helper genes (CD4-1) in spleen and kidney after bacterial challenged. However, other BLf diets did not enhance T helper gene expression in both spleen and kidney on D35, D51 and D54. On the other hand, after short term nutrients test (D35), the low dose of BLf diet (Lac 0.1%) induced the highest alternative complements activity. Moreover, alternative complement pathway (ACH50) of BLf diets significantly increased on D35 and D51. On D54, there was a significantly higher of ACH50 activity of Lac 1% compared to those of the control while, those of Lac 0.1% had no effect. After 35 days and 51 days nutrient tests, total immunoglobulin of BLf diets were more abundant than those of the control. Especially for total blood plasma immunoglobulin (Ig) of the low dose BLf diet (Lac 0.1%) on D51, which exhibited the highest level of total Ig and reached the peak of 480 µg/mL. After bacterial injection (D54), total Ig levels of the control remained at the low level of 25 µg/mL. Meanwhile, there was a significant increased of total Ig in BLf diets compared to those of the control. Furthermore, proportion of lymphocyte + thrombocyte was significantly higher in BLf diets than those of the control on D35, while no difference of this proportion was observed on D51. Due to high individual variations, no difference of monocyte proportion was observed on D35, while those of BLf diets displayed a higher proportion than those of the control on D51. Bacterial injected for the challenge test on D52. The fish mortality from control group started on the 2nd day after bacteria injection and sharply increased during the first of 5 days and stopped on the 9th day, reached the peak of 81% on the 9th day, while the mortality of low and high doses of BLf diets started on the 2nd day - stopped on the first 5th day and 7th day and reached a maximum of 64% and 62% respectively.

Overall, after 35 and 51 days of nutrient tests, BLf diets (Lac 0.1%, Lac 1%) were activated the immune system of rainbow trout juveniles to induce significant high level of alternative complements pathway (ACH50), total blood plasma immunoglobulin (Ig), increased the lymphocyte + thrombocyte and monocyte proportion, released a significant high levels of pro-inflammatory genes (il-1,il-6, il-8), gene coding for the antibacterial enzyme (lysozyme), macrophage activity gene (MCSFRa), T helper (CD4-1) gene, cytotoxic T cell (CD8) and anti-inflammatory genes (TGFβ1, il-10) expression in the spleen and kidney samples. Meanwhile, BLf diets were less effected to trout immune system during short term nutrient test (D35), but they were remarkable up regulation effected to innate immune system of trout juveniles after the long term nutrient test (D51) and protected the BLf diets (Lac 0.1%, Lac 1%) mortality of 64% and 62% after bacteria challenge respectively, compared to 81% of the mortality of the control.

Keywords: Bovine lactoferrin, disease resistance, immunity, rainbow trout juveniles (*Oncorhynchus mykiss*).

IN VITRO INVESTIGATION OF ANTIOXIDANT CAPACITY OF HERBAL EXTRACTS AND COMMERCIAL PRODUCTS USED TO IMPROVE AQUACULTURE PRODUCTS QUALITY

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ABSTRACT

This study was conducted to determine the in vitro antioxidant activities of 20 herbal extract samples and 3 commercial products for initial selection of potential application in seafood and aquaculture products storage. The methods of Folin-Ciocalteu (FC) and 2,2-diphenyl-1-pycrylhydrazyl radical scavenging (DPPH) were applied to evaluate the antioxidant activity. The results showed that eleven out of twenty three plant extracts displayed antioxidant activity, with concentrations able to inhibit half of the maximum response (IC_{50}) ranging from 6 to 49 µg/mL. Phyllanthus amarus Schum. et Thonn. showed the strongest radical scavenging effect (IC_{50}=6 µg/mL). The remaining 4 samples showing a high antioxidant activity (i.e. IC_{50} < 30 µg/mL) were in the following order: Piper betle L. > Psidium guajava L. > Euphorbia hirta L. > Mimosa pudica L.. A group of six samples, including Zingiber officinale Rosc., Hepamin, Eclipta prostrata (L.) L., Alba, Annona reticulata L., Houttuynia cordata Thunb., showed an intermediate antioxidant capacity (i.e 30 µg/mL < IC_{50} < 50 µg/mL). There was a positive correlation between total phenolic content (expressed as gallic acid equivalents) and antioxidant activity (expressed as 1/ IC_{50}) (R^2=0.9137). The next step will be to test compounds with high in vitro antioxidant activity for their capacity to inhibit fatty acid oxidation in feed used in aquaculture and aquaculture products.

Keywords: Antioxidant activity, Folin-Ciocalteu, herbal extracts, plant extracts, 2,2-diphenyl-1-pycrylhydrazyl radical scavenging.

EFFECT OF FISH MEAL REPLACED BY SOYBEAN MEAL ON GROWTH PERFORMANCE AND FEED UTILIZATION OF BLACK CARP (*Mylopharyngodon Piceus*)

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ABSTRACT

In this study, we investigated growth performance and feed utilization of black carp *(Mylopharyngodon piceus*) fed four iso-nitrogenous (crude protein 35%) and iso-lipidic (crude lipid 10%) diets, whereas fishmeal (FM) was replaced by soybean meal (SBM) at 0 (control), 10, 20 and 30% (diets designated as SBM0, SBM10, SBM20 and SBM30). The trial was conducted in a closed re-circulation culture system in a series of 12 composite tanks (3 per treatment) with a volume of about 500 L per tank, each contained 10 homogenous black carp of 10g/individual. The result showed that black carp had relatively high survival rate (SR, %) (96.29 – 100%) and there was no significant difference among experimental diets. Compared to control (SBM0), replacement of 10% (SBM10), 20% (SBM20) of FM by SBM did not significantly differ on growth performance (DWG, SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER) ($P > 0.05$), meanwhile the significant reduction in these parameters occurred when 30% FM was replaced by SBM ($P < 0.05$). The data in present study indicates that SBM could efficiently included in diets from black carp as replacement for FM at maximum ratio of 20%.

Keywords: Black carp, Feed utilization, Fishmeal, Growth*, Mylopharyngodon piceus*, Soybean meal.

1. INTRODUCTION

Black carp (Mylopharyngodon piceus) is an improtant freshwater fish in either China and Northern of Vietnam, due to its the high meat yield, favorable meat quality and medical value(Leng and Wang, 2003; Phuong et al., 2009; Kim et al., 2010). Being carnivorous feeding habit, it requires relatively high dietary protein, ranged 35 – 40% at juvenile griwth stage (Van and Thu, 2013; Hu et al., 2014). In diets for farmed fish, expecially for carnivourous species, fish meal (FM) is commonly main dietary protein source, accounting for 20 – 60% (Glencross et al., 2007; Hardy 2010; FAO, 2012). However, trash fish from natural resource has been dramatically declined over past decades, resulting in fluactuating FM price in global market. In additional, increasing use of FM is not a sustainable way in long term strategy and to some extents, it may lead to extinction of marine resources, thereforce, search for alternative feed ingredients for FM is considering a urgent solution nowadays (Glencross et al., 2007; Lim et al., 2008; Hardy, 2010; Burr et al., 2012).

Among potential alternatives, soybean meal (SBM), a plant protein, is regarded as suitable finding, due to its high protein and fatty acid content, balance in amino acid, suitable price and lower photphorate level than in FM (Hien and Tuan, 2009; Lim et al., 2011; Lin and Li, 2011). Moreover, availablitity of nutrients and energy in

SBM is relatively high in black carp (Hung et al., 2015). So far, SBM has been sucessfully replaced FM in diets for various fish species such as Chitala chitala hamilton (Dan et al., 2013), Rachycentron canadum (Hung and Mao, 2009), snakehead (Be and Hien, 2010; Hien et al., 2010), Pseudobagrus ussuriensis (Wang et al., 2015), Carassius auratus gibelio (Liu et al., 2015).

The aim of this study was to evaluate growth performace and feed utilization of black carp fed four diets whereas FM was replaced by SBM.

2. MATERIALS AND METHODS

2.1. Experiment design

A series of 12 composite tanks was connected in a water recycling system with presence of pysical and chemical filtering tanks. The system was place outdoor without directly exposing to sunlight and was aerated through the days.

Black carp was purchased from Research Institute of Aquaculture No.1 Bac Ninh, Vietnam and acclimated in a 2-m^3-composite tank for 1 month ahead of experiment. During this stage, the tank was aerated whole day and fish were fed commerical feed twice daily to apparent satiety. At the beginning of experiment, black carp of 10.03 ± 0.07 g/individual was randomly assigned in 12 500L-composite tanks at density of 10 individual/tank. Fish was fed by hand for 30 mins at 8:00h and 16:00h.

Four isonutrogenous (35%) and lipidic (10%) diets, whereas control feed (SBM0) containing FM as main protein source, other three diets was formulated by replacing FM by SBM at levels of 10, 20 và 30% (abbriviated as SBM10, SBM20 và SBM30) (Table 1).

Table 1. Composition of experimental diets (% DM)

	SBM0	SBM10	SBM20	SBM30
Fish meal	40.0	36.0		28.0
SBM	0.0	5.8		32.0
Meat bone meal	7.0	7.0	7.0	11.6
Corn meal	12.0	12.0	12.0	12.0
Hipro 70	5.0	5.0	5.0	5.0
Wheat gluten	5.0	5.0	5.0	5.0
Cassava powder	18.41	16.61	14.81	12.91
Fish oil	10.00	10.00	10.00	10.00
Premix mineral & vitamin	2.00	2.00	2.00	2.00
Anti-fungi	0.07	0.07	0.07	0.07
Anti-oxidase	0.02	0.02	0.02	0.02
Binder	0.50	0.50	0.50	0.50
Total	100	100	100	100
Proximate composition (%)				
DM	96.46	96.71	95.89	95.10
Crude protein	35.21	35.04	35.18	34.89
Crude lipid	9.83	9.80	10.10	10.10

2.2. Experiment management

Feeds comsumed and dead fish (if any) were daily managed. Temperature, dissolved oxygen and pH were masured twice daily; NO_2^- and NH_3 were checked twice a week using test kit SERA (Germany).

2.3. Experimental parametters

The following variables were calculated:

$$\text{Survival rate (\%)} = 100 \times \frac{TFf}{TFi}$$

$$\text{Specific growth rate (SGR, \%)} = 100 \times \frac{Ln(Wf) - Ln(Wi)}{T}$$

$$\text{Weight gain (WG, \%)} = (Wf - Wi) \times 100/Wi$$

$$\text{Daily weight gain (DWG, g/fish/day)} = \frac{Wf - Wi}{T}$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Total protein intake (g)}}$$

$$\text{Protein intake (g/fish/day)} = \frac{\text{Feed intake (g)}}{\text{Percent protein in diet}}$$

$$\text{Feed intake (FI, g/fish/day)} = \frac{\text{Total feed intake (g)}}{\text{Number of fish}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total feed intake (g)}}{\text{Total wet weight gain (g)}}$$

Where:

TFf is total number of fish at the end of experiment

TFi is total number of fish at the beginning of experiment

Wf and Wi are the average final weight and the average initial weight, respectively

T is the experimental period (day)

2.4. Data analysis

All data were analyzed by one-way analysis of variance (ANOVA) with Tukey's post hoc test for individual comparisons. A significance level of 0.05 was used for all comparisons.

3. RESULTS

3.1. Water quality parameters in experiment

Within experimantal period, water temperature, dissolved oxygen, pH and NO_2^- were in rank of 28.6 – 31.2°C; 5.22 – 5.42 mg/L, 7.69 – 8.02 and 0.63 – 0.70 mg/L, respectively. NH_3 concentration was less than 0.1 mg/L. These factors provided favorable habitat for black carp.

3.2. Survival rate

The data analysis reults showed that there was no significant difference on SR among fish fed experimental diets ($P > 0,05$) and this value was relatively high, ranged 96.29 - 100% (Table 2). The similar results on Cobia (Hung and Mao, 2009), giant snakehead (Hien et al., 2010), Tilapia (Oreochomis niloticus x Oreochomis aureus) (Lin and Li, 2011) confirmed that replacement of FM by SBM had no negative effect on SR.

3.3. Growth performance of black carp

At the termination of experiment, black carp fed SBM30 diet present lowest body weight (15.18 g/individual) and it was significant lower than that fed SBM0 and SBM10. Meanwhile, this parametter was not statistically different from other experimental diets. Data on SGR and DWG showed similar trend as final body weight. Therefore, FM replaced by SBM at level of 20% did not affect growth performance and feed utilization of black carp, while the increasing replacement level of 30% had negative effect on these parametters. This result was in good agreement with that in Nibea miichthioides (Wang et al., 2006), Seriola quinqueradiata (Shimeno et al., 1993) and in other carnivorous fish species, where SBM replaced for FM was in range of 10 - 30% (Tantikitti et al., 2005; Lam et al., 2012; Dan et al., 2013).

3.4. Feed utilization of black carp

FI of fish fed diets was in narrow variation of 0.384 – 0.408 g/individual/day (Table 2). The highest FI was seen at SBM0 diet (0,408), followed by SBM10 (0,401) and there was no satistic difference in FI among diets ($P > 0.05$). No significant difference was also observed at PI, where its value ranged 0.134 – 0.144 g/fish/day.

FCR in this study ranged 2.50 – 4.04 and follow the trend of FI and PI in statistic analyse ($P > 0.05$. PER presents ability of aquatic animal in using protein source for body construction (Hien and Tuan, 2009), this data differed between SBM30 (0.71) with SBM0 (1.14) and with SBM10 (1.01) (Table 2).

Domingues et al., (2003) reported that the main challenge of using alternative feed ingredient in aquafeeds is the palatibility and feed acceptance, however, no sinificant difference was observed on FI of fish offered four diets, therefore, replacement of FM by SBM do not affect these criteria on black carp.

The lower growth performance and PER seen in SBM30 diest, could refer to some anti-nutrition factors of SBM (Pereset et al., 2003; Gatlin et al., 2007), which was derived from commerical source withour any process. On the other hand, the reason could be due to lack of some amino acids, such as Methionine (Chong et al., 2003; Chou et al, 2004; Tantikitti et al., 2005) in SBM. The difficientcy of this amino acid was proved to reduce growth and feed utilization of drum (Wang et al., 2006).

Table 2. Growth performance and feed utilization of black carp

	SBM0	SBM10	SBM20	SBM30	SEM	P-Value
BWi (g)	10.49	10.49	10.22	10.24	0.09	0.65
BWf (g)	18.02^{a}	17.63^{a}	17.03^{ab}	15.18^{b}	0.39	0.01
DWG (g)	0.16^{a}	0.14^{a}	0.12^{ab}	0.10^{b}	0.01	0.001
SGR (%)	1.18^{a}	1.13^{a}	1.11^{ab}	0.85^{b}	0.05	0.02
FCR	2.50^{c}	2.83^{bc}	3.20^{ab}	4.04^{a}	0.18	0.001
PER	1.14^{a}	1.01^{ab}	0.89^{bc}	0.71^{c}	0.17	0.001
PI (g/fish/day)	0.144	0.140	0.140	0.134	0.002	0.185
FI (g/fish/day)	0.408	0.401	0.398	0.384	0.004	0.275
SR (%)	100	96.29	96.59	96.29	1.57	0.49

Note: SEM (Standard error of the mean);
Means with different superscript letters within rows are significantly different ($P < 0.05$).

4. CONCLUSIONS

In diets for black carp, SBM could be used as replacement for FM at 20% without negative effect on growth and feed utilization, while PER value reduced and FCR increased when feeding black carp with diet where replacement level of 30%.

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INFLUENCE OF FISH OIL REPLACEMENT BY DIETARY PLANT OIL ON GROWTH, FATTY ACID PROFILE AND IMMUNE RESPONSES IN COMMON CARP (*Cyprinus carpio*)

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ABSTRACT

This study was conducted to evaluate the influence of total fish oil replacement by plant oil on growth, fatty acid profile and immune responses in common carp *Cyprinus carpio* juveniles. Six isolipidic diets containing different lipid sources were formulated: fish oil (FO), linseed oil (LO), sunflower oil (SFO), sesame oil (SO) and two mixtures of plant oil (SLO – Sesame and linseed oil, SSFO – Sesame and sunflower oil. A set of 370 juvenile fish with an average weight of 28.6±1.3g was randomly allocated into 18 aquariums (3 aquariums per diet). Fish were fed to apparent satiation during 96 days and then challenged with Aeromonas hydrophyla for 10 days to determine their resistance to bacterial contamination. Husbandry variables were recorded during the rearing period. At the end of experiment and after 3 days of challenge test, plasma and other lymphoid tissues (spleen, kidney) were sampled to lysozyme activity and complement, while liver was used for fatty acid analyses at the end of the rearing period. The results indicate a slight but significant difference of final body weight (FBW) between experimental diets ($P < 0.05$). However, we did not observe any difference in specific growth rate and feed conversion ratio. We observed that lipid source affected the immune response (lysozyme activity, ACH50) and fatty acid profile in liver ($P < 0.05$). In conclusions, lipid source influences growth, immune response and fatty acid profile in common carp. Linseed oil appears as a good lipid source for carp juveniles, especially in terms of some essential fatty acid contents and w3/w6 ratio.

Keywords: Common carp, fatty acid, fish oil, plant oil.

ISOLATION AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF ENDOPHYTIC ACTINOBACTERIA ON MAY CHANG TREE (*Litsea cubeba*) AGAINST PATHOGENIC BACTERIA CAUSING DISEASES ON COMMON CARP AND TILAPIA

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ABSTRACT

Tilapia and common carp are two main cultured species with high production annually in freshwater aquaculture in northern Vietnam, however, there are serious problems caused by bacterial infection. The use of antibiotics is not sufficient to mitigate the outbreaks due to antibiotic resistance rate are increasing. Therefore, to overcome the challenges of antibiotic resistance, antimicrobial compounds with a new mechanistic approach should be urgently sought. This study aimed isolate and evaluate antimicrobial activity of endophytic actinobacteria from May Chang tree (Litsea cubeba) against three pathogenic bacterial species Aeromonas hydrophila GL14, Aeromonas caviae HD60 and S. agalactiae HY10. The results showed that 9/32 (28.2%) endophytic actinobacteria isolates could inhibit at least one target pathogenic bacteria. Three isolates MTR711, MTR622 and MTL121 showed the highest antibacterial response with minimium inhibitory concentration (MIC) ranged from 93.3 to 300 µl/mL, amongst these the lowest value is for MTR711 and MTR622 without significant difference. When combining three individual actinobacteria mentioned above for fractional inhibitory concentration (FIC) test, the synergistic effect was found for the pair of MTR711-MTR622 against three tested pathogenic bacteria chosen with ∑FIC ≤0.5. The combination of two actinobacteria MTR711 and MTR622 improved bacterial inhibitory effect at least 4 times compared to individual treatment. The results are motivating enough to conduct further studies on use of endophytic actinobacteria for treating pathogenic bacteria in aquatic animals.

Keyword: Actinobacteria, Aeromonas, common carp, May Chang, tilapia.

1. INTRODUCTION

In Vietnam, optimized use of geography and environment have improved aquaculture significantly in recently years. In particular, production of freshwater fish has increased considerably, contributing significantly to increase export and local consumption of fishery products (Nguyen Xuan Hao and Ngo Sy Van, 2001). There has been expansion in the fishing area and enhancement in the level of intensification. However, the aquaculture industry is facing serious problems such as environmental pollution and disease outbreaks. Several studies suggested that these antimicrobial effects have arisen due to the overuse of antibiotics and has led to the phenomenon "antibiotic resistance". Therefore, many countries around the world have regulated the use of antibiotic in aquaculture (Cabello, 2006).

The use of products from plant origin to replace antibiotics is being considered for both humans and animals in order to avoid antibiotic resistance. A lot of herbal plants contain antibacterial compounds such as tannin, phenol, citral, quinone (Reverter et

al., 2014). Numerous studies have shown that antimicrobial activity of the herbal plants are related to the beneficial actinobacteria as endophytic symbionts. They synthesize biological compounds which inhibit the bacteria and safe for human. Therefore the selection of potential actinobacteria from herbal plants is a promising solution (Wang and Liu, 2010).

May Chang tree (Litsea cubeba) is an herbal plant that grows in Asian countries including Vietnam. It contains many antimicrobial components (Anil Kumar et al., 2012). Although Litsea cubeba oil is in use in daily life, but so far no studies have investigated the existence of the endophytic actinobacteria in May Chang tree. Also their antimicrobial activity against pathogenic bacteria causing diseases on fish in particular and on other aquatic animal in general is yet to be understood. This is reason that our research has focused on isolation and evalution of endophytic actinobacteria on microbial resistance against Aeromonas hydrophila; Aeromonas agalactiae causing disease for tilapia and common carp.

2. MATERIALS AND METHODS

2.1. Materials

- Pathogenic bacteria

Tested isolates Aeromonas hydrophila GL14; Aeromonas caviae HD60 causing red spot disease on common carp and S. agalactiae HY10 causing pop eye disease on tilapia were provided from Environmental and fish pathology Department, Faculty of Fisheries, Vietnam National University of Agriculture.

- Medium

Nutrient Agar (NA) and Nutrient Broth (NB) (Merck) were prepared in condition of 121°C at 15 minutes. The composition of medium Gause I includes starch powder - 20; K_2HPO_4 – 0.5; $MgSO_4.H_2O$ - 0.5; NaCl - 0.5, KNO_3 - 0.5, $FeSO_4$ - 0.01 (g/l), pH = 7 - 7.4. The composition of the antibiotic producing medium A4-H includes Glucoza - 15; Soybean powder - 15, NaCl - 5; $CaCO_3$ - 1 (g/l), pH = 7 - 7.4.

2.2. Methods

- Endophytic actinobacteria (EA) isolation

Roots, stems and leaves of May Chang tree were collected from Yen Bai, Son La, Ha Tay, Ninh Binh province, Vietnam. After collection, the surface of samples were disinfected following the process of Justin and Christopher (2003) and then cultured on Gause I with complementing nalidixic acid (25 mg/l), nystatin (50 mg/l) and $K_2Cr_2O_7$ (50 mg/l) to inhibit the growth of negative bacteria and fungi. After incubation 4 days at 30°C, EAs were sub-cultured 3 times before screening antibacterial activity against tested pathogenic bacteria. Classification of EAs were based on system of color wheels of Tresner and Buckus (1963).

- Screening of EAs antibacterial activity

After isolation from May Chang tree, EAs were determined for antimicrobial activity with pathogenic bacteria A. hydrophila GL14, A. caviae HD60 and S. agalactiae HY10 by agar diffusion method (Dhanasekaran *et al.*, 2012). In particular, EAs were inoculated in medium Gause I and incubated in condition of saking 200 rpm, 28°C, 7 days and afterthat centrifuged at 6000 rpm in 10 minutes to get crude supernatant of each isolated EA strain. Tested bacteria were cultured on NB at 30°C, 24h and then adjusted to 10^8 CFU/mL by measuring at 600nm wavelength with a spectrophotometer and confirmed by colony counting method on NA medium. Bacteria were spread and

inoculated onto sterile NA medium in separate plates using sterile glass stick. Sterile paper discs (6mm) were placed on agar where bacteria have been placed. Crude supernatant of each EA strain (50 µl) was added separately into each disc and incubated at room temperature for 24 h, bacterial growth was observed and the zone of inhibition was measured (Kafur *et al.*, 2011).

- Determination of minimium inhibitory concentration (MIC) of EAs supernatant

Isolated EAs indicating antimicrobial activity were selected for determination of MIC (Dore *et al.*, 1999). EAs were inoculated shakely in antimicrobial producing medium A4-H at 200 fpm, 30°C. After 7 days of incubation, crude supernatant was separated by centrifuging at 6000 rpm, 10 minutes and then serially 2 folder diluted. Briefly, 100 µl of EAs crude supernatant at dilluted concentrations was separately added to 900 µl NB which have been mixed tested bacteria at 10^8 CFU/ml and incubated at 30°C, 24h before plating inoculum on NA plate and being examined after 24h. The MIC was defined as the lowest concentration of EAs crude supernatnant preventing visible growth. All tests were performed in duplicate and analysed by software SPSS 20 and assessed the differences by Turkey test.

- Evaluation of interaction between endophytic actinobacteria (∑FIC)

From MICs EAs supernatant, the interaction between EA metabolites was evaluated by determining the fractional inhibitory concentration (∑FIC) based on the method of Gutierrez *et al.* (2008). The test was carried out on 96 plates with 270 µL of each tested bacteria suspension containing 10^8 CFU/mL and 15µL crude supernatants of each EA. After that, the plates were incubated at 30°C, 24h before plating on NA to check the growth of bacteria. A combination of crude supernatant of two EAs at different concentration was presented in table 1.

3. RESULT AND DISCUSSION

3.1. Isolation of endophytic actinobacteria (EA)

There were 32 EA strains being isolated (table 2). Based on system of color wheels of Tresner and Buckus (1963) and the color of sporulating aerial mycelium, EAs were classified into 4 color groups as White, Grey, Pink and Brown. Within 32 EA strains, White group stand the biggest position with 13 strains (40.6%), and followed by Grey group (28,1%) and Pink group (9.3%). This result was in agreement with the study of Le Thi Hien et al (2014) which showed 37.1% of total 43 EA strains from soil belongs to White group. Apart from that, the isolation of EA in herbs was carried out by many previous studies. In particular, Gangwar et al. (2011) was isolated 40 EA strains from roots, stems and leaves of three medicinal plants viz. Aloe vera, Mentha and Ocimum sanctum.

3.2. Screening of antimicrobial activity of EA strains in May Chang tree

All 32 EA strains were tested for antimicrobial activity against 3 isolates of pathogenic bacteria A.hydrophila GL14; A.caviae HD60; S.agalactiae HY10 causing diseased on common carp and tilapia. From table 2, the result was displayed that nine in the 32 strains (28.2%) exhibited inhibitory activity against at least one of the pathogenic microorganisms tested. While as, six out of the nine strains exhibited antimicrobial activity with both three bacteria at different level (table 3). The result showed that two strains MTR711 and MTR622 have inhibitory zone ranged from 22.6 to 29,2 mm with 3 pathogenic bacteria. In following, the value

of MTL121 fluctuated from 109 to 15.4 mm. The inhibitory activities of these strains against a variety of pathogens suggested that these endophytic actinobacteria may be potential candidates for the production of bioactive compounds. Although six other EA strains showed antimicrobial capacity, inhibitory zone was small and unstable, only 3 stains MTR711; MTR611 and MTL121 were selected for futher tests.

Table 1. Combination of EAs crude supernatant at different concentration of MICs

∑FIC		EA 1						
		2 MIC	1,5 MIC	1 MIC	1/2 MIC	1/4 MIC	1/8 MIC	1/16 MIC
EA 2	2 MIC	4.00	3.50	3.00	2.50	2.25	2.13	2.06
	1,5 MIC	3.5	3.00	2.50	2.00	1.75	1.63	1.56
	1 MIC	3.00	2.50	2.00	1.50	1.25	1.13	1.06
	1/2 MIC	2.50	2.00	1.50	1.00	0.75	0.63	0.56
	1/4 MIC	2.25	1.75	1.25	0.75	0.50	0.38	0.31
	1/8 MIC	2.13	1.63	1.13	0.63	0.38	0.25	0.19
	1/16 MIC	2.06	1.56	1.06	0.56	0.31	0.19	0.13

Note: ∑FIC was determined as a minimum combination of two EAs crude supernatant which can inhibit the growth of bacteria. So, ∑FIC was calculated as $FIC_{EA1} + FIC_{EA2}$*; whereas* $FIC_{EA1} = MIC_{EA1\ in\ combination}/MIC_{EA1\ in\ single}$ *and* $FIC_{EA2} = MIC_{EA2\ in\ combination}/MIC_{EA2\ in\ single}$*. The result was interpreted the combination of EA1 and EA2 as: synergy with* $\Sigma FIC \leq 0.5$*; addition with* $0.5 < \Sigma FIC \leq 1$*, indifference with* $1 < \Sigma FIC \leq 4$*, antagonism with* $\Sigma FIC > 4$*. The test was carried out in triplicate.*

Table 2. Color classification and antimicrobial activity and endophytic actinobacteria

Color group of EAs	Number of EAs	Percentage of EAs (%)	Antimicrobial activity	
			Number of EAs	Percentage of EAs (%)
White (Allbus)	13	40.6	4	12.5
Grey (Griseus)	8	25	2	6.3
Pink (Roseus)	3	9.3	1	3.1
Grey (Chromogenes)	9	28.1	2	6.3
Total	32	100	9	28.2

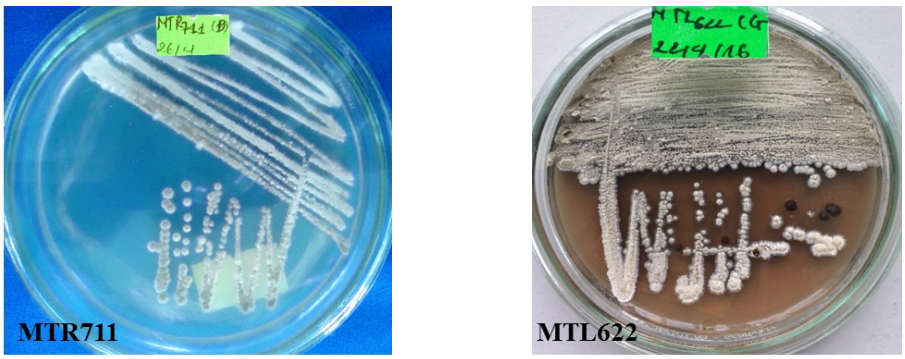

Figure 1. EA strains MTR711 and MTL622 in medium Gause I

Table 3. Antimicrobial activity of endophytic actinobacteria (EA) in May Chang tree

No	EA strains	Inhibitory zone (mm)		
		A. hydrophila GL14	*A. caviae* HD60	*S. agalactiae* HY10
1	MTR711	29.6 ± 2.5	22.6 ± 0.9	23.2 ± 1.3
2	MTL721	6.5 ± 0.8	5.2 ± 1.3	7.8 ± 1.9
3	MTL214	4.3 ± 1.4	6.3 ± 2.3	8.3 ± 1.3
4	MTR622	23.0 ± 2.1	25.6 ± 0.9	26.2 ± 0.8
5	MTT723	-	-	7.1 ± 1.0
6	MTL121	15.4 ± 0.7	12.5 ± 0.9	10.9 ± 0.5
7	MTR611	12.2 ± 1.8	1.8 ± 1.3	1.6 ± 1.6
8	MTR101	-	-	7.5 ± 0.8
9	MTT112	5.4 ± 3.3	4.0 ± 4.2	-

Note: (-) None of antimicrobial activity

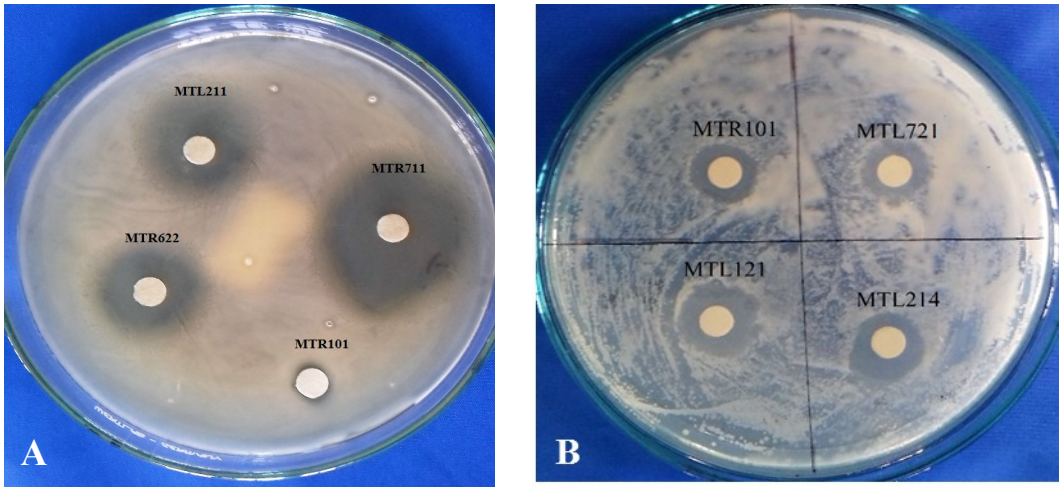

Figure 2. Inhibitory zones of EA trains against pathogenic bacteria: (A) *A. hydrophila* GL14; (B) *S. agalactiae* HY10

Table 4. Minimium inhibitory concentration of endophytic actinobacteria (EA) in May Chang tree(µl/mL)

No	EA strains	A. hydrophila GL14	A. caviae HD60	S. agalactiae HY10
1	MTR711	126.7^{a} ± 3.3	126.7^{a}± 8.8	93.3^{a} ±3.3
2	MTR622	143.3^{a} ± 3.3	100.0^{a} ± 5.7	130.0^{b} ±5.7
3	MTL121	240.0^{b} ± 5.7	283.3^{b} ± 6.7	300.0^{c} ±10.0

Note: Values followed by different letters within a column are significantly different Turkey ($p \leq 0.05$)

In nature, there are many endophytic actinobacteria being capable of production of bioactive compounds against pathogenic micro organisms such as fungi, bacteria. Therefore, many of them have been used as materials for extraction, synthetic of drug and chemical to mitigate disease for human and animals. Many studies proofed antimicrobial activity of EAs. Zhao et al. (2012) reported that there were 26 out of total 560 EA strains being isolated from 26 medical plants in Panxi, China exhibiting inhibitory activity with at least (10.7%). Similarly, Li et al. (2008) isolated 41 EAs belonging to Streptomyces, including 65.9% and 24.4% of total EAs against E.coli and Staphylococcus aureus, respectively. In spite of many researchs on antimicrobial activity of EAs on human pathogenic microorganisms, there are a lack of studies carrying out on aquatic animals.

3.3. Minimium inhibitory concentration (MIC) of EA strains in May Chang tree

From the result above, three EAs strains MTR711, MTR622 and MTL121 showing the biggest inhibitory zone were chosen for MIC determination. The result from table 4 presented that MIC of MTR711 and MTR622 ranged from 93.3 to 143.3 µl/mL and was not significantly different ($p \leq 0.05$) against *A.hydrophila* GL14 and *A.caviae* HD60. The MIC of strain MTL121 showed the highest value with the range of 240 - 300 µl/mL. The result proved that antimicrobial effect of the strains MTR711 and MTR622 were higher than that of MTL121. Our result was in agreement with Nguyen Hai Van *et al.* (2016) which MIC of endophytic actinobacteria named MPT28 in May Chang tree arranged 50 - 333 µl/mL against human pathogenic bacteria.

3.4. Interaction effect of EA strains (∑FIC) on antimicrobial activity

The interaction effect of 3 EA strains in pair combination was presented in table 5. The result indicated that the combination of MTR711 and MTR622 showed synergy effect of antimicrobial activity against all three tested bacteria (∑FIC ≤0.5). The combination of MTR711 and MTL121 has resulted in addition effect with ∑FIC in range of 0.5 - 1.0. Indifference effect of MTR622 and MTL121 was observed with ∑FIC > 1.0. Therefore, the combination of MTR711 and MTR622 could decrease concentration at least 4 times comparing single treatment.

Table 5. Interaction effect of EA strains on antimicrobial activity

Combination of EA strains	Pathogenic bacteria	∑FIC	Interaction*
MTR711-MTR622	*A.hydrophila*	0.3	Synergy
	A.caviae	0.5	Synergy
	A.agalactiae	0.5	Synergy
MTR711- MTL121	*A.hydrophila*	0.7	Addition
	A.caviae	1.0	Addition
	A.agalactiae	1.0	Addition
MTR622-MTL121	*A.hydrophila*	1.6	Indifference
	A.caviae	1.1	Indifference
	A.agalactiae	1.8	Indifference

*Note: *Synergy (ΣFIC ≤0.5); Addition (0.5 < ΣFIC≤ 1); Indifference 1 < ΣFIC≤ 4; Antagonism (ΣFIC > 4)*

The interaction effect of antimicrobial compounds have conducted by some studies. Cai et al. (2007) reported that MIC of allicin alone was 512 µg/mL, but it facilitated antibacterial activity of all three β -lactams tested at subinhibitory concentrations. In particular, ∑FI của cefazolin was 0.5 ($1/4MIC_{allicin\ alone}$ và $1/4MIC_{cefazolin}$), ∑FIC of oxacillin was 0.375 ($1/8MIC_{allicin\ alone}$ and $1/4MIC_{oxacillin}$). The study of Zafar Ahmed et al. (2013) showed that Amoxicllin and Cefadroxil have synergy effect against 47 isolates Staphylococcus aureus with value ∑FIC ranged in 0.14 – 0.5, while as, Streptomycin and Cefadroxil synergized on antimicrobial activity against 44 isolates S.aureus ($∑FIC_{min}$ 0.03 - 0.5). The study of Nguyen Hai Van et al. (2016) on interaction between EAs and May Chang oil indicated that synergy effect of the oil and EA strain named MPT28 against 4 isolates of human pathogenic bacteria.

4. CONCLUSIONS

There were 9 out of total 32 EA strain in May Chang tree exhibiting antimicrobial effect with three pathogenic bacteria A. hydrophila GL14, A. caviae HD60 and S. agalactiae HY10 causing diseases on common carp and tilapia. Three EA strains MTR711, MTR622 và MTL12 have wide inhibitory zones ranged from 10,9 to 29,2mm. MICs of 2 strains MTR711 and MTR622 displayed no significant difference in a range of 93.3 to 143.3 µl/mL against both three tested bacteria. The combination of MTR711 and MTR622 showed synergistic effect against 3 tested bacteria to enhance antimicrobial activity at least 4 times comparing with single test. This result could be a potential and promising application for sustainable therapy in aquaculture.

ACKNOWLEDGEMENT

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FISH PASTE PRODUCTION FROM MARINE FISH EXPLOITED IN KIEN GIANG PROVINCE

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ABSTRACT

Fish paste production from marine fish exploited in Kien Giang province was studied in order to diversify fish paste products and enhance the efficient use of marine resources to improve the economic value of fisheries. The study included three contents: (i) evaluate the possibility and quality of marine fish used for fish paste production, (ii) fish paste production from different marine fish sources, (iii) change the quality of fish paste during cold storage. The results showed that sixteen fish species belong to 15 families in five different orders were identified, in which four most popular fish species are red tail, threadfin bream, mackerel and silver conger eel. Nine common fish species were selected for fish paste production. TVB - N value of marine fish was less than 50 mg/100g. High meat yield was found in marine fish which is appropriate for fish paste production. Fish paste gel property and sensory properties from marine fish collected from Ha Tien and Rach Gia towns were better than fish collected from Ba Hon town. The fish paste production can be stored in temperatures 0 - 5°C for at least two weeks.

Keywords: Fish paste, Kien Giang, marine fish, production.

A PATHWAY TO CLIMATE CHANGE ADAPTATION FOR AGRICULTURE PRODUCTION: SHRIMP CASE IN MEKONG DELTA

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ABSTRACT

Mekong Delta of Vietnam has a total area of 4.05 million hectares (accounting for 12.2% of the total Vietnam territory) and 17.27 million people (accounting for 19.87% of the Vietnam population). Most of land area of the Mekong Delta is used for agricultural purposes (62.95%), especially the inland provinces. Mangrove forest land accounted for 8.18% mainly concentrates in coastal provinces, particularly in Ca Mau, Kien Giang, Bac Lieu, Ben Tre.

Aquaculture is one of the key economic sectors in the strategic economic development of Vietnam, in which shrimp and pangasius are considered as key species. The Mekong Delta is annually producing 2.4 million tons of aquaculture products worth about USD 3.8 billion, compared with 6.2 million tons of rice worth USD 2.7 billion.

Shrimp production in the Mekong delta is considered as key coastal economic production, which has some models such as extensive, improved extensive and semi-intensive farming which have been mainly applied for tiger shrimp (P. monodon); whereas P. vannamei was to stock intensive farming for easily controlling diseases spread-out. The alternative shrimp - rice model is also found where soil is affected by sea-water in dry season. This model is characterized by land use for rice planting in rainy season and shrimp farming (both semi-intensive and improved extensive farming) in dry season. This model is now considered as good option for certain areas, where nutrient accumulation after shrimp crop is used as sources of fertilizer for rice planting. Land is used for different purpose round year can also eliminate disease infection for shrimp or rice.

Climate change is a dynamic process which is impacting on the agriculture production in the Mekong Delta region, by which, large parts of the delta are no more than one metre above sea level in the most vulnerable coastal region. As such this region is very subject to rising sea level, intrusion and lack of fresh water.

Increased salinity intrusion arising from rising sea levels is already impacting on traditional cropping patterns in the region, most notably on rice production, which also mean having opportunity for another production such as shrimp.

This foresees significant reduction in rice growing in favour of alternative production systems that are economically viable as well as environmentally sustainable, to support dependent families and communities. In this transition, farmers and communities will need to be encouraged and supported in successfully changing and adapting, which shrimp is considered as a good option.

However, the debate is how to develop sustainable production systems to which they will be really opportunity for strengthening of Vietnam shrimp competitive advantage to the global market.

Keywords: Coastal, competitive advantage, intrusion, sea level rise, Shrimp.

BIOTECHNOLOGY

EFFECTS OF THE POLYMORPHISMS OF FUT1 GENE ON BODY WEIGHTS AT BIRTH AND WEANING OF YORKSHIRE PIGLETS

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ABSTRACT

The objective of this research is to identify the polymorphisms of FUT1 gene in the Yorkshire pigs under intensive farm condition and the effect of this gene as well as of the gender on body weight at birth and at weaning. A total of 263 Yorkshire piglets (143 females and 120 intact males) were randomly selected at birth. Piglets were tattooed on the ear and their weight was recorded individually at birth. At weaning, the piglets were notched by an ear tag and individual weights were noted. The three genotypes of FUT1 (GG, GA and AA) were observed in the studied population. From 263 piglets, AA genotype was found on 3 individuals (1%), GG on 199 (76%) and AG on 61 (23%). Consequently, the allelic frequencies for G and A are respectively 0.87 and 0.13. The genotype frequencies of FUT1 were found to be in Hardy-Weinberg equilibrium (P=0.45). The effects of FUT1 and gender were not significant (P > 0.05). Nevertheless, the piglets with the AA genotype had tended to be heavier than those with GG and AG genotypes. This result suggest that a marker-assisted selection program using FUT1 alleles could not only improve PWD resistance, but also affect positively growth traits.

Keywords: Body weight at birth, body weight at weaning, FUT1 gene, Yorkshire pigs.

1. INTRODUCTION

The alpha (1,2) fucosyltransferase gene (FUT1) is located on pig chromosome 6. A single nucleotide polymorphism (SNP) within this gene, with two alleles (A and G), is associated to a phenotype of porcine post-weaning diarrhoea (PWD) resistance. The animals with AA genotype have been shown to be resistant to ETEC E18 while those with AG and GG are sensitive (Meijerink *et al.,* 1997). Marker-assisted selection might therefore help to control the disease in the targeted population by using preferably animals with genotype AA (Wang *et al.* 2012). For example, a reduction of mortality from 22% to 1% in pigs with 2 resistant alleles has been observed (Mellencamp *et al.,* 2008)

The effect of FUT1 gene on production and reproduction performance of pigs was reported in previous researches (Jiang *et al.,* 2005; Bao *et al.*, 2011; Shiping Zhu *et al.,* 2014). The gilts with AA genotype grow faster than the AG and GG ones while sows with AA genotype have more piglets born alive than those with AG and GG genotypes (Shiping Zhu *et al.,* 2014). In contrast, there was no effect of FUT1 gene on growth rate of males in Duroc and Landrace breeds (Huang *et al.,* 2008).

AA genotype is absent in Asian local pigs breeds (Yan *et al.,* 2003; Bao *et al.,* 2008; Bao *et al.,* 2011; Cuong *et al.,* 2012) although this genotype is present in European local pigs breeds (Klukowska *et al.,* 1999). All of the above studies were conducted separately for adult males and females; no gender effect was mentioned. The objective of this research is to identify the polymorphisms of FUT1 gene in the Yorkshire pigs under intensive farm condition and the effect of this gene as well as of the gender on body weight at birth and at weaning.

2. MATERIAL AND METHODS

A total of 263 Yorkshire piglets (143 females and 120 intact males) were randomly selected at birth at Dabaco Nucleus Breeding Pig Company, Dabaco Group, Bac Ninh province (40 km north from Hanoi) from March to June 2016. Piglets were tattooed on the ear and their weight was recorded individually at birth. At weaning (23.7 day ± 2.42 (sd)), the piglets were notched by an ear tag of 28 mm diameter and individual weights were noted.

The tails were docked at birth and then these docked tails were stored in keeping sample boxes and transported to the laboratory where the samples were stored at -20°C until genomic DNA was extracted. Genomic DNA was isolated from the porcine tail sample following standard procedures (Sambook *et al.,* 1998).

Forward and reverse primers sequence to amplify the FUT1 polymorphism described above were: 5′-CTTCAGCCAGGGCTCCTTTAAG-3′ and 5′-CTGCCTGAACGTCTATCAAGACC-3′ (Meijerink etal., 1997). The PCR reaction was performed on a 25µl volume, including 20 ng genomic DNA, 0.25µM of each primer, 2.5µl 10×PCR buffer (containing 1.5 mM Mg^{2+}), 0.2mM dNTPs, 1.25U *Taq* DNA polymerase, and 18.5 µl dd H_2O.

PCR was carried out on a PCR system PTC-100 with the following procedures: an initial denaturation of 3 min at 94°C, followed by 35 cycles of 45 sec at 94°C, 30 sec at 58°C, 45 sec at 72°C, and then a 5 min final extension at 72°C. The amplified DNA (8µl) was digested at 37°C with 1 unit of Hin6I for 8h. The digests were separated by electrophoresis on 3% poly-acrylamide gel, where genotypes could be extracted (Figure 1).

The obtained data was analysed using SAS software. The general linear model procedure was used to identify the effects of the FUT1 gene polymorphism and gender on body weights at birth and weaning. The least-square means were compared using Tukey's test. Hardy-Weinberg equilibrium was tested using a chi-square test. P-values < 0.05 were considered as significant.

3. RESULTS AND DISCUSSION

A 421 bp DNA fragment from the *FUT1* gene was amplified and the G/A mutation at position 307 was eliminated the polymorphic site. For the enzyme Hin6I, the fragment had two restriction sites. A monomorphic site presented in both alleles (G and A) and give two fragments (328bp and 93bp) while a polymorphic site presented only in allele G and has caused the 328 bp fragment to be digested to 241 bp and 87 bp, respectively. The three different genotypes (GG, GA and AA) at the Hin6I-RFLP site of the *FUT1* gene are illustrated in Figure 1.

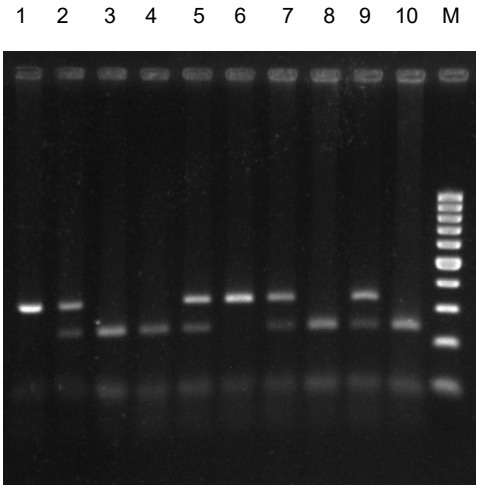

Figure 1. Genotyping result of *FUT1* gene by PCR-RFLP

Noete: Lane M: 100bp ladder, lane 1 and lane 6: genotype AA, lane 3, 4, 8 and 10: genotype GG, lane 2, 5,7 and 9: genotype AG

The genotype and allele frequencies of FUT1 gene are presented in Table 1. The three genotypes (GG, GA and AA) were observed in the studied population. From 263 piglets, AA genotype was found on 3 individuals (1%), GG on 199 (76%) and AG on 61 (23%). Consequently, the allelic frequencies for G and A are respectively 0.87 and 0.13 (Table 1). Note that the AA genotype was absent in Asian local pigs breeds (Yan *et al.* 2003; Bao *et al.* 2008; Bao *et al.* 2011; Cuong *et al.* 2012) although this genotype was present in European local pigs breeds (Klukowska *et al.* 1999). The relative low frequency of the A allele, which is the favourable allele for PWD resistance, should be questioned: selection on weight gain and/or on number of piglets might lead to reduce the frequency of the allele A in the population, possibly indicating a deleterious effect of this allele on pig growth or reproduction, which would of course raise questions on the use of this allele in the selection process. Cuong *et al.* (2012) inferred that PWD has a negative relationship with growth rate. The research of Cuong *et al.* (2012) on Yorkshire pigs in Vietnam under industrail condition also found the three genotypes but with a frequency of AA estimated at 0.13. This might indicate that the selection performed on these pigs has led to an increase in the frequency of A allele. In our study, the genotype frequencies of FUT1 were found to be in Hardy-Weinberg equilibrium (P = 0.45).

Table 1. Genotype and allele frequencies of FUT1 gene in Yorkshire pigs

Item	Genotype			Allele		P-value
	GG	GA	AA	G	A	
Observed count	199	61	3			
Expected count	199.9	57.8	5.3			
Observed frequency	0.76	0.23	0.01	0.87	0.13	
Expected frequency	0.76	0.22	0.02			0.45

Table 2. Body weight (kg) at birth and at weaning of Yorkshire piglet according to FUT1 genotype and gender

Factor	Body weight at birth			Body weight at weaning		
	n	LSM	SE	n	LSM	SE
FUT1 gene						
GG	199	1.66	0.02	198	6.82	0.13
AG	61	1.60	0.04	60	6.24	0.24
AA	3	1.80	0.16	3	6.94	1.07
P-value		0.23			0.11	
Sex						
Female	143	1.68	0.06	142	6.64	0.38
Male	120	1.69	0.06	119	6.69	0.39
P-value		0.80			0.81	

The body weights at birth and at weaning are shown in table 2. In the present research, the effect of FUT1 and gender were not significant ($P > 0.05$). Nevertheless, the piglets with the AA genotype had tended to be heavier than those with GG and AG genotypes. This result is consistent with previous researches (Huang *et al.,* 2008; Bao *et al.,* 2011): the gilts with AA genotype grow faster than those with AG or GG genotype (Bao *et al.,* 2011). In contrast, there was no effect of FUT1 gene on the growth performance of males in Duroc and Landrace breeds (Huang *et al.,* 2008). Among surviving pigs, the resistant pigs (AA) demonstrated a 30% improvement of average daily gain compared to susceptible ones (Mellencamp *et al.*,2008).

Body weights at birth and at weaning were not significantly different between female and male ($P > 0.80$). The body weights at birth were 1.68 and 1.69 kg for female and male respectively. At weaning, these values were 6.64 and 6.69 kg respectively (Table 2). The effect of gender on growth of pigs was reported by Leach *et al.* (1996), Latorre et al (2003), Peinado *et al.* (2008) and Do Duc Luc *et al.* (2015). However, these authors concluded that males grew faster than females in the fattening pigs at the weight around 100kg. In the present study, the effect of gender was not found. It might relate to the early growth period where sexual function was not expressed.

5. CONCLUSIONS

Three genotypes (GG, GA and AA) of FUT1 were recorded in studied Yorkshire pigs. However, the favourable genotype AA frequency was low. There was no effect of FUT1 on body weights at birth and weaning, although a favourable effect of AA can be suspected. This result suggest that a marker-assisted selection program using FUT1 alleles could not only improve PWD resistance, but also affect positively growth traits.

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GENETIC STRUCTURE OF CANDIDATE GENES FOR LITTER SIZE IN LANDRACE AND YORKSHIRE SOWS

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ABSTRACT

The aim of this research is to identify polymorphisms of IGF2, RBP4 and RNF4 genes in Landrace (L) and Yorkshire (Y) sows. A total of 361 ear tissue samples, including 175 L and 186 Y sows were collected from the Dabaco Nucleus Breeding Pigs company, Bac Ninh province, Vietnam. Polymorphisms of IGF2, RBP4 and RNF4 genes were indentified by PCR-RFLP with specific primers, and the restriction enzymes were *NciI, MspI* and *SacII*, respectively. The results indicated that the polymorphic sites of these genes were found in two populations, except genotype AA for IGF2 in Y sows. For the polymophism of IGF2, the frequency of allele A was low in both populations (0.21 and 0.04 for L and Y sows, respectively). Genotypes AA, AB and BB of IGF2 in L sows were 0.04, 0.34 and 0.62, respectively. In Y sows, no homozygous dominant AA was detected, two genotypes of AB and BB were detected with frequencies of 0.08 and 0.92, respectively. For RBP4-*Msp*I gene, two different alelles (A and B) were identified: allele A (0.69; 0.52), allele B (0.31; 0.48) and three genotypes: AA (0.58; 0.32), AB (0.23; 0.39) and BB (0.19; 0.29) for L and Y sows, respectively. For RNF4-*Sac*II gene, the frequencies of alelles T and C were 0.84 and 0.16 in L sows, 0.31 and 0.69 in Y sows. The frequencies of TT genotypes were 0.76 and 0.16, TC were 0.17 and 0.30, CC were 0.07 and 0.54 for Landrace and Yorkshire sows, respectively. The genotype frequency of IGF2 gene were in Hardy-Weinberg equilibrium ($P > 0.05$) but not for RBP4 and RNF4 ($P < 0.05$).

Keywords: IGF2, Landrace, Polymorphism, RBP4, RNF4 gene, Yorkshire.

1. INTRODUCTION

Litter size is one of the most improtant traits in pig industry (Rothschild, 1998). Increasing the number of pigs weaned per sow will increase economic returns for pig producer with minimal additional inputs. Improvement of reproductive traits by traditional selective breeding program has proved to be difficult due to the low heritability for litter size trait. The candidate gene approach employed in identifying the polymorphisms in genes likely to cause phenotypic variation based on physiological and biochemical evidence, could accelerate the improvement of porcine reproductive traits (Niu *et al.*, 2009). With the development of candidate gene and comparative mapping approaches, major genes or gene markers affecting important reproductive traits in pigs have been successfully identified, including Insuline-like growth factor (IGF2), Retinol Biding Protein 4 (RBP4) and Ring Finger Protein 4 (RNF4).

In pigs, the IGF2 gene, localised on chromosome 2, appears maternally imprinted and expressed only via the sire

(NEZER *et al.*, 1999). This gene was marked as a candidate gene for muscle mass (skeletal and cardiac) and fat deposition (Jeon *et al.*, 1999; Nezer *et al.*, 1999). However, Horák *et al.* (2001) reported that IGF2 could be played a role in fertility, as Black Pied Pøeštice sows of the genotypes AB and BB had larger litters. The RBP4 gene is localized in chromosome 14 in pigs. Harney *et al.* (1993) and have shown that there is an increasing RBP4 gene expression in gravid porcine endometrium from day 10 to 12. Their results support an important role for this vitamin A transport protein in uterine and conceptus physiology during the establishment of pregnancy. This suggests that RBP4 may be an interesting candidate gene for litter size in pigs.

The RNF4 is a steroid receptor coregulator, which can activate transcription from steroid-independent promoter (Kaiser *et al.*, 2003; Poukka *et al.*, 2000). RNF4 can stimulate the rat Luteinizing Hormone - (LH) promoter (Curtin *et al.*, 2004), and overexpression of RNF4 can enhance the transcription of glucocorticoid, progesterone, and estrogen receptors as well (Saville *et al.*, 2002). Expression study of RNF4 during murine fetal gonad development and postnatal ovarian folliculogenesis suggested that RNF4 play a role in fetal germ cell development as well as in oocyte and granulosa cell maturation (Hirvonen-Santti *et al.*, 2004). There are very few works have mentioned the effect of IGF2 and RNF4 genes or many different views on the effect of RBP4 on litter size. Therefore, to use these markers on selection breeds, it is necessary to verify whether these markers are associated with the traits in the specific populations under selection. However, as a preliminary step, it is important to identify polymorphism of IGF2, RBP4 and RNF4 genes in Landrace and Yorkshire populations.

2. MATERIALS AND METHODS

2.1. Sample collection

Ear tissues samples from 361 sows, including 175 Landrace and 186 Yorkshire sows kept under industrial conditions were collected from the Dabaco nucleus breeding pigs company, Bac Ninh province, Vietnam. The tissue samples were collected and kept in a centrifuge tube (1,5ml), and stored at -80°C for DNA extraction.

DNA extraction, PCR amplification and Genotyping

DNA was extracted from ear tissues using QIAamp DNA Tissue Kit. Amplification of fragments of RBP4 and RNF4 and genotyping were performed primarily according to Kolaoikova *et al.* (2003), Rothschild *et al.* (2000) and Niu *et al.* (2009), however systems and conditions of reaction slightly different from what were previously reported. Information on primer sequences, restriction enzyme are shown in table 1.

Table 1. Primers, endonuclease and allele sizes of RBP4, RNF4 and IGF2 genes

Gene	Primer sequence (5'-3')	Region	Enzyme	Source
IGF2	F: CACAGCAGGTGCTCCATCGG R: GACAGGCTGTCATCCTGTGGG	Intron 7	*NciI*	Kolaoikova *et al.* (2003)
RBP4	F: GAGCAAGATGGAATGGGTT R: CTCGGTGTCTGTAAAGGTG	Intron 4	*MspI*	Rothschild *et al.* (2000)
RNF4	F: CGAAATGCCAGGGAAGAG R: CCATGCAGATCGGACAACT	Intron 5	*SacII*	Niu *et al.* (2009)

The PCR amplification was performed using 50ng of genomic DNA, 1.5mM $MgCl_2$, 0.2 mM dNTPs, 0.5 µM primers and 2U of Taq DNA polymerase and PCR buffer in a 25 µl final volume. Amplification conditions of: (1) IGF2 was at 95°C for 2 min, followed by 30 cycles of 95°C for 20 sec, 62°C for 30 s, 72°C for 60 sec, with a final extension at 72°C for 7 min; (2) RBP4 was following temprature program: 93°C for 3 min followed by 35 cycles of 93°C for 30 sec, 56°C for 45 sec, 72°C for 45 sec and ending with a final step of 72°C for 5 min; and (3) RNF4 was 94°C for 4 min followed by 35 cycles of 94°C for 45 sec, 50°C for 45 sec, 72°C for 2 min and ending with a final step of 72°C for 10 min. The length of the PCR product are 336 bp, 550 bp and 939 bp for IGF2, RBP4 and RNF4, respectively. The amplified fragment was digested by 1 U of particular restrictase by *NciI, MspI* and *SacII* respectively at 37°C overnight. The obtained fragments were separated on 2% agarose gel. After that the gels were analyzed in UV rays transillumination.

2.2. Statistical analysis

Genotypic and allelic frequencies were calculated for each marker. The Hardy-Weinberg equilibrium in each population was tested by comparing the expected and observed genotype frequencies using a chi-squared test (χ^2). The data was analyzed by SAS software.

3. RESULTS AND DISCUSSION

3.1. Genotyping

IGF2 gene

The IGF2 genotypes of 71 Landrace and 65 Yorkshire sows were detected. In Landrace herd, two allele (A and B) and three genotypes (AA, AB and BB) were indentified while there were only two genotypes (AB and BB) in Yorkshire. The allele A is characterized by digestion of the 336 bp PCR product to fragments 308 and 28 bp, while allele B with a polymorphic restriction site is represented by fragments 208, 100 and 28 bp (Figure 1).

RBP4 gene

All 361 sows, including 175 Landrace and 186 Yorkshire sows were sucessfuly genotyped of RBP4 gene. Two alleles (A and B) and three genotypes, including AA (190, 154 and 136bp), BB (190, 136 and 125bp) and AB (190, 154, 136 and 125bp) were identified (Figure 1). Banding patterns of RBP4 was not entirely consistent with study of Rothschild et al. (2000) who reported that the length of restriction fragments were AA (190, 154, 136 and 70bp); BB (190, 136, 125, 70 and 29bp); AB (190, 154, 136, 125, 70 and 29bp). However, it was consistent with previously reported of Wang et al. (2006).

RNF4 gene

For RNF4, the genotypes of 87 Landrace and 97 Yorkshire sows were detected. Two alelles specific patterns obtained after SacII digestion were an uncut 937bp fragment for allele T and two fragments of 545bp and 392bp for allele C; and consequently three genotypes, TT (937bp), CC (545 and 392bp) and TC (937, 545 and 392bp) (Figure 1). This result was similar with reported of Niu *et al.* (2009).

3.2. Polymorphism distribution

IGF2 gene

The polymorphism distributions of IGF2 are presented in Table 2 and Figure 2. A low frequency of allele A was observed in both Landrace (0.21) and Yorkshire (0.04). In Landrace sows, the genotype frequencies for AA, AB and BB were 0.04, 0.34 and 0.62, respectively, while in Yokshire sows only two genotypes of AB

and BB were observed with frequencies of 0.08 and 0.92 respectively; no homozygous AA was detected. In present study, the B allele frequencies in both populations were greater than A. This results were similar to those reported by Kolaoikova et al. (2003) and Horák et al. (2001). When studied on 75 Large White sows, Kolaoikova et al. (2003) indicated that A allele was appeared with low frequency (0.18); and the frequency of genotypes AA, AB and BB were 1.65 (2 sows), 33.88 (41 sows), and 64.4 (78 sows), respectively. Horák et al. (2001) examined the IGF2 gene polymorphism in 90 Black Pied Pøeštice sows showed that the frequency of allele A was 0.33. In present study, in Landrace and Yorkshire population, the most frequent genotype is the BB genotype. In both breeds, the IGF2 genotypes were in Hardy-Weinberg equilibrium.

RBP4 gene

In both sow herds, the A allele frequencies were considerably higher than B allele (Table 3). Particularly, the allele A occurred with the frequencies 0.69 and 0.52; and allele B were 0.31 and 0.48 in Landrace and Yorkshire sows, respectively. Previous research showed that a higher frequency of allele A was observed in the breed of German Landrace (Drogemuller *et al.*, 2001), and German Landrace x Duroc crossbred sows (Rothschild *et al.*, 2000) and other breeds (Short *et al.*, 1997). However, a lower frequency of allele A compared to allele B were also observed by Wang *et al.* (2006) and Linville *et al.* (2001).

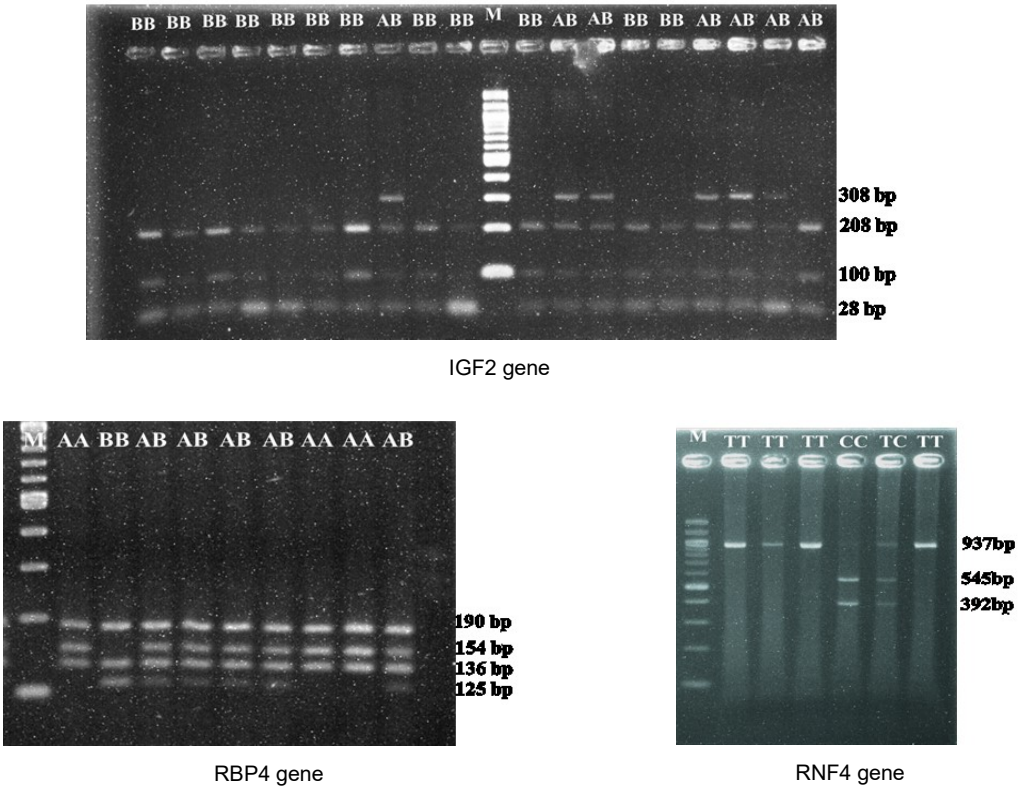
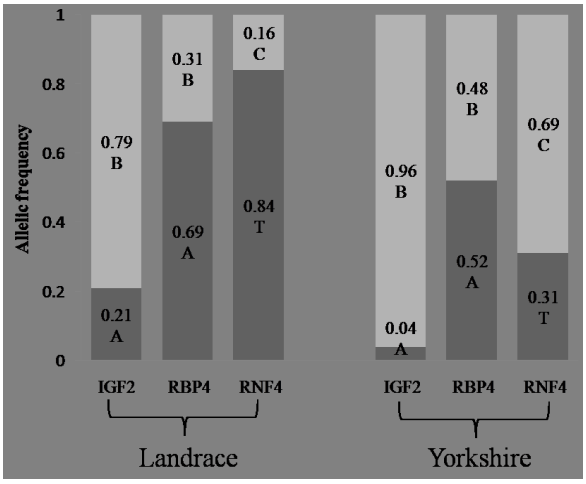


Figure 1. 2% agarose gel electrophoresis for IGF2, RBP4 and RNF4 genes product digested by *NciI, Msp*I and *SacII,* respectively showing genotype. The genotypes are shown at the top. M: DNA molecular maker

Table 2. The polymorphism distributions of IGF2 and HW equilibrium detection in Landrace and Yorkshire sows

Item	Landrace sows			Yorkshire sows		
	n	Observed frequency	Expected frequency	n	Observed frequency	Expected frequency
Genotype						
AA	3	0.04	0.05	0	0	0.01
AB	24	0.34	0.33	5	0.08	0.07
BB	44	0.62	0.62	60	0.92	0.92
Allele						
A		0.21			0.04	
B		0.79			0.96	
H-W equilibrium detection						
Chi-square		0.09			0.07	
P-value		0.95			0.96	



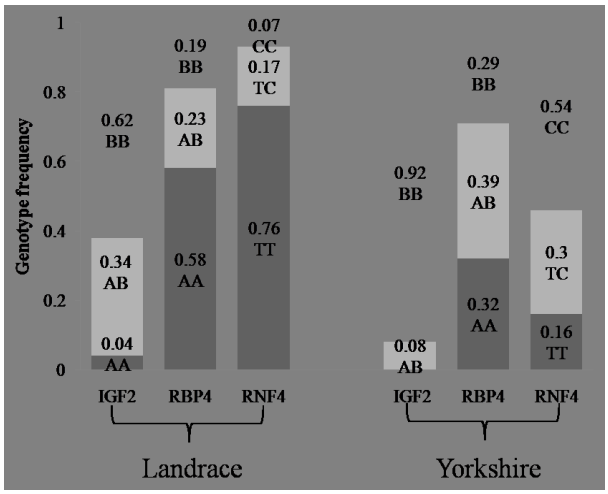


Figure 2. Genotypic and allelic frequency of IGF2, RBP4 and RNF4

Table 3. The polymorphism distributions of RBP4 and HW equilibrium detection in Landrace and Yorkshire sows

Item	Landrace sows			Yorkshire sows		
	n	Observed frequency	Expected frequency	n	Observed frequency	Expected frequency
Genotype						
AA	101	0.58	0.48	60	0.32	0.27
AB	41	0.23	0.43	72	0.39	0.50
BB	33	0.19	0.09	54	0.29	0.23
Allele						
A		0.69			0.52	
B		0.31			0.48	
H-W equilibrium detection						
Chi-square		37.92			9.58	
P-value		< 0.001			< 0.01	

All three genotypes AA, AB and BB were observed for both breeds (Table 3). The favourable genotypes were AA in both Landrace and Yorkshire sow population. However, in Yorkshire the genotype frequency of BB (0.29) was close to AA (0.32). In this study, the genotype frequency distribution of RBP4 was not in Hardy - Weinberg equilibrium ($P < 0.05$).

RNF4 gene

Allele and genotype frequencies of RNF4 are presented in Table 4. The frequency of allele C were 0.16 and 0.69 for Landrace and Yorkshire sows, respectively. When studied allele frequencies of RNF4 in different pig breeds. Niu *et al.* (2009) indicated that the allele C frequencies were from 0.47 to 0.69; and the frequency was high in some breeds as Meishan (0.69), Tongcheng (0.61). In present study, Yorkshire sows had high frequency of allele C.

All three genotypes TT, TC and CC occurred in the genotyped populations. The frequencies of the CC genotypes were 0.07 and 0.54 for Landrace and Yorkshire sows, respectively. The result of present study indicated that Yorkshire population had high frequency of C allele and CC genotype. According to Niu *et al.* (2009), CC genotype had more piglets born (+1.74) and piglets born alive (+2.02) than sows with TT genotype. The allele and genotype frequency of RNF4 were not distributions in Hardy – Weinberg equilibrium. It may due to the analyzed populations had a impact of previous selection process of breeders for other traits.

4. CONCLUSIONS

The polymorphic sites of IGF2, RBP4 and RNF4 genes were found in Landrace and Yorkshire sow populations, except genotype AA for IGF2 in Yorkshire sows. Higher frequency of allele B of IGF2 gene in both populations; and higher frequencies of allele B of RBP4 and allele C of RNF4 in Yorkshire sows were observed. The genotype frequencies distributions were in Hardy - Weinberg equilibrium for IGF2 but not for RBP4 and RNF4. Based on these results, the polymorphism in the IGF2, RBP4 and RNF4 can be considered suitable markers for association studies of litter size in Landrace and Yorkshire sows.

Table 4. The polymorphism distributions of RNF4 and HW equilibrium detection in Landrace and Yorkshire sows

Item	Landrace sows			Yorkshire sows		
	n	Observed frequency	Expected frequency	n	Observed frequency	Expected frequency
Genotype						
TT	66	0.76	0.72	16	0.16	0.10
TC	15	0.17	0.26	29	0.30	0.43
CC	6	0.07	0.02	52	0.54	0.47
Allele						
T		0.84			0.31	
C		0.16			0.69	
H-W equilibrium detection						
Chi-square		13.18			8.86	
P-value		< 0.01			< 0.05	

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AUTOSOMAL MARKERS AND MITOCHONDRIAL SEQUENCES: DO THEY TELL THE SAME PHYLOGENETIC STORY

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ABSTRACT

The current study aims at the molecular assessment of genetic diversity within and between Vietnamese local chicken populations. On average, a total of 32 individuals per Vietnamese local chicken population was randomly sampled. Nine Vietnamese chickens breeds and 2 The exotic chicken breeds originating from China were used. The DNA polymorphism was assessed using a set of 29 microsatellite markers recommended by Granevitze et al. (2007). A fragment of 455 bp from the mtDNA D-loop region was amplified. The results showed that at the autosomal level, the Vietnamese local chicken breeds from different agro-ecological zones represent genetically distinct populations. The northern breeds are clearly separated from breed of the South Central Coast and from breed of the Mekong Delta. The Vietnamese local chicken breeds are highly polymorphic and originated from eight maternal lineages. These lineages are present across the country. Two chicken breeds of Chinese origin, Tam Hoang and Luong Phuong, kept in the National Institute of Animal Sciences are genetically distinct from the Vietnamese local breeds. The Vietnamese chicken breeds are genetically separated from the Chinese chicken gene pool.

Keywords: Genetic diversity, Microsatellite, mtDNA, Vietnamese chicken breed .

1. INTRODUCTION

Microsatellites and mitochondrial DNA (mtDNA) sequences have already proved to be useful for assessing genetic variability, while single nucleotide polymorphisms (SNPs) are becoming more and more popular due to their very high density and availability of high throughput genotyping techniques. Microsatellites are tandem repeats in the genomic DNA with very short (1-5bp) simple sequence motifs, and hence they are autosomally inherited. They are considered to be evenly distributed in the genome (Tautz, 1989). Unlike microsatellite markers, mtDNA is maternally inherited. The mtDNA is a circular molecule of 16,785 bp in size (Desjardins and Morais, 1990). The displacement loop (D-loop) region of the mtDNA contains elements that control the replication of the molecule and is highly polymorphic.

A combination of these two markers is a complementary approach that combines the highly polymorphic microsatellites whose high mutation rates allow for small-scale resolution of more recent demographic events with mtDNA which shed light on phylogeographic events dating further back in time (Feulner et al., 2004). An assessment of genetic structure based on these two markers with different modes of inheritance provides more insights into the evolutionary forces shaping genetic diversity.

Eleven Vietnamese local chicken breeds have been reported (MARD, 2004) but the definition of these breeds is not fully standardized. It appears unlikely that comprehensive survey based on large scale phenotypic characterisation can be achieved considering the wide range of local chicken breeds and the diversity of local production systems. Therefore, the assessment of genetic relationships within and between populations using different molecular markers is a useful prerequisite for the development of effective conservation programs. The current study aims at the molecular assessment of genetic diversity within and between Vietnamese local chicken populations.

2. MATERIALS UND METHODS

This work was carried out in 11 villages of four agro ecological zones located in both the northern and southern part of Vietnam. In this study, a set of nine Vietnamese local chicken breeds and two exotic breeds of Chinese origin kept in Vietnam, were studied.

On average, a total of 32 individuals per Vietnamese local chicken population was randomly sampled where on average one male and one female per household were used (Table 1).

The exotic chicken breeds originating from China have been kept as conservation flocks at the National Institute of Animal Husbandry since 1995 and 2003, respectively. Here, 32 (16 males and 15 -17 females) per each breed were selected. Altogether, a total of 353 individuals were sampled. The DNA polymorphism was assessed using a set of 29 microsatellite markers recommended by Granevitze *et al.* (2007).

Table 1. Information of blood samples

Breed	Agro-ecological zone	Study area	No of blood samples
Vietnamese			
H'Mong	Northwest	Mai Son, Son La	31
Mia	Red River Delta	Duong Lam, Son Tay	32
Ri		Hoai Duc, Ha Tay	32
Ho		Thuan Thanh, Bac Ninh	32
Dong Tao		Khoai Chau, Hung Yen	32
Te		Ba Vi, Ha Tay, NIAS	32
Choi	South Central Coast	Ninh Hoa, Khanh Hoa	33
Ac	Mekong Delta	Tan An, Long An	32
Tau Vang			33
Chinese			
Luong Phuong		NIAS	32
Tam Hoang			32

Note: NIAS = National Institute of Animal Sciences

2.1. Microsattelite variability

Observed and expected heterozygosity and inbreeding coefficients (F_{IS}) for each population were calculated. STRUCTURE analysis (Pritchard *et al.,* 2000) was used to cluster individuals to $2 \leq K \leq 9$ assumed clusters with 100 runs for each K value. This K values are used based on a assumption that each Vietnamese chicken breed was genetically different from the others. Comparisons of 100 runs were done by using SIMCOEFF (Rosenberg *et al.*, 2002). Solutions with a similarity higher than 95% were considered as identical. The most frequent solution was visualised using DISTRUCT (Rosenberg, 2004).

To determine the clustering that best classifies, the ΔK statistics was applied as recommended by Evanno *et al.* (2005). Phylogenetic network analysis was done by transforming the kinship matrix (Eding and Meuwissen, 2003) into a distance matrix and used as input for the SPLITSTREE software (Huson and Bryant, 2006).

2.2. Mitochondrial variability

A fragment of 455 bp from the mtDNA D-loop region was amplified using primers mtGlu-F (5'-GGCTTGAAAAGCCATTGTTG-3') and mtGlu-R (5'-CCCAAAAAGAGAAGGAACC-3'). Due to their circular nature, these primers are positioned at bases 16739–16775 (forward primer) and 649–668 (reverse primer) of the complete mtDNA sequence of domestic chickens (X52392, Desjardins and Morais, 1990). PCR amplifications and sequencing were done as described by Muchadeyi et al. (2008). To align DNA sequences, AlignIR software was used (LICOR Inc. Nebraska, USA). The list of sequences used in this study and the corresponding GenBank accession numbers are provided in Table S1.

The position and number of polymorphic sites as well as corresponding haplotypes were calculated using MEG v.3.1 (Kumar et al., 2004). The distribution of haplotypes in the samples was computed using TCS v.1.21 (Clement *et al.,* 2000). Median joining networks of haplotypes were constructed following the algorithm of Bandelt *et al.* (1995) and using NETWORK v.4.5.1.0 (http://www.fluxus-engineering.com/sharenet.htm). As reference, network analysis was used first to create a skeleton which was based on the most frequent haplotypes of the nine clades of Liu's network (Liu *et al.,* 2006) and the three additional clades (D, G and F) of Oka *et al.* (2007). This skeleton assigns clades to suggested regions of domestication in chickens, which were Yunnan and/or surrounding areas (Liu's clades A, B, F and G), South and Southwest China and/or surrounding areas and Southeast Asia (Liu's clade C, D, H, I and Oka's clade D, F, G), and the Indian subcontinent (Liu's clade E). Nomenclatures of the nine clades reported by Liu *et al.* (2006) were used as reference for the clade notation in this study. The sequences used for alignment consisted of 455 bp. Various networks were constructed by using different epsilon (ε) values ranging from zero to 20. There were no considerable differences among the different networks except a slight increase in the network connections where clades joined. The median network presented used an epsilon value of 5. The haplotype and nucleotide diversities of breeds were computed using ARLEQUIN v.3.1 (Excoffier *et al.,* 2006).

To analyse if mtDNA clades also differed at the autosomal level, the data obtained from genotyping 29 microsatellite markers was used. These individuals were labelled according to their clade affiliation based on mtDNA sequences. The microsatellite genotyping data were used in

the Bayesian model-based clustering as implemented in STRUCTURE v.2.3.1 to cluster individuals to a varying number of K clusters ($2 \leq K \leq 8$) (Pritchard *et al.*, 2000). Runs within each K-value showing a similarity coefficient of 0.95 and higher were considered as identical.

3. RESULTS AND DISCUSSIONS

3.1. Microsattelite variability

3.1.1 Genetic diversity

Expected heterozygosity of Vietnamese local chicken breeds varied considerably ranging from 0.573 (± 0.035) in Dong Tao chicken to 0.696 (± 0.021) in Tau Vang chicken (Table 2).

The observed heterozygosity of the Tau Vang chickens is much lower than expected one (0.563 ± 0.016) resulting in high F_{is} estimate in this population. This result indicates that a level high of inbreeding occurred in this breed. Similar observations were made in some other breeds as well (Ho, Te and Ri), while in Choi chicken an excess of heterozygosity was found indicating that mating between closely related chicken has avoided in this breed.

Analysing a wide range of chicken populations originating from various continents and management systems, Granevitze et al. (2007) found mean allele numbers and expected heterozygosity estimates per population varying from 2.30 to 6.72 and 0.28 to 0.67, respectively, with the Vietnamese H'mong breed being the most variable one. Berthouly et al. (2009) reported that the mean expected heterozygosity of Vietnamese chickens in the Ha Giang province was 0.62, while the corresponding values for Red Jungle Fowl, Chinese and commercial breeds were 0.60, 0.47 and 0.40, respectively.

3.1.2. Genetic structure and genetic difference

Analyzing the rate of change in log likelihood of the population structure from K = 2 to K= 9 suggested an optimal clustering at K = 6. This result is in agreement with the results from the pairwise comparison of runs for individual values of K using SIMCOEFF. The results of the STRUCTURE analysis are depicted in Figure 1.

Table 2. Expected, observed heterozygosity and F_{is} value for Vietnamese populations

Breeds	Abbreviation	N	HE ± SD	Ho ± SD	F_{IS}
H'Mong	HM2_VN	31	0.657 ± 0.028	0.633 ± 0.016	0.038
Mia	DT_VN	32	0.646 ± 0.033	0.610 ± 0.016	0.058
Ri	Ho_VN	32	0.648 ± 0.031	0.606 ± 0.016	0.065
Ho	Mia_VN	32	0.618 ± 0.034	0.564 ± 0.016	0.088
Dong Tao	Ri_VN	32	0.573 ± 0.035	0.548 ± 0.016	0.046
Te	Te_VN	32	0.635 ± 0.029	0.595 ± 0.016	0.065
Choi	Choi_VN	33	0.623 ± 0.035	0.645 ± 0.016	-0.027
AC	TV_VN	32	0.610 ± 0.033	0.608 ± 0.016	0.003
Tau Vang	Ac_VN	33	0.696 ± 0.021	0.563 ± 0.016	0.193
Luong Phuong	TH_VN	32	0.680 ± 0.023	0.657 ± 0.017	0.034
Tam Hoang	LP_VN	32	0.627 ± 0.023	0.606 ± 0.016	0.033

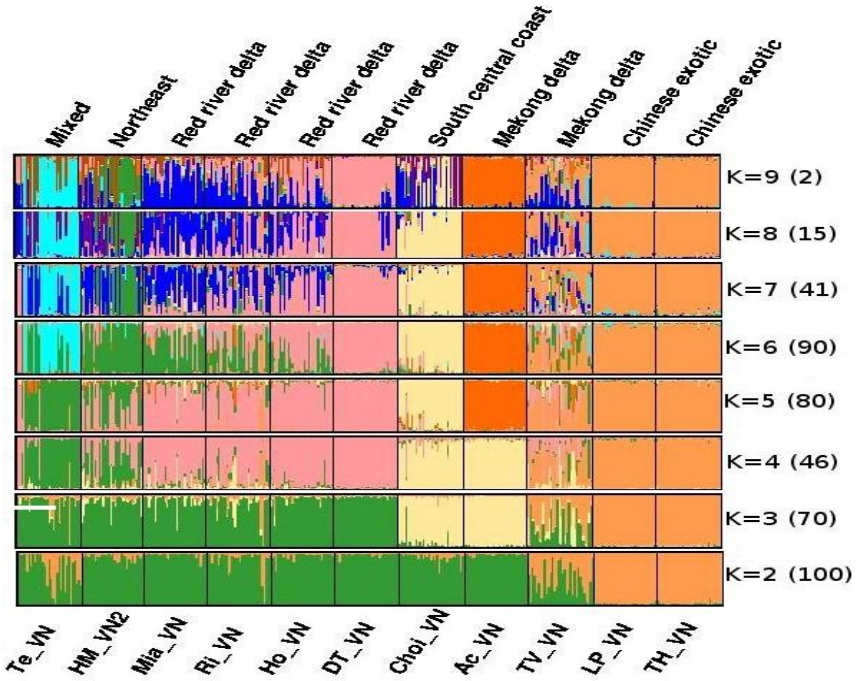

Figure1. Clustering of Vietnamese chicken populations
(Number in parenthesis is the number of identical)

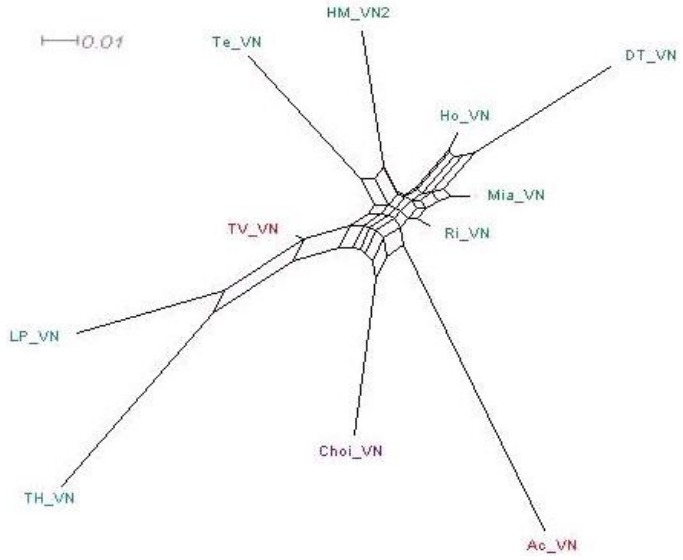

Figure 2. Phylogenetic network of 11 studied populations

At K = 6 the following pattern is found: There are four clearly distinct clusters: the two exotic breeds of Chinese origin form one cluster, and Ac, Choi, and Dong Tao breed formed individual clusters, respectively. Less clearly distinct are the clusters formed by the H'mong and the Te breeds. All other breeds are mixtures of the basic clusters, where the red river delta breeds (Mia, Ri and Ho) are mixtures of the H'mong- and Dong Tao-type, while the Tau Vang breed in the Mekong delta has a

strong influence both of local Vietnamese breeds and of breeds with Chinese origin. This structure is also reflected in the phylogenetic network (Figure 2), in which the distinct clusters found in the STRUCTURE analysis also form clearly distinct branches, while the 'mixed' populations are positioned close to the central node, indicating similar distances to the distinct clusters.

3.2. Mitochondrial variability

3.2.1. Within-population diversity

Eight clades (A-G and I) were formed by 37 haplotypes. The lowest haplotype diversity (0.62 ± 0.105) was estimated in the Ho breed, while the highest corresponding value (0.942 ± 0.034) was observed in the Tau Vang breed (Table 3 and Supplement Table S2).

Although the majority of the Vietnamese chicken breeds in this study were assigned to clade A and B, the Vietnamese breeds were found to be highly polymorphic in the mtDNA D-loop region. Estimates of haplotype diversity ranged from 0.62 to 0.94 in this study and were higher than reported previously. Muchadeyi *et al.* (2008) found the haplotype diversity ranging from 0.61 to 0.73 and from 0.27 to 0.78 in Zimbabwean chickens and purebred lines, respectively. Liu *et al.* (2004) pointed out three of 12 Chinese breeds with only one haplotype. The high degree of diversity of the Vietnamese breeds is in agreement with previous reports showing high diversity at the autosomal level analysing microsatellites. (Granevitze *et al.*, 2007; Berthouly *et al.*, 2009 and Cuc *et al.*, 2010).

Table 3. Polymophic sites, haplotype and nucleotide diversity of chicken breeds under study

Breed	Agro ecological zone	Study area	N	No. Polymophic sites	No. of Haplotypes	Haplotype diversity (±SD)
H'mong	Northwest	Mai Son, Son La	20	23	6	0.778 ± 0.055
Mia	Red River Delta	Duong Lam, Ha Tay	20	10	7	0.737 ± 0.094
Ri		Hoai Duc, Ha Tay	20	22	12	0.911 ± 0.045
Ho		Thuan Thanh, Bac Ninh	20	8	4	0.615 ± 0.105
Dong Tao		Khoai Chau, Hung Yen	20	20	7	0.768 ± 0.080
Te		Ba Vi, Ha Tay and NIAS	20	14	5	0.716 ± 0.086
Choi	South Central Coast	Ninh Hoa, Khanh Hoa	19	15	4	0.754 ± 0.053
Ac	Mekong Delta	Tan an, Long An	21	13	5	0.767 ± 0.053
Tau Vang			20	24	13	0.942 ± 0.034
Luong Phuong	(imported from China)	NIAS	21	19	8	0.852 ± 0.053
Tam Hoang			21	11	7	0.705 ± 0.095
Total			222	43	37	0.849 ± 0.184

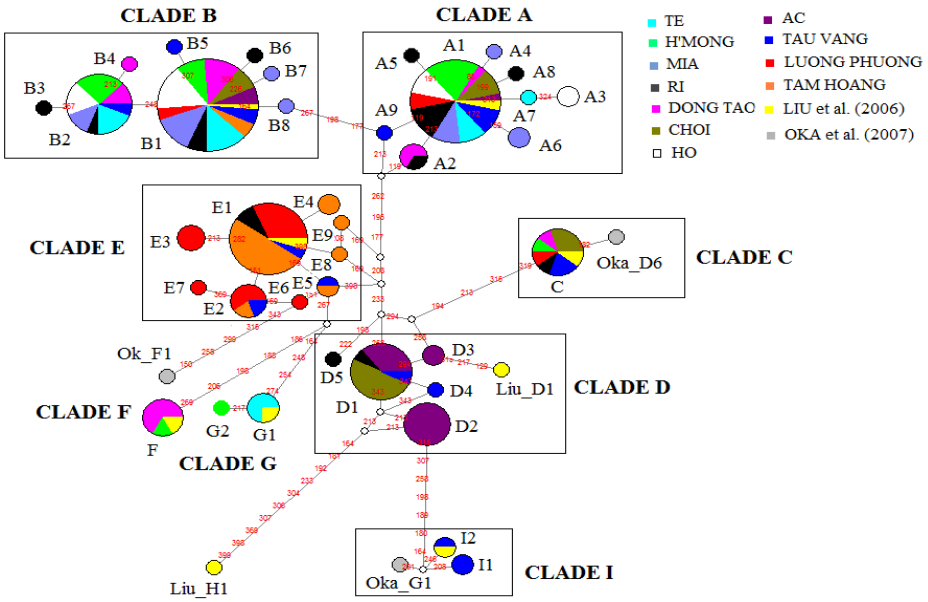

Figure 3. Median network profile of the mtDNA D-loop haplotypes observed in the current study

Note:Data merged with sequences of major haplotypes reported by Liu et al. (2006) and Oka et al. (2007). The circle size corresponds to haplotype frequency, and the numbers on the line correspond to mutational positions connecting haplotypes. Empty circles are median vectors used in connecting indirectly related haplotypes

3.2.2. Breed distribution within clades

The Vietnamese local chickens were found in all eight clades (Figure 3). The distribution of the Vietnamese breeds into clades was not related to their geographical distribution. The most frequent clades A and B included all nine Vietnamese breeds. A considerable proportion of Vietnamese local chickens belonged to clade D while only a small number of Vietnamese chicken was assigned to the five remaining clades (C, E, F, G and I). In contrast, the majority (76%) of Chinese chickens were found in clade E whereas no Chinese chickens were observed in clades D, G and I. The majority of the Vietnamese local chickens carried mtDNA haplotypes that clustered in clades A and B. Based on the skeleton of supposed regions of domestication, this finding suggests the existence of two maternal lineages dominating in the Vietnamese local chickens which presumably originate from Yunnan and surrounding regions in China (Liu *et al.*, 2006). Fourteen percent of Vietnamese chickens were found in clade D indicating that this clade also contributed considerably to the Vietnamese local chickens. Liu *et al.* (2006) and Oka *et al.* (2007) suggested that this clade has its root in Southeast, South and Southwest China and/or surrounding areas (i.e. Vietnam, Burma, Thailand, and India). This finding would be in agreement with historical records of human immigration from southern China to Vietnam. Yüeh people are inhabitants in the Southeastern coast of China and are the ancestors of the Cantonese, i.e. Guangzhou and Guangxi Southern Chinese people. By the 3rd century B.C., Yüeh people emigrated from Southern China to the Red River Delta of Vietnam and mixed with the indigenous Van Lang Vietnamese population (Taylor, 1983). Additionally, Southern Chinese people from Yunnan, Guangzhou and

Guangxi Provinces arrived at the North of Vietnam and moved to the South from the 17th to the 19th century A.C. (Nyiri and Saveliev, 2002). Descriptions of immigration always state that people of a family moved together with their animals which could result in the introduction of chickens from Southern China into the North and the South of Vietnam. While Yunnan, South and Southwest China might be seen as region of origin of the Vietnamese chicken breeds, the majority of individuals of Chinese breeds in this study were not assigned to these maternal lineages. This finding indicates that two Chinese breeds kept at NIAS do not represent the breeds of Yunnan, South and Southwest China.

The high proportion of haplotype D1 found in the Choi chickens is in agreement with findings of Liu *et al.* (2006), who reported that clade D mainly consisted of game birds. On the other hand, the clustering of the remaining Choi chickens in clades A and B is consistent with the study of Oka *et al.* (2007) who found game birds assigned to their clades B and E. Consequently, our findings suggest that the game breed Choi is a mixture of multiple maternal lineages.

A small number of Vietnamese chickens distributed in clades C, F, G and I indicate that these clades have little contribution to Vietnamese chickens. A small portion (2%) of Vietnamese local chickens was observed in clade E, originating from the Indian subcontinent (Liu *et al.*, 2006), which otherwise harboured mainly the Chinese chickens studied. Vietnamese local chickens in this clade included the Ri and Tau Vang breeds. This observation may indicate a possible exchange of genetic material between the Ri and Chinese chickens due to the wide distribution of the Ri chickens, while the Chinese origin of the Tau Vang breed (Linh, 2008) is known and explains the distribution of this breed in both Vietnamese and Chinese clades. This finding is also in agreement with the analysis at the autosomal level in which the Tau Vang breed showed clear admixture between the Chinese and Vietnamese gene pools.

3.3. Relationship between mtDNA and autosomal genetic structure

The results of the STRUCTURE analysis from K = 2 to K = 6 are shown in Figure 2. The repeatability, i.e. the number of runs giving result with similarity coefficient ≥ 0.95, varied from 34 to 100 from K= 2 to K=6, while no identical runs were found at K = 7 and 8 (data not shown). For all K values, the mtDNA defined clade E was found as a pure cluster at the autosomal level while the other seven mtDNA defined clades were mixed to different degrees (Figure 4).

Comparing results of phylogenetic relationship using mtDNA polymorphism and autosomal microsatellites it becomes obvious, that the Chinese breeds cluster together and are separated from the Vietnamese local breeds using both genetic marker systems, indicating a clear genetic differentiation between them and the Vietnamese breeds. Although Tieu et al. (2008) assumed that the Chinese chickens from NIAS have introgressed into local Vietnamese chickens, our results do not support this hypothesis, except for the Ri and Tau Vang chickens. In contrast to microsatellite analyses, which found that clustering of Vietnamese local breeds using microsatellites has a relationship to their geographical distribution, no sub-structuring was found between the Vietnamese local breeds at the mtDNA level.

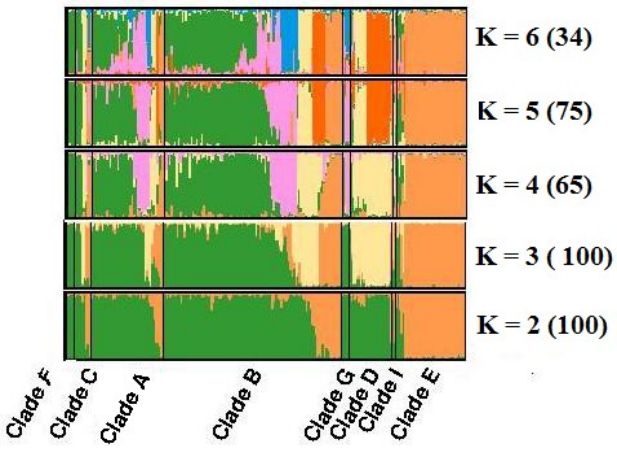

Figure 4. Structure based clustering using microsatellite genotypes of chicken groups assigned to eight mtDNA clades. Individuals are labelled according to their clade affiliation based on mtDNA sequences. Number in parenthesis is the number of runs giving an identical result (similarity coefficient ≥ 0.95)

The different results obtained in both types of markers could be due to the different mode of inheritance. Microsatellites are highly polymorphic markers with their locus specificity, abundance and random distribution over the genome, co-dominant inheritance (Weigend and Romanov, 2001). Unlike microsatellite markers, mtDNA is maternally inherited. MtDNA is used to infer regions of domestication and to identify the number of maternal lineages and their geographic origins (FAO, 2007). Unlike autosomal genetic markers, mtDNA transferred from mother to offspring is not rearranged due to recombination and less affected by gene drift (Johnson et al., 2003). In addition, mtDNA has a lower mutation rate than microsatellite as argued by Feulner *et al.* (2004).

4. CONCLUSIONS

At the autosomal level, the Vietnamese local chicken breeds from different agro-ecological zones represent genetically distinct populations. The northern breeds are clearly separated from breed of the South Central Coast and from breed of the Mekong Delta. The Vietnamese local chicken breeds are highly polymorphic and originated from eight maternal lineages. These lineages are present across the country. Two chicken breeds of Chinese origin, Tam Hoang and Luong Phuong, kept in the National Institute of Animal Sciences are genetically distinct from the Vietnamese local breeds. The Vietnamese chicken breeds are genetically separated from the Chinese chicken gene pool.

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Supplement Table S1. Haplotype names and accession numbers of chicken mtDNA sequences used in this study

Haplotype name	Accession number	Reference
A1 – A9	GU564361 - GU564369	This study
B1 – B8	GU564370 - GU564377	This study
C	GU564378	This study
D1 – D5	GU564379- GU564383	This study
E1 – E9	GU564384- GU564392	This study
F	GU564393	This study
G1 – G2	GU564394- GU564395	This study
I1 – I2	GU564396- GU564397	This study
Liu_A1	AB114069	Liu et al. (2006) haplotype A1
Liu_B1	AB007744	Liu et al. (2006) haplotype B1
Liu_C1	AB114070	Liu et al. (2006) haplotype C1
Liu_D1	AY588636	Liu et al. (2006) haplotype D1
Liu_E1	AB114076	Liu et al. (2006) haplotype E1
Liu_F1	AF512285	Liu et al. (2006) haplotype F1
Liu_G1	AF512288	Liu et al. (2006) haplotype G1
Liu_H1	D82904	Liu et al. (2006) haplotype H1
Liu_I1	AB009434	Liu et al. (2006) haplotype I1
Oka_D6	AB268535	Oka et al. (2007) haplotype D6
Oka_G1	AB268545	Oka et al. (2007) haplotype G1
Oka_F1	AB268543	Oka et al. (2007) haplotype F1

Supplement Table S2. Variable sites for 37 mtDNA haplotypes observed in 11 chicken populations

	111111111	1111111222	2222222222	2222333333	333
	8155667788	8899999001	1223445566	6789001112	469
	5919492706	8914589683	7263684827	9424673594	398
A1	TCTAGTTTCC	TAAGTCATTC	CTAACCCTCC	ATCATTACTA CCC	
A2	....	.........T	....	....	
A3	....		....T..G ...		
A4	...G..C...		....		
A5	....	..G		....	
A6	C.........		....		
A7	....		....T	...	...
A8	....	.....T...		....	
A9	.T........		....		
B1	.T..A..C..	.....T....	.........T	....	...
B2	.T..A..C..	.....T....	.....T ...T	....	...
B3	.T..A..C..	.....T... T	.....T ...T	....	...
B4	.T..A..C..	.....T....	.....T	....	
B5	.T..A..C..	.....T....	.........T	.....C.....	...
B6	.T..A..C..	.....T....	.........T	....C.....	...
B7	.T..A..C..	.....T....	..T..... T	....	...
B8	.T..... C..	.....T...	.........T	....	...
C	.T..... C..	...AC... C.	...G.... T. ...G...	TC. ...	
D1	.T..... C..	....C... CT	...G... CT.		
D2	.T..... C..	....C... C.	...G... CT. .... T..		
D3	.T..... C..	....C... CT	...G... CT. ...G.....	...	
D4	.T..... C..	....C... CT	...G... CT. .... G..		
D5	.T..... C..	....CT..CT	.C.G.... T.		
E1	.T... C.C..	....C... CT	..... T. ....T		
E2	.TC..C.C..	....C... CT	..... T. ....T		
E3	.T... C.C..	....C... C.	.....T. ..T.....	..T	
E4	.T... C.C..	....C... CT	..... T. ...G.....	..T	
E5	.T..... C..	....C... CT	..... T. ....T		
E6	.TC..... C..	....C... CT	..... T. ....T		
E7	.TC..C.C..	....C... CT	..... T. .... .TT		
E8	.T... C.C..	....C..... T	..... T.		
E9	.T... C.C..	....C... CT	..... T.		
F	.T..... C.T	C... CT.CCT	..... TT	C.....	..T
G1	.T..A..C..	....C... CT	.....AT.TT	.C.....	..T
G2	.T..A..C..	....C... CT	T.... AT.TT	.C.....	..T
I1	.T..A..CT.	.G..CT....	...G.... T.	.....C.T.. T..	
I2	.T..A..CT.	.G..CT..C.	...GT ... T.	.....C.T.. T..	

Note: Dots indicate nucleotide positions identical to those of Haplotype A1;
Numbers at the top refer to variable sites and correspond to the nucleotide positions of Haplotype A1.

MOLECULAR IDENTIFICATION OF *EIMERIA* SPECIES IN CHICKENS AT SURROUNDING AREAS OF RED RIVER DELTA IN VIETNAM

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ABSTRACT

The prevalence of avian coccidiosis in Vietnam has long been neglected, especially the identification of Eimeria at species level. Therefore, the present study was conducted to determine the molecular identification of Eimeria species infection on chicken in a small - scale and a commercial - scale farm in surrounding areas of the Red River delta of Vietnam. A total of 72 chicken fecal samples were collected from two farms above (36 samples/farm). Oocysts were collected from the microscopically positive samples and identification Eimeria at species by Polymerase Chain Reaction (PCR) method. The results showed that Eimeria were found in 32/72 (88.89%) and 29/72 (80.56%) samples which were collected from small - scale and commercial - scale farm, respectively. The prevalence of Eimeria tenella, E. acervulina, E. brunetti, E. maxima, E. mitis and E. necatrix were 93.7, 90.6, 84.4, 78.1, 59.4 and 37.5%, respectively in the small - scale farm. In commercial - scale farm , the prevalence of E. tenella, E. acervulina, E. maxima, E. brunetti, E. mitis and E. necatrix were 96.55, 89.66, 86.21, 75.86, 48.28 and 31.03%, respectively (E. praecox was not identified). The results indicated that the infection of Eimeria species in this area of Vietnam was common.

Keywords: Eimeria species; chicken; Coccidiosis; The Red River delta; Vietnam

1. INTRODUCTION

The Red River delta is a flat plain formed by the Red River and its distributaries joining in the Thai Binh River in northern Vietnam. The delta measuring 15,000 km^2 is well protected by a network of dikes. It is characterized by a strong monsoon influence, a considerable amount of sunny days, and with a high rate of rainfall and humidity. Average temperature in a year ranges from 22°C to 27°C. Poultry farming is quite common in this area, contributing the majority to the income to the people, especially large - scale farms. In previous investigations on infection of Eimeria species on chicken in Vietnam, five species (E. tenella, E. necatrix, E. maxima, E. acervulina and E. mitis) were found in the northern provinces of Vietnam by morphological methods (Luc *et al.*, 2003), of which two species (E. tenella and E. necatrix) were most pathogenic (Luc *et al.*, 2011). However, there was no report about epidemiology of *Eimeria* spp. on chicken in the Red River delta of Vietnam.

Coccidiosis is caused by protozoan parasite with high prevalence of mortality, morbidity and weight loss in chicken (Jensen *et al.*, 2000). It is one of the most commonl and economically impacted parasitic diseases in poultry industry all over the world (Shirley *et al.*, 2005). Nine different species of Eimeria have been identified. Out of them E. acervulina, E. necatrix, E. tenella, E. maxima and E. brunetti are the major species infected

among chicken (Donal and Elizabeth, 2007). These infections lead to digestion disorder resulting from damage to the intestinal epithelium, mal - absorption of nutrients, changes in protein metabolism after absorption, inefficiency of feed conversion, and reduction in weight gain (Conway *et al.*, 1993).

PCR method has recently been applied for the detection of coccidial parasites in the people and poultry. A number of approach methods have proved to be both specific and highly sensitive for isolation either of parasites grown in vitro or present in samples and clinical material (Gautam *et al.*, 2010).

The development of molecular techniques has allowed precise detection of Eimeria species, investigation of the genetic variability of the pathogen, and a search for molecular characteristics associated with phenotypical characteristics that may constitute the use of molecular markers (Costa *et al.*, 2001; Schnitzler *et al.*, 1999). Molecular techniques may also contribute to the development of new vaccines and selection of anti - coccidial drugs which to be used in control programs (Lee *et al.*, 2010; Morris and Gasser, 2006; Sun *et al.*, 2009).

The study was conducted to identify of Eimeria spp. in chicken feces samples by PCR method and also to detect Eimeria species preponderance in the Red River delta of Vietnam.

2. MATERIALS AND METHODS

2.1. Sample collection

A total of 72 fecal samples of chickens were collected randomly from individual scavenging native chickens of a small - scale farm and individual floors of a commercial - scale farms at surrounding areas of the Red River delta of Vietnam (36 samples in each).

The samples were collected between the period months of August, October, December 2013 and February, April 2014. Fresh fecal droppings were collected in sterile universal bottles and chicken carcasses were collected in polyethylene leather bags and transported to the laboratory immediately for processing.

2.2. Laboratory examination

Laboratory examination was done by wet mount smears of the fecal droppings as described by (Fleck and Moody, 1993). Concentration technique and microscope using × 10 objectives were applied for counting oocyst. Oocysts were collected from microscopically positive samples as per method developed by Daugschies et al., 2002 for molecular test. The sporulation was performed at 24 - 26°C in a 2.5% aqueous solution of potassium dichromate ($K_2Cr_2O_7$). The sporulated oocyst were concentrated by centrifugation and stored in potassium dichromate at 4°C.

2.3. PCR identification

DNA was extracted from sporulated oocysts (Zhao *et al.,* 2001). The Eimeria species were detected by PCR using primer sequences in Table 1.

The volume of 25µl extracted DNA was used for PCR amplification. Thermo - cycling condition reaction was as follows 1 cycle at 95°C, for 7 min; 35 cycles at 95°C for 20 sec, 44 to 60°C for 30 sec, 72°C for 1 min; 1 cycle at 72°C for 5 min (Gautam et al., 2010; Jenkins *et al.*, 2006a, b). 200 nM dNTP (Amersham, Piscataway, NJ), 20 mM Tris pH 8.4, 50 mM KCl, 3.0 mM $MgCl_2$, 1 U rTaq polymerase (New England Bio - labs, Ipswich, MA) in a PTC200 Mini - cycler™ (MJ Research, Watertown, VA) (Haug *et al.*, 2007).

Table 1. Primers sequences, annealing temperatures and predicted sizes of products for PCR amplification of *Eimeria* spp.

Eimeria species	Primer[1]	PCR product sequence (5'–3')	Annealing temperature (°C)	Size (nt)
E. acervulina	EaF	GGCTTGGATGATGTTTGCTG	60	321
	EaR	CGAACGCAATAACACACGCT		
E. brunetti	EbF	GATCAGTTTGAGCAAACCTTCG	45	310
	EbR	TGGTCTTCCGTACGTCGGAT		
E. maxima	EmaF	CGTTGTGAGAARACTGRAAGGG	51	144
	EmaR	GCGGTTTCATCATCCATCATCG		
E. mitis	EmiF	TATTTCCTGTCGTCGTCTCGC	54	306
	EmiR	GTATGCAAGAGAGAATCGGGA		
E. necatrix	EnF	GTCAGCTTTTTGCCTGGGTG	55	285
	EnR	ACAGACCGCTACACAACACG		
E. praecox	EpF	CATCATCGGAATGGCTTTTTGA	54	368
	EpR	AATAAATAGCGCAAAATTAAGCA		
E. tenella	EtF	AATTTAGTCCATCGCAACCCT	60	271
	EtR	CGAGCGCTCTGCATACGACA		

Note: [1]F - Forward primer and R - Reverse primer.

PCR products were separated by 1.0% agarose gel electrophoresis (Bio - metra, Göttingen, Germany). The gels were stained in an aqueous ethidium bromide solution (0.5 μg/ml) and DNA bands were visualized under UV light (transillumi - nator; UV wavelength, 254 nm; TFX - 20 M, Vilber Lourmat, France) and photographed by a digital camera (CSE - 0028, Cybertech, Berlin, Germany) (Tsuji *et al.*, 1997).

3. RESULTS

3.1. The microscopic examination of mixed Eimeria spp oocysts in chicken fecal samples

The results were described in Figure 1.

3.2. Detection Eimeria spp and species by PCR method

Eimeria spp were detected by PCR method in 32/36 (93.75%) and 29/36 (80.56%) chicken fecal samples collectected from small - scale and comercial - scale farms, respectively.

The prevalence of E. tenella detection by PCR was high, as followed by E. acervulina, E. brunetti, E. maxima, E. mitis and E. necatrix (Table 2).

The same trend was also found in the commercial poultry farm, E. tenella was most prevalent as 96.55% followed by E. acervulina, E. brunetti, E. maxima, E. mitis and E. necatrix (Table 3) and E. praecox was not found in both small - scale and commercial - scale poultry farms.

In small - scale chicken farms, the infection of E. tenella (100%) was recorded as highest prevalence in color broiler, E. tenella and E. acervulina (91.67%) were more prevalent in broiler and in case of layer chicken, E. acervulina was found with the highest infection rate (100%).

In the commercial chicken farm, the high infection rate of E. tenella, *E. acervulina* and *E. maxima* (90%) were found in color broiler, *E. tenella* (100%) was preponderant species in broiler, whereas in layer chickens E. brunetti and E. tenella were found more prevalent (100%).

All positive samples had multiple infections with 2 - 6 species of Eimeria. None of them had infections with single species of Eimeria, or all seven species.

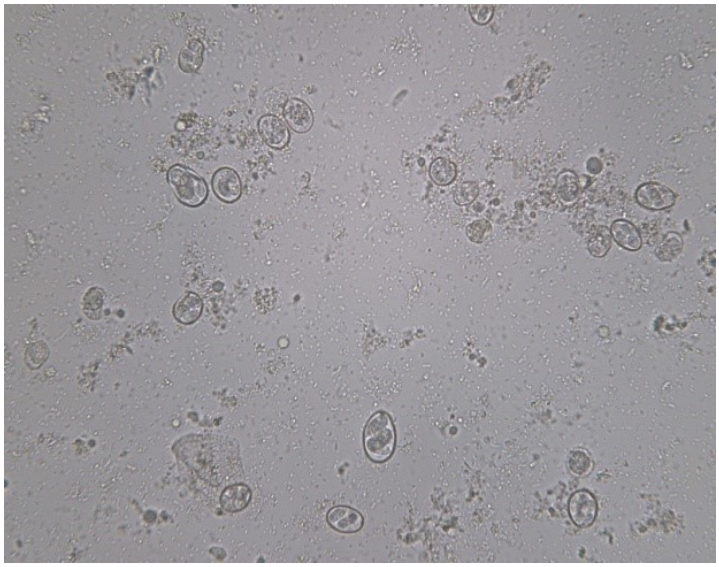

Figure 1. Microscopic examination of mixed Eimeria oocysts from chicken feces (×40 microscopic)

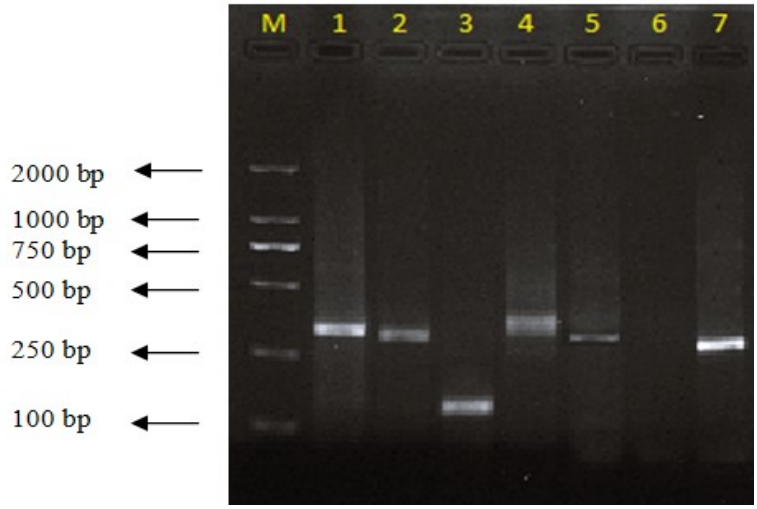

Figure 2. Identification of Eimeria species in chickens from the area of Red River Delta in Vietnam by PCR method

Note: Lane M 100 bp DNA ladder, Lane 1 E. acervulina primers (321 bp), Lane 2 E. brunetti primers (310 bp), Lane 3 E. maxima primers (144 bp), Lane 4 E. mitis primers (306 bp), Lane 5 E. necatrix primers (285 bp), Lane 6 E. praecox primers (368 bp), Lane 7 E. tenella primers (271 bp)

Table 2. Identification of Eimeria species by PCR amplification in small - scale chicken farm

Type of bird	No. of examined samples	Microscopically positive (%)	Molecular identification by PCR (%)						
			E.a	E.b	E.ma	E.mi	E.n	E.p	E.t
Colour broiler	12	10 (83.3)	9 (90)	8 (80)	9 (90)	4 (40)	2 (20)	0	9 (90)
Broiler	13	11 (84.6)	10 (90.9)	9 (81)	8 (72.7)	5 (45.5)	5 (45.5)	0	11 (100)
Layer	11	8 (72.7)	7 (87.5)	8 (100)	5 (62.5)	5 (62.5)	2 (25)	0	8 (100)
Total	36	29 (80.6)	26 (89.7)	25 (86.2)	22 (75.9)	14 (48.3)	9 (31.1)	0	28 (96.6)

Note: E.a, Eimeria acervulina; E.b, Eimeria brunetti; E.ma, Eimeria maxima; E.mi, Eimeria mitis; E.n, Eimeria necatrix; E.p, Eimeria praecox; E.t, Eimeria tenella

Table 3. Identification of Eimeria species by PCR amplification in commercial chicken farms

Type of bird	No. of examined samples	Microscopically positive (%)	Molecular identification by PCR (%)						
			E.a	E.b	E.ma	E.mi	E.n	E.p	E.t
Colour broiler	14	12 (85.7)	10 (83.3)	11 (91.7)	8 (66.7)	8 (66.7)	5 (41.7)	0	12 (100)
Broiler	13	12 (84.6)	11 (91.7)	10 (83.3)	10 (83.3)	7 (58.3)	5 (41.7)	0	11 (91.7)
Layer	9	8 (88.9)	8 (100)	6 (75)	7 (87.5)	4 (50)	2 (25)	0	7 (87.5)
Total	36	32 (88.9)	29 (90.6)	27 (84.4)	25 (78.1)	19 (59.4)	12 (37.5)	0	30 (93.8)

Note: E.a, Eimeria acervulina; E.b, Eimeria brunetti; E.ma, Eimeria maxima; E.mi, Eimeria mitis; E.n, Eimeria necatrix; E.p, Eimeria praecox; E.t, Eimeria tenella.

4. DISCUSSION

The overall prevalence of Eimeria spp. was 88.89% in the small - scale farm and 80.56% in the commercial - scale farm. The results showed that the infection of Eimeria species in the Red River delta of Vietnam was prevalent. The infection rate at scavenging native chickens in small - scale and commercial - scale farms in surrounding areas of the Red River Delta of Vietnam was not significantly different. This indicated that difference in breed did not influence on the infection of Eimeria spp. in chickens. Similarly, variety of infection rate among chickens with the same Eimeria species was not significant. However, the rates among Eimeria species in small - scale and commercial - scale farms were considerably different.

Six species of Eimeria, including *E. tenella, E. acervulina, E. maxima, E. brunetti, E. mitis* and *E. necatrix* were identified and Eimeria tenella was the most detecting species in the small - scale farm (93.75%) and commercial farms (96.55%) in surrounding areas of the Red River Delta of Vietnam. These results were not coincide with previous reports relying on prevalence estimates or individual species identification about the preponderance of *Eimeria* spp. (*E. tenella, E. necatrix, E. acervulina, E. maxima* and *E. mitis*) on chickens in Vietnam (Luc *et al.,* 2005). In another report, five species of oocysts in Eimeria were found in broiler chicken and the highest infection rate appeared in *E. acervulina* (44.66%) followed by *E. necatrix* (31.62%), E. tenella (21.76%), *E. maxima* (10,67%) and *E.*

brunetti (7.82%) (Hung, 2010). These reports were different with our report in both species and infection rate.

Regarding to Gari *et al.*, (2008), the prevalence of coccidian parasite such as the Eimeria species infecting chickens mostly in Ethiopia showed *E. tenella*, *E. acervulina, E. maxima, E.brunetti, E. mitis and E. necatrix* (Gari *et al.,* 2008). In China, the infection rate of identified Eimeria spp. in the farms was 90, 88, 72, 68, 60, 26, and 8% for E. tenella, E. praecox, E. acervulina, E. maxima, E. mitis, E. necatrix, and E. brunetti, respectively (Sun *et al.,* 2009). On other hand, the latest report in Nigeria showed that the prevalence of multi - infection was 71 and 57.7% and for the single infection was 29 and 42.3% in layer and broiler respectively (Jatau *et al.,* 2012). These results are similar to our results of the prevalence of Eimeria species in chicken in Vietnam.

The present study also showed that there was poor agreement between PCR and traditional identification for diagnosis of Eimeria species. Traditional methods are not sufficiently reliable for specific diagnosis of Eimeria species in chickens. Alternatively, occurrence of multiple infections in a single bird and the fact that Eimeria species with low oocyst frequency in the mixture may be missed indicates that PCR based amplification of DNA sequence of parasite, could resolve this problem and overcame the limitation in analysis of small amounts of oocyst in mixed infections. Hence, in the future, sufficiently reliable method for specific diagnosis of Eimeria species in chickens and PCR based amplification of DNA sequence of parasite would have been developed in replacement of traditional methods.

In conclusion, the initial molecular identification of Eimeria species infection in the Red River Delta of Vietnam was succesfully conducted by using PCR assay. The results also recommend a more effective method over conventional ones in molecular diagnosis of livestock diseases in Vietnam.

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MONOCLONAL ANTIBODIES SPECIFIC TO WATER BUFFALO *(Bubalus Bubalis)* MYXOVIRUS RESISTANCE PROTEIN 1

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ABSTRACT

Recombinant water buffalo myxovirus resistance protein 1 (Mx1) was successfully expressed by an *Escherichia coli* expression system. After hyperimmunization of BALB/c mice and cell fusion, 9 mouse hybridomas producing mAbs to Mx1 were established. The isotypes of the monoclonal antibodies were tested to be IgG1 (hybridomas FD4, OB8, PE6, RD5, 10B4, 11A7, 10F5, 9D1) or IgG2a (7B3). We are currently testing whether the 9 mAbs can also specifically recognize buffalo Mx1 protein in various formats by Western blot analysis, immunofluorescent staining, and immunoprecipitation assay. We further intend to examine whether they could be used to detect Mx1 expression induced by type I interferon in water buffalo's cell lines and buffalo peripheral blood mononuclear cells by Western blot or ELISA, in a dose-dependent manner.

Keywords: Hybridomas, Mx1 protein, monoclonal antibodies, recombinant protein, water buffalo.

1. INTRODUCTION

Myxovirus resistance proteins (Mx) belong to GTPases that are induced in cells of many vertebrates on exposure to type I interferons (INF-α/β); and play important roles in response of the host to viral infections (Baise *et al.,* 2004, Horisberger, 1992). Recently, some of those proteins such as MxA in human or Mx1 in animals have been widely demonstrated to be a specific biomarker for several viral infections. Furthermore, the ELISA format is suitable for detecting the biomarker reliably (Kawamura *et al.*, 2012). In paediatrics, MxA has been used successfully to distinguish viral infections from bacterial infections in young children (Halminen *et al.*, 1997, Haller *et al.*, 2010). In animals, Mx1 protein has been found to accelerate in response to some RNA virus infections such as FMD, pneumovirus, and influenza virus in several species including bovine, porcine and canine (Shi *et al.*, 2014, Dermine and Desmecht, 2012). These findings resulted in several ELISA-based tools, rapidly detecting Mx proteins, which play important role in screening of viral infections and disease control. However, there is not any similar data available on water buffalo, even this is crucially socioeconomic livestock specie in Southeast Asia. Particularly, the area has been facing several viral disease outbreaks such as FMD due to the lack of rapid screen tests for viral diseases, particularly in health control and management of the animal transportation. To deal with this circumstance, it is feasible to create an ELISA device for screening viral infections

by detecting Mx1 protein in buffalo blood; and therefore production of fundamental elements including recombinant buffalo Mx1 (buMx1) protein and its specific monoclonal antibodies are the crucial first steps. In this study recombinant water buffalo myxovirus resistance protein 1 (buMx1) was successfully expressed by *Escherichia coli* and mammalian cells expression systems. After hyper immunization of BALB/c mice and cell fusion, 9 mouse hybridomas producing mAbs to buMx1 were established. The isotypes of the monoclonal antibodies were tested to be IgG1 (hybridomas FD4, OB8, PE6, RD5, 10B4, 11A7, 10F5, 9D1) or IgG2a (7B3). We are currently testing whether the 9 mAbs can also specifically recognize buffalo Mx1 protein in various formats by Western blot analysis, immunofluorescent staining, and immunoprecipitation assay. We further intend to examine whether they could be used to detect Mx1 expression induced by type I interferon in water buffalo's cell lines and buffalo peripheral blood mononuclear cells by Western blot or ELISA in a dose-dependent manner.

2. MATERIALS AND METHODS

2.1.Materials

Animal and materials including: BALB/c mice, E. coli BL21 (DE3), P3-X63Ag8 myelomas were purchased from Charles River Laboratories, Sigma-Aldrich and ATCC, respectively. Reagents and kits used for constructing recombinant buMx1 expression vectors included PCR primers, the BD Advantage™ kit (provided by BD Bioscience); pCRII – TOPO, pET-28b, Lipofectamine 3000 reagent and TRIzol (from Invitrogen) and Ni-NTA His-Bind resin (EMD Bioscience, Inc., Milwaukee, WI).

2.2. Construction of buffalo Mx1 protein (buMx1) expression vectors

Total RNA of buffalo cells exposed to poly (I)-poly (C) for 48 hours (100 mg/mL) was extracted with TRIzol (Invitrogen) and reverse transcribed using the Improm-II (Promega) technology. PCR was carried out by using primers 5'-CGTCACAGCGTCAAAGAAAAGGA-3' and antisense 5'-GTTGGCGGGGCTCATT CAAG-3 '; and the BD Advantage ™ kit provided by BD Bioscience. In principle, the target cDNA was amplified by PCR according to the following protocol: a step at 94°C for 5 min, then 35 cycles each consisting of three step: 94°C for 30sec, 63°C for 30 sec and 69°C for 2 min 30sec, and finally at 68 ° C for 3 min. Then, the PCR amplicons are TA- cloned into pCRII - TOPO vector (Invitrogen), which was used to transform E. coli TOP 10. Ten clones will be sequenced in both directions and a clone 'win' will be identified. Subsequently, the XbaI / BamHI fragment of pCRII - TOPO corresponding (containing cDNA that encodes buMx1) is directionally subcloned into the expression plasmid pET - 28b prokaryotic. Recombinants will be selected after transformation into E. coli Top 10 and via ampicillin selection and confirmation by sequencing.

2.3. Production of recombinant buffalo Mx1 protein (buMx1)

The pET- 28b plasmids containing full-length cDNA encoding buMx1 protein was transformed into E. coli BL21 (DE3) by using Lipofectamine 3000 reagent protocol (Invitrogen), cultured at 37^0C for 4h in 200 ml of Lysogeny broth (LB) medium containing 50 μg/ml of ampicillin, followed by the addition of 0.4 mmol/l of iso-propylthiogalactoside and subsequently, cultured at 37^0C for 3h as described previously (Kohno *et al.*, 1994). Then, the E. coli was

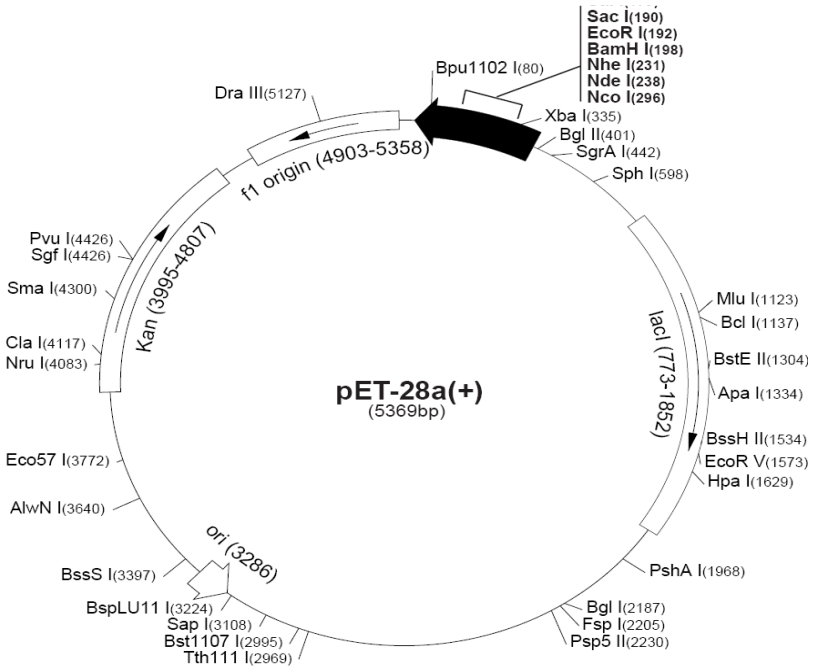

Figure 1. BuMx1 expression vector

collected from 200 ml of the cultured by centrifugation at 3,000 rpm for 15 min, washed with phosphate-buffered saline (PBS), and suspended in 20 mmol/l Tris-HCl buffer (pH7.9) containing 5 mmol/l imidazole and 0.5 mol/l NaCl, and then lysed by ultrasonic treatment. Subsequently, the suspension was centrifuged, the supernatant was extracted, and the residue was added to the binding buffer (20 mmol/l Tris-HCl buffer (pH7.9) containing 6mol/l urea, 5mmol/l imida-zole, and 0.5mol/l NaCl) and Ni-NTA His-Bind resin (EMD Bioscience, Inc., Milwaukee, WI) to purify full- length buMx1–polyHis tag. The resins were then eluted to produce a raw buMx1 solution.

2.4. Production of mAbs against buMx1

Healthy female BALB/c mice (CD1) were hyper-immunised by four intraperitoneal injections with recombinant buMx1 at the age of 8, 10, 12 and 14 weeks old, respectively as described previously (Kohno et al., 1994). Briefly, the mice were initially vaccinated with recombinant buMx1 in Complete Freund's Adjuvant - (CFA) (0.2mL) at 1:1 v/v ratio to boost the immune response. Then the three subsequent injections were carried out with buMx1 in Incomplete Freund's Adjuvant - (IFA) (1:1 v/v ratio) at two-week intervals. Six days after the final injection, blood samples from each mouse were tested for immunological response by ELISA, using buMx1 protein-coated plates. The best hyper-immunized mice were humanly euthanized to extract splenocytes (expressing antibody against buMx1) for fusion partners. In order to produce hybridomas, secreting monoclonal antibodies specific for the buMx1, $2x10^8$ splenocytes were incubated with $2x10^7$ P3-X63Ag8 myelomas (from BALB/c mice) in 50% (w/v) poly- ethylene glycol (PEG 1500 solution). The fused cells were incubated with HAT medium (GIT medium supplemented with 0.1mM hypoxanthine, 0.4μM aminopterin, and 16μM thymidine)

in 96-well plates at 37^0C in a humidified atmosphere of 5% CO_2. Two weeks post-fusion, cell culture supernatants were tested for antibody activity by ELISA using a buMx1 protein-coated plates as described previously by (Kohno et al., 1994). Hybridomas from positive wells were further selected on the basis of specificity and subclass, and were cloned twice by limiting dilution.

2.5. Monoclonal antibody isoptying

Isotypes of the monoclonal antibodies against buMx1 were classified by using mouse immunoglobulin isotyping ELISA kit (RD-Biotech) in accordance with the instructions from the provider. Briefly, the cell culture supernatant was diluted in dilution buffer (1:20 v/v), then 20µl aliquot of the diluted sample was added in each well, immediately add 100 µl of peroxidase conjugated anti-mouse Ig to each well. Mix gently until obtaining an homogeneous purple colour. Incubate the plate for 15 min at room temperature. After incubation, remove the solution and wash the microwells three times with 300 µl of the wash solution. Subsequently, add 100 µl of TMB substrate in each well. Incubate for 10 min at room temperature. Then stop the reaction with 100 µl of STOP solution. Results can be directly seen. The absorbance can also be read with a microplate reader at 450nm.

3. RESULTS AND DISCUSSION

3.1. Recombinant buffalo Mx1: production and characterization

After 3h expression, all of the E.coli cells were collected for buMx1 extraction and purification. The results were shown in figure 2 to 6.

Figure 2 shows the electrophoresis of the whole E.coli lysate after three hours expression. The results indicated that Iso-propylthiogalactoside (IPTG) tightly regulated the translation of cDNA encoding buMx1 as only transformed E.coli cultured in media supplemented with IPTG expressed recombinant buMx1 protein, which appears obviously at about 82 KDa (lane W), meanwhile the protein was not found in the control condition, which was cultured in media without IPTG (lane Ctl).

In order to examine the location of the recombinant protein within the expression host, the inclusion body lysate was analyzed independently to the other cellular components (Figure 3) and the results show that a large amount of buMx1 was found in both of inclusion body lysate (lane W) and the supernatant (lane "Sup"), meanwhile just a small amount of the protein remained in the precipitation in water (lane P). These results indicated that, expression of recombinant buffalo Mx1 protein leaded to formation of several inclusion bodies in the cell cytoplasm. In addition, the expressed recombinant proteins were also partially diffused in cell cytoplasm.

Protein purification

The recombinant buMx1 protein was purified by two different method including Ni^{2+} Affinity chromato-graphy and Superdex 200 (Figure 4). The results show that the latter was more effective than the former. By using Ni^{2+} affinity chromatography, majority of unwanted proteins were expelled, however a large amount of the interested proteins still remained in the by-pass filtration product (lane FT). In the elution of buMx1, either in reducing (E-R) or non-reducing conditions (E-N) the size and the density of buMx1 band was the most obvious in comparison to those in the supernatant (lane S) and the by-pass filtration product (lane FT). In

addition, almost all of the unwanted protein bands, which appeared in the S- and FT- lanes, disappeared.

Protein sequencing

After purification, the recombinant buMx1 protein was sequenced, the amino acid sequence are presented in Figure 6.

Recombinant buffalo Mx1 protein contains a total of 673 a.a, including a polypeptide of 653 a.a (in blue colour), fused with MVP tag - MGSSHHHHHHSSGLVPRGS at N-terminal. This sequence is absolutely matched with its the cDNA sequence constructed in the expression vectors, indicating that there was no modification or mutation during translation, production and purification of the protein. The total molecular mass of the protein is about 82 kDa, which is longer and heavier than bovine Mx1 (648 a.a and 77kDa).

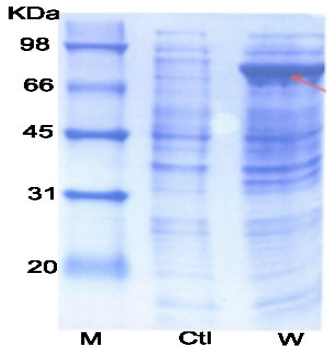

Figure 2. Recombinant buMx1 protein after 3h expression in E.coli (BL21-DE3)

Note: M: marker; Ctl: control (whole bacterial protein cultured without IPTG); W: whole bacterial protein cultured with IPTG (0,1mM).

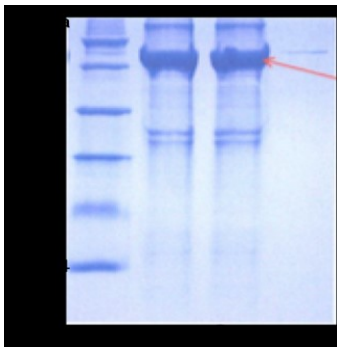

Figure 3. Dissolving of inclusion body

Note: M: marker; W: inclusion bodies; Sup: supernatant in 20mM Tris-HCl (pH 8,0), 20mM Imidazole, 7M GuaHCl, 20mM beta-Me; P: precipitation in water.

Figure 4. Purification of buMx1 by Ni^{2+} Affinity chromatography

Note: M: marker; S: supernatant after dissolving of inclusion bodies; FT: Flowthrough; E: Elution by 20mM Tris-HCl (pH 8,0), 300mM Imidazole, 300mM NaCl, 8M urea, 20mM beta-Me; R: Reducing; N: Non-reducing.

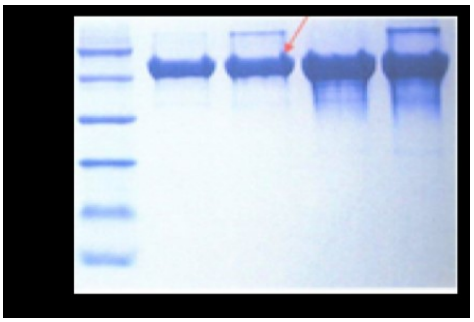

Figure 5. Purification of buMX1 by superdex 200

Note: M: marker; F1-F2: Fraction 1 to 2; R: Reducing; N: Non-reducing. Buffer: 20mM Tris, 0,5mM Imidazole, 500mM NaCl, 6M urea, 0,1mM DTT, pH 7,9.

***MGSSHHHHHH*SSGLVPRGSH*MVHSDLDIKEPNSPESSLNGSDDV**
VREHETESKSNLYSQYEEKVRPCIDLIDSLRALGVEQDLALPAIAVI
GDQSSGKSSVLEALSGVALPRGSGIVTRCPLVLRLKKLGNEDQWK
GKVSFLDKEIEISDASQVEKEISEAQIAIAGEGTGISHELISLEVSSPH
VPDLTLIDLPGITRVAVGNQPPDIEYQIKSLIRKYILRQETINLVVVP
ANVDIATTEALRMAQEVDPQGDRTIGILTKPDLVDKGTEDKVVD
VVRNLVFHLKKGYMIVKCRGQQDIKHRMSLDKALQRERIFFEDHA
HFRDLLEEGKATIPCLAERLTSELIMHICKTLPLLENQIKETHQRITEE
LQKYGKDIPEEESEKMFSLIEKIDTFNKEIISTIEGEEFVEQYDSRLFTK
VRTEFCKWSAVVEKNFEEGFEAIRKEIKQFEKRYRGRELPGFVNYK
TFETIIKKQVRVLEEPAVDMLRTVTDIIRNTFTNVSGRHFNEFFNLH
RTAKCKIEDIRSEQENEAEKSIRLHFQMEQLVYCQDQVYRRALQQ
VREKEAEEEKNKKSNHYFQSQVSEPSTDEIFQHLTAYHQEVSTRIS

Figure 6. Amino acid sequence of buMx1α

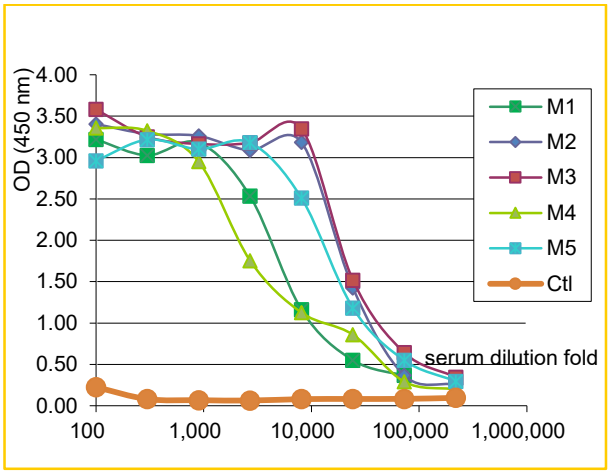

Figure 7. Serum titer buMx1-hyperimmunized mice

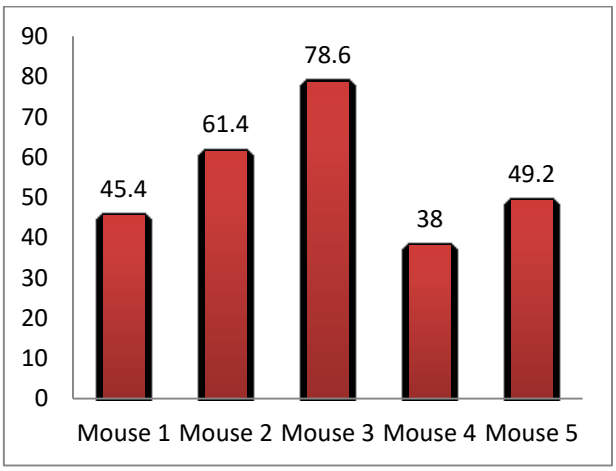

Figure 8. Number of spleenocytes extracted from superimmunized mice (in million)

Table 1. Hybridomas and buMx1 mAb Isotype screening

N^0	Hybridomas	Screening fusion P48	Confirmation P24		Isotype
		BuMx1	BuMx1	HisTag	
1	7B3	3.146	2.972	0.097	IgG1 / IgG2a
2	6A3	2.901	3.289	0.095	IgG1 / IgG2b
3	6F5	2.97	3.098	0.085	IgG2a / IgG2b
4	7C6	3.018	2.786	0.079	IgG1 / IgG2b*
5	FD4	2.575	2.441	0.092	IgG1
6	OB8	2.962	2.733	0.086	IgG1
7	8B2	2.996	3.074	0.089	IgG2a* / IgG2b
8	8C3	2.881	1.973	0.078	IgG2b
9	9D1	2.488	3.104	0.082	IgG1
10	10A2	2.3	3.077	0.083	IgG1 / IgG2b
11	10A8	3.159	3.116	0.088	IgG1* / IgG2b
12	10B4	2.852	3.173	0.087	IgG1 / IgG2a
13	10C1	2.74	3.039	0.086	IgG1 / IgG2b
14	10D8	2.993	3.003	0.093	IgG2b
15	10F5	2.594	3.058	0.084	IgG1
16	11A7	2.155	2.991	0.084	IgG1
17	11C3	2.736	2.866	0.081	IgG1 / IgG2b*
18	11C7	1.068	2.865	0.073	IgG1
19	11D4	2.811	2.283	0.079	IgG1 / IgG2a*
20	18A1	2.282	3.063	0.085	IgG1 / IgG2a
21	18B4	2.968	3.096	0.08	IgG2b
22	PE6	2.207	3.036	0.127	IgG1
23	8D6	2.633	2.936	0.096	IgG2a
24	RD5	2.383	2.992	0.105	IgG1
25	9B3	1.244	3.093	0.121	IgG1 / IgG2a
26	9E1	2.28	3.057	0.097	IgG2b

3.2. Production of monoclonal antibodies against buMx1 protein

3.2.1. Superimmunization of BALB/c mice with recombinant buMx1

Four days after the final vaccination; blood samples of superimmunized mice were tested for immunological response by ELISA, using buMx1 protein-coated plates. The results were shown in Figure 6. It was clear that all of the 5 immunized mice responded very well to the immunization procedures. Their serum antibody against buMx1 titration was quite high as it was still detected at the dilution of one million fold; meanwhile there was no signal in blood sample of the control mouse, injected with the same procedures of the superimmunized group but without buMx1. The results indicated that recombinant buMx1 protein was highly immunogenic to BALB/c mice. Consequently, all of the 5 mice met the conditions for hybridoma fusion. Subsequently, the animals were humanely euthanized for extraction of activated B lymphocytes (splenocytes). The result shows that number of splenocytes extracted from each mouse were tightly proportional to their serum antibody titer. As can be seen from Figure 8, the mouse 2 and mouse 3 provided the highest number of splenocytes, while whose serum antibody against buMx1 protein titration were also the highest values (Figure 7). In contrast,

mice 1 and 4 simultaneously had low serum antibody titration and splenocytes. Thus, in order to save time in this stage we decided to give the first priority to fusion and screening hybridomas derived from mouse 2, 3 and 5.

Table 2. Hybridomas cloning and buMx1 mAb Isotype screening

Hybridomas	Clone 1	Screening fusion P96	Confirmation P24		Clone 1 Isotype	Clone 2 Isotype	Clone 3 Isotype
		BuMx1	BuMx1	HisTag			
FD4	AC3	2.904	2.708	0.100	IgG1	IgG1	IgG1
	AC12	2.94	2.716	0.104	IgG1	IgG1	IgG1
	AD2	2.88	2.726	0.106	IgG1	IgG1	IgG1
	AF4	3.04	2.697	0.114	IgG1	IgG1	IgG1
	AG2	2.901	2.786	0.105	IgG1	IgG1	IgG1
	AG6	2.98	2.713	0.126	IgG1	IgG1	IgG1
OB8	CC1	3.04	3.145	0.168	IgG1	IgG1	IgG1
	CD2	3.219	3.032	0.139	IgG1	IgG1	IgG1
	CF4	2.89	3.017	0.203	IgG1	IgG1	IgG1
	CG7	3.224	2.906	0.239	IgG1	IgG1	IgG1
	CH3	3.403	3.044	0.173	IgG1	IgG1	IgG1
	DG3	3.161	3.15	0.133	IgG1	IgG1	IgG1
PE6	ED11	3.083	2.62	0.145	IgG1	IgG1	IgG1
	EE7	2.95	2.522	0.087	IgG1	IgG1	IgG1
	EG9	2.927	2.691	0.172	IgG1	IgG1	IgG1
	EH5	2.947	2.62	0.084	IgG1	IgG1	IgG1
	FA3	3.088	2.616	0.12	IgG1	IgG1	IgG1
	FA8	3.014	2.603	0.158	IgG1	IgG1	IgG1
RD5	GE10	2.87	3.025	0.095	IgG1	IgG1	IgG1
	GF12	2.857	3.048	0.109	IgG1	IgG1	IgG1
	GG9	2.853	2.923	0.087	IgG1	IgG1	IgG1
	GG11	2.896	2.956	0.085	IgG1	IgG1	IgG1
	HB9	2.983	2.921	0.064	IgG1	IgG1	IgG1
	HC9	2.963	2.912	0.102	IgG1	IgG1	IgG1
9D1	JC1	3.199	2.761	0.04	IgG1	IgG1	IgG1
	JC2	3.009	2.934	0.036	IgG1	IgG1	IgG1
	JC3	2.632	2.057	0.025	IgG1	IgG1	IgG1
10B4	MC6	2.793	2.852	0.036	IgG1 / IgG2a	-	-
	MD5	2.83	2.858	0.033	IgG1	IgG1	IgG1
	ME1	3.002	2.805	0.062	IgG1 / IgG2a	-	-
10F5	OE1	3.061	2.582	0.036	IgG1	IgG1	IgG1
	OE2	3.067	2.693	0.041	IgG1	IgG1	IgG1
	OE3	3.048	2.547	0.03	IgG1	IgG1	IgG1
11A7	QD1	2.777	2.852	0.036	IgG1	IgG1	IgG1
	QD2	2.796	2.756	0.037	IgG1	IgG1	IgG1
	QD3	3.077	2.766	0.035	IgG1	IgG1	IgG1
11C7	TC1	3.01	2.472	0.035	IgG1	IgG1	IgG1
	TC2	2.938	2.441	0.037	IgG1	IgG1	IgG1
	TC3	2.994	2.417	0.035	IgG1	IgG1	IgG1

3.2.2. Screening hybridomas and IgG – isotyping

After fusion of millions splenocytes and myeloma cells, just about 50% of the wells presented with hybridomas. However, only 26 hybridomas have been detected for secreting IgG1; IgG2a or IgG2b monoclonal antibody against buMx1 (as presented in table1). Interestingly, there wasn't any antibody against His tag protein, which was designed as an indirect marker for determination and purification of recombinant buMx1 in the earlier stages. Isotypes such as IgM and IgG3 were not found in this study.

Besides ability of high specific binding, the isotype of the monoclonal antibodies was also an important factor for selection of hybridomas. By using mouse immunoglobulin isotyping ELISA kit (RD-Biotech protocol: Catalogue number RDB 3255), the isotypes of the monoclonal antibodies against buMx1 derived from the 26 hybridomas were identified. Based on these results, the first choices for this study were hybridomas secreting IgG1 monoclonal antibody including: FD4, OB8, 9D1, 10F5, 11A7, 11C7, PE6, RD5, and 10B4 secreting high level of IgG1/IgG2a. In order to ensure if the hybridomas consistently secreted high level of IgG1 monoclonal antibody against buMx1, these parent cells were sub – cloned and repeated with the same screening procedure as described previously. The results show that all of the sub-clones (from clone 1 to 3) derived from FD4, OB8, 9D1, 10F5, 11A7, 11C7, PE6 and RD5 persistently produced quite high levels of mAb against buMx1. Interestingly, one of the first sub-clones (MD5) of hybridoma 10B4 persistently secreted quite high level of IgG1 mAb while IgG1/IgG2a mAb were found in the other two first sub-clones including MC6 and ME1. These cells were cryopreserved for further study and the results were not displayed in this topic.

4. CONCLUSIONS

The pET - 28b is a suitable expression vector for production of recombinant buffalo Mx1 protein. Majority of the expressed protein was located in inclusion bodies and cytoplasm. Superdex 200 was the better method for purification of recombinant buMX1, compared to Ni^{2+} Affinity chromato-graphy. The total molecular mass of the recombinant buMx1 protein was about 82 kDa, whose sequence was absolutely matched with its the cDNA sequence constructed in the expression vectors. The mixture of recombinant buMx1 and Freund's Adjuvant was highly immunogenic to BALB/c mice as administered via intraperitoneal injection. Nine hybridomas, secreting mAbs to Mx1 were obtained including 8 hybridomas producing IgG1 mAbs (FD4, OB8, PE6, RD5, 10B4, 11A7, 10F5, 9D1) and another producing IgG2a mAbs (7B3).

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OCCURRENCE OF EXFOLIATIVE TOXIN GENES AND ANTIMICROBIAL SUSCEPTIBILITY OF *Staphylococcus Hyicus* ISOLATED FROM PIG FARMS IN THANH HOA PROVINCE

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ABSTRACT

This study was conducted to investigate the occurrence of exfoliative toxin genes and antimicrobial susceptibility of *Staphylococcus hyicus (S. hyicus)* in pigs with and without exudative epidermitis (EE) in Thanh Hoa province. Twelve pig farms were chosen in this study. The *S. hyicus* was isolated from different parts of the pigs with and without EE and was identified by biochemical test, PCR test. Four exfoliative toxin genes (exhA, exhB, exhC, and exhD*)* were detected by multiplex PCR test. Thirty toxin producing strains and thirty non - toxin producing strains from isolated strains were selected for their susceptibility test to 7 antimicrobial agents by using the by broth microdilution method. Nighty four isolates from collected samples were positive with *S. hyicus.* Exfoliative toxin genes (exhA, exhD, and exhA and exhD*)* were detected in 31 (32,97%) isolates. The exhA gene was the highest prevalent in these toxin genes with 14 isolates (45,16%)*,* following by exhD gene with 13 isolates (20.63%), eight (12.69%) isolates were positive for the combination of *exhA* and exhD genes. Only one isolate was positive with *exhB*. The highest susceptibility rate was trimethoprim - sulfamethoxazole (81.25%), followed by methicillin (80%). The highest resistant rate was penicillin (75%), followed by cephalexin (27.5%). In CONCLUSIONS, the prevalence of exfoliative toxin genes was higher in pigs with EE than that of the pigs without EE. Antimicrobial susceptibility was not significantly different between toxigenic strains and non - toxigenic strains.

Keywords: Antimicrobial susceptibilities, PCR, pig, *staphylococcus hyicus*, toxin gene.

1. INTRODUCTION

S.hyicus is divided into virulent and avirulent strains. The virulent strains cause exudative epidermitis in pigs, particularly in suckling and weaned pigs. Exfoliative toxin products are the main factors to cause this disease. These toxins induce EE with special clinical signs; therefore, the virulent strains are named by toxin producing or toxigenic strains. The virulent strains of *S. hyicus* produce several different exfoliative toxins; these toxins are distinguished by amino acid structures. However, they all seem to have the same functions in causing the disease with particular skin lesions (Andresen et al., 1997). Either crude or purified exfoliative toxins from culture supernatants of *S. hyicus* also caused the disease when piglets were injected subcutaneously in the pig skin (Sato et al., 1991; Andresen et al., 1993). The exfoliative toxins of the virulent *S. hyicus* have been isolated from the affected piglets in Japan and Denmark, and these

exfoliative toxins were named as SHETA and SHETB in Japan (Sato et al., 2000) and ExhA, ExhB, ExhC, and ExhD in Denmark (Andresen and Ahrens, 2004).

Base on pathognomonic lesions on skin of affected piglets, the disease is easily recognized and continuously identified in pig farms in Thanh Hoa province. The veterinarian and pig producers normally use antimicrobial agents in treatment in Thanh Hoa pig farms. Antimicrobial resistance also occurred in pig farms because of the antibiotic use as a growth promoter in feed and control and prevention. Moreover, autogenous vaccine for this disease was not widely applicable. Therefore, the result of the treatment was not high, the economic lossen still continuously occur in Vietnam pig producing industry. The important aspects for the control and prevention this disease in Thanh Hoa are that which toxin genes are more prevalent and also patterns of antimicrobial susceptibilities of *S. hyicus* in Thanh Hoa pig farms.

Therefore, determination of the encoding gene of exfoliation toxins of *S. hyicus* from infected pigs might be benefit in prevention this disease in future time. Autogenous vaccine will be made from toxin producing strains of *S. hyicus* in order to inject to sows prior to farrowing. On the other hand, determination of the antimicrobial susceptibilities from the isolated pathogens will be useful for choosing right antibiotics in treatment EE. Regarding the above benefits, the current research was conducted to get more information not only about the disease but also the disease prevention method in future time. This could be a valuable guideline for a designation of autogenous vaccine, and perhaps a further application for antimicrobial selection regarding to the toxin producing isolates.

2. MATERIALS AND METHODS

2.1. Sampling collection

Twelve pig farms (two farms with EE pigs and ten farms without EE pigs) in Thanh Hoa, were purposively selected for the study. Sows on production varied from 100 to 300 sows per each farm. Thirty samples per each farm were randomly chosen (10 suckling pigs, 10 weaning pigs, and 10 sows).

In the sucking and nursing pigs, the sterile cotton swabs were dipped in 0.9% sterile sodium chloride and applied to approximately 10 cm^2 area behind the right ear of the EE pigs and non EE piglets. Then, the swab samples were placed in liquid Stuars medium. In sows, Vaginal swabs were collected by rigorous rubbing with a sterile cotton swab inserted approximately 10 cm into sow vagina. The swab tips were then put into a plastic tube containing 5 ml Stuart's medium. Then, the samples were submitted to the bacterial laboratory in Hong Duc University

2.2. *S. hyicus* identification and further biochemical characterization

All of the collected samples were inoculated directly onto 5% sheep blood agar (Oxoid Ltd, Basingstoke, Hampshire, England) and McConkey agar (Merck KGaA, 64271 Darmstadt, Germany) and then incubated at 37°C for 18 - 24h. If bacterial culture heavily contaminated with other species, the colonies were subcultured on mannitol - salt agar (Oxoid Ltd, Basingstoke, Hampshire, England). After incubation, three single colonies with size of about 3 - 4mm, slightly opaque white, and non - hemolytic were selected for gram staining. Continuously, the gram staining was positive; the colonies were subcultured on sheep blood agar and tested

for further other biochemistry tests such as catalase, oxidase, and oxidative fermentation test.

The identification of *S. hyicus* follows the method polymerase chain reaction mediated amplification of species specific sequences of superoxide dismutase A encoding gene (sodA) (Voytenko *et al.,* 2006). The bacteria genomic DNA were extracted by boiling method. Briefly, in each isolate, three colonies of freshly subcultured strain on sheep blood agar to be investigated were suspended in 400 µl PBS, pH 7.4. The bacteria suspension was boiled at 100°C for 10 minutes. After centrifugation at 13,000 rpm for 3 minutes, the supernatant was carefully collected in a new tube and keep in - 20°C until used for PCR.

2.3. Detection of exh genes by multiplex PCR test

Primers described in the work of Andresen and Ahrens (2004) were used to detect the exfoliative toxins ExhA, ExhB, ExhC, ExhD. The DNA was amplified by an initial denaturation at 94°C for 180s followed by 30 cycles of 60s at 94°C, 60s at 56°C and 60s at 72°C. The PCR reaction was completed by a 10min incubation at 72°C to ensure full extension of the PCR products.

2.4. Antimicrobial susceptibility test

A total of 60 *S. hyicus* strains, including 30 exfolitative toxigenic isolates and 30 non - toxigenic isolates from exudative epidermitis and healthy pigs, isolated from pig farms in ThanhHoa, were included in this study. Minimal inhibitory concentration (MIC) determination was done by broth microdilution method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines (2007). The following antimicrobials were tested penicillin (0.06 - 8 µg/ml), gentamicin (0.5 - 128 µg/ml), cefotaxime (0.5 - 64 µg/ml), cephalexin (0.5 - 128 µg/ml), methicillin (0.5 - 128 µg/ml), trimethoprim - sulfamethoxazole (0.06 - 8 and 1.187 - 152 µg/ml, respectively), and vancomycin (0.5 - 128 µg/ml). *Staphylococcus aureus* JCM 2874 (ATCC29213) was included as controls.

3. RESULTS AND DISCUSSIONS

3.1. *S. hyicus* identification and further biochemical characterization

Based on criteria to identify *S. hyicus* in previous study, 139 isolates that grown on sheep blood agar with no pigment, non hemolysis, positive for catalase, O - F, and negative for oxidase reaction were collected.

The API Staph strip test was also used for identification of randomly selected 30 *S. hyicus* isolates. All *S. hyicus* strains fermented glucose, fructose, mannose, trehalose, saccharose, and N - acetylglucosamine, but maltose, mannitol, xylitol, melibose, raffinose, xylose, and alpha - methylglucoside were not fermented.

The identification of *S. hyicus* was further performed using sodA PCR. Totally, 139 *S. hyicus* isolates identified by biochemical identification were further identified by sodA PCR technique. Of 139 isolates, only 94 (67,62%) isolates were positive with *S. hyicus*. Of 94 positive isolates, 52 and 42 isolates were taken from suckling and nursing pigs, respectively. None of 94 samples from sow vagina was positive with sodA PCR. 24 out of 94 positive isolates collected from two EE pigs farms and 70 positive came from non EE pig farms.

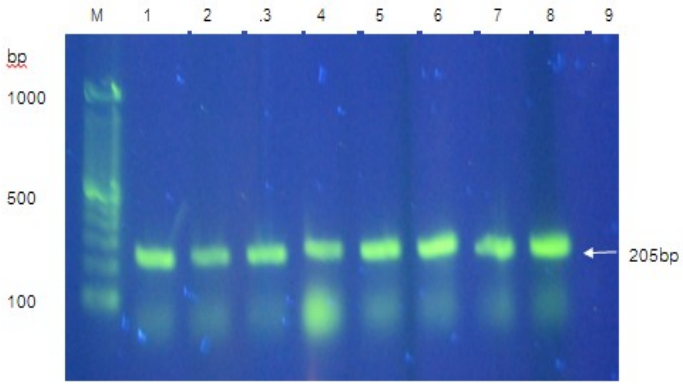

Figure 1. Typical agarose gel electrophosis pattern of the sodA PCR detecting *S. Hyicus*

Note: Lane M: 100 bp plus DNA marker (GeneRuler™, Fermentus, Canada); Lane 1. Pos: Positive control; Lane 2 - 8: Sample isolates; Lane 9: Negative control

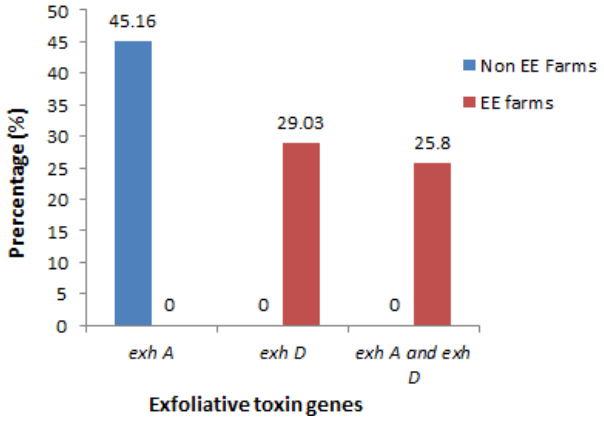

Figure 2. Distribution of exfoliative toxin genes in the pigs with and without skin lesion from 12 pig farms

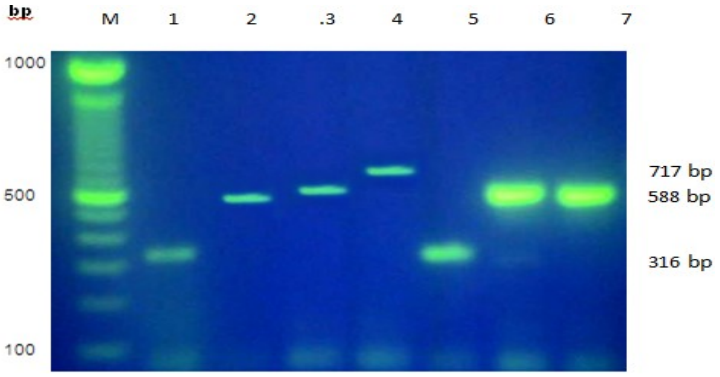

Figure 3. Typical amplicons of the multiplex PCR detecting the genes encoding exfoliative toxins from *S. Hyicus*

Note: Lane M: 100 bp plus DNA marker (GeneRuler™, Fermentus, Canada); Lane 1 - 4: positive control exhA, exhC, exhD, exhB; Lane 5 - 7: samples

3.2. Detection of ET genes

The occurrence of exh genes of *S. hyicus* from EE pigs was higher non - EE pigs (Figure 2). A total 31 isolates from 12 farms was toxin producing strains, including 17 isolates from EE pigs and 14 isolates from non EE pigs. Of 17 toxigenic isolates from EE, 9 (52.50%) isolates were positive for exhD and 8 (47.05%) isolates were positive for combination of exhA and exhD. All of 14 (100%) toxigenic strains from non EE pigs ere positive for exhA. None of the strains harboured genes for exhB or exhC.

A variable prevalence of toxin types among *S. hyicus* isolated in different countries has been reported. Among the isolates from Russia, Belgium, Germany and Slovenia, exhD - positive were the most predominant (Andresen, 2005; Kanbar *et al.,* 2006). In Denmark, ExhA - , ExhB - , ExhC - and ExhD - producing *S. hyicus* were isolated respectively from 20%, 33%, 18% and 22% of pigs with exudative epidermitis (Andresen and Ahrens, 2004). Whereas, in a recently published study from Japan, the corresponding genes were present in 42.9%, 23.6%, 0.6% and 20.5% of 161 *S. hyicus* strains from diseased pigs (Futagawa - Saito *et al.,* 2007). Moreover, these authors found no significant differences between strains from diseased and healthy pigs with regard to the carriage of toxin types. However, The isolation rate of toxigenic *S. hyicus* was four times higher in the pigs with exudative epidermitis than the healthy pigs (87.6% versus 19.6%; $p < 0.01$)" was described by Futagawa - Saito et al. (2007).

3.2. Antimicrobial susceptibility

Antimicrobial susceptibility results of 60 *S. hyicus* strains are shown in Figure 5. For total *S. hyicus* strains, the highest frequency of susceptibility was found with trimethoprim - sulfamethoxazole (81.25%), followed by methicillin, cephalexin, 80%, 57.5%, respectively. The highest resistance was observed with penicillin (75%), followed by cephalexin, methicillin 27.5%, 20%, respectively. Vancomycin was sensitive and intermediate to all strains tested. The largest sensitivity rate of toxigenic and non - toxigenic isolates was against trimethoprim - sulfamethoxazole, methicillin, gentamicin, vancomycin, cephalexin, cefotaxime and penicillin, respectively.

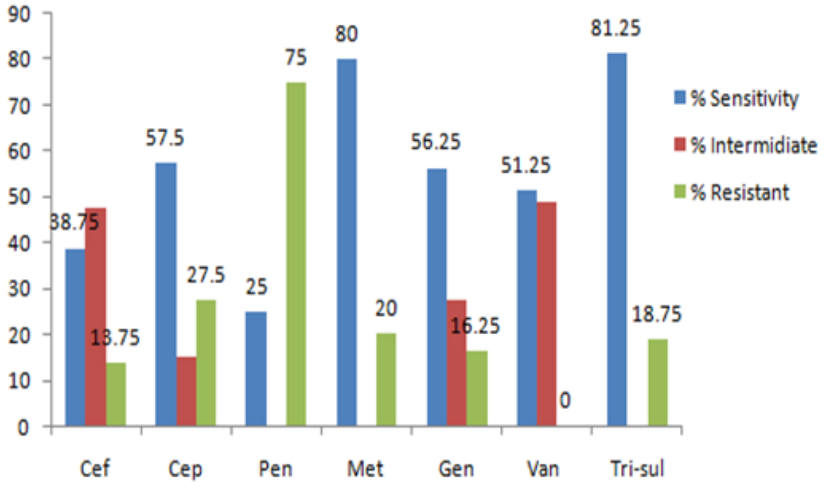

Figure 5. Antimicrobial susceptibility of 80 *S. hyicus* isolates

The difference of antimicrobial susceptibility between toxigenic strains and non - toxigenic strains was found in this study. The highest resistant rate was found with penicillin and cephalexin to both toxin and non - toxin producing strains, but higher in toxin producing than non - toxin producing (90.00% and 35.00% for toxigenic isolates, and 87.50% and 20.00% for non - toxigenic isolates). The lower frequency of resistance was found with trimethoprim - sulfamethoxazole, follow by methicillin, cefotaxime, gentamicin to toxin and non - toxin producing strains, vancomycin was not resistant to both toxin and non - toxin producing strains. But this was not significantly different between toxigenic strains and non - toxigenic strains. The intermediate was found highest with cefotaxime and vancomycin 52.50%, 50.00% to toxigenic strains and 42.50% and 47.50% to non - toxigenic strains, respectively.

Comparable data are not available as the two previous studies dealing with antimicrobial susceptibility of *S. hyicus* were based on strains isolated from pigs with exudative epidermitis (Aarestrup and Jensen, 2002; Wegener *et al.*, 1994). However, the favourable resistance results in *S. hyicus* are in accordance with the situation in *S. aureus* strains isolated from pig carcasses in Switzerland (Nitzsche *et al.,* 2007).

4. CONCLUSIONS

In CONCLUSIONS, the exfoliative toxin genes were detected with relative higher prevalent rate in isolates from skin in the pigs with lesions of EE than isolates from skin of healthy pigs. The exhA was the most common toxin gene in the pigs in Thanh Hoa. In one isolate of *S. hyicus* can carry two of exfoliative toxin genes (exhA and exhD). For antimicrobial susceptibility, trimethoprim - sulfamethoxazole and methicillin appear to be the most sensitive antimicrobials among 80 *S. hyicus* isolates. Susceptibility to all antimicrobials, except vancomycin was higher in toxigenic strains than non - toxigenic strains. No correlation between antimicrobial resistance and toxin gene carriers was found. Multiple drug resistance was also observed (data not shown). Therefore, antimicrobial susceptibility should be evaluated as guideline for antimicrobial use in treatment of exudative epidermitis in pig farms.

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OCCURRENCE OF EXTENDED SPECTRUM BETA - LACTAMASE PRODUCING *Escherichia Coli* IN CHICKENS FROM SLAUGHTER HOUSES IN THE MEKONG DELTA OF VIETNAM

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ABSTRACT

The aim of the study was a preliminary determination of occurrence of extended spectrum beta - lactamaseproducing *Escherichia coli* (ESBL - producing *E. coli*) in chickens collected from slaughter - houses located in different regions in the Mekong Delta of Vietnam. A total of 720 samples from 180 chickens were tested, comprising 180 liver samples, 180 lung samples, 180 meat samples, and 180 fecal samples. Isolated and identified *E. coli* strains were examined for the ESBL production by the use of combination disc methods. The presence of the CTX - M, TEM, and SHV genes were examined by Polymerase Chain Reaction method. The results showed that 48.33% chickens contained ESBL *E. coli*; the presence of ESBL *E. coli* on liver, lung, meat, and fecal samples were 5%, 11.67%, 11.11%, and 48.33% respectively. The presence of the genes encoding ESBL was identified in 40 isolates recognised as ESBL positive in phenotypic tests. All isolates showed the presence of the antibiotic resistance genes including 87.5% CTX - M gene, 75% TEM gene, and 70% SHV gene.

Keywords: Chicken, *Escherichia coli,* ESBL, slaughter - houses

1. INTRODUCTION

Escherichia coli (*E. coli*) is one of the most important bacterial diseases affecting the poultry industry and contributing significantly to economic losses in chickens (Raji *et al.,* 2003). In recent years, the bacterial resistance to beta - lactam antibiotic has risen dramatically (Katayama *et al.,* 2004). Extended spectrum β - lactamases (ESBLs) are plasmidencoded enzymes that are commonly produced by *E. coli* (Paterson and Bomono, 2005). ESBLs are grouped into four classes A, B, C and D enzymes. Temoneira (TEM), sulfhydryl variable (SHV) and cefotaximase (CTX - M) are class A ESBLs (Shahid *et al.*, 2011). In recent years, the prevalence of ESBL - producing *E. coli* and their resistance is increasing. More than 400 ESBLs have been described with 183 TEM, 134 SHV and 103 CTX - M variants (Barguigua *et al.,* 2011). *E. coli* strains expressing CTX - M have presently replaced TEM and SHV as the most common types of ESBLs (Oteo *et al.,* 2010). Beside, *E. coli* is also an important cause of morbidity and mortality in foodborne diseases. Foods contaminated with antibiotic resistant bacteria could be a major threat to public health because they may be transferred genes encoding antibiotic resistance to other bacteria of human (Van *et al.,* 2008). Therefore, this study was conducted to provide a preliminary investigation of the occurrence

of ESBL - producing *E. coli* in chickens from slaughter - houses in the Mekong Delta of Vietnam and to better understand the epidemiology of genes coding for ESBL.

2. MATERIALS AND METHODS

The study randomly collected 180 chickens from 12 slaughter - houses in Vinh Long, Tra Vinh, Hau Giang, and Soc Trang provinces of the Mekong Delta. Samples of lungs, livers, muscles and faeces were collected from each chicken. A total of 720 samples were tested. The samples were plated on MacConkey agar supplemented with ceftazidime 2 mg/l, incubated at 37°C for 24 hours. Followingr incubation, *E. coli* will produce acid, which lowers the pH of the agar below 6.8 and results in the appearance of pink colonies. From each positive sample, 10 typical *E. coli* colonies were selected to do biochemical tests, including indole, methyl red, voges proskauer and citrate. Identified *E. coli* isolates were examined for ESBL production by a combined disk method (CLSI, 2014).

The presence of genes encoding extended - spectrum beta - lactamases was determined by PCR. DNA was extracted by the boiling lysis method. The study used primers F: 5' - ATGAGTATTCAACATTTCCG - 3 'and R: 5' - TTACTGTCATGCCATCC - 3' to amplify a 351 bp fragment of TEM gene (Rasheed *et al.*, 2000); F primers: 5' - ACTGAATGAGGCGCTTCC - 3 'and R: 5' - ATCCCGCAGATAAATCACC - 3' to amplify a 297 bp fragment of SHV gene (Gniadkowski *et al.*, 1998); and primers F: 5' - CGCTTTGCGATGTGCAG - 3' and R: 5' - ACCGCGATATCGTTGGT - 3' to amplify a 550 bp fragement of CTX - M gene (Bonnet *et al.*, 2000). DNA amplification reactions are performed in a thermal cycle (Lucena *et al.*, 2012).

3. RESULTS AND DISCUSSION

The results in Table 1 showed that the prevalence of ESBL - producing *E. coli* in chickens from slaughter - houses was high (48,33%). In this study, ESBL - producing *E. coli* was detected in lungs, livers, muscles and faeces, and mainly detected in faeces of chickens (Table 2). ESBL - producing *E. coli* bacteria are highly prevalent in poultry, and chicken meat has been implicated as a source of ESBL - producing *E. coli* present in the human population, as well as flies acquire ESBL - producing *E. coli*, warranting further evaluation of the contribution of flies to dissemination of ESBL - producing *E. coli* in the community (Blaak, 2014). Therefore, the slaughter - houses should be done good hygiene and disinfection regularly. Besides, wastewater of the slaughter - houses need to be treated properly to limit the spread of ESBL - producing *E. coli* in the community.

Table 1. Prevalence of ESBL - producing *E. coli* in chickens

Provinces	No. chickens	No. positive chickens	% positive chickens
Tra Vinh	45	24	53.33
Vinh Long	45	23	51.11
Soc Trang	45	21	46.67
Hau Giang	45	19	42.22
Total	180	87	48.33

Table 2. Prevalence of ESBL - producing *E. coli* in various tissues and faeces

Samples	No. samples	No. positive samples	% positive samples
Livers	180	9	5.0
Lungs	180	21	11.67
Muscles	180	20	11.11
Faeces	180	87	48.33

This study results showed that the presence of genes encoding extended - spectrum beta - lactamases (Table 3) was very high with CTX - M (87.5%), TEM (75%), and SHV (70%). The importance of this study is the finding that ESBL - producing *E. coli* bacteria on chickens from slaughter - houses in Vietnamese are significant reservoirs of antibiotic resistance genes. Food may be an important source of *E. coli* in human who develop urinary tract infections (UTIs) and antimicrobial resistant UTIs in humans may be associated with chicken consumption (Manges *et al.,* 2007). A comparison of ESBL - producing *E. coli* derived from chicken meat and hospitalized patients in the Netherlands showed a high degree of similarity of resistance genes. Genotype $bla_{CTX-M-1}$ was the most frequent drug resistance gene in chicken meat and humans (Overdevest *et al.,* 2011).

In recent years, the results of a study on *E. coli* isolates from chicken meat (n = 14) and chicken faeces (n = 7) in Vietnam showed that there were 84.2% of the tested isolates which contained TEM gene. In contrast, SHV gene was not detected in any isolates in this study (Van *et al.,* 2008). Therefore, research on antibiotic resistance genes of ESBL - producing *E. coli* in chickens in the different regions in Vietnam may be needed to be able to build a map of the epidemiology of these genes.

Table 3. Distribution of CTX - M, TEM and SHV genes

Gene	No. isolates (n=40)	
	No. positive isolates	% positive isolates
CTX - M	35	87.5
TEM	30	75.0
SHV	28	70.0

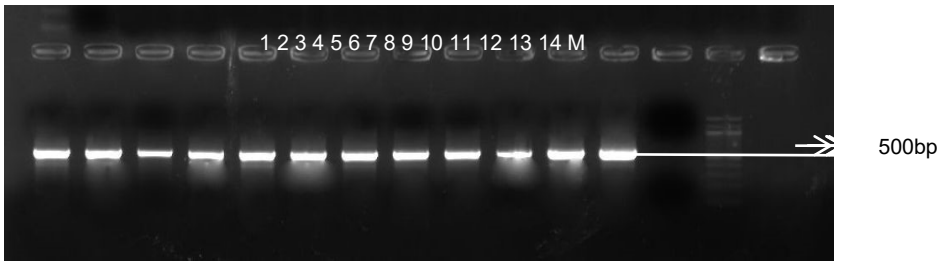

Figure 1. PCR amplification of CTX - M gene

Note: Well 14: ladder, well 13: negative control, well 12: positive control, well 1 to well 11: VL1W2D, VL1P2A, VL2W18G, VL2G18A, VL3P39A,VL3G39A, VL3T39C, HL1T1C, HL2W26C, TL2T19B, TL3W33A

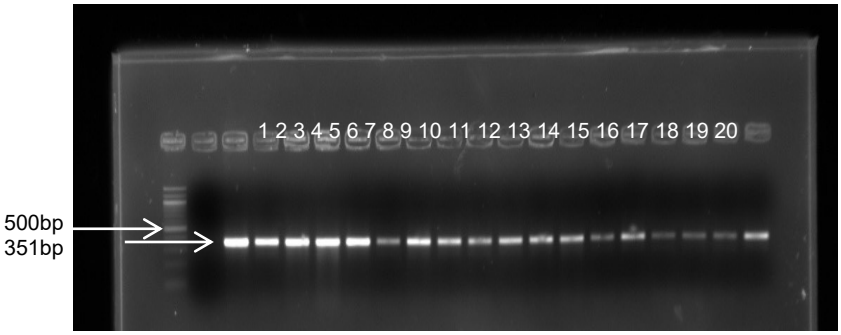

Figure 2. PCR amplification of TEM gene

Note: Well 1: ladder, well 2: negative control, well 3: positive control, well 4 to well 20: VL1W2D, VL1P2A, VL2W18G, VL2G18A, VL3P39A,VL3G39A, VL3T39C, HL1T1C, HL2W26C, TL2T19B, TL3W33A, TL3P33A, SL3G34A, TL2W26A, SL2W16C, SL3G34A, TL1W9A)

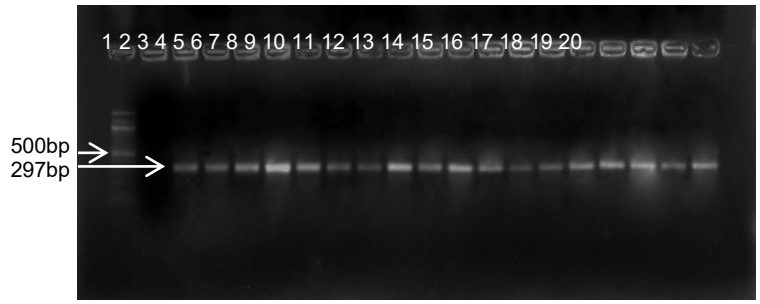

Figure 3. PCR amplification of SHV gene

Note: Well 1: ladder, well 2: negative control, well 3: positive control, well 4 to well 20: VL1W2D, VL1P2A, VL2W18G, VL2G18A, VL3P39A,VL3G39A, VL3T39C, HL1T1C, HL2W26C, TL2T19B, TL3W33A, TL3P33A, SL3G34A, TL2W26A, SL2W16C, SL3G34A, TL1W9A)

4. CONCLUSIONS

This is the first study on prevalence ESBL - producing *E. coli* in chickens from slaughter - houses in the Mekong Delta of Vietnam. The study confirms the large prevalence of ESBL - producing *E. coli* and antibiotic resistance CtX - M, TEM, SHV genes in chickens from slaughter - houses. Therefore, it is important to increase efforts to monitor and control the spread of antimicrobial resistant isolates in community.

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DEVELOPMENT OF *Arthrobacter Globiformis CODA* GENE IN IMPROVING PLANT RESISTANCE TO DISADVANTAGEOUS CONDITIONS

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ABSTRACT

Plants are often severely affected even dead when exposed to adverse factors of the environment such as drought, salinity, water logging or high temperature... as they lost osmotic pressure balance. Glycinebetaine is one of the compatible solutes that accumulate in the cell of halotolerant plants when these plants are exposed to salty conditions. The *CodA* gene for choline oxidase, the enzyme that converts choline into glycinebetaine, has previously been cloned from *Arthrobacter globiformis* bacteria. This study presents the results of changing genetic code of *Arthrobacter globiformis CodA* gene, encoding *choline oxidase* which could stimulate biosynthesis of glycinebetaine in some plants. The new *CodA* gene also was used to design transfer vector for producing transgenic plants (such as tobacco) which were capable of withstanding to extreme conditions via *Agrobacterium tumefaciens* - mediated transformation method. Some *CodA* transferred tobacco lines had very high accumulation of glycinebetaine, and it was confirmed that they had resistance to *in vitro* stress such as culturing in high temperate conditions, 42° C; medium added 2.5% *PEG6000* (artificial drought*)*, medium supplemented *NaCl 250mM/l* (salty).

Keywords: *Arthrobacter globiformis*, *CodA* gene, non - biological disadvantage, plant tolerance, transgenic plants.

1. INTRODUCTION

Glycinebetaine (GB), an amphoteric quaternary amine, plays an important role as a compatible solute under various stresses, such as salinity, water deficit or high temperature.

Capacity to synthesize GB under stress conditions differs from species to species (Ashraf and Foolad, 2009). In some plant, they can stimulate GB. In contrast, many plant species naturally can not produce GB (McCue and Hanson, 1990; Sakamoto and Murata, 2002; Quan *et al.*, 2004; Wahid and Close, 2007).

GB is one of the most compatible solutes which can stabilize proteins and integrity structural of cell membranes, against dehydration and contraction of protoplasm in adverse environmental conditions. GB could keep water balance and macromolecules that were an important physiological role related to osmotic pressure which reduces stress in cells (Ikuta *et al.*, 1997).

The GB enhanced tolerance to abiotic stress that appears to involve the GB - mediated enhanced expression of stress tolerance - related genes (Chen and Murata 2011). Kathuria found that levels of expression of 165 genes were higher in *CodA* - transgenic rice than in wild - type (WT) rice (Kathuria *et al.,* 2009). These genes have been shown to be involved in

stress responses, regulation of gene expression, signal transduction, transport across membranes, cellular metabolism and the general growth and development of plants.

CodA gene, was found and isolated from *Arthrobacter globiformis,* directly code *choline oxidase* which is a key enzyme in oxidation of choline to quickly form glycinebetaine (GB) (Ikuta *et al*., 1997).

GB can be accumulated in some plants under stress conditions but with fairly complex mechanism (McCue & Hanson 1990; Rhodes & Hanson 1993; Bohnert *et al.* 2006). Therefore, genetic engineering has allowed the introduction of GB - biosynthetic pathways into GB - deficit species (Sakamoto and Murata, 2002; Quan *et al*., 2004). There are some publish showed obtained results of transgenic lines concludes bacteria *CodA* been the improved resistant to environmental conditions adverse such as cold and frost, salt and mustard oxidation (Alia *et al*., 1998; Sakamoto and Murata, 2001; Prasad *et al*., 2000 ; Ahmad *et al*., 2008; Yu *et al*., 2009). However, the resistance had been strengthened a little bit and amount of GB calculated is still low.

This report presents the result of transcoding of bacteria's *CodA* genes into compatible plant genetic code, designing of transgenic vectors carrying the target gene and the initial data showed the potential modified genetic tobacco plants that could have some special characteristics withstand adverse conditions.

2. MATERIAL AND METHODS

2.1. Material

Choline oxidase (*CodA*) sequence AY304485 in NCBI of *Arthrobacter globiformis* with the size of 1641 base pairs (bp), encoding *choline oxidase* included 547 amino acids.

Receptor vector (pBI121 containing *Gus* gene, marker gene and the activitives zone of *Xba*I and *Sac*I restriction enzymes (Fermentas, Korea); Bacterial strains (*E. coli* DH5α, *A. tumefaciens* EHA101); and some commonly chemicals used from of Fermentas, Invitrogen, Merck, Sigma company.

Seedlings of C9.1 tobacco variety with 3 to 4 *in vitro* leaves were used to conduct transformation experiments, supplied by the Institute of Biotechnology, Vietnam Academy of Science and Technology.

2.2. Methods

2.2.1. Modifying and cloning CodA genes

CodA gene sequence AY304485 of *Arthrobacter globiformis* in the GenBank (NCBI) comprised 1641 bp, 547 amino acids encoded of *choline oxidase* that was ordered to appropiate conduct changing the code for expression in plants. In addition, at the 3' end of the gene, there was a nucleotide segment encoded a peptide with 30 nucleotides in length named *cmyc* which help to check the transgene expression by Western blot experiment. Moreover, at the 5' end of the gene, a sequence was added a 216 nucleotide segment encoding transited peptide (TP) leading transportation of the enzyme into the chloroplast.

Two target genes, with and without TP segment were ordered to artificially synthesized by the Fermentas company from USA, located in pBluescript II SK vector (Figure 1).

The artificial genes with two ends of restriction enzymes (*Xba*I / *Sac*I) were amplified by PCR with specific primers synthesized from the Macrogen Corporation (South Korea). The PCR was controlled under thermal cycling as 94ºC / 3 min, 94ºC / 30 seconds, (57 - 60ºC) / 30 seconds, 72ºC / 1 minute 30 seconds; 72ºC / 7 min, 4ºC / 30 min, 25 cycles.

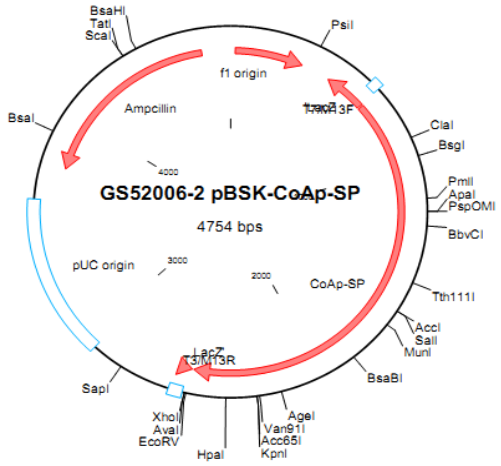


Figure 1. pBluescript 2pB SK vector with modified CodA gene

The artificial CodA genes sequence was analyzed by automated sequencing ABI PRISM 3100Avant Genetic Analyzer with principles of Sanger and BigDye Terminator kit v. 3.2 Cycle Sequencing. DNA and protein sequence data were processed by using the PackageLasergene program, version 4,5 of DNASTAR Inc., USA and BLAST (Basic Local Alignment Search Tool (Altschul *et al.*, 1990).

2.2.2. Design vector carrying the target gene

Receptor vector carrying *Gus* gene and target gene segments were simultaneous treated with two restriction enzymes *Xba*I/*Sac*I. Products were electrophoresis tested on 0.8% agarose gel and purified from the gel by using a Gel Purification kit AccuPrep (Bioneer). Recombination the receptor vector reaction and the gene fragment by ligation reaction used T4 ligase (Bioneer).

Recombined vector was transformed into variable *E. coli* DH5α with heat shock method (Cohen *et al.*, 1972). Plasmid DNA was extracted and purified with method of Sambrook (Sambrook *et al.*, 2001).

2.2.3. Transformation, regeneration and evaluation of transgenic plants

Recombinant vector was transferred into tobacco through *A. tumefaciens* followed by the method of Topping (Topping., 1998). The transgenic tobacco lines were examined by PCR and evaluated stress tolerance as culturing them on culturing medium supplemented with 250 mM NaCl/l (Wang *et al.*, 2003; Barunava *et al.*, 2010); PEG6000 at a concentration of 2.5% (La *et al.*, 2012) or at high temperature (42°C) based on survey experiments determined resistance of non transfered tobacco.

GB content in transgenic plants was determined by the method of Grieve and Grattan (Grieve and Grattan,1983).

3. RESULTS AND DISCUSSION

3.1. Modifying and clonling *CodA* genes

Genetic information based on nucleotide sequences of synthetic gene segments in pBluescript II SK vector was used to design specific primers concluding two necessary restriction enzymes *Sac*I and *Xba*I as followed table 1.

The nucleotide sequence and amino acid sequence of synthesized *CodA* gene was sequenced and compared with *A. globiformis CodA* gene coded AY304485 on NCBI. The results showed that the nucleotide sequences of the modified *CodA* gene was different with nucleotide sequences of *A. globiformis CodA* gene, but their amino acids were similar (Figure 3 and Figure 4).

The modified *CodA* gene could make protein translated from changed genetic code which are suitable for plant to produce *choline oxidase*, the key enzyme functions synthesis glycinebetaine.

3.2. Designing recombination vector carrying the target gene

Recombination vector can be formed when mixing the segment concluded target gene and receiving vector which were both treated with the similar restriction enzymes,

XbalI/SacI. The result showed in the fig 5, 6 demonstrated that they were ready to make the recombinant vectors. The vectors were inserted to E.coli DH - 5α and tested by colony – PCR and restriction reaction that showed in fig 7.

3.3. Transformation the target gene to tobacco through *A. tumefaciens*

After transformation, there were several pieces of *in vitro tobacco* leaves which had formed new shoots and most of these shoots had formed roots (Table 2).

Some survival of the full shoots, planets was grown in greenhouse and tested the appearance of target genes by PCR (Figure 8).

3.4. Stress tolerance of tobacco lines transferred artificial *CodA* gene

The transgenic tobacco lines were tested some stress tolerance such as heat tolerance, drought tolerance and salinity tolerance. The original results shown in table 3, table 4 and 5 demonstrated that they almost expressed the desired tolerances. In contrast, the non - transferred tobacco had not the tolerance or a little.

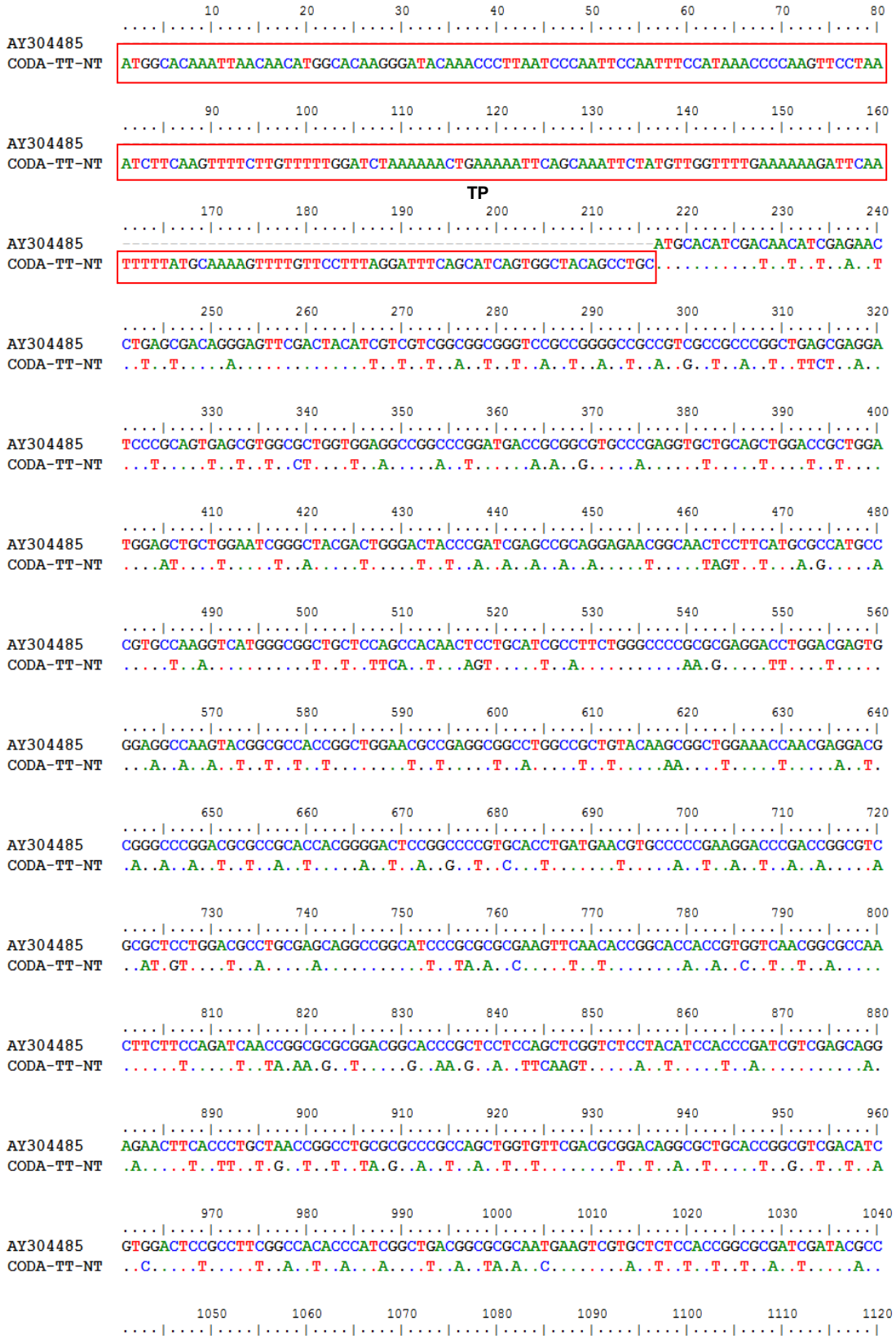
The data provided that all the transferred lines had ability in facing to disadvantage *in vitro* environments such as high temperature (heat), drought, salty. It was expressed in the survival rate and rooting rate of the shoots when they exposed to these conditions.

Table 1. Characterictis of specific primers for cloning *CodA gene and TP - CodA* gene

Number	Name of primer	Nucleotide sequence of primer	Target gene	Tm (°C)	Size (bp)
1	F/*TP - Xba*I	5'GCTCTAGAATGGCACAAATTAACA3'	*TP - CodA*	51	1904
	R/*cmyc - Sac*I	5'CGAGCTCTCAATTCAGATCCTCTTC3'		53	
2	F/*CodA - Xba*I	5'GCTCTAGAATGCACATCGATAATATTGA3'	*CodA*	55	1688
	R/*cmyc - Sac*I	5'CGAGCTCTCAATTCAGATCCTCTTC3'		53	

Figure 2. Electrophoresis photo of PCR products

Note: A: TP - CodA; B: CodA


TP
AY304485
CODA-TT-NT

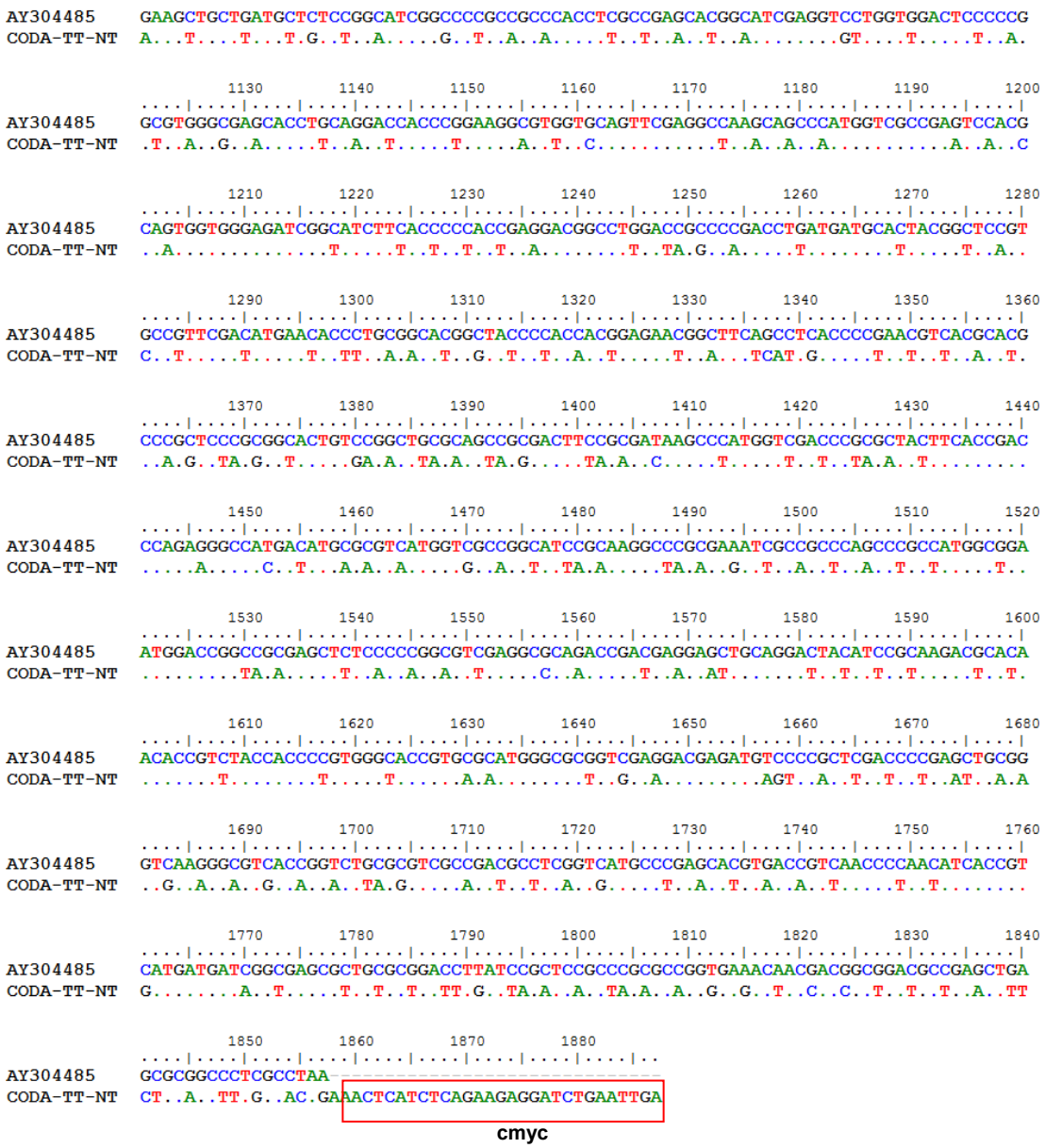


Figure 3. The nucleotide sequence of synthesized *CodA* gene and *A. globiformis'CodA* gene

Note: AY304485: CodA genes of A. globiformis (1641bp, code AY304485);
CODA - TT - NT: Modified TP - CodA - cymc gene

In vitro culturing in the heat condition, 42 °C, transgenic lines had survival rate higher than non - transgenic lines (WT) at the time of 24 day culturing, higher rooting rate than WT at the time 6 days, 12 days and 24 days culturing, significantly. Specifically, the rooting rates were different from 54,32 (± 2,14)% (20.1A line) to 82,72 (± 2,14)% (29A line), meanwhile the rooting rate of WT was 40,74 (± 3,70) %. It originally proved that *CodA* had improved the heat tolerance of transgenic lines.

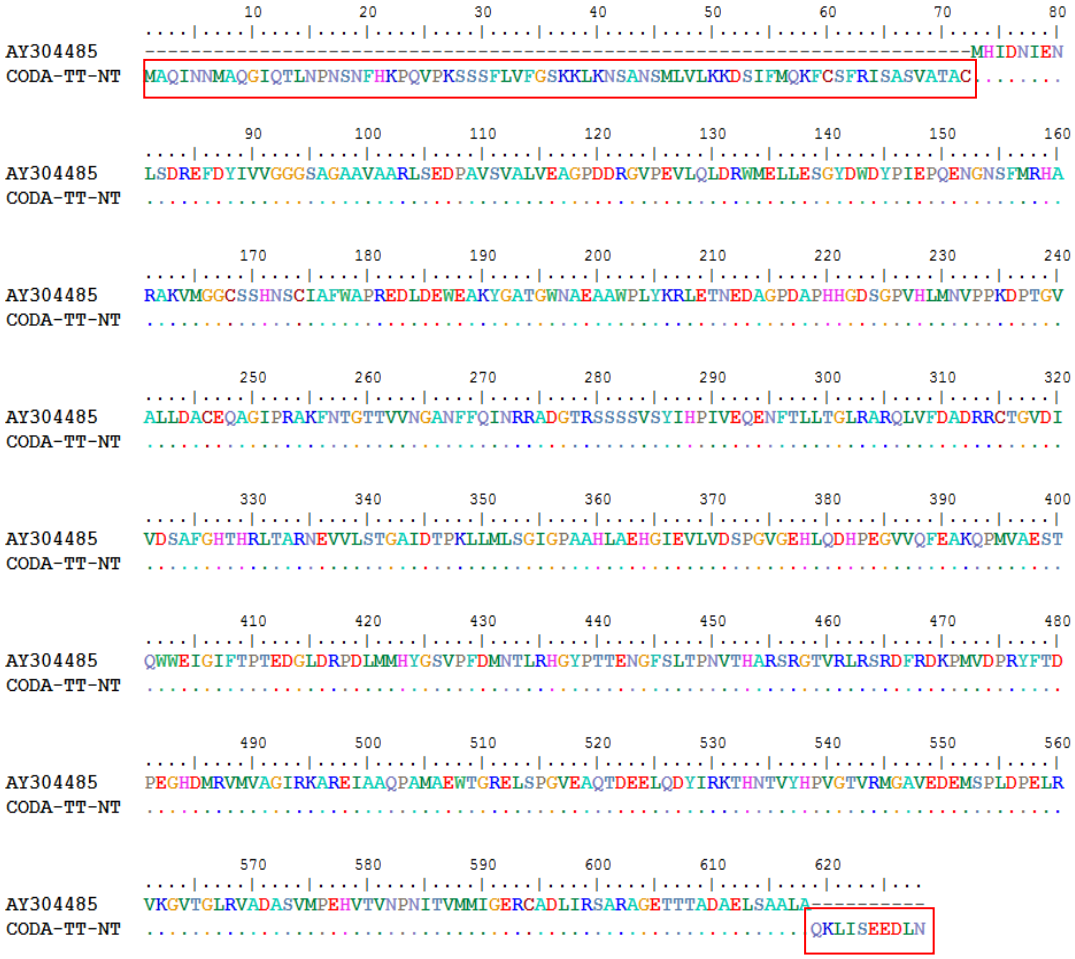
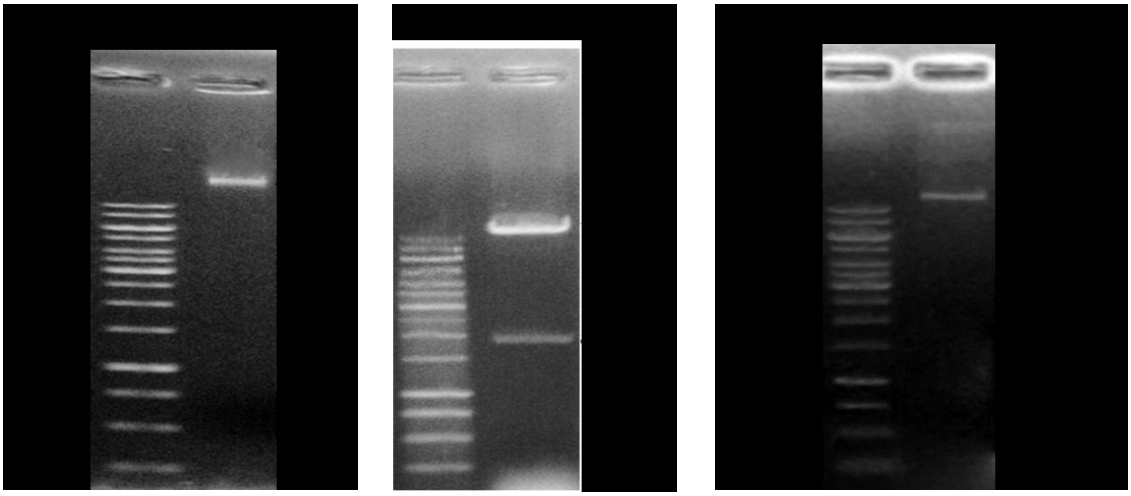


Fig 4. The amino acid sequence of synthesized *CodA* gene and *A.globiformis CodA* gene

Note: AY304485: CodA genes of A. globiformis (1641bp, code AY304485); CODA - TT - NT: Modified TP - CodA - cymc gene

A B

Figure 5. Electrophoresis photos of purified product of *TP - CodA - cmyc* gene sized 1904 bp (A), *CodA - cmyc* gene sized 1688 bp (B) after being treated by *XbaI*/*Sac*I



A B C

Figure 6. Electrophoresis photos of receptor pBI121 plasmid sized 13 kb (A); pBI121 plasmid treated by *Xba*I/*Sac*I(B); purified pBI121 sized 11,2 kb without *Gus* gene (C)

Table 2. Shooting and rooting of transferred tobacco lines

Experiment	Shooting (*)		Rooting (**)	
	Number of sample (piece)	Shooting rate (%)	Number of sample (shoot)	Rooting rate (%)
TN1	110	77,73	198	77.43
TN2	107	77.67	186	86.6
WT1	36	0	-	-
WT2	36	100	112	92.1

Note: (*)MS + 1 mg/l BAP + 30 g/l sucrose + 8 g/l agar + 500 mg/l cefotaxime + 50 mg/l kanamycine, pH = 5,8
(**)MS + 0,1 mg/l IBA + 30 g/l sucrose + 8 g/l agar + 500 mg/l cefotaxime + 50 mg/l kanamycine pH = 5,8
TN1: Leaves were tranferred TP - CodA - cmyc; TN2:Leaves were transferred CodA - cmyc; WT1. Leaves were transferred on culturing added antibiotic; WT2. Leaves were transferred on culturing without antibiotic

Table 3. Heat tolerance of transferred tobacco lines

Line	Survival rate (%)			Rooting rate (%)		
	6 days after	12 days after	24 days after	6 days after	12 days after	24 days after
20.1A	100	100 ± 0,00^{a}	98,77 ± 2,14^{a}	0	38,27 ± 2,14^{b}	54,32 ± 2,14^{c}
43B	100	100 ± 0,00^{a}	100,00 ± 0,00^{a}	0	55,56 ± 3,70^{a}	74,07 ± 3,70^{b}
29A	100	100 ± 0,00^{a}	97,53 ± 4,28^{a}	0	34,57 ± 2,14^{b}	82,72 ± 2,14^{a}
2.1B	100	100 ± 0,00^{a}	95,06 ± 4,28^{a}	0	54,32 ± 4,28^{a}	72,84 ± 2,14^{b}
26A	100	100 ± 0,00^{a}	98,77 ± 2,14^{a}	0	49,38 ± 2,14^{a}	71,60 ± 2,14^{b}
WT	100	72,84 ± 2,14^{b}	69,14 ± 2,14^{b}	0	22,22 ± 3,70^{c}	40,74 ± 3,70^{d}

Note:Condition of culturing: MS + 3% sucrose + 0,1mg/l IBA; 10h light / 14h dark, 420 C,

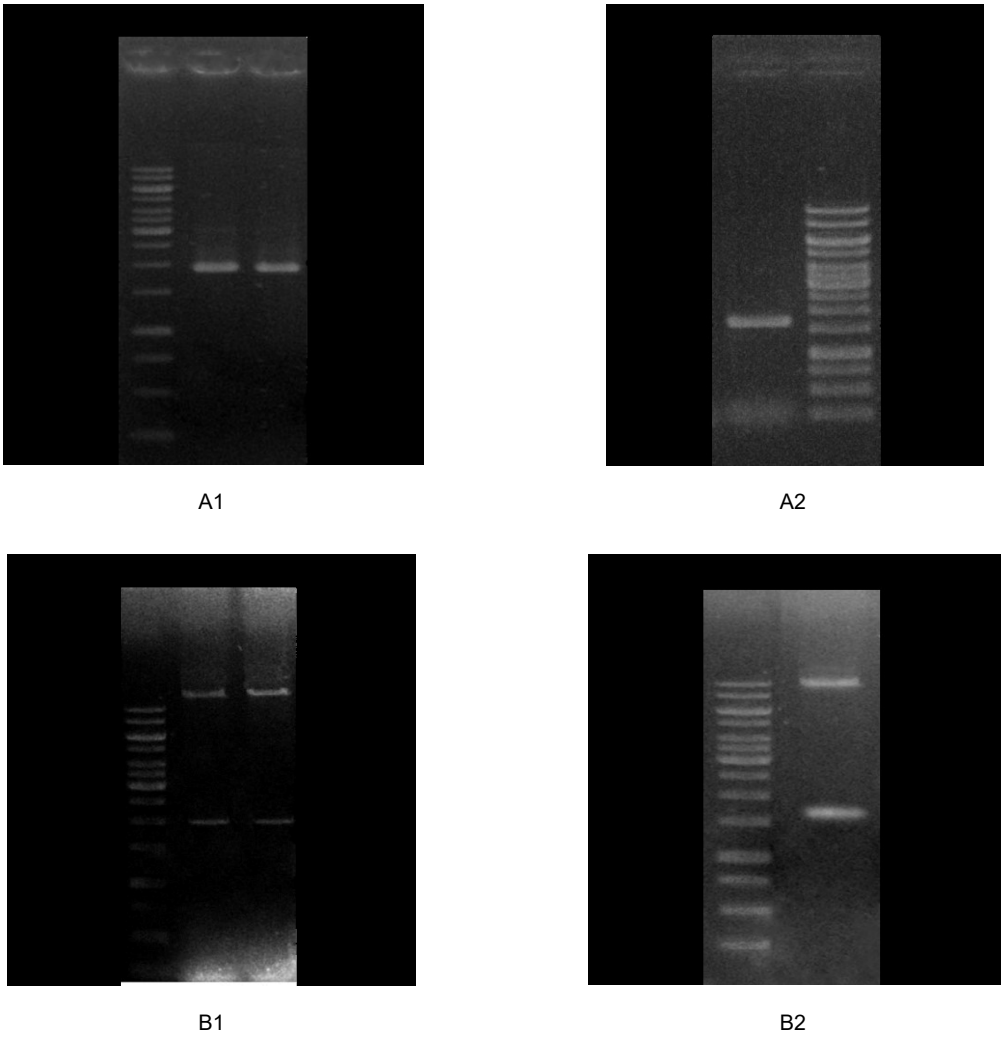


Figure 7. Electrophoresis photo of colony - PCR (A1, A2), recombinant vector treated by *Xba*I/*Sac*I (B1, B2)

Note: A1: 2 colonies, line1 and line 2, carried TP - CodA gene sized 1904 bp; A2: 1colony, line1, carried CodA gene sized 1688bp; B1: 2 recombinant vector, line1 and line 2, carried TP - CodA gene were treated by XbaI/SacI; B2: 1 recombinant vector, line1, carried CodA gene was treated by XbaI/SacI

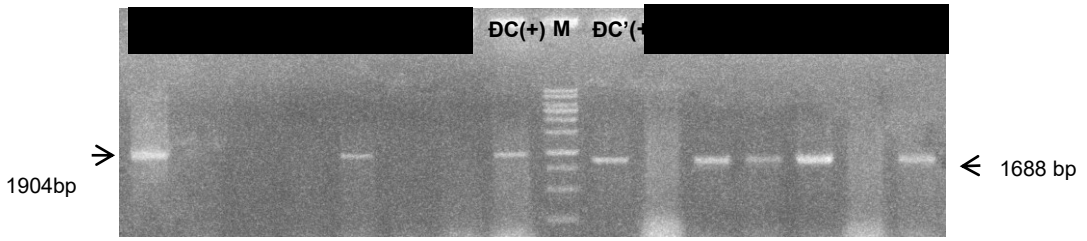


Figure 8. Electrophoresis photos of PCR products of transferred tobacco lines

Note:Line 1 - 7: transferred tobacco lines with TP - CodA - cmyc; Line 1' - 6': transferred tobacco lines with CodA - cmyc; ĐC, ĐC': TP - CodA - cmyc gene and CodA - cmyc gene

Table 4. Drought tolerance of transferred tobacco lines

Line	Survival rate (%)			Rooting rate (%)		
	6 days after	12 days after	24 days after	6 days after	12 days after	24 days after
20.1A	100	100	100 ± 0,00[a]	25,93 ± 3,70[a]	66,67 ± 3,70[a]	81,48 ± 3,70[a]
43B	100	100	100 ± 0,00[a]	0,00 ± 0,00[c]	18,52 ± 3,70[c]	37,04 ± 3,70[d]
29A	100	100	100 ± 0,00[a]	14,81 ± 3,70[b]	51,85 ± 3,70[b]	70,37 ± 3,70[b]
2.1B	100	100	100 ± 0,00[a]	0,00 ± 0,00[c]	6,17 ± 4,28[d]	38,27 ± 4,28[d]
26A	100	100	100 ± 0,00[a]	0,00 ± 0,00[c]	25,93 ± 3,70[c]	51,85 ± 3,70[c]
WT	100	100	88,89 ± 3,70[b]	0,00 ± 0,00[c]	3,70 ± 3,70[d]	27,16 ± 2,14[e]

Note:Condition of culturing: MS + 3% sucrose + 0,1mg/l IBA + 2.5% PEG6000; 25^0 C, 10h light / 14h dark

Table 5. Salinity tolerance of transferred tobacco lines

Line	Survival rate (%)			Rooting rate (%)		
	6 days after	12 days after	24 days after	6 days after	12 days after	24 days after
20.1A	100	65,43 ± 2,14[a]	65,43 ± 2,14[a]	0	2,47 ± 2,14[a]	27,16 ± 2,14[a]
43B	100	32,10 ± 2,14[b]	14,81 ± 3,70[c]	0	1,23 ± 2,14[b]	2,47 ± 2,14[b]
29A	100	62,96 ± 3,70[a]	53,09 ± 2,14[b]	0	2,47 ± 2,14[a]	29,63 ± 3,70[a]
2.1B	100	60,49 ± 4,28[a]	55,56 ± 3,70[b]	0	1,23 ± 2,14[b]	8,64 ± 4,28[b]
26A	100	62,96 ± 3,70[a]	19,75 ± 4,28[c]	0	1,23 ± 2,14[c]	6,17 ± 2,14[b]
WT	100	32,10 ± 4,28[b]	3,70 ± 0,00d	0	0,00 ± 0,00d	1,23 ± 2,14[c]

Note: Condition of culturing: MS + 3% sucrose + 0,1mg/l IBA+ NaCl 250mM/l; 25^0 C, 10h light / 14h dark

Drought tolerance of transgenic lines was also tested *in vitro.* They were cultured *in vitro* drought condition (medium added 2.5% PEG6000), the tolerance to drought were expressed by the rate of survival and rooting in the stress. Specifically, the rooting rate of two lines such as 29A and 20.1A line were 70.37 (± 3.70) % and 81, 48 (± 3.70) %; while the rooting rate of WT was 27.16 (± 2.14) % at the time of 24 days culturing (Table 4).

The transgenic tobacco lines were clearly proved that they had salt tolerance *in vitro* higher than the control, WT as they cultured in medium supplemented NaCl 250 mM/l. Especially, 29A lines and 20.1A line had rooting rate at the time of 24 day culturing were 29.63 (± 3.70) and 27.16% (± 2.14)%, respectively. It were nearly 20 times higher than it of WT, which the rooting rate was 1.23 (± 2.14) % (Table 5).

It was difficult to explain the stress tolerance of plant because of that it depends on several genes with complicated mechanisms (Kazuo *et al.*, 2003). However, it was said that in some plant which have the stress tolerance by the way intensive synthesis and accumulation compatible solutions such as some glucose, glycinebetaine, proline... These solutions can maintain osmotic pressure in cell (Hasegava and Bressan, 2000).

Table 6. Accumulation of GB of transgenic tobacco lines

Order	Line	GB (mM/g)
1	20.1A	2,91 ± 0,10a
2	29A	2,53 ± 0,05b
3	43B	1,77 ± 0,10c
4	2.1B	1,06 ± 0,15d
5	26A	0,73 ± 0,15e
6	WT	0

As some previous research, *Arabidopsis thaliana* were transferred bacteria's CodA gene could stimulate GB but small amount. For example, there were 1,0 µM GB/g in leaf and fresh seed (Hayashi *et al.*,1997; Hayashi *et al.*, 1998); 12,0 - 18,0 µM GB/g in dry seed (Alia *et al.*, 1998); 0,7 - 0,9 µM GB/g in shoot (Sakamoto & Murata, 2000). Some other plant was transferred bacteria *CodA* gene as persimmon, *Diospyros kaki,* accumulated GB with 0,1 - 0,3 µM/g of leaf (Huang *et al.*, 2000); tomato, synthesized GB with 0,1 - 0,3 µM/g in leaf (Park *et al.*, 2004); red gum, *Eucalyptus camaldulensis,* accumulated GB with 0,17 - 0,29 µM/g in leaf (Yu *et al.*, 2009).

In this result, some *CodA* or *TP - CodA* gene transgenic tobacco lines as 20.1A; 26A; 29A; 2.1B; or 43B could accumulate remarkable amount of GB with a mount from 0.73 to 2.91 mM/g, meanwhile the WT almost could not stimulate GB (table 6). GB can gradually strengthen the resistance to environmental stress. Therefore, the artificial *CodA gene* had made plants being capable of enhanced accumulation GB that is necessary to help them be able to adapt to hard climate.

There were several publications that have proclaimed about transforming *CodA* to chloroplast (Hayashi *et al.*, 1997; Hayashi *et al.*, 1998; Alia *et al.*, 1998; Sakamoto & Murata, 2000; Mohanty *et al.*, 2002; Ahmad *et al.*, 2008; Park *et al.*, 2004). One of the reasons is that GB can protect the chloroplast from adverse environment (Park *et al.*, 2007). Additionally, GB stabized chloroplast membrane as it was in hard circumstance that means it could guarante PSII electron transport (Hamilton & Heckathorn, 2001). Beside, some researcher prefer the approach which made the *CodA* facilitate the expression in cytoplasm that it improved the stress tolerance in cell easily (Huang *et al.*, 2000; Park *et al.*, 2007). In our experiment, there were not initially differences between *TP - CodA* transgenic plants and *CodA* transgenic plants. It need to study more to conclude which approach is better.

4. CONCLUSIONS

Succeeded changing the genetic code of *Arthrobacter globiformis' CodA* gene, the modified *CodA* gene which could translate make protein been suitable for plant to produce *choline oxidase* leading synthesis glycinebetaine. Also, the synthesized gene were added to the 5 'end a 216 nucleotide segment encoding a transited peptide (TP) which help transporting enzyme in to chloroplasts.

Transgenic vector carrying *CodA - cmyc* or *TP - CodA - cmyc* gene were successfully designed and transferred into tobacco through *A. tumefaciens* bacteria.

The transgenic tobacco lines accumulated remarkable amount of glycinebetaine and were able to withstand unfavorable conditions such as heat, drought and salinity.

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FOOD SCIENCE

BACTERIOCINS AS FOOD PRESERVATIVES

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ABSTRACT

Hurdle technology is defined as a combination of food preservation methods ensuring the microbial safety as well as the nutritional and organoleptic quality of food products. More than 60 hurdles can be used for food, among which the most effective are temperature (low or high), acidity, water activity, preservatives and microbial biocontrol. Despite these systems, 340.000 human cases of food poisoning are still reported each year within the European Union. This major health issue is also challenged by many other factors, such as the globalization of the food market or the increase of the more susceptible populations, which may increase the risk of food outbreaks.

In this context, the preservation of foods by natural, biological methods is an interesting approach that may solve some of the current food-related issues. Among others, bacteriocins, ribosomally synthesized antimicrobial peptides or proteins are investigated as innovative biopreservation tools given (1) their antimicrobial spectrum, (2) their stability under the heat and pH conditions used in food processing and (3) their safety for humans due to their protease sensitivity. Even tough research in this area is usually oriented towards Lactic Acid Bacteria, increasing attention is paid to bacteriocins produced by *Bacillus subtilis* subsp. *subtilis*. Indeed, their Generally Recognized As Safe (GRAS) status and their ability to produce a wide variety of antimicrobial compounds make them promising candidates for industrial applications.

Within this framework, the main objective of this study is to identify and characterize antimicrobial peptides active against the major food poisoning and food spoilage pathogens (e.g. *Campylobacter* spp., *Salmonella* spp., pathotypes of *E. coli*, *Bacillus* spp. and *Listeria* spp.). More than 300 *B. subtilis* strains have been isolated from different environmental samples and Vietnamese fermented food. The antimicrobial activity of their culture supernatant against numerous pathogens has been investigated by the Spot On Plate method. This screening step allowed the selection of 40 strains showing an inhibition against at least three pathogens. This work is an encouraging first step in the development of natural food biopreservatives for which the market has rapidly grown in the past decade.

Keywords: *Bacillus subtilis,* bacteriocins, biopreservation, Foodborne intoxications.

ULTRASOUND-ASSISTED EXTRACTION AND ANTICANCER ACTIVITY OF PICEATANNOL FROM SIM (*Rhodomyrtus Tomentosa*) SEED

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ABSTRACT

The present study focused on the optimization of the extraction conditions of piceatannol from sim (*Rhodomyrtus tomentosa*) seed by using ultrasonication. The effects of ethanol concentration, temperature, ultrasonication time, and number of extractions on ultrasound-assisted extraction were investigated. The optimized extraction conditions were as follows: ethanol concentration of 60%, temperature of 70ºC, number of extractions of 2 times (30 min for the first time and 15 min for the second time). Under these optimal conditions, the yield of piceatannol was 9.99 ± 0.16 mg per g of dry matter. In comparison with those of conventional liquid-solid method (78.8% ethanol, 85.3ºC and extraction time of 78.8 min), the assistance of ultrasound enables a higher yield of piceatannol with lower ethanol consumption, lower temperature, and shorter time. The freeze-dried piceatannol extract powder exhibited anticancer activity against two cancer cell lines including Panc1 and HT29. This report should be considered as a first step for the production of piceatannol-rich products used as nutraceuticals from sim seed, a by-product of sim wine production.

Keywords: Anticancer activity, piceatannol, *Rhodomyrtus tomentosa* seed, ultrasound-assisted extraction.

1. INTRODUCTION

Sim (*Rhodomyrtus tomentosa* (Ait.) Hassk.) is a shrub of the Myrtaceae family, originating from South-East Asia. It wildly develops in many countries like China, Taiwan, Philippines, Malaysia, Indonesia, Vietnam and has been used in traditional medicine for a long time (Do, 2011; Agro Forestry Tree Database, http://www.worldagroforestrycentre.org). A recent study performed by our group showed that the total phenolic content and antioxidant capacity of the sim fruit were high and similar to those of other berry fruits, which are well known as rich sources of antioxidant phenolic compounds (Lai *et al.*, 2015). More interestingly, the sim fruit contains piceatannol, a potent bioactive compound, with a concentration being 1000-2000 times higher than that of red grape, a major source of stilbenes in human diet (Lai et al., 2013). In the sim fruit, nearly 95% of piceatannol concentrated in the seed part (Lai *et al.*, 2014) which is by-product of the sim wine or sim juice production. The sim seed may thus be used as starting material to produce piceatannol extracts, which could be further used in the food or pharmaceutical industries.

In recent years, ultrasound-assisted extraction has attracted growing interest, as it is an effective method for the rapid extraction with high efficiency of bioactive compounds from plants (Bandar *et al.,* 2013). This technique have successfully been used to extract phenolic compounds

from areca husk (Chen *et al.,* 2014; Wang *et al.*, 2013), antioxidant compounds from *Morus alba* L. (Thong *et al.,* 2014), flavonoids from *Eriobotrya japonica* Lindl. flowers (Zhou *et al.,* 2011), and piceatannol from passion seeds (Lai *et al.*, 2016).

The present study had two purposes: the first one was to optimize the ultrasound-assisted extraction parameters of piceatannol from sim seed and the second was to investigate the anticancer activity of the piceatannol extract freeze-dried powder against some cancer cell lines.

2. MATERIALS AND METHODS

2.1. Sample collection and preparation

The mature sim fruits (*Rhodomyrtus tomentosa*) were collected in Sao Do, Hai Duong province in August 2013. The fruits were placed in a plastic box, kept on ice and transported to the laboratory on the same day. In the laboratory, the seeds were isolated from the fruits and washed by tap water then dried under sunlight. The dried seeds were ground using a TecatorCyclotec 1093 sample mill (Sweden), kept in a sealed plastic bag and stored at -53°C until extraction. For the production of piceatannol extract powder used in anticancer test, piceatannol in the sim seed was extracted two times (30 min for the first time and 15 min for the second one) by using ethanol 60% (*v/v*), at 70°C with assistance of ultrasound of 37 kHz/600W. The extract was then centrifuged, concentrated, freeze-dried and stored at 4°C for further anticancer test.

2.2. Chemicals and reagents

Piceatannol standard, ethylene diamine tetraacetic acid (EDTA), dihydro ethidium (DHE), and propidium iodide were purchased from Sigma–Aldrich (St. Louis, MO). Ethanol of analytical grade, acetonitrile and acetic acid of HPLC grade were obtained from Merck (Darmstadt, Germany).

2.3. Ultrasound-assisted extraction of piceatannol and determination by HPLC

Ultrasound-assisted extraction was performed in an ultrasonic cleaning bath (Elma S60H, Germany) with a useful volume of 6 L. Working frequency and power were fixed at 37 kHz and 600 W, respectively. Approximately 0.25 g of powdered dried sample was mixed with 5 mL of solvent (ethanol at different concentration) in a Falcon 15 mL conical centrifuge tube. The tube then was placed in the bath and sonicated for different times at the required temperatures. After centrifugation at 3,642 *g* (6,000 rpm) for 10 min at 4°C, the supernatant was collected. The solution was filtered through a 0.42 μm syringe filter (Phenex™-NY, Utrecht, The Netherlands) before analysis by using HPLC-UV/VIS.

Quantification of picetananol in the extract was performed by HPLC using a Shimadzu system (Japan) equipped with a LC-10Ai pump, a DGU-20A3 degasser, a SPD-20A UV/VIS detector, and a CBM-20A interface as described in our recent study (Lai *et al.,* 2016). Piceatannol in the extract was identified by its retention time as compared to authentic standard and was quantified using five-point calibration curves ($y = 10.03x – 807.91$; $R^2 = 1$).

2.4. Anticancer activity analysis

- Analysis of reactive oxygen species (ROS) and cell cycle arrest

Analysis of ROS, cell cycle arrest and apoptosis were done as described by Kitanovic *et al.,* (2009). Two humanPanc1 (pancreatic carcinoma) and HT29 (colon

adenocarcinoma) cell lines were purchased from Sigma-Aldrich (Germany). Cancer cells were plated in 12-well plate at a density of 200,000 cells/well and cultivated in standard condition for 24 h before cells were treated with extract as described in the text. Cells were collected by trypsinization and centrifugation with 200 g, and resuspended in 2 mL of FACS (fluorescence activated cell sorting) buffer (1% BSA in phosphate buffered saline (PBS)). For ROS determination, cell suspension was supplemented with 5 µM dihydroethidium. After 15 min incubation at room temperature in the dark cells were washed with FACS buffer. For cell cycle arrest analysis, cell suspension was incubated with RNase A (50 µg/mL) for 30 min at 37°C and sequentially stained with propidium iodide (PI, 50 µg/mL) for 1 h and analysed by FACS (fluorescence activated cell sorting). At least two-independent experiments were performed. After staining with specific chemicals and incubation, all aliquots of cell suspension were immediately analysed using a FACSCalibur flow-cytometer (Becton Dickinson) and CellQuest Pro (BD) analysis software.

- Cell cytotoxicity assay

Effects of the piceatannol extract powder (0.02 mg/mL) on cell growth were determined using the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assay (MTT assay, ATCC company, Germany) at an initial cell density of 5,000 cells/well in a 96-well plate. The protocol was performed according to the instructions of the manufacture (ATCC, USA).

- Immunoblot

Immunoblot assay was performed according to protocol of Cheng et al., (2014). Cell extracts were homogenized in urea-lysis buffer (1 mM EDTA, 0.5% Triton X-100, 5 mM NaF, 6 M Urea, 1 mM Na_3VO_4, 10 µg/mL Pepstatin, 100 µM PMSF and 3 µg/mL Aprotinin in PBS). For this 40 µg of total protein was resolved on 10% SDS-PAGE gels and immunoblotted with specific antibodies, Poly (ADP-ribose) Polymerase (PARP, cat: #4855, Sigma Aldrich, Germany). Primary antibodies were incubated at a 1:1,000 dilution in TBS (pH 7.5) with 0.1% Tween-20 and 5% BSA/non- fat milk with gentle agitation overnight at 4°C. The secondary antibodies were incubated in TBS (pH 7.5) with 5% BSA/nonfat milk and 0.1% Tween-20 at a 1:5,000 dilution for 1 h at room temperature. Finally, enhanced chemiluminescence (ECL) immunoblot analysis was performed.

2.5. Statistical analysis

All extractions were performed in triplicate. The apparent content of piceatannol obtained at different conditions were analyzed by the SAS 9.0 software (SAS Institute, Cary, NC) and expressed as mean ± standard deviation. One way analysis of variance (ANOVA) and Duncan test were used to determine the differences amongst the means. p-values < 0.05 were considered to be significantly different.

3. RESULTS AND DISCUSSION

3.1. Ultrasound-assisted extraction of piceatannol from Rhodomyrtus tomentosa seed

- Effect of ethanol concentration

Ethanol concentration showed significant effect on apparent piceatannol content of the sim seed (p < 0.0001). Indeed, the apparent piceatannol content mounted up with an increase in ethanol concentration, reached its highest value (8.84 ± 0.07 mg/g DW) at 60% ethanol and then began to decrease (Table 1).

Table 1. Effect of the ethanol concentration on the apparent piceatannol content of sim seeds

Ethanol concentration (%)	Piceatannol (mg/g DW)	% of piceatannol quantity obtained by optimised conventional extraction
0	1.14 ± 0.05 e	12.74
20	3.07 ± 0.16 d	34.42
40	8.51 ± 0.07 ab	95.44
60	8.84 ± 0.07 a	99.11
80	8.34 ± 0.09 b	93.53
100	7.83 ± 0.44 c	87.78

Note: Values marked by the same letter are not significantly different ($p < 0.05$). Ultrasound-assisted extraction conditions: extraction temperature, 50°C; extraction time, 30 min; ultrasound frequency and power, 37 kHz and 600 W.

An effect of the ethanol concentration in the extraction medium on the phenolic compounds (in general) and on piceatannol (in particular) yield has been observed in various studies. The best ethanol concentrations for the ultrasound-assisted extraction of phenolic compounds from areca husk (*Areca catechu* L.) and from loquat (*Eriobotrya japonica* Lindl.) flowers were 41% and 60%, respectively (Chen et al., 2014; Zhou et al., 2011). The impact of the ethanol concentration is due to its effect on the polarity of the extraction solvent and the resulting solubility of the phenolic compounds. The general principle is "like dissolve like", which means that solvents only extract those phytochemicals, which have a similar polarity to that of the solvent (Lai et al., 2014). As the highest apparent piceatannol content reached maximum when ethanol concentration was of 60%, this concentration was chosen and used in further extraction.

- Effect of extraction temperature

The temperature had significant effect on the piceatannol extraction from sim seed (p = 0.0002) (Table 2). An increase in the apparent piceatannol content was observed over the extraction temperature range (30-70°C). This effect of temperature was in accordance with studies on piceatannol extraction from passion seeds (Lai et al., 2016), and on phenolic extraction areca husk (Chen et al., 2014). An increase in the extraction temperature may increase the solubility of piceatannol in the solvent and decrease the viscosity of the solvent. The combination of these to phenomena enhanced the overall extraction efficiency (Chen et al., 2014). However, the phenolic yield, after having highest value, decreased when the extraction temperature increased due a possible concurrent decomposition of phenolic compounds. In this study, because of the low capacity of the ultrasonic cleaning bath, the extraction temperature could not be higher than 70°C. As the highest apparent piceatannol content of the sim seed was obtained at 70°C, this temperature was chosen for the piceatannol extraction from sim seed with assistance of ultrasound.

- Effect of extraction time

The amounts of piceatannol extracted from sim seeds as a function of sonication time are presented in Table 3. Apparent piceatannol content of the sim seed increased during the first 30 min with the rate of 0.32 mg/g DW per minute before became stable. This result agreed with other researches on the phenolic extraction from plant materials. For example, Chen et

al., (2014) found that total phenolics extracted from areca husk increased markedly up to 30 min, then, remained constant at 40 min. Ultrasonic extraction of flavonoids and phenolics from loquat flowers showed that the extraction rate became slow after 80 min (Zhou et al., 2011). In this study, extraction time of 30 minutes was chosen for the sake of saving time and energy.

- Effect of time of extraction

Piceatannol in sim seed was extracted one time, two times and three times. The results presented in Table 4 and showed that two time of extraction permitted to obtain nearly total quantity of piceatannol in the sim seed. Thus, two steps of extraction with 30 and 15 minutes were chosen. In comparing with the conventional extraction method previously optimized by our group (78.8% ethanol, 85.3°C and extraction time of 78.8 min), the use of ultrasound permitted to have a higher yield of piceatannol with lower ethanol consumption, lower temperature, and shorter time.

Table 2. Effect of the extraction temperature on the apparent piceatannol content of sim seeds

Temperature (°C)	Piceatannol (mg/g DW)	% of piceatannol quantity obtained by optimised conventional extraction
40	8.19 ± 0.20 c	91.85
50	9.03 ± 0.07 b	101.21
60	9.13 ± 0.29 ab	102.35
70	9.46 ± 0.06 a	106.01

Note: Values marked by the same letter are not significantly different ($p < 0.05$). Ultrasound-assisted extraction conditions: ethanol concentration, 60% (v/v); extraction time, 30 min; ultrasound frequency and power, 37 kHz and 600 W.

Table 3. Effect of the extraction time on the piceatannol content of sim seeds

Time (minutes)	Piceatannol (mg/g DW)	% of piceatannol quantity obtained by optimised conventional extraction
5	8.32 ± 0.26 c	93.23
15	8.84 ± 0.13 b	99.10
30	9.46 ± 0.06 a	106.01
60	9.21 ± 0.10 a	103.30

Note: Values marked by the same letter are not significantly different ($p < 0.05$). Ultrasound-assisted extraction conditions: ethanol concentration, 60%; extraction temperature, 70°C; ultrasound frequency and power, 37 kHz and 600 W.

Table 4. Effect of the time of extraction on the piceatannol content of sim seeds

Time of extraction	Piceatannol (mg/g DW)	% of piceatannol quantity obtained by optimised conventional extraction
1 (30 minutes)	9.15 ± 0.22 b	102.63
2 times (30 and 15 min)	9.99 ± 0.16 a	112.01
3 times (30, 15 and 15 min)	10.13 ± 0.14 a	113.58

Note: Values marked by the same letter are not significantly different ($p < 0.05$). Ultrasound-assisted extraction conditions: ethanol concentration, 60%; extraction temperature, 70°C; ultrasound frequency and power, 37kHz and 600W.

3.2. Anticancer activity of the piceatannol extract powder

- ROS formation, cell cycle arrest and cell cytotoxicity assay

High intracellular ROS levels pose a significant threat to cellular integrity and can lead to mitochondrial DNA damage, and subsequent induction cell cycle arrest for DNA repair and for programed cell death (apoptosis). Apoptosis plays a crucial role in the normal development of the cell and in the inhibition of tumor growth and development (Kim et al., 2011). Apoptosis could be induced by intrinsic or extrinsic pathways, and a variety of agents including biological or chemical compounds. Of which are various cytotoxic substances. The results illustrated in Figure 1, calculated, and summarized in Table 5 showed that the piceatannol extract powder from sim seed induced high intracellular ROS level in two cancer cell lines, Panc1 and HT29. The ROS level generated by Panc1 and HT29 cells upon treatment with piceatannol extract powder were 4.2 ± 0.21 and 5.1 ± 0.32 times higher than that formed by untreated cancer cell, respectively. To cope with the damage of high ROS level, cell cycle arrest was triggered at G2/M phase before cell division, thereby inhibiting cancer cell to develop. Moreover, the anti-proliferative or cytotoxic effect of piceatannol extract powder on Panc1 and HT29 was also indicated by IC_{50} values of 8.1 ± 0.03 and 7.2 ± 0.01 µg/mL in the respective cell lines. Thus, piceatannol extract powder induced significant high ROS level to induce cell cycle arrest (Table 5).

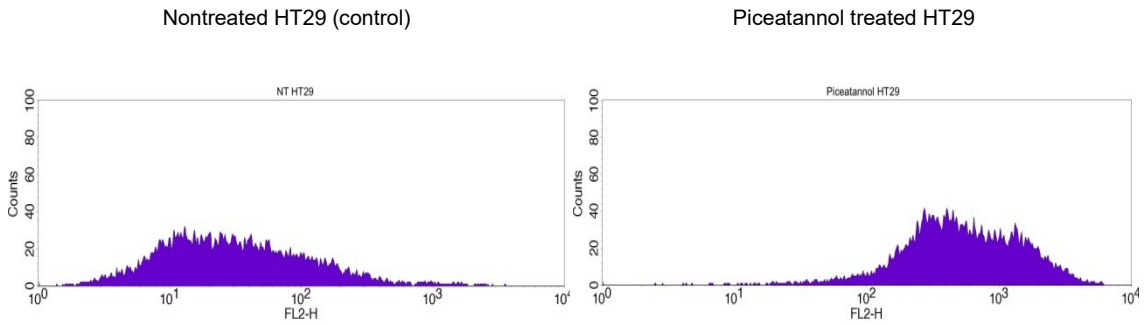

Figure 1. ROS formation of HT29 cell line without treatment (left panel) and upon treatment with piceatannol (right panel)

Table 5. Anticancer activity of piceatannol in some human cancer cell lines

Cell line	Cytotoxicity* (IC_{50}, µg/ml)	ROS** (Fold)	Cell cycle arrest*** (Phase)
Panc1	8.1 ± 0.03	4.2 ± 0.21	G2/M
HT29	7.2 ± 0.01	5.1 ± 0.32	G2/M

*Note: *Half maximal inhibitory concentration (IC_{50}) of compound in inhibiting cell growth was calculated from dose-response curves in three independent experiments;*
***ROS expressed by fluoresence intensity was calculated by normalisation of ROS level of treated cells to that of untreated cells (control) giving folds or times;*
****Cell cycle at which certain phases are arrested in the specific point of cell division cycle*

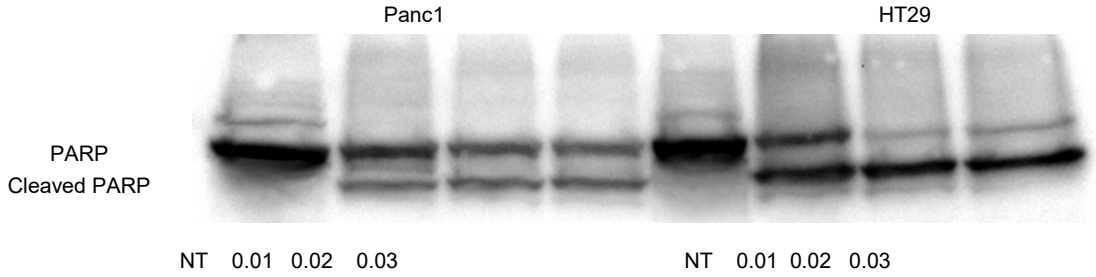

Figure 2. Induction and degradation of PARP of two cell lines in response to treatment with different concentrations of piceatannol extract powder (0.01–0.03 mg/mL)

- Immunoblot assay

In addition, the anticancer activity of piceatannol extract powder was performed and confirmed by induction and degradation of poly (ADP-ribose) polymerase (PARP, Figure 2). PARP belongs to a family of proteins involved in DNA repair and programmed cell death (apoptosis). The role of PARP is to detect single-strand DNA breaks (SSB). PARP can be activated or highly expressed in cells experiencing stress and/or DNA damage. After repairing or expression, the PAR chains are degraded via poly (ADP-ribose) glycohydrolase (PARG). Cleavage of PARP by intracellular enzymes such as caspases or cathepsins forms smaller fragments. The size of the cleaved fragments can give insight into the cells and determine that cell death pathway has been activated. Indeed, treatment of Panc1 and HT29 cell lines with different concentrations of piceatannol leads to induction and degradation of PARP (Figure 2). This could be said that PARP is activated or expressed when responsible for DNA repair and apoptosis, and inactivated or degraded into shorter fragments by caspase cleavage after repairing and induction of apoptosis.

In comparison of the results from this study with those from other, the piceatannol extract was also effective in showing anticancer activity against Panc1 cell line as compared with the synthetic compounds, gold(I)NHC complex (MC3) (Cheng *et al.,* 2014; Ewton *et al.,* 2011). However, to understand and evaluate the overall anticancer activity of piceatannol, this compound or its derivative will be tested with other main human cancer cell lines, UM-UC-10 (bladder), SVCT (breast), MDST8 (colon), thereby the action mechanism of piceatannol could be more elucidated. Additionally, in order to use piceatannol as functional food products or to develop pharmaceutical materials, further studies, such as bioavailability, bioequivalence, safety, tolerability, and clinical trials should be investigated.

4. CONCLUSIONS

Ultrasound enhanced piceatannol extraction from the sim seeds. Ultrasound-assisted extraction conditions were determined and were as followings: ethanol concentration, 60% (*v/v*); extraction temperature, 70°C; ultrasound frequency and power, 37 kHz and 600 W; and extraction time, 30 minutes. The piceatannol freeze-dried extract powder from sim seed showed anticancer activity on two cancer cell lines, Panc1 and HT29. This study provided first bases for the production of piceatannol-rich products to be used as nutraceuticals from this by-product of food technology.

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SCREENING AND CHARACTERIZATION OF β – GLUCANASE PRODUCED BY *Bacillus* spp. ISOLATED FROM MUONG KHUONG CHILI SAUCE

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ABSTRACT

β-glucanase is produced by a large diversity of microorganisms including Bacillus species. In this study, 46 strains of Bacillus spp. isolated from Muong Khuong chili sause were used to screen enzyme activity and characterize enzyme production. Screening of β - glucanase producing bacterial isolates was modified based on the description of Khatiwada *et al*., (2016) and β - glucanase activity assay was done as described by Khianngam *et al.,* (2014). The result showed that from 46 strains of Bacillus spp. isolated, TO40.38, TO47.3, TO64.4 strains produced highest beta - glucanase activity. The growth of Bacillus strains was proportional to their enzyme production. Temperature, time and pH of culture medium suitable to production of enzymes were 37°C, 30h and pH 7 respectively. Besides, the enzyme activity of TO64.4 was strongly affected by heat treatment condition, pH and metal ions. Highest enzyme activity reaches at 60°C, pH 6. Enzyme activity was inhibited or inactivated by Ca^{2+}, Cu^{2+}, Fe^{2+}, K^{+}, Zn^{2+}.

Keywords: β – glucanase, *Bacillus* spp., enzyme production, enzyme activity, Muong Khuong chili sauce.

1. INTRODUCTION

β-glucanase which hydrolyzes β-glucan to glucose monomer or saccharide oligomer is an important enzyme in food industry (Dewi *et al*., 2016). β-1,4-endoglucanase was applied in beer brewing and animal feed by decreasing the viscosity of β-glucan solution (Pandey *et al.,* 2014). In addition, this enzyme also was used as a biological control agent against fungal diseases (Dewi *et al*., 2016).

β-glucanase is produced by a large diversity of microorganisms such as fungi, bacteria and invertebrates (Padilha *et al*., 2015). However, production of β-glucanase from bacteria has more advantages than that from fungi (Seo *et al*., 2013). In which, the cost of fungal enzyme production is very high (Amore *et al.,* 2012). Meanwhile, bacteria have high growth rate and short generation (*Seo et al*., 2013; Khatiwada *et al.,* 2016). Hence, many researches on the ability of production of β-glucanase by bacteria have been doing. Several bacteria appear β-glucanase activity including Bacillus specie such as Bacillus clausii (Aono *et al.,* 1995), B. halodurans (Akita *et al*., 2005), B. licheniformis (Chaari *et al*., 2012), B. subtilis (Manjula and Podile., 2005; Tang *et al*., 2004; Leelasuphakul *et al.,* 2006; Narasimhan *et al.,* 2013).

β-glucanase produced by Bacillus species isolated from different sources have dissimilar characteristics (Khatiwada et al., 2016; Seo *et al.,* 2013; Padilha *et al*., 2015). Special material sources can isolate strains with exceptional abilities. Hence, in this study, Muong khuong chili sauce - a local dish of mountainous region with very

spicy taste, was used for isolation of *Bacillus* spp.

Besides, secretion of β-glucanase from bacteria was strongly influenced by time cultivation, and pH and temperature. The enzyme activity was also affected by heat treatment, metal ions (Khatiwada *et al.*, 2016). The objective of this study is to do screening and characterization of β-glucanase production by *Bacillus* spp. isolated from Muong Khuong chili sauce.

2. MATERIAL AND METHODS

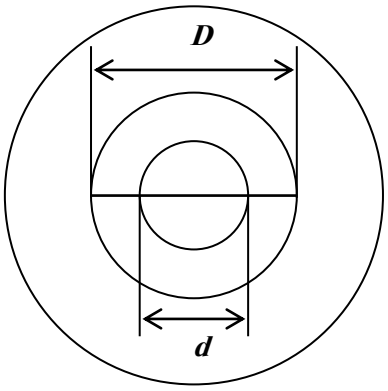
2.1. Materials

46 strains Bacillus spp. isolated from 34 Muong Khuong chili sause sample were stored in glycerol-stock 50% at -80°C for screening and characterization of β-glucanase.

2.2. Methods

2.2.1. Screening of β-glucanase producing bacterial isolates

Screening of β-glucanase producing bacterial isolates was modified based on the description of Khatiwada *et al.* (2016). The isolate was incubated in basal media (g/l) CMC (10), KH_2PO_4 (1), K_2HPO_4 (1), $MgSO_4$ (0.4), NaCl (0.05), and $FeSO_4$ (0.00125), pH 7.0 at 37°C with agitation 150 rpm in 24h for β-glucanase production. The cell free supernatant was obtained by centrifugation at 8000 rpm for 15 min at 4°C. 100 µl of cell free supernatant was poured on wells with diameter of 6mm which already have made in carboxymethyl cellulose agar plates (g/l) CMC (2); yeast extract (0.25); KH_2PO_4 (3.5); $MgSO_4$ (0.625); 5g KNO_3 (5); agar (20); pH 7. The plates were incubated at 4°C and 37°C for 30 minutes and 24h respectively. After incubation time, plates were flooded with Lugol's iodine solution. A clear zone formed around the wells indicates the enzyme hydrolysis of CMC. The highest β-glucanase activity assumed by the largest clear zone.



The β-glucanase activity is determined through the ability of CMC hydrolysis: D – d (mm)

With D: diameter of clear zone;

d: diameter of agar well.

2.2.2. β - glucanase activity assay

β-glucanase activity assay was followed as described by Khianngam *et al.* (2014). β-glucanase activity was determined by estimating the reducing sugar produced during enzymatic reaction by using dinitrosalicylic acid (DNS). A reaction mixture composed of 0.5ml of enzyme, 0.5 ml of 0.05 M citratephosphate buffer (pH 7.0) and 1.0 ml of 1% (w/v) CMC in 0.05 M citrate - phosphate buffer (pH 7.0) was incubated at 37°C, 150rpm for 15 min. The reaction was terminated by adding 1.5 ml of DNS reagent. After boiling the mixture for 5 min, the color was developed and the samples were measured using a colorimeter as the absorbance at 540 nm. One unit (IU) of β-glucanase activity was defined as the amount of enzyme required to release 1 µmol of glucose per min under assay condition.

2.2.3. Effect of time, temperature incubated, pH medium on growth of Bacillus spp. *isolates and β-glucanase production*

To study the effects of time, temperatureof cell cultivation and pH medium on growth of *Bacillus* spp. and β-glucanase production, isolates were cultured in basal media with different pH (5, 6, 7, 8, 9) at different temperatures of incubation for various time. Enzyme activity was measured based on clear - zoneson agar plates as above, the growth of bacteria was calculated by absorbance at 600nm.

2.2.4. Effect of temperature, pH, metal ions on β - glucanase activity produced by Bacillus spp.

The effect of temperature, pH, metal ions on β - glucanase activity produced by Bacillus spp. was investigated. The isolate was incubated in basal media at 37°C, 150rpm in 24h. The cell free supernatant was obtained by centrifugation at 8000 rpm for 15 min at 4°C. Crude enzyme were incubated at a range of temperatures (20, 30, 37, 40, 50, 55, 60, 65, 70, 80°C), pH was adjusted at 3, 4, 5, 6, 7, 8, 9. The metal ions used in this research include Ag^{+}, Ca^{2+}, Cu^{2+}, Fe^{2+}, K^{+}, Zn^{2+}, Ni^{+}. Enzyme activities were assayed as described previously. Residual activity was calculated as relative (%) value to control.

3. RESULTS AND DISCUSSIONS

3.1. β-glucanase screening of isolated *Bacillus* spp.

From 34 chili sauce samples, 46 strains suspected to be *Bacillus* spp. which got all positive results with Gram stain, spore formation, catalase, motility, starch hydrolysis and methyl red reaction were collected for screening of β-glucanase activity. Results of enzyme screening in Table 1 showed that TO40.38, TO47.3, TO64.4 strains gave the better enzyme activity.

3.2. Effect of incubation time, temperature of cell cultivation, pH medium on β-glucanase production of *Bacillus* spp.

3.2.1. Effect of incubation time

There is a correlation between the growth rate and β-glucanase production from Bacillus spp (Table 2). As the time from 18h, enzyme production was increased. However, it was started to decline as time increased above 30 hour. After 30 hours of incubation, all of three strains produced a higher amount of β - glucanase compare to other incubation time.

This might be explained that 30 hours of incubation was sationary phase time of Bacillus spp. and maximum number of viable bacteria reached , therefore enzymes were produced more at this time. Similar finding was also reported by Do Thu Ha (2004). The optimal time for incubation of three strains was achieved at 30 hours.

3.2.2. Effect of pH

At examine pH, all three strains grew and produced enzyme. The range of pH at which the bacterial strains had good enzyme activity was from 6 to 8. It was found that these three strains were capable of producing the enzyme at a broad pH range (Table 3). This made it easy to adapt to environmental conditions. The highest β-glucanase production was found at pH 7. At pH 5 and 9, the enzyme activity was decreased. This is probably explained, at pH too high or too low, the growth of bacterial strains was inhibited. If bacteria survive, the enzyme secreted by them will be inhibited by extreme pH and loss their activity.

Table 1. β - glucanase production of collected bacterial strains

No.	Code of strains	Clear zone diameter (mm)	No.	Code of strains	Clear zone diameter (mm)
1	TO32.4	5	24	TO48.5	0
2	TO32.11	1	25	TO49.5	3
3	TO32.13	0	26	TO51.2	5
4	TO34.5	2	27	TO51.4	4
5	TO35.3	4	28	TO51.5	6
6	TO35.12	0	29	TO52.1	2
7	TO35.18	9	30	TO52.3	5
8	TO36.35	6	31	TO52.5	7
9	TO39.5	2	32	TO53.2	4
10	TO40.38	15	33	TO53.4	6
11	TO41.5	6	34	TO53.6	0
12	TO41.11	3	35	TO54.3	11
13	TO41.14	5	36	TO54.4	6
14	TO41.15	8	37	TO56.2	11
15	TO43.10	6	38	TO56.3	5
16	TO43.13	11	39	TO59.2	0
17	TO44.10	3	40	TO59.3	6
18	TO46.5	4	41	TO59.5	8
19	TO46.6	6	42	TO60.4	11
20	TO46.7	10	43	TO61.1	3
21	TO47.3	14	44	TO61.11	9
22	TO47.10	5	45	TO64.4	15
23	TO48.4	8	46	TO64.6	6

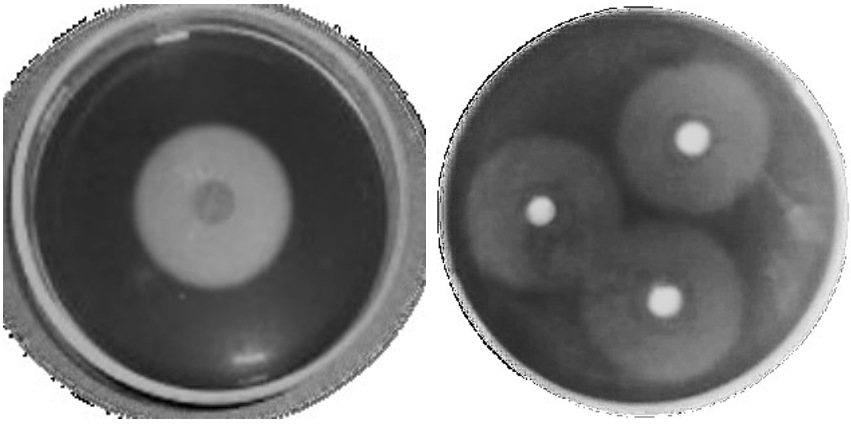


Figure 1. Clear - zones of TO40.38 and TO64.4

Table 2. Effect of incubation time on β-glucanase production from Bacillus spp.

Time (h)	TO40.38		TO47.3		TO64.4	
	OD_{600}	Clear - zones diameter (mm)	OD_{600}	Clear - zones diameter (mm)	OD_{600}	Clear - zones diameter (mm)
18	$1.762^{e} \pm 0.001$	11^{d}	$1.511^{c} \pm 0.000$	12^{d}	$1.522^{d} \pm 0.007$	11^{d}
24	$2.254^{b} \pm 0.002$	17^{c}	$1.834^{b} \pm 0.007$	15^{c}	$1.987^{b} \pm 0.026$	14^{c}
30	$2.521^{a} \pm 0.010$	24^{a}	$2.162^{a} \pm 0.002$	23^{a}	$2.365^{a} \pm 0.010$	25^{a}
36	$2.312^{c} \pm 0.162$	20^{b}	$1.922^{b} \pm 0.037$	20^{b}	$2.052^{b} \pm 0.079$	21^{b}
42	$1.932^{d} \pm 0.055$	16^{c}	$1.763^{b} \pm 0.128$	16^{c}	$1.821^{c} \pm 0.127$	15^{c}

Table 3. Effect of pH on β-glucanase production from Bacillus spp.

pH	TO40.38		TO47.3		TO64.4	
	OD_{600}	Clear - zones diameter (mm)	OD_{600}	Clear - zones diameter (mm)	OD_{600}	Clear - zones diameter (mm)
5	$2.321^{c} \pm 0.038$	14^{b}	$1.543^{c} \pm 0.001$	12^{b}	$2.076^{d} \pm 0.156$	12^{c}
6	$2.745^{b} \pm 0.012$	12^{c}	$1.642^{b} \pm 0.010$	13^{b}	$2.396^{b} \pm 0.005$	15^{b}
7	$2.922^{a} \pm 0.008$	17^{a}	$1.842^{a} \pm 0.010$	18^{a}	$2.676^{a} \pm 0.012$	17^{a}
8	$2.716^{b} \pm 0.031$	11^{c}	$1.695^{b} \pm 0.008$	12^{b}	$2.571^{a} \pm 0.009$	14^{b}
9	$2.385^{c} \pm 0.004$	13^{b}	$1.531^{c} \pm 0.004$	13^{b}	$2.206^{c} \pm 0.026$	12^{c}

Table 4. Effect of temperature on β-glucanase production from Bacillus spp.

Temperature (°C)	TO40.38		TO47.3		TO64.4	
	OD_{600}	Clear - zones diameter (mm)	OD_{600}	Clear - zones diameter (mm)	OD_{600}	Clear - zones diameter (mm)
23	$1.555^{c} \pm 0.009$	7^{d}	$1.302^{c} \pm 0.009$	6^{d}	$1.085^{d} \pm 0.010$	8^{c}
30	$2.371^{b} \pm 0.017$	13^{b}	$1.584^{b} \pm 0.026$	14^{b}	$2.281^{b} \pm 0.099$	13^{b}
37	$2.881^{a} \pm 0.071$	23^{a}	$1.905^{a} \pm 0.003$	23^{a}	$2.677^{a} \pm 0.008$	25^{a}
44	$1.535^{c} \pm 0.068$	9^{c}	$1.423^{b} \pm 0.047$	9^{c}	$1.735^{c} \pm 0.032$	8^{c}
51	$0.571^{d} \pm 0.053$	2^{e}	$0.631^{d} \pm 0.024$	3^{e}	$0.681^{e} \pm 0.038$	3^{d}

3.2.3. Effect of temperature

The cultivation temperature has major influenced on the growth rate as well as on the β-glucanase activity. Each enzyme has an optimum temperature at which it performs the best activity. Below or above this temperature, the enzyme activity is reduced or loss. Acording to Table 4, as the temperature increased from 23°C, enzyme activity was increased, however,it was started to decline as temperature increased above 44°C. β-glucanase enzyme acts well at temperature ranging from 30°C to 37°C. The optimal temperature of cell cultivation was achieved at 37°C.

3.3. Effect of temperature, pH, metal ions on β-glucanase activity produced by TO64.4 strain

3.3.1. Effect of temperature on β-glucanase activity

The results showed that the highest enzyme activity was achieved at 55 - 65°C. Too low or too high temperature made enzyme be reduced its activity. The enzyme activity got maximum of 0.0131 U/ml at

60°C and decreased when temperature increased or reduced. The achieved optimal temperature in this research was higher than that reported by Otajevwo *et al.* (2011) (*Bacillus* subtilis and *Bacillus* circulans recorded optimal cellulolytic activities at 35°C) and a broad range of optimum temperatures from 20 - 40°C was achieved for Bacillus licheniformis JK7 (Seo *et al.*, 2013).

This could explain that too high or too low temperature was not suitable for enzyme activity. The optimal temperature of the enzyme β-glucanase produced by TO64.4 strain is about 55 - 65°C.

3.3.2. Effect of pH on β-glucanase activity

It was found that, at pH = 6, the enzyme β - glucanase activity was at the highest (0.0092 U/ml) and was used as standard activity (100%) for residual activity measurement of enzyme incubated at other pH. With a pH of 5 is relatively high activity accounted for 76% compared to pH = 6. Similarly in an acidic or basic pH, the residual activity of the enzyme β-glucanase was remained only 9%, at pH = 9 residual activity was 16%. In a neutral pH, the residual activity of the enzyme was 55%. So optimal pH of the β-glucanase is 6.

3.3.3. Effect of metal ions on β - glucanase activity

Metal ions play important roles in the biological function of many enzymes. The various modes of metal-protein interaction include metal, ligand- , and enzyme-bridge complexes. Metals can serve as electron donors or acceptors, Lewis acids or structural regulators. β - glucanase was incubated with the same amount of metal ions: Ca^{2+}, Cu^{2+}, Fe^{2+}, K^{+}, Zn^{2+} 5 mM concentration at 37°C for 30 minutes. Then residual activity was measured using the DNS method.

Table 5. Effect of temperature on β-glucanase activity of TO64.4 strain

Temperature (°C)	Enzymatic actyvity (U/ml)	Residual activity (%)
20	0.0019^{g} ± 0.00001	14
30	0.0038^{f} ± 0.00003	29
37	0.0082^{d} ± 0.00002	63
40	0.0091^{d} ± 0.00003	69
50	0.0107^{c} ± 0.00003	82
55	0.0123^{b} ± 0.00004	94
60	0.0131^{a} ± 0.00003	100
65	0.0130^{a} ± 0.00005	99
70	0.0105^{c} ± 0.00005	80
80	0.0044^{f} ± 0.00003	34

Table 6. Effect of pH on β-glucanase activity of TO64.4 strain

pH	Enzymatic actyvity (U/ml)	Residual activity (%)
3	0.0008^{f} ± 0.00003	9
4	0.0022^{d} ± 0.00001	24
5	0.0070^{b} ± 0.00003	76
6	0.0092^{a} ± 0.00010	100
7	0.0051^{c} ± 0.00008	55
8	0.0043^{c} ± 0.00001	47
9	0.0015^{e} ± 0.00004	16

Table 7. Effect of chemical additive on β-glucanase activity of TO64.4 strain

Metal ions	Enzymatic activity (U/ml)	Residual activity (%)
Control	0.0094 ± 0.00002	100
Fe^{2+}	0.0068 ± 0.00003	72
Cu^{2+}	0.0059 ± 0.00002	63
Zn^{2+}	0.0020 ± 0.00001	21
K^{+}	0.0046 ± 0.00005	49
Ca^{2+}	0.0064 ± 0.00004	68

As the results showed in Table 7, the enzyme activity was reduced by all examined metal ions. This result was comparable with the of Nema *et al.*, (2015). However, the records of Padilha *et al.*, (2015) showed that only Cu^{2+} inhibited enzyme activity. Meanwhile, β-glucanase activity was strongly activated by Fe^{2+} Ca^{2+}.

4. CONCLUSIONS

From 46 strains of *Bacillus* spp. isolated, strain TO40.38, TO47.3, TO64.4 produced beta-glucanase with highest enzyme activity. The study showed that the growth of Bacillus strains was proportional to their enzyme activity. Temperature, pH suitable culture medium and cultivation time for enzyme production were 37°C, pH 7 and 30 h respectively.

Besides, the activity of the enzyme produced by TO64.4 is strongly affected by heat treatment conditions, pH and metal ions. Highest enzyme activity reached at 60°C, pH 6. Among the metal ion survey includes Ca^{2+}, Cu^{2+}, Fe^{2+}, K^{+}, Zn^{2+}. All of ions inhibited or inactivated enzyme activity.

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THE EFFECT OF HIGH SALINITY CONCENTRATION ON PROTEOLYSIS OF SARDINE (*Sardina pilchardus*) WITH COMMERCIAL ENZYMES

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ABSTRACT

Fish sauce production is a very long process and there is a great interest in shortening it. Among different strategies to speed up this process, the addition of external proteases might be a solution. Therefore, studies on hydrolysis of sardines, one of fish species traditionally used to produced fish sauce, at its natural pH and high concentration of NaCl have been described. In this study, fish substrates were hydrolyzed by endogenous enzymes or/and in combination with one of four different commercial proteases, namely Protamex TM, Protex 51FP, Protex 6L and Fungal Protease. Hydrolysis reactions were conducted with fresh fish at 30°C without using pH adjustment methods. Hydrolysis was performed by hydrolyzing device PH-start and ended at several times by increasing the temperature in order to inactivate enzyme's activities. Hydrolysis was assessed by the most important index - Degree of Hydrolysis (DH). In experiments conducted with four enzymes, result of hydrolysis was highest at 10% then down at 20% and reached lowest at 30% of NaCl concentrations ($P < 0.05$). Hydrolysis activities of endogenous enzymes in sardines among fish with 25% NaCl (w/w) and 20% water (w/w), the value of DH received was 7.55% lower than samples that adding enzymes from outside ($P < 0.05$). Protamex 51FP brought the highest degree of hydrolysis at all levels of salt experiment ($P < 0.05$), then the protamex, Protex 6L and lowest fungal protease. This study demonstrates that the addition of commercial proteases could be useful for fish liquefaction and cleavage of peptide bonds that occur during fish sauce production and thus speed up the production process.

Keywords: fish sauce, hydrolysis Sardines, endogenous enzymes, production process.

ENVIRONMENT

THE POTENTIAL USES OF SEWAGE SLUDGE AND SEWAGE SLUDGE COMPOST AS SOIL IMPROVEMENT MATERIALS FOR SUSTAINABLE AGRICULTURE

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ABSTRACT

This study was carried out to determine the potential of using sewage sludge (SS) and sewage sludge compost (SSC) as soil improvement materials through determining their effects on plant growth and post - harvest soil properties, in which their environmental hazards associated with heavy metal accumulation and N leaching losses were also examined. A pot experiment with Water Spinach (Ipomoea aquatic) was carried out on sandy soil with daily sufficient water - sprinkling in the greenhouse. The experiment consisted of four N sources (raw SS, SSC 14 days, finished SSC and urea fertilizer). The results showed that (i) the content of organic matter, N and P in sewage sludge was high; (ii) The sewage sludge may be considered as a slow - release N source which can provide long - term benefits for crop production. In addition, the risk of sludge and its compost amendments based soil N to be leached was low in comparison with urea fertilizer;(iii) the application of sewage sludge or compost had also significantly positive effects on soil pH, total N, total C, C/N ratio and available P of amended soils; (iiii) The levels of heavy metals in sewage sludge and sludge compost used in this study were far below the limit values, therefore none of heavy metal elements in both plant tissues and post - harvest soil reached either phytotoxic or toxic levels for human health, livestock, or plant growth. These findings, therefore, showed that recycling of sewage sludge or sludge compost as soil improvement materials could be an efficient method to improve the fertility of agricultural land and a feasible option for sludge disposal problems.

Keywords: Compost, heavy metals, nitrogen, sewage sludge.

1. INTRODUCTION

Agriculture use of sewage sludge has been considered as an attractive practice for alleviating the disposal pressure, and, at the same time, recycling the sources of plant nutrients in this waste. Nevertheless, the negative effects resulting from usage of sewage sludge such as elevated heavy metal levels in soil – plant systems or risk of nitrogen leaching losses into underground water often restrict its uses and require more sufficient knowledge on the impact of its utilization. In view of those facts, this study was carried out to determining the potential of using sewage sludge and its compost as a soil improvement materials through: i) characterizing sewage sludge by changes of its chemical and physical properties during composting process; ii) quantifying the effects of sewage sludge, sludge compost, mineral fertilizers on plant nutrient uptake; iii) evaluating changes in soil properties after harvest; iiii) accessing environmental hazards of sewage sludge associated with heavy metal accumulation in soil - plant system and N leaching losses into underground water.

2. MATERIALS AND METHODS

A pot experiment with Water Spinach (Ipomoea aquatic) was carried out on sandy

soil with daily sufficient water - sprinkling in the greenhouse. The experiment consisted of four N sources (raw sludge (C0), sludge after composting for 14 days (C14), finished compost (Cp) and urea (U)). Sewage sludge at different stages of composting was collected from a composting plant in Okayama prefecture, Japan.

2.1. Experimental design

The method of Nitrogen application was base dressing, in which different kind of nitrogen sources and dose were uniformly mixed throughout the soil to a 15cm depth at the time of sowing seeds. Superphosphate (P2O5 17.5%) and KCl were also added to all treatments together with Nitrogen at the rate of 2.54 N: 3P:5K for 50kg N/ha treatments and 5.08 N: 3P:5K for 100kg N/ha treatments. The actual amount of SS, SSC and Urea applications was based on the amount required to supply 50 and 100 kg N ha^{-1}.

There were 7 treatments, including:

U_50: control, Urea applied at rate of 50 kg N ha^{-1}

U_100: control, Urea applied at rate of 100 kg N ha^{-1}

C0_50: Sewage sludge Compost raw material applied at rate of 50 kg N ha^{-1}

C0_100: Compost raw material applied at rate of 100 kg N ha^{-1}

C14_50: Sewage sludge Compost 14 days applied at rate of 50 kg N ha^{-1}

Cp_50: Finished Sewage sludge Compost applied at rate of 50 kg N ha^{-1}

Cp_100: Finished Sewage sludge Compost applied at rate of 100 kg N ha^{-1}

2.2. Planting and plant sampling

Twenty water spinach (Ipomoea aquatica) seeds were sown to each plastic pot (0.5 m^2 in area). Seeds were planted in rows across the bed, at spacing between seeds of 5 cm and at 1 - 2 cm depth. The distance between rows was 15cm.

One week after emergence, five seedlings were randomly chose and marked in each pot for plant height and biomass measurement. During growing season, plant height was measured three times at two, four and six weeks after seeding.

Plants were sprinkled two times per day to ensure that the plant have enough supply of water for their growth. The leached water was collected from every pot daily for total - P, NO_3 - N and NH_4 - N measurement.

The plants were first harvested at 46 days and second harvested at 91days of growing. Maximum height of five water spinaches were recorded before cutting, the plant shoots and roots were separately harvested and oven - dried at 500°C for 24 - 48 hours for total N, total P, total nutrient and heavy metal analysis, a part of plant shoot was freeze - dry for NO_3 - N, NH_4 - N analysis.

2.3. Soil sampling

Sampling was conducted prior to soil amendment and at the end of the plant growth cycle. At final harvesting, five surface (0 - 10 cm) and five subsurface soil samples (10 - 15 cm) were randomly (following zig - zag way) collected from every pot. All samples were oven - dried and ground before being stored in plastic bags and kelp in a dry storage at room temperature until analysis.

2.4. Plant, sewage sludge and soil analyses methods

Typical parameters were measured including T - N, T - C, T - P, available P, Ca, K, Mg, total heavy metals elements (Cu, Zn, Cd, and Ni), exchangeable cations (K^+, Ca^{2+}, Mg^{2+}, Na^+), NH_4 - N, NO_3 - N, pH. Analytical methods are as the following:

The total nitrogen and carbon contents were determined by dry combustion method using CN - corder (MT - 700) analyzer.

The sample extraction for measurement of total phosphorus, total mineral nutrients (Ca, Mg, K, Na) and heavy metal (Cu, Zn, Cd, Pb) were carried out by dry ashing of oven - dried sample in a muffle furnace at 500°C for 8h25minutes. Then after these ashes were dissolved in hydrochloric acid (HCl) 50% , heating and filtered, total P was determined by Molybdenum blue method using spectrophotometer analyzer (Uv - Vis) at 710 nm absorbance, total mineral nutrients and heavy metals concentrations were determined using flame atomic absorption spectrophotometer (FAA) and flameless graphite furnace atomic absorption spectrophotometer (GFAA).

The sample extraction for available P were carried out by shaking in 0.002 N H_2SO_4 solution about 30 minutes, filtered and determined concentrations using Uv - Vis.

Exchangeable K, Ca, Na and Mg were measured by FAA after an ammonium acetate extraction.

The samples for ammonium nitrogen (NH_4 - N) and nitrogen (NO_3 - N) measurement was extracted by shaking in 2M KCl solution. After that, the concentrations of NH_4 - N of those filtered samples were determined by Indophenol blue method using Uv - Vis at 635 nm, and NO_3 - N by Colorimetric Vanadium chloride method using Uv - Vis at 510 nm.

The pH of the soil was measured in water suspension using a 1: 5 soil: deionized water ratio (w: w) after 60 - min equilibrium time.

2.5. Data analysis

Statistical analysis was performed using Microsoft Excel and R (i386 3.0.1) software. An analysis of variance (ANOVA) and Tukey's Honesty Significant Difference (HSD) pot hoc test, at 95 % confidence level, were performed to detect significant differences in soil and plant characteristics between treatments.

3. RESULTS AND DISCUSSION

3.1. Change in chemical characteristic of sewage sludge compost during composting process

Table 1 and 2 show that: The chemical properties of sewage sludge changed markedly during the composting process. Total nitrogen (T - N) and phosphorus (T - P) concentrations increased from 40.8 and 13.6 mg kg^{-1} in raw sludge to 42.4 and 21.9 mg kg^{-1} in composted sludge, respectively.

The moisture content of sludge compost decreased from 77% in raw sludge to nearly 30% in finished compost, this moisture value is considered lower than the recommended optimum water content for practical mature compost that ranged from 40 to 60% on a mass basis (Mary, 2011). High moisture levels cause nutrients leaching losses, reduce oxygen and decomposition rate. Low moisture levels can result in temperatures rising too high, bacterial activity will slow down. Therefore, to maintain optimum microbial activity, optimum moisture content must also be maintained.

C: N ratio of sludge reduced from 8.2 to 7.3 at the end of composting process. C:N ratio has been used as an index of compost maturity. The "ideal" range of C:N ratios of raw materials should be from 25 - 35:1, the best is 30:1. At this C:N ratio, microorganisms can decompose organic material quickly. When the C:N ratio is too high, there is too little nitrogen and decomposition slows. When the C:N ratio is

too low, there is too much nitrogen and it will likely be lost to the atmosphere in the form of ammonia gas and can lead to odor problems. As composting proceeds, the C:N ratio gradually decreases from 30:1 to 10 - 15:1 for the end - product (Chen, 2011).

The order of concentration of elements in sewage sludge compost at all stages showed the following trend: Zn > Cu > Pb > Cd. During composting, all amounts of heavy metals studied increased except for Zn. The increase of total metal content was due to weight loss in the course of composting following organic matter decomposition, release of CO_2, water and mineralization processes. The decrease in content of Zn could be explained by metal loss through leaching in the course of composting. This loss mainly occurred during the thermophilic phase and could be related to metal release from decomposed organic matter (Soumia, 2005). In general, the heavy metals concentrations in sludge compost at all stages were below the environmental quality standard for agricultural land use.

3.2. Effects of sewage sludge and sludge compost application on plant Nitrogen

Table 3 shows that the total amounts of nutrients taken up and accumulated in plant tissues of sewage sludge and composted sludge - amended soils were comparable with those of urea treatments. Another finding was that while NUE of plant in urea treatments trended to decrease over time (NUE: 25.1% at first harvest deduced to 9.0% at second harvest), raw sludge with high content of nitrogen but slow mineralization capacity, generally had greater influence on N uptake of plants in the second season (NUE: 29.6 % at first harvest increased to 13.3 % at second harvest). This finding confirm the results from other experiments with the sewage sludge (Osmetullah, 2011b; Sigua, 2009). The sewage sludge, therefore, may be considered as a slow - release source of N which can provide long - term benefits for crop production (Pishdar, 2007).

Table 1. Chemical properties of sewage sludge compost at different stages

Raw material and compost	Exchangeable Cations				Total N	Total C	C/N	Total P	Available - P	Moisture content
	K^+	Na^+	Ca^{2+}	Mg^{2+}						
	cmol kg^{-1}				g kg^{-1}					%
Raw Sludge	6.2	4.8	7.9	4.3	40.8	335.4	8.2	13.6	4.0	77.1
Compost (after 14 days of composting)	10.7	5.1	9.7	4.3	41.9	314.7	7.5	20.6	3.8	47.4
Compost (finished product)	11.3	5.5	8.5	4.4	42.4	310.0	7.3	21.9	5.1	21.9

Table 2. Heavy metal concentrations of sewage sludge and sludge compost (*mg kg^{-1}*)

	Cu	Zn	Pb	Cd
Raw Sludge	145	432	3.62	0.41
Compost (after 14days of composting)	298	458	9.59	0.77
Compost (finished product)	304	381	5.49	0.80
*Limit value	1500	2500	750	20

Note: (*) The limit values for allowable metal content in sludge when used on arable soil, recommended by European Economic Commission (EEC) Council

Table 3. Effects of different N sources on plant N content, N uptake, N Uptake Efficiency (NUE)

Treatments	First Harvest			Second Harvest		
	N content* g kg^{-1}	N uptake* kg ha^{-1}	NUE* %	N content* g kg^{-1}	N uptake* kg ha^{-1}	NUE* %
U	20.9 ± 2.31 a	12.5 ± 1.38 a	25.1 ± 2.76 a	12.4 ± 0.26ns	4.5 ± 0.10 af	9.0 ± 0.19 a
C0	19.7 ± 0.81 a	4.8 ± 0.20 b	9.6 ± 0.40 b	14.0 ± 0.79 ns	6.7 ± 0.38 bf	13.3 ± 0.75b
C14	13.7 ± 0.10 b	2.1 ± 0.01 c	4.1 ± 0.03 c	15.5 ± 0.78 ns	2.3 ± 0.11 ac	4.5 ± 0.23 a
Cp	13.2 ± 0.40 b	1.7 ± 0.05 c	3.3 ± 0.10 c	14.6 ± 1.04 ns	1.2 ± 0.09 c	2.5 ± 0.18 c

*Note: The means in the column followed with the same letter are not statistically significant difference from each other (Tukey HSD test, p≤0.05); *Significant at p ≤ 0.001*

Table 4. Ammonium (NH_4 - N), nitrate (NO_3 - N) leaching losses

	NO_3 - N		NH_4 - N	
	mgL^{-1}	kgha^{-1}	mgL^{-1}	kgha^{-1}
U	66.48	1.50	3.45	0.29
C0	7.14	0.31	1.80	0.13
C14	10.12	0.41	1.69	0.06
Cp	11.09	0.23	1.30	0.06

Table 5. Selected chemical properties of post - harvest soils

	pH	Total N		Total C		C/N Ratio		Available - P	
		0~10 cm	10~15 cm	0~10 cm	10~15cm	0~10 cm	10~15 cm	0~10 cm	10~15 cm
		g kg^{-1}						mg kg^{-1}	
U	7.1 ± 0.25 ab	0.2 ± 0.01b	0.2 ± 0.02ns	0.9 ± 0.04a	1.1 ± 0.09ns	4.0 ± 0.17	5.5 ± 0.33	27.6 ± 3.6ns	18.0 ± 2.9ad
C0	7.0 ± 0.68a	0.3 ± 0.02ab	0.2 ± 0.01ns	1.0 ± 0.08a	0.9 ± 0.08ns	3.9 ± 0.39	5.2 ± 0.2	30.3 ± 4.9ns	16.0 ± 1.0a
C14	7.7 ± 0.23b	0.3 ± 0.02a	0.2 ± 0.02ns	1.4 ± 0.27b	1.0 ± 0.09ns	5.3 ± 0.63	5.3 ± 0.13	30.2 ± 2.5ns	19.8 ± 1.8ab
Cp	7.2 ± 0.32ab	0.3 ± 0.02ab	0.2 ± 0.04ns	1.2 ± 0.25ab	0.9 ± 0.19ns	4.9 ± 0.64	5.4 ± 0.22	32.5 ± 8.1ns	20.8 ± 2.8bd
Original Soil		0.10		0.40		4.40		8.0	

Note: The means in the column followed with the same letter are not statistically significant difference from each other (Turkey HSD test, P≤0.05), ns: not significant

3.3. Ammonium (NH_4 - N), nitrate (NO_3 - N) leaching losses

Table 4 shows There was considerable variation in nitrate concentrations in leached water due to the fertilization. The fertilizer, especially urea had a strong effect on NO_3^- concentrations in leached water, and significant greater than sludge or compost treatments. The average nitrogen leach in the form of NO_3 - N was greater than NH_4 - N. This was explained

by a positive charge of ammonium which can be absorbed by any negative charges in the soil. The total amount and concentration of ammonium in leachated water, followed the similar trend as nitrate, were highest in urea treatment. Majority of the nitrogen in sewage sludge exists in organic forms. Thus, the sludge' nitrogen is released at a much slower rate as compared to the chemical fertilizer nitrogen, which makes it less likely to leaching to groundwater (Borken, 2004).

3.4. Effects of sewage sludge and sludge compost application on post – harvest soil properties

Total N increased from 1.7 to 2.6 - fold the fertilizer amended soils in comparison to original soil. The application of sewage sludge or compost had also highly significant positive effects on soil pH, total N, total C, C/N ratio and available P of amended soils. At all the treatments with sewage sludge and compost slightly increased N concentrations in surface soil with respect to chemical fertilizer, although this increase was not significant in some cases. This fact points out that sewage sludge or composted sewage sludge have a slow rate of N releases (Sigua, 2009). In both soil depths, soil analysis showed more pronounced increases in levels of available phosphorus for sludge and compost treatments compared to urea, but not statistically significant.

3.5. Accumulation of heavy metals in the soil - plant system

3.5.1. Heavy metals accumulation in post - harvest soils

Table 6 shows the metal (Cu, Zn, Cd and Pd) concentrations in the sludge, compost and urea amended soils at final harvesting stage. Zn was the most abundant metal, followed by Pb, Cu and Cd. Heavy metal concentrations were varied between treatments and soil depths, nevertheless, none of them indicated excessive limit values for allowable concentration in comparison with the EU limits and TCVN 7209 - 2002.

Table 6. Content of heavy metal elements (Cu, Zn, Pb, Cd) accumulation in post - harvest soils

Treaments	Zn		Pb		Cd		Cu	
	0~10cm	10~15 cm	0~10cm	10~15cm	0~10cm	10~15 cm	0~10cm	10~15cm
	mg kg^{-1}							
C0	38.6 a	34.0 a	1.70ns	1.63 a	0.01 a	0.01 ns	2.09	1.16ns
C14	37.8 ac	34.7 ab	2.41ns	2.05 ab	0.25 bc	0.01 ns	2.10	1.10ns
Cp	34.5 c	38.8 b	1.45ns	2.20 b	0.30 c	0.02 ns	1.88	1.39 ns
*Limit value	300.0		100.00		3.00		100.00	
TCVN 7209 - 2002	200.0		70.00		-		50.00	

Note: The means in the column followed with the same letter are not statistically significance different from each other (Turkey HSD test, P≤0.05), ns: not significant;

(*)The limit values origin from a proposal to European Economic Commission (EEC) Council Directive No. 86/278/EEC (Council of the European Communities 1986 on the protection of the environment, and in particular of the soil, when sewage sludge is used in agriculture.

Table7. Content of heavy metal elements (Cu, Zn, Pb, Cd) accumulation in the plant at harvesting stages

Treatments	Shoots							Roots		
	First Harvest				Second Harvest					
	Cu	Zn	Pb	Cd	Cu	Zn	Pb	Cu	Zn	Pb
	mg Kg^{-1}									
U	0.66	29.3	0.23	0.01	1.04	28.94	0.05	1.02	32.1	0.00
C0	1.74	36.9	0.22	0.01	0.94	40.71	0.07	1.96	48.4	0.05
C14	2.29	29.6	0.22	0.02	1.61	43.30	0.06	4.94	49.5	0.08
Cp	2.58	26.5	0.20	0.09	4.08	55.58	0.08	4.20	56.6	0.10
*WHO - ML	73.00	100.0	0.30	0.10						

*Note: *Recommended Maximum levels for vegetables by World Health Organization Model List (WHO – ML) (CODEX, 2001) (Mohsen, 2008; Oti, 2013)*

3.5.2. Heavy metal uptake by plant tissues

Sewage sludge and sludge compost are higher in heavy metal levels in comparison to other substrates. To avoid the potential exposure hazards from contacting the plant, it is important to evaluate the content of heavy metal accumulated in the plants grown in soil - amended sewage sludge whether or not exceeding the allowable levels (Pishdar, 2007).

The total metal concentrations in shoots and roots of plant were analyzed at harvesting stages. The total metal concentration followed the trend, Zn > Cu > Pd > Cd (Table 7). Although the content of Cu, Zn, Cd in water spinach plants grown in soil amended with sewage sludge and sludge compost were generally higher than those in plant from urea application, (in several cases, the contents were lower in pots applied by sludge or compost than in the urea pots), no values of those exceeded the permissible limit for vegetables established by FAO/WHO (Mohsen, 2008). The levels of heavy metal in sewage were relative low, therefore content of those accumulated in leaves and grains did not indicate excessive contamination that could be considered a serious health hazard to consumers (Akdeniz, 2006).

In general, the results revealed that the plant tissue grown in the soil - amended with sewage sludge and sludge compost contain only the trace amount of these toxic metals. The concentrations found were within the permissible limits and safe in consumption point of view.

4. CONCLUSIONS

The content of organic matter, N and P in sewage sludge was high. The heavy metals concentrations in sludge compost at all stages were below the environmental quality standard for agricultural land use.

The total amounts of nutrients taken up and accumulated in plant tissues of sewage sludge and composted sludge - amended soils were comparable with those of urea treatments. While NUE of plant in urea treatments trended to decrease with time, raw sludge with high content of nitrogen but slow mineralization rate had greater influence on NUE of plant in the second

harvest. The sewage sludge, therefore, may be considered as a slow - release N source which can provide long - term benefits for crop production. In addition, the risk of sludge and its compost amendments based soil N to be leached was low in comparison with urea fertilizer, especially at the initial stage after N application.

The application of sewage sludge or compost had also highly significant positive effects on soil pH, total N, total C, C/N ratio and available P of amended soils. Furthermore, none of heavy metal elements in both plant tissues and post - harvest soil reached either phytotoxic or toxic levels for human health, livestock, or plant growth.

These findings, therefore, showed that recycling of sewage sludge or sludge compost as soil improvement materials could be an efficient method to improve the fertility of agricultural land and a feasible option for sludge disposal problems.

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LOSSES OF GREENHOUSE GASES FROM FEEDLOT AND HOW FEEDLOT MANAGEMENT REDUCES THESE LOSSES

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ABSTRACT

Greenhouse gas (GHG) emissions from a directly emitted enteric fermentation contribute to the loss of 6 to 10% of gross energy intake, depending on level of feed intake, diet composition and digestibility of dietary energy. The consequences of the loss may result in a low animal productivity and a negative impact on the sustainability of the feedlot production. Enteric methane emissions from beef cattle are lower than dairy cattle, but N_2O emissions produced from fresh manure and manure storage of beef cattle are higher than that of dairy cattle, and N_2O emissions from field crops after spreading composted beef and dairy manure also need to account for feedlot operation impact on the environment. A single change of feedlot operation may decrease or increase one part to another of a system, thus a whole feedlot system needs to be considered from feeding to manure management and cropping system to reduce a feedlot impact on the environment. The goal of this paper was to extensively review the losses of GHG from an enteric fermentation and manure compost in a feedlot operation. The review also discussed feedlot management changes can increase its production and decrease the losses of GHG from a feedlot operation.

Keywords: Enteric fermentation, feedlot, greenhouse gas, manure compost.

1. INTRODUCTION

The major cause of global warming is due to the increase in the emissions of the GHG including carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). Cattle feedlot operation, increasingly common worldwide in the beef industry, is significantly contributing to the increase in CO_2, CH_4 and N_2O. Greenhouse gases emitted by ruminants are paid much attention to CH_4 because CH_4 has 21 times global warming potential of CO_2 and it is produced in much greater amounts than N_2O (United Nations Framework Convention on Climate Change, 2006). In 1990, the greatest source of CH_4 emissions (45%) came from agricultural sector in EU with an estimate of 10.2 million tons per year. Of these emissions, enteric fermentation represents roughly two-thirds and livestock manure represents one-third of total CH_4 production (Moss *et al.*, 2000). However, CH_4 emissions from livestock manure are paid little attention. In global scale CH_4 emissions from livestock manure accounts for 5 - 6% of total CH_4 emissions and N_2O accounts for 7% of total N_2O emissions (Hogan *et al.*, 1991; Khalil and Rasmussen, 1992). Global warming largely affects feedlot systems in a way that crop and forage yields are reduced as negative effects of water shortage and extreme weather conditions (Olesen and Bindi, 2002). Global warming reduces subtropical areas where C3 grasses can be grown and is associated with reduction of pasture nutritive values (C4 grasses are less digestible than C3 ones) which potentially lead to decreases in animal productivity (Dryden, 2008). Thereby, feedlot systems and the global warming have a strong

correlation and they interact with each other. This paper extensively reviewed potential GHG emissions from feedlot systems and discussed possible management strategies to mitigate feedlot impacts on the environment.

2. LOSSES OF GREENHOUSE GASES FROM FEEDLOT

Greenhouse gas emissions from feedlot systems comprise CH_4 directly emitted from enteric fermentation, CH_4 and N_2O produced from fresh manure and manure storage, and N_2O emissions from field crops after spreading composted manure (Figure 1).

Direct CH_4 emissions from feedlot cattle are associated with feed intake and production. Mills (2008) reported that CH_4 emissions represent an inefficiency of energy intake and reduction of feed conversion ratio. Approximately from 6 to 10 % of gross energy intake is lost through ruminal fermentation, varying on the level of feed intake, diet composition and digestibility of dietary energy (Soliva and Hess, 2007; Ushida *et al.*, 1997). Direct CH_4 emissions from enteric fermentation contribute large amounts of CH_4 emissions to GHG. Therefore, understanding possible pathway and mechanism of CH_4 production from fermentation is necessary to manipulate rumen fermentation and mitigate methane emission output from feedlot cattle.

3. GREENHOUSE GAS EMISSIONS FROM ENTERIC FERMENTATION

In ruminal fermetation, rumen methanogenic bacteria utilize hydrogen (H_2) and CO_2 to form CH_4 (Moss, 1993). Buddle *et al.*, (2010) illustrated a diagram of rumen process as CH_4 generated in rumen is formed by H_2 and CO_2 (Figure 2). Therefore, acetate or H_2 and CO_2 are believed to be important substrates of CH_4 production in rumen.

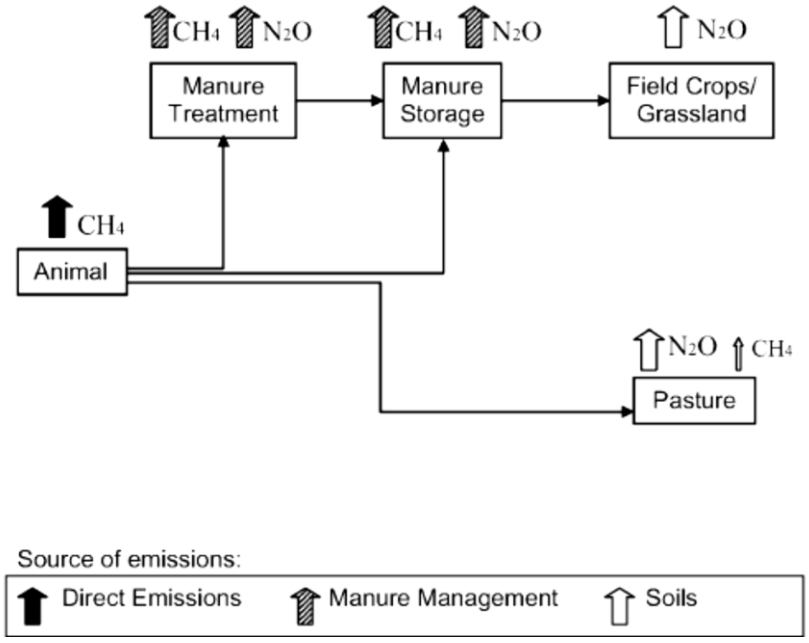

Figure 1. Greenhouse gas emissions from a feedlot system

Source: Kebreab et al., 2006

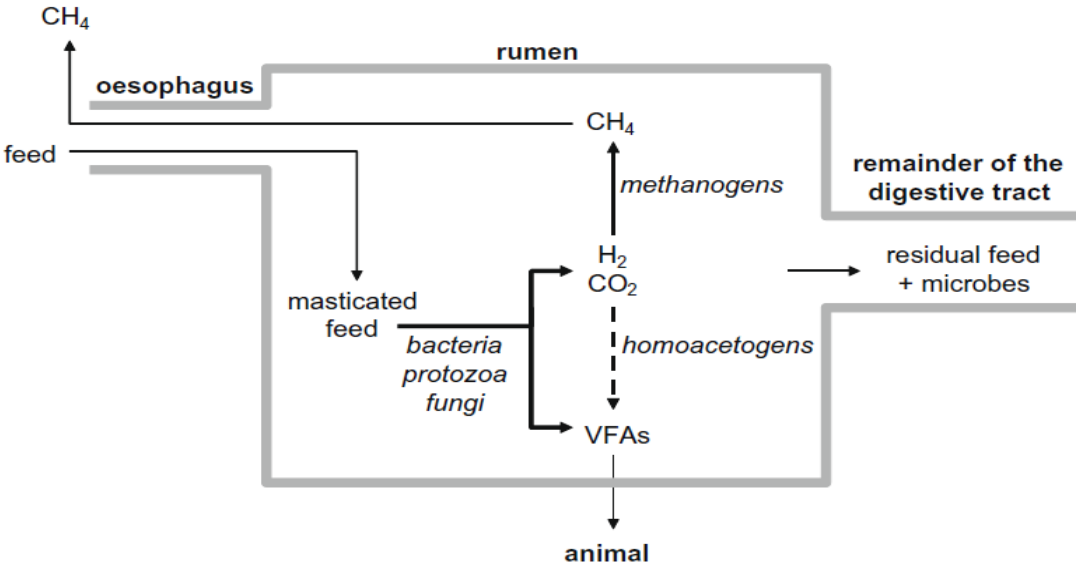

Figure 2. Methane formation in ruminal digestion process

Source: Buddle et al., 2010

In the rumen fermentation process as presented in Figure 2, the microbial bacteria ferment eaten feeds to volatile fatty acids (VFA) , H_2 and CO_2. The VFA are then absorbed across the rumen wall to provide carbon and energy sources for the ruminants, while the H_2 is utilized by methanogenic bacteria to produce CH_4 ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$), which is finally belched by the animal into the atmosphere (Buddle *et al.*, 2010). Furthermore, Van Soest (1994) showed an equation of breakdown carbohydrates process into VFA including acetic, propionic, butyric, and methane and CO_2.

57.5 $C_6H_{12}O_6$ → 65 Acetic acid + 20 Propionic acid + 15 Butyric acid + 35 Methane + 60 CO_2 + 25 H_2O (Van Soest, 1994)

Volatile fatty acids, major energy sources to animals, are produced during ruminal fermentation but CH_4 production, created by methanogens, has a significant restraint on the amount of VFA and affects the animal feed - efficiency. It has been already reported widely in the literature that fermentable feeds being rich in starch and protein lead to lower CH_4 formed in rumen. On the other hand, slowly digestible feeds high in celluloses and hemicelluloses result in higher CH_4 emissions (Janssen, 2010).

Janssen (2010) showed the glucose fermentation from starch and cellulose leads to different amount of CH_4 formed (Figure 3), and also the amount of CH_4 formed per unit of feed digested is dependent upon the amount of H_2 formed ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$).

An example of enteric methane production from US livestock is presented in Table 1. Agriculture sector contributed 6.3 percent of US total greenhouse gas emissions. In 2010, the US livestock produced 6,728 Gg of enteric methane emissions, while beef cattle were mostly responsible for methane emissions from enteric fermentation, accounting for 72 percent.

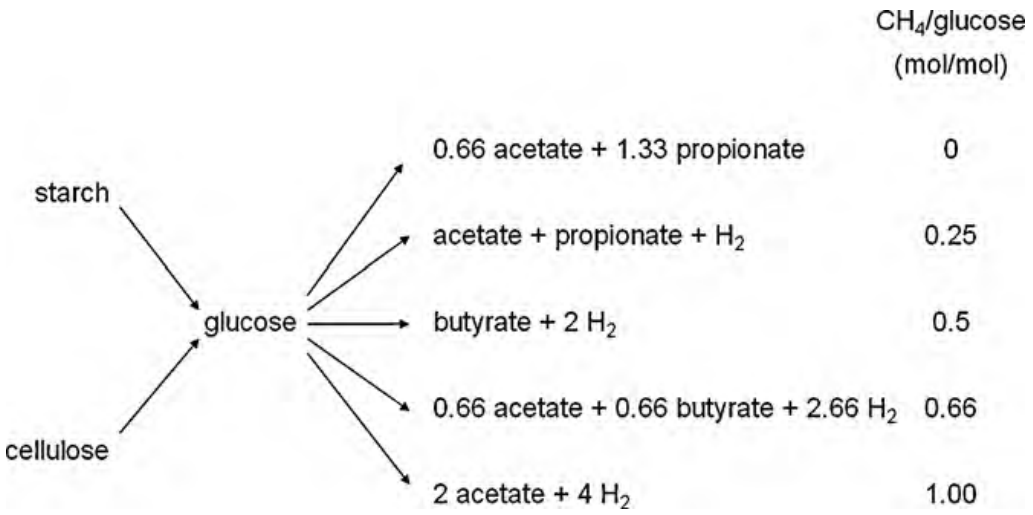

Figure 3. Cellulose and starch fermentation via glucose producing acetate, propionate, butyrate, and H2

Source: Janssen, 2010

Table 1. Methane emissions from enteric fermentation

Livestock Type	1990	2005	2006	2007	2008	2009	2010
Beef Cattle	4,581	4,829	4,904	4,953	4,909	4,857	4,812
Dairy Cattle	1,513	1,449	1,479	1,544	1,564	1,581	1,569
Horses	91	166	171	171	171	171	171
Swine	81	92	93	98	101	99	97
Sheep	91	49	50	49	48	46	45
Goats	13	14	15	16	16	16	16
American Bison	4	17	17	16	17	17	16
Mules, Burros, and Donkeys	1	2	2	3	3	3	3
Total	**6,373**	**6,618**	**6,731**	**6,850**	**6,829**	**6,788**	**6,728**

Note: Totals may not sum due to independent rounding.

Source: United States Environmental Agency, 2012

4. GREENHOUSE GAS EMISSIONS FROM MANURE

Greenhouse gases such as CH_4 and CO_2 from livestock manure are products of organic waste decomposition under anaerobic conditions.

Burton and Turner (2003) described three stages of an anaerobic digestion process: hydrolytic, acid forming and methanogenic (Figure 3). Total complex organic materials in manure, in the first stage, are broken down by the enzymes of hydrolytic bacteria. Then, anaerobic bacteria involve in reducing the simple sugars produced in stage 1 to simple organic acids. After the process of breakdown carbohydrates, acetic acids propionic acid and butyric acid are formed and acetic acid is a major product of carbohydrate digestion. At the same time, H_2 and CO_2 are also produced. In final stage, CH_4 and CO_2 are formed by methanogenic bacteria.

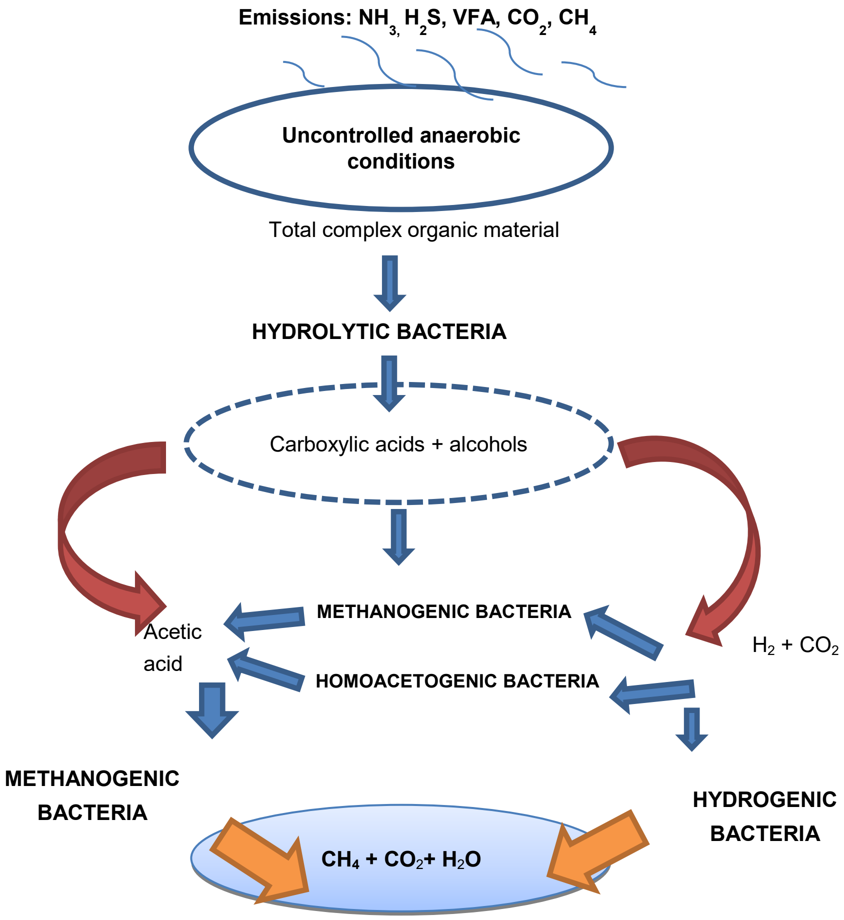

Figure 4. Biochemical mechanisms related to uncontrolled anaerobic stage of livestock manure (Burton and Turner, 2003)

Besides CH_4 production from microbial degradation in manure, N_2O emissions, which is formed by nitrification and detrification processes in manure storage, also contribute to GHG. The nitrification process happens under aerobic conditions, two steps involving ammonium oxidization to nitrite and then nitrite is converted to nitrate. Denitrification is the reduction of nitrite to di-nitrogen gas. These process are presented according to equations below (Kebreab *et al.*, 2006 cited in Galbally (1989)).

$$NH_4^+ \rightarrow NO_2^- \rightarrow NO_3^- \quad (NH_4^+ \rightarrow N_2O)$$

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

In a range of temperatures between 2 and 50°C, the process of denitrification naturally occurs, but the rate of denitrification would be doubled if the substrate temperature increased in every 10°C rise. Mahimairaja et al., (1995)found that the ration of N_2O to N_2 ranged from 0.09 to 0.21 and denitrification process might be relatively slow in fresh manures as NO_3^- was low. An example of CH_4 and N_2O emissions from US livestock manure is presented in Table 2. Beef cattle contributed less manure CH_4 emissions compared with swine, and dairy cows throughout the period from 1990 to 2010. However, N_2O emissions from beef cattle

manure were higher than that of dairy cows and swine.

5. EEDLOT MANAGEMENTS TO MITIGATE GREENHOUSE GAS EMISSIONS

Feedlot management is important to reduce GHG emissions which in turn contribute to a mitigation of global warming and climate change. Janzen et al., (2005) demonstrated a strategy of practice change on GHG emissions from a whole feedlot farm (Figure 5). The authors proposed to increase the area of legume forages, which increase soil C and reduce fertilizer, thereby reducing CO_2 and N_2O. However, legume forages fed to feedlot cattle produce more CH_4 emissions from the enteric fermentation and more emissions of CH_4 and N_2O during manure storage and manure compost adding to the land. The use of manure as organic fertilizer would reduce reliance on chemical fertilizer produced in manufactures, leading to less N_2O and CO_2 emissions from manufacturing processes. Thus, one single change may affect GHG from one part to another of a system, thus a whole system of feedlot needs to be integrated.

In the illustration above (Figure 5) GHG emissions from enteric fermentation and manure decomposition are inevitable. However, feedlot managements would mitigate GHG emissions by integrating a whole system change.

6. MANURE MANAGEMENTS

The diversity of feedlot practices including diets, manure treatment and building design lead to a complex issue dealing with GHG emissions and opportunities to mitigate GHG (Mathot *et al.*, 2012). Yamulki (2006) reported that adding straw in manure would provide an opportunity to reduce N_2O and CH_4 emissions from stored solid manure. The study showed

Table 2. Methane and N_2O emissions from manure

Gas/Animal Type	1990	2005	2006	2007	2008	2009	2010
CH_4[a]	**1,511**	**2,280**	**2,303**	**2,508**	**2,465**	**2,416**	**2,478**
Dairy Cattle	599	1069	1101	1224	1238	1233	1239
Beef Cattle	128	135	138	136	132	131	134
Swine	624	914	901	982	938	896	948
Sheep	7	3	3	3	3	3	3
Goats	1	1	1	1	1	1	1
Poultry	131	129	131	134	129	128	129
Horses	22	28	27	27	24	24	24
N_2O[b]	**48**	**57**	**59**	**60**	**59**	**59**	**59**
Dairy Cattle	17	18	19	19	19	19	19
Beef Cattle	21	25	27	27	26	26	27
Swine	4	6	6	6	6	6	6
Sheep	+	1	1	1	1	1	1
Goats	+	+	+	+	+	+	+
Poultry	5	5	5	5	5	5	5
Horses	1	1	1	1	1	1	1

+ Less than 0.5 Gg.
[a]Accounts for CH_4 reductions due to capture and destruction of CH_4 at facilities using anaerobic digesters.
[b]Includes both direct and indirect N_2O emissions.
Note: Totals may not sum due to independent rounding.

Source: United States Environmental Agency, 2012

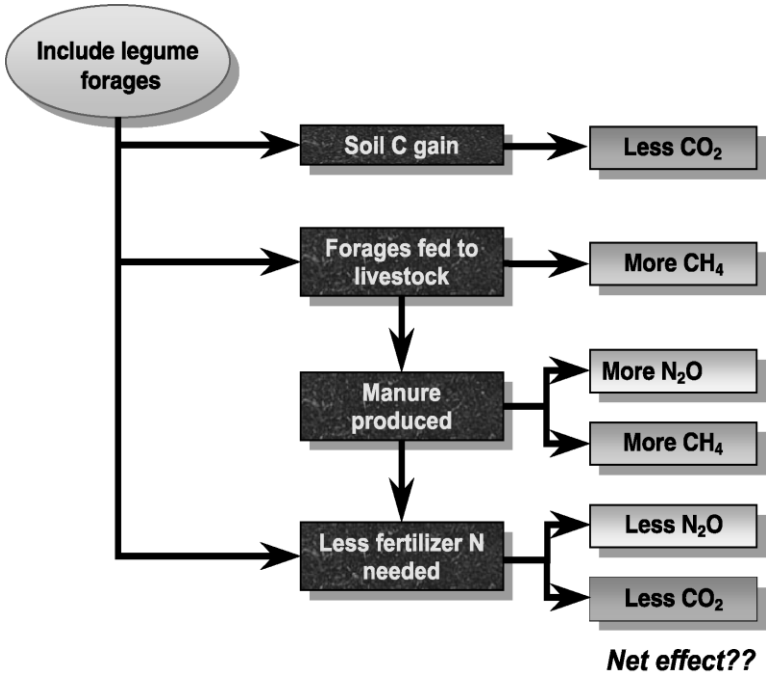

Figure 5. An illustration of a practice change to greenhouse gas emissions from a feedlot farm

Source: Janzen et al., 2005

that total emissions of CH_4 and N_2O were lower in stored solid manure with straw (32 g N t^{-1}) compared with control without straw (47 g N t^{-1}). Straw, rich in C molecules, being added in solid manure results in promoting aerobic decomposition, increasing dry matter content and C:N ratio, and therefore decreasing GHG emissions. Mathot et al., (2012) studied CH_4 and N_2O emissions from two feedlot systems: tie-stall system (barn + manure storage) and stored solid manure (farmyard). Barn + manure storage produced more than 11% of GHG emissions compared with stored solid manure, emphasizing that building design and facility also influence the total GHG outputs from feedlot cattle.

Furthermore, manure composting technology is an effective practice in feedlot systems where a large numbers of cattle density producing a great amount of solid manure in a very small area of land. Composting is an alternative to spread manure to soil, instead of fresh manure, thus both weight and volume are reduced, leading to considerably reduced hauling cost (Larney *et al.*, 2001). In addition, composted manure provides N available for soil to reduce reliance of chemical fertilizer from manufactures, thus less N_2O and CO_2 emitted from manufacturing industries (Janzen *et al.*, 2005).

However, the emissions of CH_4 and N_2O from feedlot manure composting are a deeply growing concern, raising a question of manure composting methods that can be used to reduce a negative benefit of composting. Larney *et al.*, (2001) reported that bedding materials are important in feedlot management because properties of fresh feedlot manure and its composted

product would be changed physically and chemically by the presence of bedding materials. The amount of GHG emission from composting is affected by dry matter content and C:N ratio in fresh manure. A study by Hao *et al.*, (2004) on GHG emissions during composting of straw bedded manure and wood chip bedded manure showed that more than 50% of CH_4 emissions occurred during the first 28 d of composting and then decreasing rapidly to near zero after 70 d. The highest rate of N_2O emissions occurred during the first 14 d, decreasing during mid-composting and a minor peak at the end. Hao *et al.*, (2001) also conducted a study to determine whether GHG emissions were influenced by composting methods of passive (no turning) and active (turned six times). The authors found that lower GHG emissions were observed in passive treatment that associated with incomplete decomposition of manure and a lower rate of gas diffusion.

Manure managements in feedlot systems are highly necessary. Besides the benefit of manure treatment to reduce the odor nuisance, the incidence of weed and pathogen and provide bio-fertilizer to soil, manure management helps protect the environment by reducing the emissions of N_2O and CH_4.

7. FEEDING MANAGEMENTS

Production of CH_4 emissions from feeding management is associated with increases in livestock productivity. Faster growth, higher milk yield and shorter dry period in lactating cows will lower CH_4 emissions (Monteny *et al.*, 2006). Because the levels of CH_4 emission is relatively related to live weight (LW), dry matter intake (DMI), milk yield (MY) and feeding regime, whereas increasing feed efficiency, live weight gain and milk production are effective approaches to reduce CH_4 emissions from cattle (Blummel *et al.*, 2005; Yan *et al.*, 2006).

Hegarty and Nolan (2007) indicated that because CH_4 is formed according to the equation ($4H_2 + CO_2 \rightarrow CH_4 + 2H_20$) and therefore it is a potentially effective way to reduce CH_4 by efficient diets. This is agreed with the study by (Monteny *et al.*, 2006). Carbohydrates are an essential element to synthesize carbon chains of rumen anaerobic microbes. This process of making carbon chain produces carbon dioxide. As a consequence, processed feeds like silage and alkaline treated roughage are suitable for increasing degradable rates of ruminal fermentation which result in less CO_2 remaining. The fermentation of carbohydrates releases ATP (adenosine-5'-triphosphate) and creates NADH dehydrogenase hence less CO_2 remaining would lead to less NADH formation and therefore free H_2 would be less as following the equation ($NADH + H^+ \leftrightarrow NAD^+ + 2H$) (Hegarty and Nolan, 2007).

Lovett *et al.* (2003) conducted a trial to investigate effect of forage and concentrate ratio and supplementation of coconut oil on CH_4 output and beef heifer performance. The experiment consisted three ratios of forage and concentrate (F/C) (0.65:0.35; 0.40:0.60 and 0.10:0.90) in silage based diets and added with two levels of coconut oil (0 or 350g/d). Methane output recorded per liter per day was significantly changed by reducing F/C ratios and supplementing coconut oil. Compared between the first F/C ratio and the third F/C ratio, methane outputs per kg DMI, kg live weight gain and percentage of gross energy intake were declined from 30.88, 301, 6.06 to 20.64, 239, 4.44 ($p < 0.01$), respectively.

Diet modification such as feed additives has been considered a potential approach in which ruminant industry can

reduce CH_4 production from animals. Unsaturated fats including sunflower oil and canola oil are commonly used to reduce CH_4 production. Beauchemin & McGinn (2006) fed feedlot cattle with high forage diets (75% whole crop barley silage, DM basis) and supplemented sunflower oil (50 g/ kg DM) and canola oil (46 g/kg DM) into two treatments and compared them with control (no additive). Sunflower oil reduced CH_4 emissions by 22% per unit of gross energy intake and 17% per unit of digestible energy intake while canola oil decreased CH_4 production by 21% per unit of gross energy intake and by 6% per unit of digestible energy intake. However, the authors found that 25 % and 70% of CH_4 reduction caused by sunflower oil and canola oil due to a restriction of dietary digestibility, respectively.

Monensin has been recently studied in rumen manipulation, which enhances ruminal fermentation and therefore improving feed efficiency (Salles *et al.*, 2008). According to Russell and Houlihan (2003) lactating dairy cows fed monensin benefit from a shift in the acetate to propionate, leading to more propionate formed in rumial fermentation than acetate and as a result CH_4 production is relatively reduced. Odongo *et al.*, (2007) showed a potential effect of feeding monensin on CH_4 production in feedlot cattle. It was reported that feedlot cattle fed 25 mg of monensin premix per kg of dry matter reduced CH_4 production by 7% (CH_4 g/d) and by 9% (CH_4 g/ kg of body weight) compared with unsupplemented cows.

Acurate feeding management pracitices in feedlot systems not only benefit from improving feed conversion ratio and effciency, leading to have better production but also have a potential effect of mitigation of CH_4 emissions.

8. CONCLUSIONS

Feedlot industry considerably represent a considerable amount of greenhouse gas emissions in total emissions of agriculture sector and methane and N_2O emissions have been paid much concern. Enteric fermentation from feedlot rumens is mostly responsible for total methane emissions while N_2O emissions from manure management represent largest amount in total N_2O emissions. Within feedlot management, there have been a number of methods to mitigate greenhouse gas emissions; however, feeding management and manure management are most important and practical.

With manure management, bedding materials are an important factor affecting manure handling and they may contribute to reduction of greenhouse gas emissions from manure. There has been concerned about net benefit of composting that is producing greenhouse gas. Therefore, more research needs to be investigated. Feeding management seems to be more applicable in an attempt to mitigate methane emissions. The basic idea is to feed cattle more fermentable feeds and less fiber in order to help animal improve feed conversion ratio and feed efficiency. Feed additives such as unsaturated fats and monensin have been recently studied and received a potential effect on reduction of enteric methane emissions. However, results vary from experiments to experiments, and thereby further research needs to be more comprehensively investigated in the future.

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STATUS OF PHOSPHORUS SOLUBILIZING MICROORGANIZISM IN SOME KIND OF ALLUVIAL SOILS CULTIVATING WET RICE

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ABSTRACT

The aim of this study was to assess phosphorus solubilizing microorganizisms in neutral, less acidic alluvial soil (Gialam district, Hanoi) and gleiy alluvial soil (Tienlu district, Hungyen province) of the system of Red River cultivating wet rice fully (2 crops per year). The results of isolation showed appearance of bacterias, actinomycetes in samples but absolutely no mould. Overall, density of phosphorus solubilizing microorganizisms in neutral, less acidic alluvial soil is much more than in gleiy alluvial soil, however, the amount of strains is less diverse. The degree of diversity of strains are not the same between the 2 types of soil, even between different samples of the same soil type. There are 4 common strains of bacteria in neutral, less acidic alluvial soil, the density ranged from 15.5 to 22.9 x10^4 CFU/g soil; meanwhile, the gleiy alluvial soil has 4 popular bacterias and 1 actinomycetes , ranged from 2.3 to 17.3 x10^4 CFU/g soil. In these 2 types of soil, density of microorganism solubilizing inorganic phosphate is higher than one of organic phosphate. However, comparition to the total of microorganisms, both of microbial groups are very low in density, up less than 1% of each. Besides, ability of phosphorus solubilization of them are not high, phosphate PO_4^{3-} released ranging from 0.70 to 5.66 ppm ($Ca_3(PO_4)_2$) and from 0.0 to 1.83 ppm (Lecithine form).

Keywords: Alluvial soil, phosphorus solubilizing microorganism.

1. INTRODUTION

Elemental phosphorus in the soil is the macronutrient for plants. Lacking of this makes plants low in growth, poor in yield and quality of harvest products.

In soil, phosphorus exist in the organic and inorganic compounds. Organic compounds include: Phytin, nucleic acids, nucleoprotein, phosphatid. Inorganic compounds contain phosphorus mainly octophosphoric acid salts with Ca, Mg, Fe and Al. All forms of inorganic and organic phosphate are in the form of indigestion for the plants. Phosphorus enters the tree as phosphorus ions, such as $PO4^{3-}$, $HPO4^{2-}$, H_2PO^{4-}. Many studies showed that, in Vietnam soil previous years, although total phosphorus reserves have been quite plentiful depended on kind of soil, such as 0,11 - 0,15% P_2O_5 in alluvial soil of Red River but concentration of available phosphorus (CAP) has been usually low to average, < 10mg P_2O_5/100 g soil (Ha and Thang, 2009). However, in recent years, CAP has tended to increase, such as 16,72-44,88mg P_2O_5 /100g gray soil due to using phosphate fertilizer much (Duc *et al.*, 2012).

Normally in natural soil, there are some kinds of microorganisms with ability of solubilizing unavailable phosphorus

forms into available one for plants. Bacterias, especially *Pseudomonas*, *Bacillus* and moulds, especially *Penicillium*, *Aspergillus* can do that strongly (Rodriquez and Fraga, 1999). However, they are often dissimilar on different soils, closely dependent on soil's properties, cultivation modes. Their existence and development greatly affects the ability of mobilizing available phosphorus in soil.

Rice is the major food crop in Vietnam and is an important exported good. According to statistics of the customs office, by the end of November 2014, the export turnover of Vietnam's rice reached 6.062 million tons, valued at 2.807 billion dollars. Rice plant is mainly cultivated on alluvial soils of two crops (systems) of the Red River Delta and Mekong River Delta. What is the reason has led the result of the research of Ha and Thang (2009) above? Is there or not the role of phosphorus solubilizing microorganism (PSM) here? The assessment of the status of PSM on alluvial soil cultivating rice plant is one of the important basis to explain the above problems as well as suggest measures appropriately to improve phosphorus nutrition in the soil.

This article only provides some analysis and assessment of the amount, composition and activity of microorganisms structure solubilizing phosphorus in the neutral, less acidic and gleiy alluvial soil cultivating rice plant in some localities.

Table 1. Location of soil sampling

NLAAS				GAS			
Sam.	Commune	Location		Sam.	Commune	Location	
		N	E			N	E
M1	Lechi	21º02.024'	106º00.839'	M17	Lexa	$20^0$41.339'	$106^0$09.376'
M2		21º02.222'	106º01.030'	M18		$20^0$41.411'	$106^0$09.409'
M3		21º02.360'	106º00.545'	M19		$20^0$41.479'	$106^0$09.331'
M4		21º02.603'	106º00.496'	M20		$20^0$41.418'	$106^0$09.296'
M5		21º02.980	106º00.463'	M21		$20^0$41.307'	$106^0$09.257'
M6	Kimson	21º02.320'	106º00.463'	M22	Cuongchinh	$20^0$41.432'	$106^0$07.210'
M7		21 º01.035'	105 º59.973'	M23		$20^0$41.330'	$106^0$07.082'
M8		21 º01.305'	105 º59.954'	M24		$20^0$41.282'	$106^0$07.096'
M9		21 º01.735'	105 º59.926'	M25		$20^0$41.171'	$106^0$07.020'
M10		21 º01.629'	105 º59.745'	M26		$20^0$41.261'	$106^0$07.426'
M11	Duongquang	21 º00.407'	105 º58.296'	M27	Minhphuong	$20^0$40.200'	$106^0$10.318'
M12		21 º00.382'	105 º58.407'	M28		$20^0$40.237'	$106^0$10.433'
M13		21 º00.144'	105 º58.373'	M29		$20^0$40.202'	$106^0$10.561'
M14		21 º00.065'	105 º58.575'	M30		$20^0$40.137'	$106^0$10.005'
M15		21 º00.218'	105 º59.038'	M31		$20^0$40.072'	$106^0$10.016'
M16	VNUA*	21 º00.030'	105 º55.538'				

Note: VNUA - Vietnam National University of Agriculture

2. MATERIALS AND METHODS

2.1. Materials

Neutral, less acidic alluvial soil of the Red River system (**now denoted NLAAS**) at the Lechi, Kimson, Duongquang communes and at the farm of Department of Microbiology, Vietnam National University of Agriculture, Gialam district, Hanoi city, Vietnam country.

Gleic alluvial soil of Red River system (**now denoted GAS**) at the Minhphuong, Lexa, Cuongchinh commune, Tienlu district, Hungyen province.

2.2. Methods

2.2.1. Soil sampling

- Time: Summer season in 2015.

- Number of samples: 31.

- Method: ISO 10381-1-2002 and ISO 10381-2-2002: Guidance on soil sampling techniques.

2.2.2. Methods of analysis microorganisims

- Determining density of microorganisms (Total of bacteria, actinomycetes, microfungi, phosphate and phospholipid solubilizing, microorganism of solubilizing organic phosphorus: cultivating microorganisms on nutrient agar medium, then counting colonies appeared (TCVN 4833 - 89) (TCVN - Standardization of Vietnamese)

- Fomular for determining density of microorganism:

$$A(CFU/ml) = \frac{N}{n_1 Vf_1 + ... + n_i Vf_i}$$

A: colony forming units per 1ml (mg) sample

N: total of colonies in all petri dishes counted

n_i: number of petri dishes at (i) constration

V: volume of solution (ml) culturing into each petri dish

f_i: level of dilution respectively

- Assesment the phosphate/ phospholipid solubilizing ability: Using 10% $Ca_3(PO_4)_2$/ Lecithine for 10ml liquid medium, culturing for 5 days at 28°C, filtering to recieve the transparent fluid, then making blue color by the reaction of Molipdate, determining the concentration of PO_4^{3-}.

3. RESULT AND DISCUSSIONS

3.1. Status of microorganism structure in the soil

Table 2 shows that density of TM is very different between samples of two types of soil and even in the samples of the same type. In NLAAS and GAS, the mean density of TM is 89.6 or 48.0 CFU/g respectively. According to the study of Nguyen Xuan Thanh (1996), the average density of TM in neutrally alluvial of Red River system cultivating rice plant (2 crops/year) was about 186.6 $x10^6$ CFU/g soil. Compared with the result of this study, it is about 52% lower. There are many causes of the decrease in density of TM, such as the temperature, soil fertility, cultivation mode...

The average density of TM and microbial groups like TB, TF, TA in NLAAS are always higher than themseft in GAS. This point can be explained due to the affect of the acidity of the soil to microorganism structure. Most of microorganisms grow well at less acidic to slightly alkaline condition. GAS often has so acidicproperty that less favorable for the development of microorganism.

Table 2. Density of some group of microorganism in the soil *(Unit: x10^6 CFU/soil)*

NLAAS					GAS				
Sam.	TM	TB	TF	TA	Sam.	TM	TB	TF	TA
M1	73	71	0.8	1.2	M17	68.2	65	1.7	1.5
M2	92.4	86	1.8	4.6	M17	52.7	48	0.9	3.8
M3	69.9	65	0.2	4.7	M19	63.3	57	1.1	5.2
M4	88.7	80	0.9	7.8	M20	36.1	34	0.5	1.6
M5	100.4	97	0.8	2.6	M21	44.9	42	0.4	2.5
M6	143.4	136	0.9	6.5	M22	45.8	42	0.6	3.2
M7	83.7	79	0.5	4.2	M23	37	34	0.8	2.2
M8	87.6	82	0.9	4.7	M24	52.3	49	0.6	2.7
M9	80.6	76	1.3	3.3	M25	24.1	22	1	1.1
M10	109.4	103	0.8	5.6	M26	31.2	29	0.7	1.5
M11	98.9	93	0.5	5.4	M27	52.8	52	0.3	0.5
M12	94.4	90	1.6	2.8	M28	64.8	63	0.2	1.6
M13	86.8	81	1.5	4.3	M29	32.7	29	1.8	1.9
M14	62.5	60	1.7	0.8	M30	49.8	46	1.6	2.2
M15	88.6	83	1.9	3.7	M31	63.7	62	0.8	0.9
M16	73.4	70	0.9	2.5	-	-	-	-	-
M	89.6	84.5	1.1	4.0	-	48.0	44.9	0.9	2.2
SE	4.7	4.5	0.1	0.5	-	3.5	3.5	0.1	0.3

Note: P values = 0.05; TM - Total of Microorganism; TB - Total of Bacteria; TF - Total of Fungi; TA - Total of Actinomycetes.

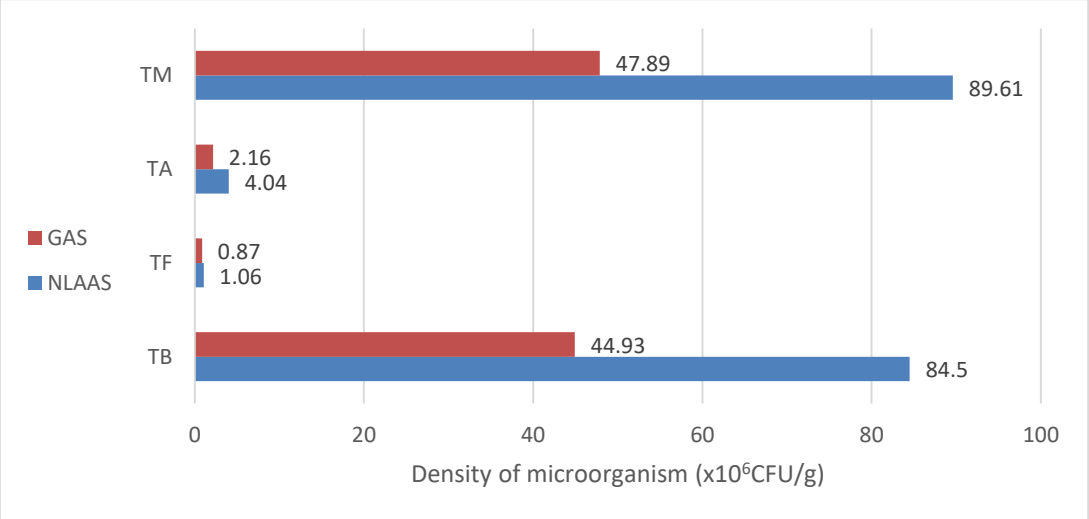

Figure1. Average density of microorganism in the soils

Table 3. Isolated result of phosphorus solubilizing microorganism

(Unit: number of strain)

Soil	$Ca_3(PO_4)_2$			Lecithine		
	TB	TF	TA	TB	TF	TA
NLAAS	6	0	1	4	0	0
GAS	5	0	2	6	0	2

Table 4. Prequency density of phosphorus solubilizing microorganism in NLAAS

$Ca_3(PO4)_2$			Lecithine		
Symbol	Frequence (/16)	Density (CFU/g)	Symbol	Frequence (/16)	Density (CFU/g)
VK1	13	15.5	VK7	5	16.2
VK2	13	19.5	VK8	12	22.9
VK3	8	21.8	VK9	3	4.3
VK4	4	1.3	VK10	1	6.0
VK5	1	3.0			
VK6	2	3.5			
XK1	4	10.8			

Note: VK - Bacteria; XK - Actinomycetes

3.2. Status of microorganism structure of phosphorus sobubilizing in the soils

3.2.1. Isolated result of phosphorus solubilizing microorganism

The number of strains isolated in NLAAS is less than in the GAS. Additionally, the same points in both of soils is the number of bacteria group more than other groups and completely no existence of the fungi. Due to the time of sampling, soil is waterlogged state. This inhibited the growth of fungi, especially mould.

3.2.2. Density of phosphorus solubilizing microorganism

The number of strains solubilizing $Ca_3(PO_4)_2$ is more than strains solubilizing Lecithine. They appeared in samples with very different frequency. Common strains are VK1, VK2, VK3, VK8. They were found in from 8 to 13 samples of 16 samples overall. Others are less common strains, especially VK5 and VK10, only appeared in 1/16 samples.On the other hand, the common strains have also a higher density, from 15.5 to 22.9 × 10^4 CFU/g soil. The remaining strains have lower density, especially strain of VK4, only 1.3 × 104 CFU/g soil.

The number of strains solubilizing Lecithine is more than strains solubilizing $Ca_3(PO_4)_2$. They also appeared in samples with very different frequency. Common strains were VK11, VK12, VK16, VK18, XK2. They were found in from 8 to 13 samples of 15 samples overall. Others are less common strains, especially VK21, only appeared in 1/15 samples. Density of strains is from 1.8 - 17.3 × 10^4 CFU/g soil, the highest - VK12, the lowest - XK4. Additionally, VK11, VK16, XK2 are quite common but not much in density.

Table 5. Prequence and density of phosphorus solubilizing microorganism in GAS

$Ca_3(PO4)_2$			Lecithine		
Symbol	Frequence (/15)	Density (CFU/g)	Symbol	Frequence (/15)	Density (CFU/g)
VK11	11	8.1	VK16	10	5.3
VK12	8	17.3	VK17	3	3.4
VK13	2	17.0	VK18	13	12.1
VK14	2	3.8	VK19	7	2.2
VK15	2	6.3	VK20	3	4.5
XK2	13	2.3	VK21	1	2.9
XK3	2	2.9	XK4	6	1.8
			XK5	5	2.1

Note: VK - Bacteria; XK - Actinomycetes

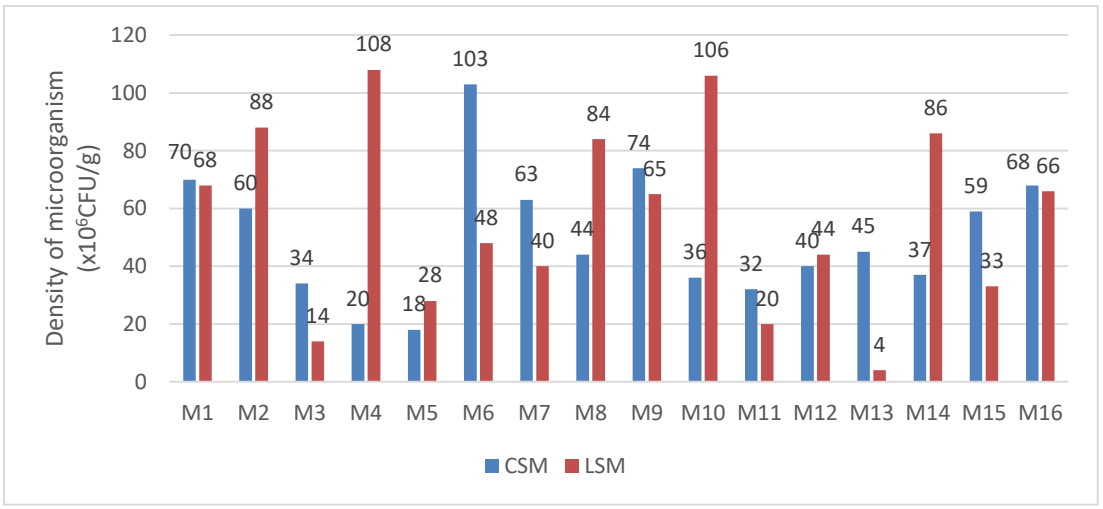

Figure 2. Density of phosphorus solubilizing microorganism in samples of NLAAS

Note: CSM - Calcium phosphate solubilizing microorganism; LSM - Lecithine solubilizing microorganism

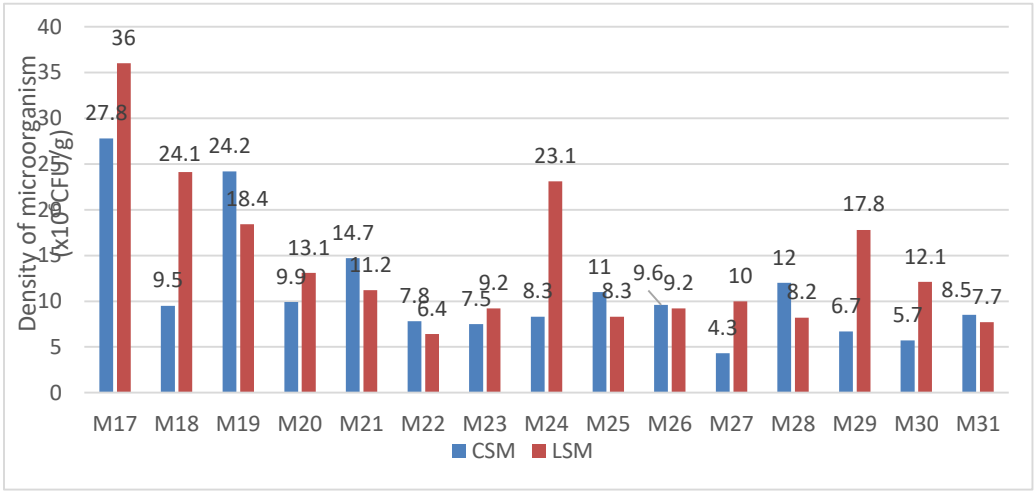

Figure 3. Density of phosphorus solubilizing microorganism in samples of GAS

Table 6. Phosphorus solubilizing ability of isolated strains *(Unit:ppm PO_4^{3-})*

Soil	Strains	$Ca_3(PO4)_2$	Strains	Lecithine
NLAAS	VK1	1.88	VK7	1.18
	VK2	1.32	VK8	0.27
	VK3	3.60	VK9	0.02
	VK4	3.82	VK10	0.42
	VK5	5.66		
	VK6	2.08		
	XK1	2.62		
GAS	VK11	1.37	VK16	1.83
	VK12	0.81	VK17	0.40
	VK13	1.93	VK18	0.27
	VK14	2.91	VK19	0.01
	VK15	2.67	VK20	0.53
	XK2	0.74	VK21	0.71
	XK3	0.70	XK4	0.28
			XK5	0.0

Figure 3 and 4 showed the average density of calcium phosphate and lecithine solubilizing microorganism for each samples. Through these 2 charts, we can see the density varying widely according to each sample. However, in general, the density in NLAAS is quite more plentiful than inGAS, respectively from 18 - 103 and 4 - 108 × 10^4 CFU/g soil, meanwhile in GAS, ranged from 4.3 to 27.8 and 6.4 to 36 × 10^4 CFU/g soil.

3.2.3. Phosphorus solubilizing ability

The data of table 6 showed that the calcium phosphate and lecithine solubilizing ability of each strains were very different, the concentration of PO_4^{3-} anion ranging from 0.70 to 5.66 with $Ca_3(PO_4)_2$ and from 0.0 to 1.83 ppm with lecithine form. Comparing this experimental result with the study of Henri et al (2008) about ability of phosphate solubilizing bacteria *Pseudomonas fluoresens* reached 15.25 ppm PO_4^{3-} after 5 days of cultivation, so the ability of strains in both of soils were much lower. In general, the ability of strains in NLAAS were higher than in GAS. Although some strains had the best ability as VK5, VK7, VK14, VK16 but their popularity and density in both of soils were not high (in table 4, 5).

4. CONCLUSIONS

Joining the process of calcium phosphate solubilizing in NLAAS were 6 strains of bacteria, 1 strain of actinomycetes; meanwhile in GAS were 5 strains of bacteria, 2 strains of actinomycetes. There were 4 strains of bacteria in NLAAS and 6 strains of bacteria, 2 strains of actinomycetes in GAS which can solubilize lecithine. Especially there was not appearance of fungi in both of soils.

In NLAAS, the most popular strains with calcium phosphate solubilizing were VK1, VK2, VK3, VK8. They also had a higher density than others. Meanwhile,in

GAS, the most popular strains were VK11, VK12, VK16, VK18, XK2.

In the both of soils, phosphorus solubilizing ability of the strains were not high. On the other hand, although some strains had the best ability as VK5, VK7, VK14, VK16 but their popularity and density were low. In general, the ability of strains in NLAAS were higher than in GAS but not much.

Should have measures to improve the microorganism structure of phosphorus solubilizing in both of soils in both of quality and quantity. Maybe researching to use microbial products matching the ecological conditions and farming mode of the location.

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FOOD WASTE AND HOUSEHOLD BEHAVIOUR: A CASE STUDY IN TRAU QUY TOWN, GIA LAM, HA NOI

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ABSTRACT

Food waste from households was a serious concern of both developed countries and developing countries due to several negative consequences on environment and food security. Household behaviour is claimed to be the main cause of food waste at consumption stage. The research carried out in Trau Quy Town to find out the current situation of food waste and household behaviour on food waste. By using correlation analysis, the research also aim to examine the relationship between food waste and household behaviour at all stages of food consumption. The results from the analysis of 83 household pointed out, Trau Quy Town has significant amount of food waste thought this problem has been aware by most of household. Two main behaviours, which show significant positive correlation to food, waste which are eating leftover and storing leftover with the correlation coefficients are 0.255 and 0.238 respectively.

Keywords: Food waste, household behaviour.

1. INTRODUCTION

According to the studies of Gustavsson et al., (2011), FAO (2013, 2014), ADB (2015) and Parfitt et al., (2010), food waste is one of the biggest challenges in this century. While food security is a major concern of many developing countries, one-third of global food is lost or wasted every year. Consumption behaviour is claimed to be the main cause of food waste at household level (Aschemann et al., 2015). The food waste due to household consumption might lead to several problems, which are the increase of household domestic waste, poverty, the environmental degradation. Household food waste is not only happen in developed countries but also in developing countries as (Gustavsson et al., 2011) reported. There were several studies were conducted in different countries. However in Vietnam hitherto there were very limited studies which mainly focus on food waste, especially at household level.

In order to provide scientific evidences for sustainable food consumption progress, we conducted a study in Trau Quy, a small town nearby Ha Noi Capital. By using questionnaire to interview 103 household and continuously recording food wastes at 83 households for one week, we explored the current situation of food waste in this town and analysed the link between this problem and household behaviour. After describing the applied methods, this paper analysis and discusses the results before drawing conclusions.

2. METHODS

2.1. Theoretical framework

In order to target study's objectives, a conceptual framework was developed in Figure 1. This framework suggested that, households' food waste is caused by a set of behaviour factors including household perception, household actions and habits through a progress of food consumption which include several stages: purchasing, processing, storing and handing food waste. We hypothesis that, food waste problem might be a consequence of households' behaviours from all mentioned stages. In addition, these behaviours would be shaped by their own perception and social context, for example culture, situation of society. However, in the scope of this paper, we only aim to explored the correlation between households' behaviour and the food waste only.

2.2. Study area and research methods

2.2.1. Study area

The research was conducted in Trau Quy Town, a peri-area nearby Ha Noi. The average income person^{-1} year^{-1} 20015 was 31.2 million VND (Trau Quy People Committee, 2015). The economic growth was 15.8% year^{-1} and mainly contributed by industry and services sector (92.3%), agriculture only takes 7.7% of total GDP. The domestic waste was roughly 4.0 to 4.5 tons day^{-1}.

2.2.2. Household interview method

From February to March 2016, 103 households in Trau Quy Town were interviewed. There was none of these households participated in special occupation related to food services, such as restaurant. The questionnaire aims to collect household perceptions on food and food waste and determine the practices and attitudes of households throughout the food consumption progress (purchase, use and storage). In order to measure the the responses of household, the study used likert five points scale. To measure the households' perception, we used likert agreement scale: strongly agree, agree, neutral, disagree and strongly disagree. To measure the response of household on food choice, we used likert important scale: very important, important, moderately important and not important. To measure household behaviour on food cooking and storage, we used likert frequency scale: always, very often, sometimes, rarely, never.

2.2.3. Diary methods

After interview 103 households, we asked them to continue recording household food waste for one week from 8th of March to 14th of March, following a structured form and full fill a designed form. There were 86 households agreed to join the research but three of them quitted after few days due to special reasons. Finally, we had food waste diary of 83 households. The dairy record focuses mainly on the the volume and types of daily household avoidable food waste. We also add additional question to collect the information about the money household spend for foodstuffs each day and reasons of wasting food as well as food waste treatments methods.

2.2.4. Data analysis

We used SPSS 15.0 software to analyse the collected data. In order to determine the relationship between food waste and household behaviour, we conducted correlation analysis to find out Pearson Correlation Coefficient from the data of 83 households who participated in the diary research.

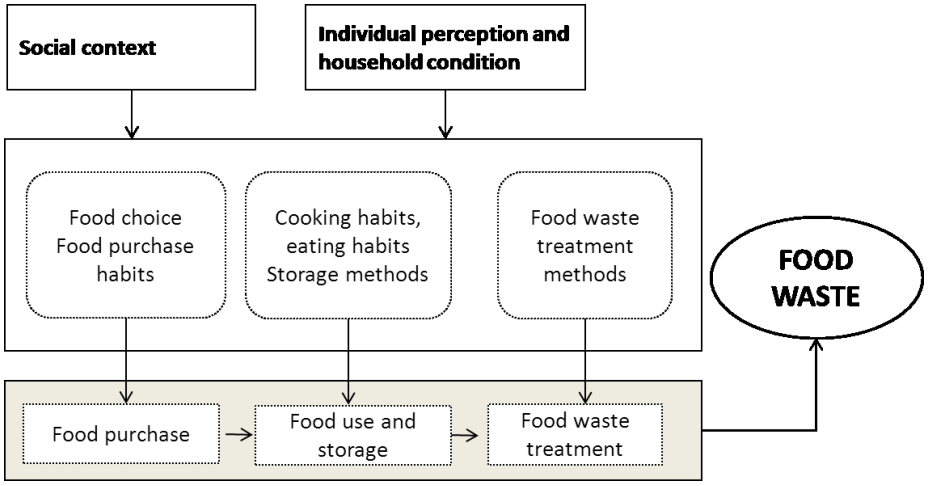

Figure 1. Conceptual framework of household behaviour impact on food waste

Table 1. Characteristics of interviewed households

No.	Interviewees and household characteristics		Frequency	%
1	Gender of interviewee	Male	34	33
		Female	69	67
2	Occupations of intervieewees	Civil servants	8	8
		Bussinessman	13	13
		Elders and retiree	22	21
		Farmers	28	27
		Workers, officers	15	15
		Others	17	17
3	Household size	< 2person	14	14
		3 persons	18	17
		4 persons	36	35
		> 5 person	35	34

3. RESULTS OF STUDY

3.1. Characteristics of sample

There was 103 household participated in the interview and 83 household participated in the diary record. The main characteristics of all interviewed households were summarised in Table 1. 67% of them were female and they have diversified occupations (civil servants, businessman, workers, officers and farmers etc.) The popular households' size was four to five people per household, taking over a haft of total samples. The results of interview also pointed out that, most of households (87%) depend on opened market as their main source of grocery. In addition to that, fridge has become the common facility, which help household storing the food and the leftover recently.

3.2. Household avoidable food waste situation in Trau Quy Town

The volume of avoidable food waste and types of avoidable food waste are presented in Figure 2 and Figure 3. From

food waste diary of 83 household, the total volume of avoidable food waste range from over 14 kg day^{-1} to over 20 kg day^{-1}. The mean value of avoidable food waste household^{-1} is roughly 200 grams day^{-1}. Some household even discharge over one kg food waste day^{-1}.

Base on the volume of food waste and the population of household, we estimated the the coefficient of avoidable food waste person^{-1} in Trau Quy Town is 0.054 kg day^{-1} and around 19.7 kg year^{-1}. In comparison to the food waste coefficient of other developed countries, the food waste coefficient in Trau Quy Town is much lower. In the UK, the food waste coefficient was 0.77 kg person^{-1} day^{-1}; the annual food waste volume of USA, Canada, Australia and New Zealand was 110 kg person^{-1}; Europe was 90 kg person^{-1} (Gustavsson *et al*, 2011). However, the food waste coefficient in Trau Quy Town is slightly higher than the average food waste volume of the Sought and Southeast Asia (15 kg person^{-1} year^{-1}) and double higher than the food waste of Sub Sahara and Africa (8 kg person^{-1}, year^{-1}).

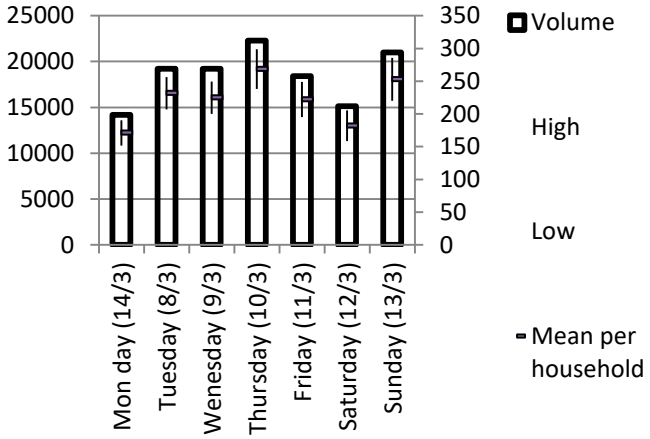

Figure 2. Daily avoidable food waste record of 83 households (Unit: Gram). The columns present the total avoidable food waste volume of 83 household each day. The cross (-) present the mean value of avoidable food waste household^{-1} and the high and low value calculated by ±SE

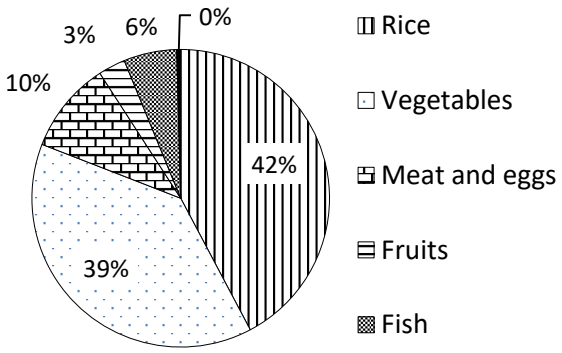

Figure 3. Type of avoidable food waste (%)

In terms of types of food waste, rice and vegetable take over four-fifths of total food waste each day. Other types include fish, meat, eggs and fruit take other one-fifth. Canned food and sweets take smallest proportion, less than one percentage of total volume. Most of these foods were wasted due to cooking too much; take nearly two-third causes of wasting food.

3.3. Household behaviour and food waste

The interview data shows that, most of interviewed households have been aware food waste problems. The proportion of people who agree and strongly agree food waste cause serious environmental problems (88%) and over three fourth of them always attempt to reduce food waste. The proportion of people who do not pay attention to the volume of food waste and economic value of food waste was small (less than 20% of interviewee). However, 83% of household agree and strongly agree that food waste is unavoidable problems.

Regarding to the factors influencing the food choice of households, the study suggested several options in the questionnaire. The response of interviewees show that, most of people pay more attention to the household member favour, household income, labelled or origin product, seasonal products, product of acquaintance and near locations when they purchase groceries. The proportion of people who thought these criteria was important and strongly important take over two third of interviewees. People pay less attention to environmental friendly and easy for processing products, only one third of interviewees. Especially, only few people considered cheap price and advertised products are the important factors, which affect their food choice decision making.

In addition to households' perception and food choice, the study also gathers the frequency of household on food purchasing, cooking and storage habits. Some frequent behaviours of households which are shopping one time day^{-1}(77%), cooking enough food for one meal (76%), purchasing enough food for a day (56%), splitting food into smaller parts for cooking and storing, checking the leftover and the remaining groceries in the kitchen (nearly 50%). There was only 27% household buying enough food for one meal and 14% of household purchasing grocery two times day^{-1}. In term of cooking and storage habits, only one third of households eat leftover and store the leftover, only 20 % of household frequently and often throw the leftover away.

The statistic review of household responses point out that, people in Trau Quy Town have many positive reactions in order to minimize food waste of households. However, to find out which behaviour has relations to the food waste volume, we use SPSS 15.0 to analyse the Pearson Coefficient testing the statistical reliable level. The results are summarised in Table 2.

The correlation analysis confirmed the presence of a significant correlation between the households' food waste coefficient and some households' specific habit and perception. The results pointed out, household food waste volume has a significant negative relationship with with habits of purchasing grocery sold at near locations ($r=-.237$, $p < 0.05$), buying food which is easy for cooking ($r=-.247$, $p < 0.05$) and the habits of buying the amount of groceries which are enough for one meal ($r=-.245$, $p < 0.01$). Nevertheless, food waste volume has a significant positive correlation to the habits of eat leftover ($r=.255$, $p < 0.05$) and store leftover ($r=.238$, $p < 0.05$). These households take one-third of total interviewed households.

Table 2. The correlation between food waste per household and its behaviours

No. of question	Opinions and habits	Pearson Correlation	Sig. (2-tailed)
Household perception (Strongly agree, agree, neutral, disagree, strongly disagree)			
Q23.1	Food waste is unavoidable	-.113	.311
Q23.2	I do not pay attention to money loss of food waste	-.091	.415
Q23.3	I do not pay attention to volume of food waste	.093	.402
Q23.5	I always attempt to reduce food waste	.123	.267
Q23.6	Food waste cause serious environmental problems	.034	.762
Food choice (very important, important, moderately important and not important)			
Q17.1	Household members favour	.053	.636
Q17.2	Near location	-.237(*)	.031
Q17.3	Products of acquaintance	-.143	.196
Q17.4	Cheap price	-.025	.822
Q17.5	Advertised products	.186	.092
Q17.6	Nutrient needs of household members	-.044	.691
Q17.7	Seasonal products	.094	.399
Q17.8	Label or origin products	-.018	.870
Q17.9	Household income	-.133	.229
Q17.10	Environmental friendly product	-.160	.149
Q17.11	Easy for processing	-.247(*)	.024
Food purchase (always, very often, sometimes, rarely, never)			
Q16.1	Meal planning	.056	.615
Q16.2	Checking the remaining food stuffs in the kitchen	.032	.774
Q16.3	Checking the leftover	.059	.594
Q15.1	Buying enough food for one meal	-.245(*)	.026
Q15.2	Buying enough food for a day	-.115	.300
Q15.3	Buying food for two days or more	.013	.907
Q14.1	Grocery shopping two times day $^{-1}$	-.383(**)	.000
Q14.2	Grocery shopping one times day $^{-}$1	-.020	.860
Cooking and storage (always, very often, sometimes, rarely, never)			
Q21.2	Cooking enough food for one meal	-.099	.375
Q21.1	Splitting food into smaller parts for cooking and storage	-.096	.388
Q21.3	Cooking enough food for two meals or more	-.058	.606
Q21.4	Eating leftover	.255(*)	.020
Q21.7	Eating all food on the table	.118	.836
Q21.5	Throwing away leftover	-.023	.836
Q21.6	Storing leftover	.238(*)	.030

*Note: * Correlation is significant at the 0.05 level (2-tailed);** Correlation is significant at the 0.01 level (2-tailed).*

Relations between food waste and others of households' perception, food choice, food purchase as well as cooking and storage are very weak, all correlation values are lower than 0.19 with p-value bigger and 0.05. Thus, the study does not have sufficient evidence to confirm to existences of these relationships.

4. CONCLUSIONS

The results of study present evidence that food waste is happening in Trau Quy Town. Although the coefficient of food waste per person in this town is much lower in comparison to the coefficient of UK and other developed countries, however, it is much higher than the mean value of other areas, which has similar condition, for instance Sub African countries and the South and Southeast Asian Countries. Most of food waste in Trau Quy Town is the groceries bought from opened markets and the types of leftover.

The collected information also presents positive behaviour situation of residents in Trau Quy Town about food waste problems. Most of interviewed people have awareness about food waste and food waste value. Some good habits of household, which are purchasing seasonal, acquaintance and origin products; buying enough food for a day, shopping frequently; and cooking enough food for one meal. As recommendation of FAO and some other research about food waste, these behaviours would contribute to the reduction of food waste from households. The correlation analysis also state that the habits of purchasing grocery at near location and especially the grocery which easy for processing, shopping two times per day and buying enough food for one meal have significant negative correlation to household food waste volume. The habits of eating leftover and storing the leftover have significant positive correlation of food waste. However, these habits actually relate to cooking too much food. These households only take one third of total interviewed household. There was no significant relationship between food waste and household perception and other behaviour.

In CONCLUSIONS, this research confirms the existence of food waste problem in Trau Quy. The study has evidences to state that, the leftover could be the main source of food waste in this town. The waste was not only lead to several environmental problems but also affect to household income. Thus, changing household behaviour to reduce food waste is essential solutions.

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THE STUDY OF BIOGAS GENERATION FROM HEIFER MANURE DIGESTION BY USING PINEAPPLE PEEL AS CRUDE FIBER FEEDING

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ABSTRACT

The objective of this research was to study of biogas generation from heifer manure in anaerobic digestion condition by using pineapple peel as crude fiber feeding. The experimental was a complete randomized design with 3 treatments. There was Treatment I: Fermented the heifer manure by feeding with hay, Treatment II: Fermented the heifer manure by feeding with hay and pineapple peel (50:50) and Treatment III: Fermented the heifer manure by feeding with pineapple peel. It was found that the Initial pH of these mixes was 5.9 – 7.5. The percentage of total solid (TS) was decreased about 11.51%, 2.11% and 0.16% respectively. The volatile total solid (TVS) was decreased about 9.15%, 6.02% and 1.83%, respectively. The COD was increased about 0.77%, 77.24% and 54.59% respectively. The ammonium nitrate was increased about 71.43% and 57.89% in Treatment I and II respectively but Treatment III was increased about 3.85%. The carbon was decreased about 12.21% and 2.42% in Treatment I and II respectively but Treatment III was increased about 3.38%. The cumulative of biogas was produced average 11.22, 2.53 and 4.59 liter respectively ($p < 0.01$). The methane was produced average 38.33%, 5.95% and 11.55% respectively ($p < 0.01$).

Keywords: Anaerobic digestion, biogas, pineapple peel.

STUDY ON THE CURRENT STATUS OF METHANE EMISSIONS AND INTEGRATED DOMESTIC WASTE MANAGEMENT IN THE CENTER OF THAI NGUYEN CITY

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ABSTRACT

Thai Nguyen city is an urban of type 1. Alonging with economic development, urbanization of Thai Nguyen City is an increase in household waste and environmental pollution. This study found out that the amount of average waste generated in 10 wards of the downtown area of Thai Nguyen City is 118.57 tons per day and collection rate is 83% (98.42 tons per day). The proportion of recyclable and reusable materials includes 62.39% of organic material, 7.36%, of rubber, plastic, nylon, 6.83% of shredded paper, 6.62% of metals, 4.91% of fabrics and cloth and 2.23% of ceramic and glass. It estimates CH4 emissions based on organic ingredients that are easy to be biodegradation in waste (DOC). Amount of CH_4 emissions generated from household waste is 4.62 tons per day corresponding with 106.26 tons CO_2 per day and a proposed model of collection, sort and processing household waste in line with the Thai Nguyen City.

Keywords: CH_4 emissions, household wastes, Thai Nguyen City .

1. INTRODUCTION

Currently, the environmental pollution caused by domestic waste is a disturbing problem of many cities in Vietnam, in which, it includes Thai Nguyen City. Thai Nguyen city is an urban of type 1 and is the center of the midland and mountainous regions in the North of Vietnam with the 10th largest population nationwide. Along with economic development and urbanization, the industrial waste, domestic waste and environmental pollution also increase. Actually, the management work of domestic waste is currently only to focus on gathering and burying wastes, it is not safe for humans and the environment. The management work on collection, classification and reuse of wastes, if implemented in a synchronous and systematic manner and appropriate processing technology, will be very significant, bring economic benefits, environmental protection and savings of natural resources. Solid waste management in general and domestic waste management in particular in a sustainable way is a top priority in the national strategy of environmental protection. With high concentration of organic matter, the anaerobic decomposition process takes place when burring. This can generate a large amount of CH_4 emissions - a gas capable of cause of the greenhouse effect with 23 times higher than that of CO_2 (Forster *et al.,* 2007). Therefore, the calculation of CH4 emissions from domestic waste in the downtown area of Thai Nguyen City will allow people who are working in waste management to have an overview of the potential of recovery of CH_4 and energy source arising from waste, etc. Stemming from the that fact, "Research on the current status of methane

emissions and measures of integrated management of domestic waste in the downtown area of Thai Nguyen City" has been studied. The study results will contribute to Thai Nguyen City's waste management getting better and better.

2. METERIALS AND METHODS

Location: Research on the current status of methane emissions and measures of integrated management of domestic waste in the downtown area of Thai Nguyen City including 10 wards of Quang Trung, Dong Quang, Phan Dinh Phung, Hoang Van Thu, Tuc Duyen, Trung Vuong, Gia Sang, Tan Lap, Thinh Dan and Tan Thinh.

Methods: An actual survey was carried out in volume of domestic waste generated by 200 households divided equally 10 wards in the downtown area of Thai Nguyen City. Of which, rich households, good income households, medium income households and poor household account for 10%, 60%, 15% and 15%, respectively. The survey was taken place once per month in a 3-month period, analyzed components of domestic solid wastes from the surveyed households. Then the current status of the management work of domestic waste in the downtown area of Thai Nguyen City was analyzed. From this the pattern of solid waste management under the orientation of the integrated management was analyzed and given.

Calculating the amount of decomposable organic carbon (DOC) (Nguyen Xuan Hoang and Vo Dinh Long, 2010) as follows:

DOC = 0.4A + 0.17B + 0.15C + 0.01D

Of which:

A: The percentage of garbage in forms of paper, carbon and cloth

B: Percentage of other garden garbage to decompose (excluding food waste)

C: The percentage of food waste

D: The percentage of other organic waste.

Calculating amount of methane (CH_4) emissions from of domestic waste (IPCC, 1995) as follows:

CH4 emissions (ton per year) = (WT *WF*MCF*DOC*DOCF*F*16/12-R)*(1-OX)

Of which: - WT: Total amount of waste generated (tons per year)

- DOCF: Error Value of DOC (the default value is 0.7)

- WF: The percentage of garbage taken to landfills

- F: Percentage of CH_4 in landfills (default value is 0.5)

- MCF: The default value of methane parameter (0.6)

- R: methane recovered (tons per year)

- DOC: The percentage of DOC in waste

- OX: Oxidation Ratio

Data processing: The data were statistically processed in MS Excel and SAS 9.0

3. RESULTS AND DISCUSSION

3.1. Current status of waste generated in the downtown area of Thai Nguyen City

The total volume of domestics waste generated by 10 wards in the downtown area of Thai Nguyen City is presented in Table 1 as follows: Factual survey results of 200 households in 10 wards of the downtown area show that the average waste discharge rate per person (in households) in the downtown area ranges between 0.53 to 0.72 kg per day and from other sources range from 0.11 to 0.34 kg/person/day. The average garbage discharge per capita of households in the 10 wards of the downtown area compared was not statistically significant at a 5% level.

Comparison of the average garbage discharge level per capita of households under income showed that there was significantly different when comparing the poor and medium income households versus good income and rich households. However, there was not significantly different when comparing the poor and medium income households versus good income households with the rich households at a 5% level.

The waste volume and quality in urban depend on the economic status. Waste in rich area is different from that in the poor area. Therefore, economic sectors impact significantly on waste discharge of residents. The actual survey results on components of domestic waste in average in the downtown area of Thai Nguyen City is clearly shown in the Figure 1.

Through Figure 1 shows that organic waste, other material, group of rubber, plastic, nylon, shredded paper, metals, group of fabrics and cloth and group of ceramic and glass account for 62.39%, 9.66%, 7.36%, 6.83%, 6.62%, 4.91%, and 2.23%, respectively. Notably, the amount of organic wastes, metal, plastic, etc. is the recyclable and reusable sources, if utilized, it will reduce the costs of treatment and bring economic benefits.

Table 1. Volume of domestics waste generated by 10 wards in the downtown area

Seq.	The downtown area	Population (people)	Waste from households (kg/person/day)	Waste from other sources (kg/person/day)	Total (tons/day)
1	Quang Trung Ward	23.383	0.66 ± 0.18ns*	0.18 ± 0.08ns*	19.64
2	Dong Quang Ward	11.369	0.67 ± 0.16ns*	0.34 ± 0.03ns*	11.48
3	Phan Dinh Phung Ward	18.533	0.72 ± 0.21ns*	0.24 ± 0.18ns*	17.79
4	Hoang Van Thu Ward	17.234	0.63 ± 0.12ns*	0.21 ± 0.06ns*	14.48
5	Tuc Duyen Ward	9.312	0.62 ± 0.22ns*	0.21 ± 0.11ns*	7.73
6	Trung Vuong Ward	8.078	0.69 ± 0.19ns*	0.26 ± 0.04ns*	7.67
7	Gia Sang Ward	12.963	0.59 ± 0.26ns*	0.13 ± 0.09ns*	9.33
8	Tan Lap Ward	12.573	0,65 ± 0,15ns*	0,14 ± 0,02ns*	9,93
9	Thinh Dan Ward	15.320	0.53 ± 0.19ns*	0,11 ± 0.05ns*	9.80
10	Tan Thinh Ward	14.667	0.54 ± 0.11ns*	0,19 ± 0.10ns*	10.71
	Total	143.432	0.63 ± 0.21ns*	0,20 ± 0.17ns*	118.57

*Note: *Mean values ± SE; and ns: non-significant difference*
Source: Survey results, 2016

Table 2: the average garbage discharge level per capita among household groups

Seq.	Household surveyed	The average garbage discharge level (kg/person/day)
1	Rich	0.74 ± 0.08a
2	Good income	0.69 ± 0.05a
3	Medium	0.41 ± 0.03b
4	Poor	0.35 ± 0.03b

Note: Mean values ± St.E, the same letters were not statistically different at a 5% level.

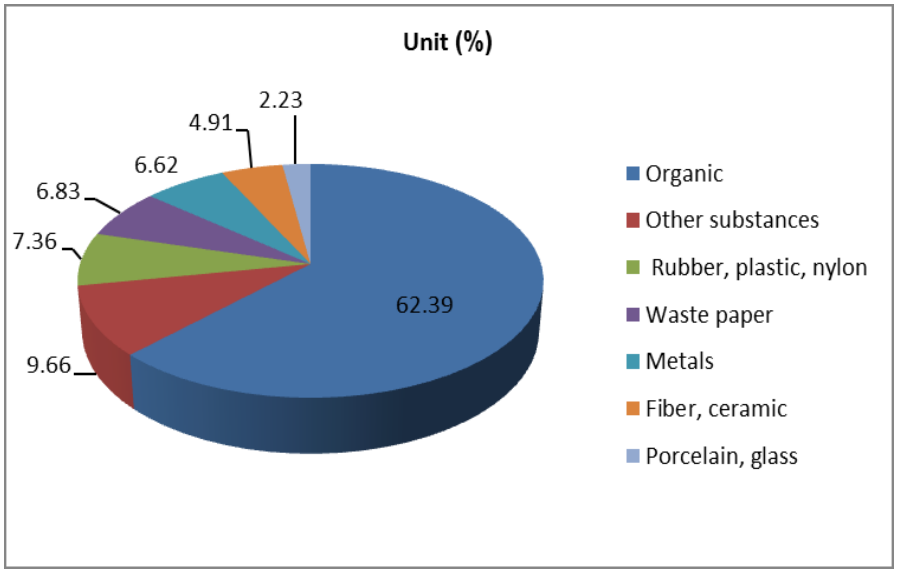

Figure 1. Ratio of components of domestic waste in average in the downtown area of Thai Nguyen City

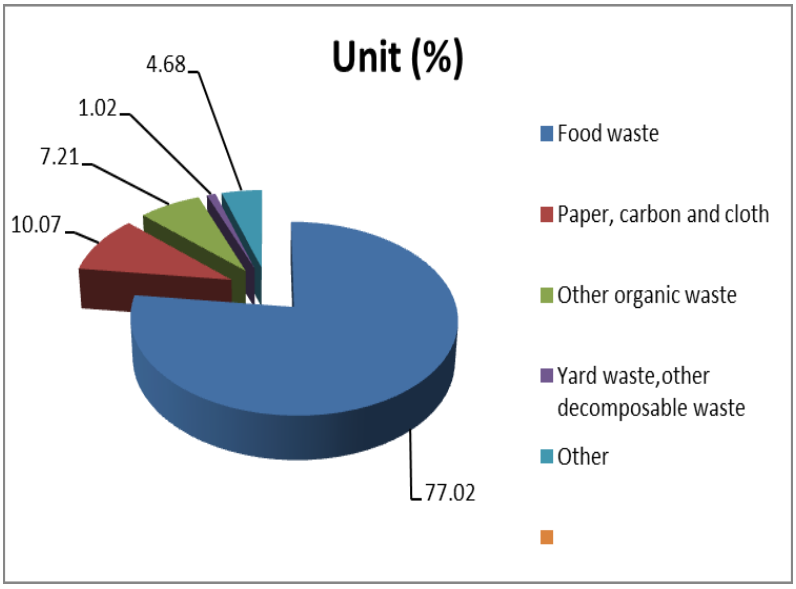

Figure 2. The amount of decomposable organic carbon in the domestic waste

3.2. Calculation on the level of greenhouse gas emissions (CH_4) and the amount of decomposable organic carbon (DOC) in conditions of domestic waste landfilled in Thai Nguyen City

Calculation of the amount of decomposable organic carbon (DOC):

From the results, the classification of components of the decomposable organic wastes in domestic waste in the downtown area of Thai Nguyen City is as follows:

According to IPCC(1995), the amount of decomposable organic carbon:

$$DOC = 0.4A + 0.17B + 0.15C + 0.01D$$

Of which: A = 10.07: The percentage of garbage in forms of paper, carbon and cloth

B = 1.02: Percentage of other garden garbage to decompose (excluding food waste)

C = 77.02: The percentage of food waste

D = 7.21: The percentage of other organic waste.

DOC = 0.4 * 10.07 + 0.17 * 1.02 + 0.15 * 77.02 + 0.01 * 7.21 = 16.79%

Thus, the amount of decomposable organic carbon in the waste to in the downtown area of Thai Nguyen City is 16.79% .

Calculation of the amount of CH_4 emissions from the domestic waste:

According to IPCC (1995), amount of recovered methane can be calculated on the basis of the total amount of waste taken to landfills. It applied the method of USEPA'S LANGEM, Methane (CH_4) conversion factors corresponding to the different types of landfills are shown in Table 3:

Through practical investigation in conjunction with the report on the situation of garbage collection of Thai Nguyen Urban Environment Company, the amount of collected waste in the downtown area of Thai Nguyen City is 118.57 tons per day, with a 83% rate of collection (98.42 tons per day).

Choose WF = 0.83, MCF = 0.6, WT = 118.57, DOC= 16.79, DOCF = 0.7, F=0.5, R=0, OX=0. Therefore, the amount of methane is calculated as follows:

CH_4 emissions (tons per day) = (WT * WF * MCF * DOC * DOCF * F * 16/12-R)*(1-OX)

=(118.57*0.83*0.6*0.1679*0.7*0.5*16/12)*(1-0) = 4.62 (tons per day).

CH_4 emissions generated from of domestic waste per day in the downtown area of Thai Nguyen City is 4.62 tons per day corresponding to 106.26 tons of CO_2 per day and amount of emissions increases directly proportional to the amount of waste annually. If there are no measures to collect or minimize CH_4 emissions, the consequences for the environment are significant.

Proposal of the pattern of the integrated management of domestic waste in wards of the downtown area of Thai Nguyen City:

Diagram of management of domestic waste of households in 10 wards of the downtown area of Thai Nguyen City proposed (Figure 3).

This is an important factor determining the whole managing process of domestic waste. Waste of households is classified under three groups:

Group 1: Biodegradable organic matter (green) as food waste yard waste, etc.

Group 2: Recyclable inorganic waste (red) as paper, rubber, metal, etc.

Group 3: Unrecyclable inorganic waste cannot be (yellow) as brick, stone, porcelain, etc.

Three groups of solid waste are contained in three separate bins or used trash model having 3 compartments with three different colors and 3R-W (3R are Reduce, Reuse and Recycle; W is Water. After wastes are collected and stored at households, wastes will be collected daily by hygiene workers with stroller available separate compartments and be taken to transit station. At this station, waste of the group 1 will be transported to the composting plant, that of group 2 will be collected and sold for recycling facilities and that of Group 3 will be transported to landfills appropriate sanitation.

Table 3. Landfills burying domestic wastes and correlation values of the parameter of methane

Seq.	Solid waste disposal sites (SWDS)	Methane conversion factors (MCFx)
1	With management	1
2	Without management - depth (≥ 5m of waste)	0.8
3	Without management - depth (< 5m of waste)	0.4
4	The default value for landfills unclassified	0.6

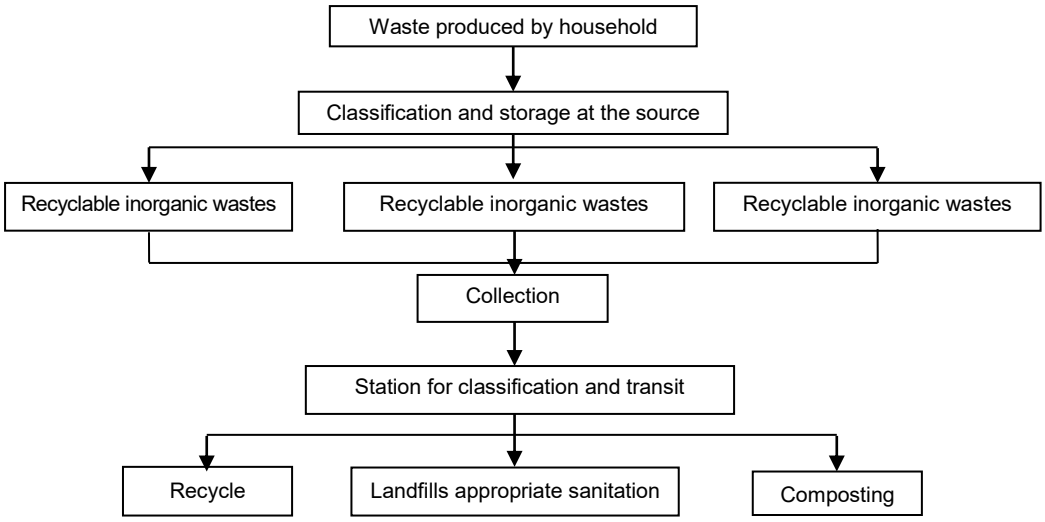

Figure 3. Diagram of proposal of management of domestic waste in households of the downtown area of Thai Nguyen City

To thoroughly treat amount of waste classified, it is necessary to invest in building synchronizing waste treatment station, including waste receiving points, composting plants, landfilled areas, wastewater treatment plants, etc. Thus, methane (CH4) emissions causing the greenhouse effect will reduce. This is not only to make sense in terms of the environment, but also bring the important significance in terms of economics (heat collection, improvement of natural air quality, etc.) and save natural resources.

4. CONCLUSIONS

The average amount of waste in 10 wards of the downtown area is 0.63 kg per day per person. The biodegradable organic component of domestic waste accounts for a high proportion is 62.39%. That of other material, group of rubber, plastic, nylon, shredded paper, metals, group of fabrics and cloth and group of ceramic and glass accounts for 9.66%, 7.36%, 6.83%, 6.62%, 4.91%, and 2.23%, respectively.

The amount of CH4 emissions generated from of domestic waste per day in the downtown area of Thai Nguyen City is 4.62 tons corresponding to 106.26 tons of CO2 per day.

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ECONOMIC AND RURAL DEVELOPMENT

THE DYNAMIC PATHWAYS OF AGRARIAN CHANGE IN THE RED RIVER DELTA OF VIETNAM

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ABSTRACT

Even though agricultural production has not been the main source of income for rural households in Red River Delta, yet farming remains an important livelihood and security for many rural people. The empirical findings from fieldwork in a village in the Red River Delta of Vietnam show how dependence on agriculture is determined by family context, including land, education and job of household members, their gender and age. Most educated young people have successfully found employment opportunities outside in the village as migratory wage labour, and have capacity to attain higher social status and become rural entrepreneurs. While less educated and older villagers consider agriculture as a more favorable alternative and valuable security. Besides, maintenance agricultural production at reasonable level and return to traditional cultivation is also considered as a rural household strategy coping with food security in industrialization and rural-urban linkage. Diverse and special extended livelihoods are contributed by historical, cultural and economic specificities which are conditioned for rural transformation.

Keywords: Agriculture, livelihoods, migration, Red, remittances, River Delta, rice production.

1. INTRODUCTION

Southeast Asia still tends to be characterized as a region where farming remains the pre-eminent occupation. Indeed, statistics shows that even Southeast Asia has recently experienced rapid industrialization and modernization which would lead to the diminishing role of agriculture (Rigg, 1998, Soda, 2007, Cramb, 2012). The region's agriculture is also influenced by a range of factors including globalisation, environmental and demographic changes and increased mobility. Despite the prediction of de-agrarianisation, agriculture is enduring (Hirsch, 2011, Peemans, 2013), but trajectories of agricultural change vary given the cultural, geographical and historical specificities of rural place (Kelly, 2011). In Vietnam, the Red River Delta region in the Northern part shares the common features of agrarian transition undergone by the *Doimoi* (Innovation Reforms) in the mid-1980s. There has been a change in the structure of GDP whereby the share of agriculture has relative declined from 42 percent in 1989 to 26 percent in 1999 and 21 percent in 2011 while the industry sector has more than doubled from 23 percent in 1990 to 47 percent in 2011 (GS0, 2011). Agricultural land has been converted toward more market and industrial orientation to gain faster economic development. According to the recent National Survey on Land, in a decade from 2000 to 2010, the non-agricultural land increased 89000 hectares while the land for rice production decreased more than 34000 hectares annually

(Nguyễn Ngọc Công, 2012). The overall number of landless farmer households in the region was 3.3 percent in 1999, 13.9 percent in 2002 rose up to 22 percent 2012 (FAO, 2014). Red River Delta historically is also the densest populated region in Vietnam with average 0.04 hectares per head[1]. Therefore, since late 1990s this region witnessed a significant increase of off-farm business ever. Rural households often adopt more than one strategy to diversify their livelihood such as intensifying agricultural production and diversifying their economic activities in non-farm business. Therefore, there is a large wave of peasant migrants moving out of agriculture to find the jobs in nonfarm sectors along rural-urban continuum (Nguyen Thi Dien et al., 2014). While there is little doubt that migration would improve the well-being of migrants' households in developing country, its impact on agriculture remains debated and less straightforward. Some hypothesized that rural out-migration could lead to land abandonment and de-agrarianization as a part of "agrarian change" in Vietnam. Negative impact of migration on agriculture was observed when peasants lost their farming skills and also the desire to farm their lands. Beside, when peasants move, their self-identifications shift (Royal et al., 2003). They see a bright future in urban areas with better social service and white collar jobs; hence they lose their interests in agriculture as well as rural areas. In such instances land may be sold, leading to disengagement and a final break between the rural household and traditional farm-based livelihoods. In some areas, severe labour shortages due to out-migration cause farmers either to leave their land, rent it out or dispose of it. However, this notion of de-agrarianisation has problems in its claiming the only posits linear trajectories of rural lives (Hirsch, 2011). Likewise, despite the emphasis on the importance of non-agricultural work, industrialisation, and migration, agricultural production endures (Brookfield, 2008, Lebailly, 2015). Furthermore, government agricultural subsidies and high food prices have helped farmers to introduce cash crops and maintain farming activities despite their engagement in non-agricultural wage labour. In other instances, people are sufficiently inspired to renew their interest in agriculture and have returned to villages to farm (White, 2012). Although out-migration of rural populations to urban centres caused labour shortages for farm households, they were often able to find substitute labour. Especially in the Red River Delta, the labour gap was filled by labour acquired through market (*wage labour*) and non-market relations (*mutual help*). Farming is also supported by off-farm jobs and remittances. (Hoang Xuan Thanh et al., 2013) argue that diversification of rural livelihood strategies actually helps to sustain family farms and keep them alive. Non-farm work and migration have potential in improving rural household's living standard and provide a endorsement against agricultural shocks (Ellis, 2000). Farmers are able to be both peasants and labourers at the same time. They can be wage labourers in the agricultural sector, and international migrants, urban labourers or small-scale traders. Some of the practices employed by farmers include switching between farm and non-farm jobs, growing non-rice cash crops and utilising social

[1] The area of land devoted to production agriculture accounts for 29% of Vietnam's total land area; Vietnam currently has only 0.11 hectares of agricultural land per person. This area however is distributed uneven distribution across regions. In the Red River Delta, land area for agricultural production accounts for 0.04 hectares per head. IN Mekong Delta, the average MRD people have land for 0.14 hectares agricultural land BUI MINH, B. Q. N., DANG THI VIET PHUONG 2012. Agricultural land, peasant and rural development. Sociology, 3, 26-33.

capital such as kinship ties in times of need. Moreover, in the era of globalization and labour flows have significant impact upon national and local economies, this paper want to emphasizes on the persistence of agriculture in contrast to the notion that rural Vietnam is shifting away from farming livelihood. This paper therefore both captures the problems of a linear interpretation of de-agrarianisationand seeks to explain the persistence of agricultural livelihoods, with particular reference to one village in the Red River Delta. The first section explains research methods and the field data collection process. The second section discusses the key findings, which focus on the importance of internal migration, remittances, land ownership and opportunities to participate in cash-crop production. This section highlights that, irrespective of how rural livelihoods have changed, farming remains a vital component. In order to understand the agrarian transformation adequately, it is necessary to consider local and geographical contexts of the rural place. The last part offers a summary and CONCLUSIONSs.

2. METHODOLOGY

This paper draws on a case study carried out in Mai Thon village, Bac Ninh province. Bac Ninh is located in Red River Delta, about 30km far north from the capital Ha Noi, along the recently upgraded National Highway 1A. Having an area of 823 km2 in total and with around 1.038 million populations, Bacninh is the smallest province of the Delta. Mai Thôn was chose as the research site because it was near Bacninh Industrial Zones, and it had a good infrastructure with Hanoi and other provinces; therefore, it created chances for their habitants to migrate out. In 2015, I had conducted a systematic household survey in Mai Thon village, using a questionnaire. According to Hokhau Record[2], Mai Thon village has 158 households in total (up to July/2015) which include 699 villagers I did interview with 128 households, equivalent with 81% of the whole village household number. This year, during January 2016 and June 2016, ethnographic research was conducted continuously in Mai Thon village. This involved participant observation, in-depth interviews, everyday conversations, and the 'walk and talk method', or observing, talking, and joining villagers' activities. In this case, I worked in the paddy fields with the villagers while carrying out conversations, especially when migrants from Hanoi and other provinces come back home at peak time of farming season.

2.1. Mai Thon, agrarian community?

Outwardly, Mai Thon village appears agrarian, with large areas of paddy fields and gardens surrounding the village. The socio-economic conditions of Mai Thôn are characterized by monoculture in rice production in the long period, and high levels of out-migration. Mai Thôn village has around 158 households and most of these households have agricultural land and home garden. Even though 97% households claim agriculture is as one source of income, only 10 percent declared agriculture as their main occupation. It is has a traditional handicraft but very limited dynamic non-farm business diversification *within the village*. However, it is considered to have the highest on economy status due to migration process. It is also located 20km from capital of province and

[2] Ho khau refers to the system of residency permits which dates back 1950s, which is closely related to other benefit such as agricultural land contribution, housing, education, administrative papers and even food in the collectivization period. A hokhau can also refer to a family register in many contexts since the household registration record is issued per family, and usually includes the births, deaths, marriages, divorces, and moves of all members in the family.

40km from Hanoi. It has a very good telecommunications, power and local road infrastructure. Nearly 30 percent of household has member migrate out of province for work. And 83.6 percent of households in the village has one member who has travelled *out of village* for off-farm job.

Apart from these four households, the remaining 83.6 percent of village households identified themselves as farmers, regardless of the amount they actually earned from agriculture. Although rice farming had become a subsidiary economic activity for the majority of households, most villagers consider themselves culturally active members of their rural community. This, therefore, contrasts with the agrarian literature where livelihood diversification and migration are considered to diminish the importance of agriculture in rural livelihoods (Rigg, 2005, Rigg, 2001, Vaddhanaphuti and Wittayapak, 2011). In the following sections, I will show how households in the Delta village manage land and labour, and continue to engage in agriculture.

2.2. Land holdings in Mai Thon village

Agrarian studies on rural transformation reveal that one of the factors leading farmers to move away from agriculture is the diminishing importance of agricultural land. Vietnam's export-oriented industrialization efforts since the mid-1980s have created large industrial estates in many provinces and increased land prices. Even though being the smallest province of the Delta. BacNinh province has been considered as prominent in term of industrial development in Vietnam. At the time of its formation in 1997, Bacninh was an agricultural province, with only several handicraft villages and no industrial zone or industrial cluster. Since 1998 the provincial government started acquiring agricultural land for industrial purposes, after which the first industrial zone has been built. To date, Bac Ninh has 15 industrial zones and more than 35 industrial clusters with more than 9400 ha agricultural land acquired[3]. This encouraged many rural households to sell their agricultural land use right. The average level of compensation for one *sào* of agricultural land in 2000 was 30 million VNĐ, it doubles in 2007, including four items of compensation and assistance. This rapid increase in land price compensation had changed some farmers become millionaires overnight. However, in sharp contrast to the land boom in BacNinh itself, this did not occur in Mai Thon. Located in Que Vo district which has the largest industrial zones within the province with 1,204 ha already converted for industrial zones, Mai Thon villagers in contrast with other villages in the same district, have no chance to convert their land for anything other than agriculture, although many wished to. There is only 0.2% of households change paddy field into other agricultural purposes (aquaculture and cash-crop growing). There is also a paradox in their own though when they on one hand waiting for a chance to have compensation for their land, on the other hand they refuse to sell their land. Mr Diep, a 54-year-old male who owned 8 sao agricultural lands, stated that:

I heard that our village will belong to Bac Ninh city in next few years and agricultural land price will rise. But it is all in future, in general, villagers here do not sell land and nobody asks to buy land from neighbours or relatives. If they need money urgently, they tend to borrow from relatives. Some might mortgage the land and continue cultivating it but, in the end, they would try to pay and get the land back.

[3] The data has been collected at the official website of Bacninh Industrial Zone (http://www.izabacninh.gov.vn/ ?page=home&portal=kcnbn accessed on 16 of Feb, 2014) and Decision 396/QĐ-UBND, issued on 31, October 2013 on the approval of the cluster planning in Bacninh province to 2020, vision 2030)

Table 1. Main occupations of rural households in Mai Thon village

Main occupations*	Number of households
Farmers	12
Farmers + Wage labour (freelance workers)	27
Farmers + Local enterprises (traders, food staplers, etc)	24
Local enterprises (no farm)	1
Salaried employees + farmers	53
Wage labour	6
Unemployed	5
Total	128

*Note: *: Major occupations are based on what villagers earn for their income*
Source: author's survey 2015;

Non-farm households who obtain majority income from non-agricultural and off-village still keep their own land and the maintenance of land rights is always their priority, even when they have other opportunities of other employment and income from elsewhere. Even when they migrate out, they are unlikely to sell their land (0.1%), but rather leasing that land (11.7%) or even leaving it fallow for certain crops (6.3%). The whole group of non-agricultural households releases their paddy field for as their brothers, cousins to produce and field supervise. It is noteworthy that even some agricultural households (7.6% its group) also release a part of their land for other household cultivation. Fieldtrip 2015 concluded that agricultural land in the village is rarely rented for longer than 3 years. Normally it is informal land transaction and based on the idea that the owner would withdraw their land whenever they want. Agricultural land remains critical for generating food and security back-up for peasants, even though agriculture was a subsidiary activity for many households.

2.3. Agricultural labour and technology in the village

The movement of people from farm to non-farm employment and from rural to urban areas typically causes agricultural labour shortages, and forces farmers to adapt their farming techniques. In the Southern of Vietnam (Mekong River Delta), farmers have mechanized rice production (Hoang Xuan Thanh et al., 2013). However, in the Red River Delta, the household division of labour and production process will easily adapt to the out-migration of one or two members, and to the subsequent relative labour shortage and decreased flexibility in production sphere. In the survey, the consequence of the loss of a household member was found to be unproblematic. A large majority of respondents (91.5 percent) indicated that their households did not suffer a negative impact due to the loss of labour. They reported that the loss of labour from a circulating member was solved easily by using externally hired labour and mutual help. This can be explained in part by the large population already mentioned in part 3.1 which resulted in a huge labour surplus and limited land. Only few households expressed that because of migration to international, or moving around the Southern part of Vietnam, the migrants cannot easily come back during the peak period of the harvest, some labour shortage occurred, but its effects have not been drastic.

There is no remarkable labour deficit occurring due to migration because among agricultural activities, only rice production orders exchanging and renting some external labour. The reason why rice production is considered as the fundamental agricultural activities in this village is because on one hand, it does not take as much time and labour care as other agricultural activities. On the other hand, unlike other agricultural activities, rice production has high demand labour at only some periods of season; therefore households could concentrate their labour members elsewhere. In cases when they cannot manage within households, then wage labour and mutual help are available and easy to access..

Mutual help is traditional important way for the farmer to adapt with labour shortage in the season time. Most households in the village got help during harvest from their neighbors and relative. All these people are farmers in Bacninh and this reflects the important role of reciprocity. In Vietnam reciprocity is expressed as "đổi công", which means human feelings and is seen as a form of ethical and expressive exchange. The concept of "đổi công" has three implications in Vietnamese culture: (1) it indicates the affective responses of an individual confronting different situations; (2) it means a resource that an individual can present to another as a gift in the course of social interaction; and (3) it implies the social norms by which people have to abide in order to get along well with other people. Owing and returning "công" are important elements in Vietnam social life. It is interesting that wage labour and mutual help both play an important role. However, it is notable that mutual help is decreasing over the years we are considering here, while paid labour is increasing. In sum, rural households in Red River Delta respond to labour constraint by keeping rice production as main agricultural activities, renting labour and using mutual help, rather than investing in machinery.

2.4. Rice production or emerging opportunity for cash crop

About the hypothesis that migration may result in the shift from rice production to other cash crops or/ and livestock due to the labour deficit; the data shows, there is also no significant shift in production patterns from rice to other cash crop. Rather, migration encourage moving back to local traditional agriculture when rice production becomes the main focus in many areas. It should notice that in Vietnam the same term is used to designate both "Rice" and "Agriculture" (Lebailly, 2015). Data shows this village does not follow the trend of many other Northern villages in Vietnam where number of households increase diversifying their agricultural activities[4] beyond the previously predominant rice production. Table 2 shows that 86.7 percent (109/128) *Mai Thon households keep rice production as the only fundamental agricultural activities*, combining with some subsistence agricultural activities such as backyard cultivation and poultry rising for household using rather than commercial agricultural production.

From peasants' perspective[5], the reason is *not* because they lack of inputs to invest in other agricultural, but they consider on one hand animal raising or cash-crop is *time and labour consuming*

[4]The popular trend in Vietnamese rural in agricultural diversification is raise large stocks of pigs and/or large flocks of poultry for sale, with intensive use of industrial animal feed

[5]From National Policy, the rural households in Red River Delta are not permitted to change agricultural land into other purposes due to National Food Security

than rice production (as discussed in the session above); on the other hand its profit is lower than migration or other non-farm business. Besides, the cost of growing rice for household consumption was considerably cheaper and higher quality than buying rice. Therefore, by far the best combination strategy of households in Mai Thon village is practicing rice production while releasing some of their family members for off-village and off-farm business like a household model presented in figure 1.

Household membership is usually defined as living "under the same roof", however, under the context of industrialization, this concept is gradually changing into *a multi-spatial household*. The figure 2 describes a model of a typical multi-spatial rural household in BacNinh which has 5 members. This household has got a couple, one eldest son, one younger daughter and a husband's mother. Their son is working in Que Vo industrial zone which is 10km from home therefore he moves back and forth daily. The wife is migrating in Hanoi as a house cleaner (and junk collector when she has time) to take care of the youngest daughter who is studying in Hanoi University. She often goes home to transplant rice seedlings or to harvest. Other activities for rice cultivation such as spraying fertilizer or pesticides would be mainly carried by the husband who also spends around 15 days per month to work as a construction worker. The husband's mother takes care of their house, and she would do votive weaving in free time. The strong commitments and obligations between rural-based and urban-based individuals and units show that this household model is well-functioning with mutual support divided across space. For example, remittances from urban areas are important income sources for rural family members, Seasonal rural-to-rural migration improves some households' daily cash flow. And when access to food is largely determined by access to cash for most Vietnamese households, in both urban and rural areas, income from non-farm activities and migration is important for rural food security. In turns, family members who stay at home and take care of agriculture will supply the migrant members with high-quality food and other kinds of food such as vegetable, eggs, fruit. These linkages are popular and crucial in the livelihood strategies of the rural households, but usually not taken into account by policy makers.

2.5. Rural differentiation in Red River Delta village

In the early agrarian studies, scholars paid attention to farmers' access to the means of production, such as land, capital, and labour, as the major factor in rural differentiation. Class was based on the ownership of means of production (White, 1989). In contemporary agrarian studies, many scholars suggest broadening the way of understanding rural communities by taking into account a range of new opportunities such as off-farm occupations, participation in commercial agriculture and migration (Rigg and Vandergeest 2012, Kelly, 2012). While the new opportunities are available, villagers again do not have equal access. Kelly (2011) and White (2012) highlight education as an important determinant of the opportunities. They point out that villagers with a low level of educational attainment are marginalised as wage labourers on the agricultural lands or in unskilled non-farm employment. Besides, Vandergeest (2012) emphasizes that agriculture is a favoured alternative for villagers lacking post-secondary

degrees, that is, people faced with the choice of either agrarian occupations or working as unskilled hired labour on construction sites and in the factories. This was partly true in Mai Thon, which saw many middle-aged people above 40 years of age return to agriculture. The decision to stay in agriculture was often influenced by generational factors, as well as their gender, family, and educational background. These factors also contributed towards differentiation within the village.

Table 2. Major agricultural activities of Mai Thon households

Agricultural activities	Number of households
Rice only	111
Rice and cash crop (potatoes, tomatoes, beans, bananas…for sale)	3
Rice and aquaculture	2
Rice, aquaculture and cash crops	0
Cash crops only	0
No farm	12
Total	128

Source: author's survey 2015

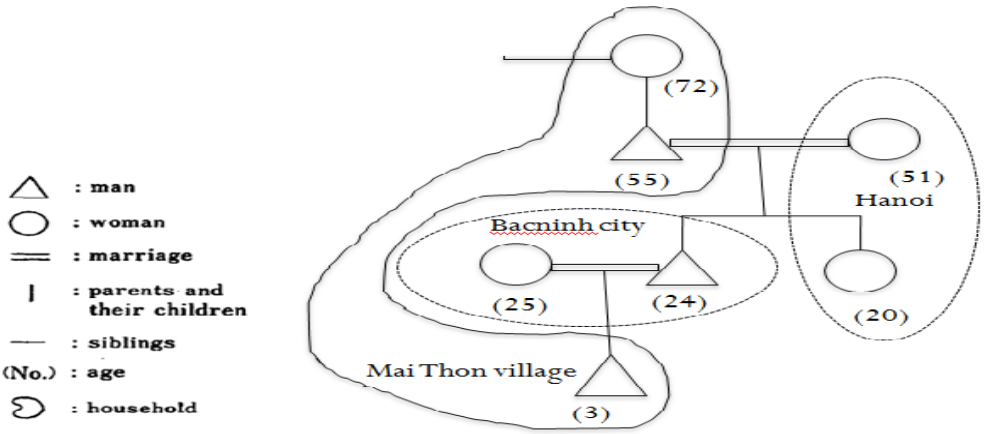

Figure 1. A model of a multi-spatial BacNinh's household

Table 3. Range of income based on main occupations in Mai Thon village

Main occupations	Number of households			Total
	Group 1	Group 2	Group 3	
	Below 5 millions VND/ month	5 - 10 millions VND/ month	10 millions VND/ month	
Agriculture	4	5	3	12
Farming and non-agricultural wage labour	6	19	2	27
Farming and local enterprise	5	14	5	24
Local enterprise only	-	1	-	1
Farming and salaried workers	-	21	32	53
Non-farm, wage labour only	-	5	1	6
Unemployment	5	-	-	5
Total	20	65	43	128

Source: author's survey 2015

Incomes varied significantly within the village. Table 3 shows household sources of main income in relation to occupations. Four households whose main occupation was agriculture had incomes below 5 millions vnđ per month. They were the least successful farming households, with access only to less productive land, or were elderly and illness people who could not effectively exploit opportunities from non-farm jobs.

The majority of households in Mai Thon fell under the income category of 5 millions – 10 millions VND/ month. They had an average land holding of 4–6 sao and had off-farm jobs to support their agricultural activities. Almost half of the agricultural and labour households and agricultural and enterprise households consisted of people aged above 40 years, who had children working in the urban centers. For most of rural household, off-farm employment and/or enterprises were the most important sources of income. More than 80 per cent of the households in the income category of group 2 had at least one household member who had completed high school to higher education. They were faced with the choice of agrarian occupations or wage labour opportunities in the industrialized zone in BacNinh cities or immigrate to Hanoi. In turn, the close relationship between the migrants and the family ensures the flow of remittance in the research site is its stability and frequency. The frequency of receiving money however was found to depend upon on the distance and the social networks which permit them to visit or send money home. Due to departures of migrants are mostly near and convenient for them to remit, 73.4 percent of households who reported that they received remittances claimed the frequency of remittance every month. It is coincidence with the wage monthly they received. The impacts of remittance on the rural society are much more complicated than the current simple economic view (Zhang et al., 2006). In Mai Thon village, remittances have a social as well as an economic function; they are not only a mean to maintain or improve economic status but also a mean to achieve higher prestige and standing in the local community and family for instance by spending part of remittances on ceremonies or local amenities. In sum, with substantial flows of remittances, in one direction or other or in both, it is likely to have affected income distribution, which has had an accelerating or facilitating technological change, altering the division of labour. However, in most cases, earnings from migration are not competitive with other sources of incomes from agriculture.

Furthermore, it is interesting to find out some case studies that also experienced working in cities, but chose to return to Mai Thon to invest in agriculture such as Nguyen Văn Phuc households who used to a truck driver for food company in the Industrial Zones. After having a net connection for potatoes and cash crops, he came back hometown to rent land for cash crop. He had used to borrowed some high land areas[6] of his neighbor in the village to grow vegetable in the winter season, which was consider very personal and informal deal among villager (2015 survey). But in 2016, following the 2016 Land Law Change he has made it become formal with Commune contract and village households' agreement who own the field in high land area to concentrate 10ha for cash crop cultivation during the whole year. In these

[6]High land area (*đong cao*) is consider as the highest fertilized land which is suitable for 2 rice seasons and one vegetable season in th winter.

contracts, he has the right of using the rented agricultural land for 10 years. His successful farming business has inspired many youth in the village for agriculture choice.

Agriculture was therefore a favorable alternative. The households that earned more than 10 millions VNĐ per month usually had two or more household members below 50 years of age who were working. Although these households allocated the majority of their time to non-farm jobs inside and outside of the village, many of them continued to farm in Mai Thon. Although farming is still the primary activity for over half of the population, most households (81.2%) now combine farming with nonfarm employment and migration. However, villagers keep working on their agricultural land due to some reasons belows: 1) Agricultural land and rice production ensure food security (both in quantity and quality) for their families. The quality of food becomes very important recently when food safety is warning in Vietnam. 2) Agricultural land assured people a job, a livelihood and somehow it is like a backup strategy. Whatever they do outside, if they fail they can always go back to their own land which is considered as "safety nest" 3) Even people may success out of village, numerous people want to go back and spend their elder time in the village doing farm and garden. Traditional agriculture, at that sense, is considered as a way of life – slowly but full of joy. 4) The agricultural land is not only the livelihood for themselves but also for their children in any circumstances. Even though in term of youth and agriculture, 2016 field works show that the majority of young migrants have absolutely no desire to do agriculture. The main causes are due to the disregard for the agricultural production and rural life coming from modern education system, traditional norms and social media; the lack of investment in infrastructure for the youth in rural areas, and even if youth want to become farmers, they also face many difficulties. One of the important strategies to handle this contradiction is the migration of young people while agriculture is always the social security. According to the survey, 90% of young people in the study area have experienced the migratory work. However, labour migration is not permanent phenomenon; instead, it's anonymous throughout life cycle. With youth, rural village is where they grow up, will leave and seek employment opportunities in urban areas, and finally back for agriculture when the land is ready for them, and they have a little available capital accumulated while migrating away. Mai Thon village demonstrated that each household had different livelihood strategies, and these differed according to age, gender and educational background. Rural villagers' livelihood trajectories were very far from uniform. It would be both impossible and meaningless to categorize rural households within a single social class. Households are fragmented as their members live across rural and urban settings, while different members of the households have their own interests and aspirations.

3. CONCLUSIONS

Through analysis of rural livelihoods in Mai Thon, this paper has demonstrated the dynamic and variegated processes of agrarian transformation. Although the basis of rural livelihoods in the village has diversified, as many farmers engage in activities outside the agricultural sector, farming remains vital to the majority of households. Farmers in Mai Thon continue to hold on their land even when they move

away from agricultural activities and stop farming, since land represents security and it can be leased out. Those villagers who possess sizeable tracts of land continue to cultivate rice and other cash crops. Villagers who have little land also continue to farm by investing their earnings from off-farm activities into agriculture. While the majority of Mai Thon village households may be engaged in activities outside the agricultural sector, villagers continue to keep one foot in agriculture. Nevertheless, livelihood reorientation, occupational adjustment, and spatial relocation of rural people away from agriculture do not occur uniformly. The case study of Mai Thon also, however, demonstrates educational and generational perspectives on agriculture. Young villagers, with high school education and lower who working in the industrial zones or migrate out still have agricultural skills, although they do not want to do small household farming, they keep practicing agriculture with family. Mostly because they considers agriculture is stable and safety net, and would be a good alternative if they have chance to work on their own way. Those villagers who were elderly, or had little access in other off-farm jobs based on agriculture as a critical source of income. Others youth with higher levels of education and who had moved away from the village viewed agriculture as an activity for their retirement. This suggests that agriculture still plays an important role in the local farmers' life. The use of agricultural land varies according to educational level, gender and age.

Finally, the case study has shown that Red River Delta contributes towards the persistence of agriculture. For a variety of reasons ranging from notions of identity, to the security of land, and to the significance of crop insurance, and the insecurity and dissatisfaction of much urban employment, a range of factors influence—in ever-changing ways—the attitudes to and success of agriculture in Mai Thon. Agriculture thus persists but increasingly as one component of more diverse and more wide-ranging livelihood strategies that are influenced by local, national and global factors.

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SHARE VALUE CREATION AND COLLABORATION AMONG SMALLHOLDER FARMERS, BUSINESS SECTORS AND ACADEMICIANS: EXPERIENCE FROM QUALITY OF DRIED CHILI PRODUCTION PROJECT IN UBONRATCHATHANI PROVINCE, NORTHEAST THAILAND

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ABSTRACT

This participatory action research (PAR) had two objectives: 1) to find out the pattern for creating collaboration among smallhoder farmers, business sectors and academicians through “Quality of Dried Chili Production Project” at Muang Samsib district, Ubonratchthani province; 2) to create sustainable occupations and incomes for the farmers. The research process composed of 4 phases namely: 1) area selection and feasibility study of good quality dried chili production of the farmers in target area; 2) making a good understanding and creating participatory learning process among the farmers, target business sectors and academicians to make the agreements; 3) promoting the knowledge and developing technology based on the participatory actions to create confidence of participants and 4) operation for good quality dried chili production and evaluation for improving production process. The main approaches of research process were participatory workshop, group discussion, knowledge management and after action review (AAR). The results found the collaboration and share value creation among the farmers, business sectors and academicians for good quality chili production for supplying the consumers, occupation creation and sustainable secure incomes for the target farmer groups. The participatory companies obtained good quality chili for their production process and the academicians could conduct the projects meaningfully. This PAR found that the consumer demand enhanced, compared to the fair price of the supply which could create the occupation, incomes and satisfaction of the target farmer groups and companies. This share value creation would elevate the target farmer groups for being one sector of supply chain of the target companies that would lead to the sustainable development in future.

Keywords: Dried chili production, knowledge transfer, participatory method, share value, smallholder farmer.

PERFORMANCE OF THE CHICKEN CONTRACT FARMING AND ITS AFFECTING FACTORS IN VIETNAM: A CASE STUDY IN HOA THACH COMMUNE, QUOC OAI DISTRICT, HANOI

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ABSTRACT

This study aims to analyze the performance and its affecting factors in the chicken contract farming in a case study of Hoa Thach commune, Quoc Oai province, Hanoi, based on the questionnaire surveyed data of 40 representative chicken contract farmers in the study site in 2016. The results showed that surveyed household's head was medium (37 years old), low educational level (80% secondary school), but they had experience (87.5% had more than 3 years of raising), with quite large raising scale (popular 3001 to 5000 heads). Feed cost was too high (87.7% total cost), led farmers to lower performance, and situation of depending on the market. The farm income was rather low, accounted for only 10.4% total revenue. Results also showed that, any decrease in labor cost, improvement in by - products revenue or egg laying rate will lead to the enhancement of chicken raising performance for contract farmers. Therefore, the farmers had better reduced the feed cost, better manage their labor, exploit by - products and improve the egg laying rate to enhance the farm performance.

Keywords: Chicken raising, farmer, contract farming, performance

1. INTRODUCTION

Chicken raising is a traditional occupation and plays an important role in the economic development of Vietnam. It ranks the second important positions, just after pigs, in the whole livestock sector (Nguyen Van Khoa, 2014). In 2014, the numbers of poultry in Vietnam reached 327.7 million heads (GSO, 2015), in which, the numbers of chicken accounts for around 95% and provides proportion of meat for the market of 14 - 15% (Nguyen Van Khoa, 2014). It is considered as an important economic sector and a great value contribution to the agricultural production, significance in the process of restructuring the rural agricultural economy in Vietnam in general, and in Hoa Thach commune, Quoc Oai District, Hanoi, in particular.

However, the chicken raising in Vietnam is still facing many risks such as the bird flu, many kinds of diseases, increasing input price, fluctuated, and reducing the output price (Bui Thi Nga and Philippe Lebailly, 2016). This significantly affected the outcome and efficiency of chicken raising farmers.

The Vietnamese government has launched a number of measures to limit risks to chicken farmers such as providing technical assistance, supporting seeds and markets, preventive vaccination. However, these solutions seemed have not overcome the obstacles (Nguyen Thai Bac, 2013). In order to reduce the risk and stabilize the chicken raising, farmers have shifted from self - livestock systems into the contract farming system. However, so far, there is not in - depth study about the performance of chicken contract farming in the North of Vietnam in general and in the study site in particular. Therefore, this study aims to evaluate the operating results of processing chicken, consider factors that affect the activity and propose some solutions to improve the results processed chicken locality.

2. METHODOLOGY

2.1. Data collection

Hoa Thach commune was selected as a case study because it is one of the first and rapid development from self – livestock system to contract farming system in the North of Vietnam as the farmers in this area well - recognized the negative impact of risk factors for the sustainable development of their farming. Therefore, many farmers in this area have joined and signed the contract farming with the Charoen Pokphand Group (CP) – a livestock feed processing company originated from Thailand.

In the study sites, most of the contract farmers (90%) raising chicken for egg, there were only 10% farmers raising the chicken for meat. Therefore, this study focused mainly on the contract farmers who rose chicken for egg.

The probability sampling method with probability proportional to size (PPS) combined with the stratified sampling was chosen to take the samples for the research. A sampling frame was established and estimates were reckoned so as to approximate real population values. Total 40 chicken contract farmers, which accounted for 50.6% the total population size in the study site. The questionnaire was used to collect primary data from these farmers in 2016.

Besides, the in - depth interviews, expert method, and observation were used to collect primary information of chicken raising in the study site.

2.2. Data Analysis

The research used OLS regression to compute the effects of some factors in the performance of contract farmers in the study sites.

The performance affecting factor equation:

$Y_{1i} = \chi_{1i}\beta_1 + u_{1i}u_{1i} \sim N(0,1)$

Where: Y_{1i} is the latent dependent variable

χ_{1i} is the vectors that are assumed to affect the performance of contract farmers in the study sites

β_1 is vectors of unknown parameters

u_{1i} are residuals that are independent and normally distributed with zero mean and constant variance.

The dependent and independent variables used in the model are described in Table 1.

3. RESULTS

3.1. Characteristics of chicken contract farmers in the study site

In the chicken contract farming, the CP Group provides farmers some inputs: chicken breed, feed, veterinary and

consumes all of their main output (eggs)[1] . They send technicians to support the farmers with necessary technique and process of chicken raising. Technicians of the company are also responsible for monitoring and dealing with related issues, especially in case of the disease to ensure the product quality. Farmers invest in hen - house, livestock equipment such as feeding, drinking, cooling, heating system, generators... and pay for other related cost such as electricity, water, gas running generators, labor...).

According to the farmers, joining the chicken contract farming will ensure them a high level of safety, low risk due to technical support, ensured chicken breed, feed and veterinary from the CP group. Especially, contract farmers were less affected by the market factors because the company provides inputs and consumes all of their main products. In addition, this type of raising also reduces the investment of the farmers as the CP group provides them some inputs. However, the farmers also reflected that, this type of raising results in lower profits compared to that of self - raising. Therefore, recently, in this commune, there are some farmers gave up the contract farming after 4 - 6 years of contract with CP for self - raising.

The farmers' characteristics are presented in table 2, in which, the average age of surveyed household's head was medium at 37 years old, the eldest was 57 and the youngest was 23 years old. The mode age range was from 30 to 40 years old. At this age, the farmers had experienced in life, could make good decisions, and still young enough to absorb, learn, and apply new scientific knowledge in animal husbandry. Average number of family members were quite high at 5.5 people, and the common laborers were from 3 to 4 people. Besides hiring labor, farmers used family labor for chicken raising. Sometimes, they exchanged labor or support each other in raising chicken.

Regarding to educational level, 80% of the farmers had just finished the secondary school level. This can be explained by the fact that the majority of people with higher qualifications have done other work such as government officials, workers in companies or migrate to other major cities for work.

The farmers had experience in chicken raising as there were 37.5% of them rose chicken more than 5 years, 50% others rose chicken from 3 to 5 years, and only 12.5% just had less than 3 years of experience.

In terms of land area, the common households land area was around 5000 to 10000m^2. There were 20% of farms that had a big land size of more than 10,000m^2, 45% of them had medium land size of 5000 to 10,000 m^2, the rest had a small land size of less than 5000 m^2. In comparison to similar previous research, the land in this area was much larger (Bui Thi Nga and Philippe Lebailly, 2016).

3.2. Performance of chicken raising in contract farms

Size of the chicken raising

The surveyed farmers raised quite a large size in comparison to the results in other similar research studies. The most popular size was from 3001 to 5000 heads, which occupied 60% total farms (Table 3). The small size in this area was even larger than that of the some previous studies (Bui Thi Nga, Philippe Lebailly, 2016).

Cost for chicken raising

Table 4 showed that, feed cost was too high, occupied 26,712 VND over the total average cost of 29,845 VND per chicken

[1] The cost of provided inputs will be recovered when the farmers provide CP group the eggs

head per month, which accounted for 87.7%, the largest share of chicken raising cost in the surveyed farms, followed by the labor cost (1,575 VND and 7.2%). The high feed cost led to lower performance and low farm efficiency. In addition, farmers almost used the bought feed for chicken raising, therefore, high feed cost also led to the situation of depending on the market of the farmers, which in turn resulted in the ability of the more vulnerable for them.

Performance of chicken raising

The survey results showed that the total revenue of the farm was not very high, at only 33,307 VND per head per month, of which, revenue from the main products of farms (egg) was 32,874 VND, accounted for 98.7%. Due to the high production cost of 29,845 VND per head per month, the farm income was rather low at only 3,462 VND per head per month, which accounted for only 10.4% total revenue of the chicken raising in farms (Table 5).

Table 1. Description of the dependent and independent variables used in the model

Variables	Description	Types	Values
SIZE	Scale of the farms	Continuous	Numbers of chicken head in the farms
HENHOUSE	Cost for hen - house	Continuous	Amount of VND invested in hen - house
LABOR	Labor cost	Continuous	Amount of VND pays for the hired labor in farms
TOOLS	Cost of tools and equipment	Continuous	Amount of VND invested in tools and equipment
FEED	Feed cost	Continuous	Amount of VND to buy feed for raising chicken
BYPRO	By - products	Continuous	Amount of VND received from selling chicken by - products such as manure, old hen
EGG	Egg laying rate	Continuous	Proportion of egg laying rate by hen
Y_{1i}	Performance of the contract farms	Continuous	The income of the contract farms counted by VND

Table 2. Characteristics of farmers

No	Criteria	Unit	Number	Ratio (%)
1	Average ages of the head of the households	Years	37	
2	Average family members	People	5.5	
3	Numbers of average labors	Labor/farm	3.1	
4	Educational level of the head of the household	People	40	100
	Secondary school	People	32	80
	High school	People	8	20
5	Experience time of chicken raising	Years		
	Less than 3 years	Farmers	5	12.5
	From 3 to 5 years	Farmers	20	50
	More than 5 years	Farmers	15	37.5
6	Land area			
	From 1000 to 5000 m^2	m^2	14	35
	From 5001 to 10,000 m^2	m^2	18	45
	More than 10,000 m^2	m^2	8	20

Source: Survey results, 2016

Table 3. Scale of raising

Scale of raising	Numbers of head	Proportion
Small size	Less than 3000 heads	20%
Medium size	From 3001 to 5000 heads	60%
Big size	More than 5000 heads	20%

Source: Survey results, 2016

Table 4. Cost for chicken raising in the study sites *(Unit: VND/head/month)*

Cost	Min	Max	Mean	Proportion
Labor cost	1,500	2,000	1,575	7.2%
Hen - house	298	383	340	1.1%
Feed	26,124	27,752	26,712	87.7%
Tools and equipment	315	450	374	1.2%
Interest	0	180	152	0.5%
Other cost (Electricity, water …)	600	740	692	2.3%
Total	28,937	31,405	29,845	100%

Source: Survey results, 2016

Table 5. Performance of chicken raising in study sites (U*nit: VND/head/month)*

Item	Min	Max	Mean
Main products	32,350	33,450	32,874
By - products	400	450	433
Total revenue	32,750	33,900	33,307
Total cost	28,937	31,405	29,845
Income	2,813	3,495	3,462

Source: Survey results, 2016

Table 6. Affecting factors to the chicken contract farming in the study site

Factors	Coefficient	P - Value
Intercept	19835	0.19
SIZE	- 0.004	0.889
HENHOUSE	0.173	0.245
LABOR	- 0.995	$2.545.10^{-7}$
TOOLS	0.034	0.714
FEED	- 0.123	0.171
BYPRO	1.144	0.001
EGG	367247	$5.574.10^{-8}$

Note: F = 7.1, number of observations (N) = 40, Multiple R = 0.6701; R - squared = 0.6441, Adjusted R - square = 0.6189
Source: Survey data, 2015

3.3. Affecting factors to the performance of chicken contract farming

In the selection equation of the regression model, three variables were found to be significant affecting the chicken contract farming in the study site (Table 6). These were labor cost (LABOR); by - products (BYPRO), and egg laying rate (EGG).

F = 7.1, number of observations (N) = 40, Multiple R = 0.6701; R - squared = 0.6441, Adjusted R - square = 0.6189.

There was one variable, labor cost (LABOR) had negative effects on the performance of the contract chicken raising, and two variables, by - products (BYPRO) and egg laying rate (EGG) had positive on the performance of the performance of chicken raising in the study site. That means, any decrease in labor cost, for example, thanks to better labor manage, and any improvement in by - products revenue or egg laying rate will lead to the enhancement of chicken raising performance for contract farmers.

Although in the regression results, feed cost did not statistically significant affect the performance of the chicken raising, the reality of high proportion feed cost (87.7% of total cost) and the deep interview showed that this is one of the factor that has most impact on the performance of the chicken raising. According to the responds of the key persons with high experience in the study site, if the feed cost could be reduced, the farmers will surely have a chance to improve their farm's performance and develop more sustainably.

4. CONCLUSIONS

The surveyed results showed that the average age of surveyed household's head was medium at 37 years old with low educational level. The farmers had experience in chicken raising as there were 87.5% of them rose chicken more than 3 years. The common households land area was around 5000 to 10000m^2 and popular scale of production was from 3001 to 5000 heads, which occupied 60% total farms.

Feed cost was too high, occupied 87.7% the total average cost of chicken raising, which led to lower performance, low farm efficiency, also led to the situation of depending on the market of the farmers. This, in turn, resulted in the ability of the more vulnerable for farmers. Total revenue of the farm was not very high, of which, revenue from the main products of farms accounted for 98.7%. The farm income was rather low, accounted for only 10.4% total revenue of the chicken raising in farms.

The results also showed that, three variables were found to be significant affecting the chicken contract farming in the study site: labor cost, by - products, and egg laying rate. Decrease in labor cost, increase in by - products revenue and egg laying rate will lead to the improvement of performance for contract farmers.

To improve the performance of chicken contract farming in the study site, there are some suggestions: (1) Firstly, it is necessary to reduce the feed cost of chicken raising. The farmers had better self - produce the feed for the chicken by mixing necessary materials, which are very available in their living area such as corn, rice, soybean, etc. This not only could help them to decrease the feed cost, but only help them not depend too much on the feed market. (2) Secondly, farmers should decrease the hired labor cost to improve their performance by using more suitable labor sources such as managing labors better, increasing the family labors. (3) Thirdly, farmers should increase their by -

products by accumulating the chicken manure to sell, which would increase their by - product revenue. (4) Finally, farmers should upgrade their knowledge and skill of chicken raising and find, buy good chicken breed to improve the egg laying rate, which in turn, would increase their performance.

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FACTORS INFLUENCE THE DEVELOPMENT OF VEGETABLE SUPPLY CHAIN: A CASE STUDY OF THUA THIEN HUE PROVINCE

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ABSTRACT

This article has built a researching model of the factors influencing development of the vegetable supply chain in Thua Thien Hue (TTH) province. It includes five factors: consumption tendency, marketing tendency, production tendency, globalization and policies of government. The research has used Exploratory Factor Analysis (EFA) to reunite a set of variables into factors that can measure the different aspects of the research topic. Regression Analysis is a statistical method used to identify the influence of the five independent variables on development of vegetables supply chain. Results from regression analysis have shown that Production Tendency has biggest impact on development of the vegetable supply chain (β = 0.305). Policies of Government ranks the second position (β = 0.286), followed by Consumption Tendency (β = 0.187) and Marketing Tendency (β = 0.154). Globalization factor has the smallest impact (β = 0.102) in the regression model. Based on regression results, some solutions that are classified by each group of factor have been proposed in order to help the development process become more effective.

Keywords: Consumption tendency, exploratory factor analysis, marketing tendency, production tendency, regression analysis.

1. INTRODUCTION

In recent years, the vegetable production sector in Vietnam has created more jobs and higher incomes for producers compared with other annual crops. Growing demands of vegetables has lead vegetable production increasing in terms of both quantity and quality. Besides, globalization has speeded up cross - culture between the developed and developing countries (Reardon et al, 2008). Hence, there has been a considerable shifting in daily diet and preferences of consumers in developing countries. This phenomenon requires more abundant types of vegetables in order to meet with the diversified needs of domestic consumers and foreign markets. Moreover, changes in production techniques and in agricultural development policies have created many opportunities as well as challenges for the development of the vegetable supply chain. All the above changes have forced the vegetable supply chain and the related stakeholders to adapt to the new situations as much as possible. Obviously, during the adaptive process, the vegetable supply chain will also change its structure as well as the stakeholders involved in. Hence, it is important to find out what are the factors influencing on development of the vegetable supply chain and what the actors should do to adapt to the development. This paper aims to answer these two questions through a case study of Thua Thien Hue province.

2. CONCEPTUAL FRAMEWORK AND METHODOLOGY

2.1. Conceptual framework

"A supply chain is a network of facilities that procure raw materials, transform them into intermediate goods and then final products, and deliver the products to customers through a distribution system" (Lee and Billington, 1995). A supply chain composes all stakeholders that directly or indirectly satisfy the demand of customers. Functions of a supply chain are not only limited by two main activities: production and distribution but also expanded by other activities such as: R&D, marketing, finance and other supporting services (Wisner et al., 2012). According to McCullough et al., (2008), a food supply chain has three levels of development, including: traditional chain, structured chain and industrialized chain. Depending on development of the economy, a food supply chain will trend to develop by three above levels.

2.2. Methodology

2.2.1. Sampling method

According to Nguyen Dinh Tho (2011b) extracted from Hair &ctg (2006), the minimum sample sizes of 50 and 100 are better and the rate of observations per items is 5:1. This study has 25 observed variables for Exploratory Factor Analysis so the required sample size is 25 × 5 = 125. However, to ensure the confidence, the authors have chosen 200 samples based on expert opinions, on overall literature related to stakeholders and on available resources.

2.2.2. Data collecting method

The primary data have been collected from semi - structure interviews and in - deep interviews with the key informants such as the experts, the researchers and the policy makers in the agricultural and economic field. In addition, relevant surveys have been carried out with questionnaires designed for each stakeholder of the vegetable supply chain in Quang Dien and Phu Vang districts. The secondary data has been collected by review of quantitative and qualitative data from the available sources

2.2.3. Data analyzing method

Data inputting, processing and analyzing are carried out by SPSS software.

2.2.4. Proposed research model (Figure 1)

3. RESULTS

3.1. Descriptions of samples

3.1.1. Farmers

Most farmers are members of cooperatives in the province such as Phu Mau II, Kim Thanh or Quang Tho 2 cooperative. Through field surveys, discussions with farmers and the information from the Department of Agriculture and Rural Development of TTH province, the research results showed that vegetable production households have a fragmented land (300 - 500 m2 /household). The fact shows that farmers cannot expand planted area because limitation of land resource and of difficulties of at production's output.

3.1.2. Collectors

Collectors of vegetable supply chain of TTH province are mostly people in villages. Surveys' results indicates that there is approximately 40% of the vegetable products harvested is sold to collectors. Collectors play an important role as a bridge between farmers and other actors in the chain. They help farmers in harvesting and selling vegetable products even they give cheaper price than the wholesale's price.

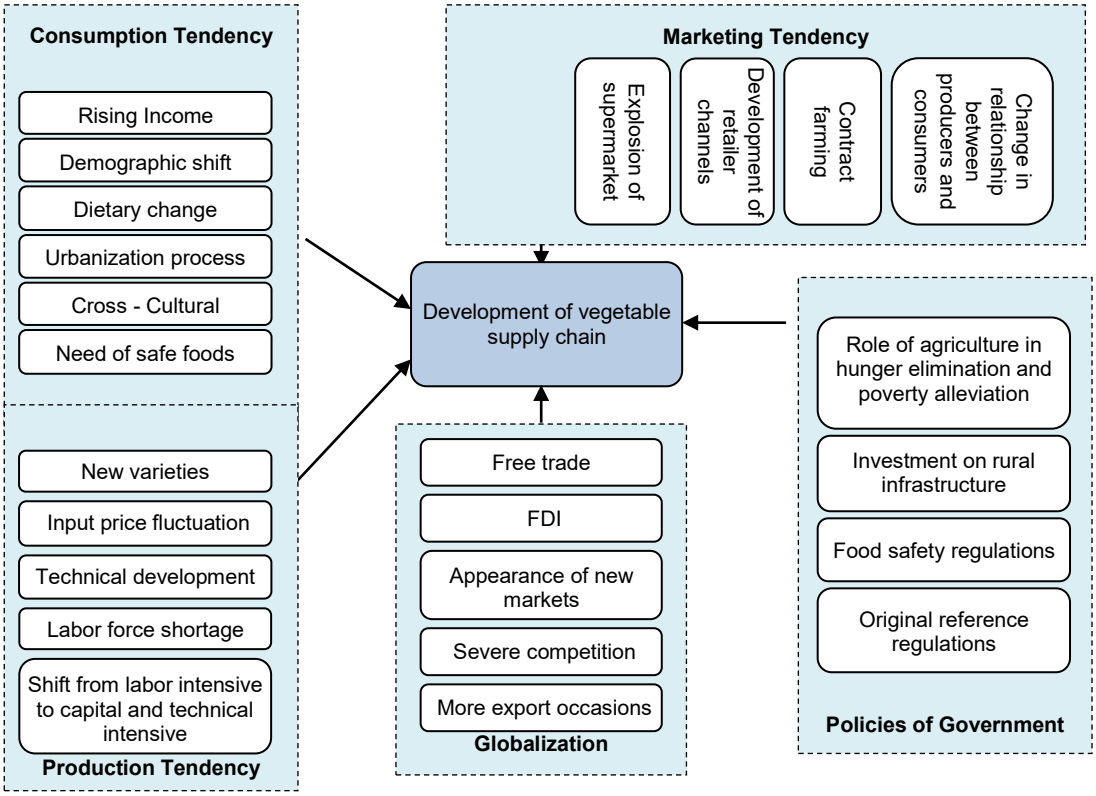

Figure 1. Proposed research model

Source: Authors' study

3.1.3. Wholesalers

Business size of wholesalers is quite large with capital fluctuates around 20 - 100 million VND and number of employees fluctuates from 2 - 10 people. The average consumption volume is around 300 - 2000 kg/day depending on season. In TTH province, the base of the wholesalers is located mainly in the wholesale market Bai Dau, Phu Hau Ward, Hue City.

3.1.4. Retailers

Because of huge number of retailers, the operating scale of each retailer is small with a capital from 1 to 5 million VND (70%) or from 5 - 10 million VND (30%). Everyday, retailers go directly to wholesale markets to buy from 10kg to 50kg different vegetables per time. This vegetable can be sold on the day or within 2 - 3 days later, depending on the characteristics of vegetables.

3.1.5. Consumers

Because of difficulties in preserving, consumers should buy vegetables every day or per 2 - 3 days at a traditional market in the area (100%). Besides, 37.5% of consumers choose reliable supermarket to buy vegetables, 25% of consumers still buy vegetables from vendors outside the traditional markets. Survey's results show that 100% of consumers pay attention to the quality of vegetables but only 70% of consumers are willing to pay higher prices for safe vegetables. The reasons for which 30% of consumers do not pay more for safe

vegetables are the limitation of income and the trust for quality of safe vegetables.

3.2. Factor Analysis Results

3.2.1. Exploratory Factor Analysis (EFA)

From reviewing both local and global relating research, combining with quantitative research and pre - test, this research has built 25 - indicator scale to evaluate Consumption Tendency, Marketing Tendency, Production Tendency, Globalization and Policies of Government. In order to classify indicators and premise for the research model of vegetable supply chain, this research will conduct the exploratory factor analysis for these 25 variables.

a. Extraction of factors affecting supply chain development

In order to check if the sample is large and eligible to conduct EFA, we use the Kaiser - Meyer - Olkin (KMO) and the Bartlett's test (Table 1). The result shows that KMO is equal to 0.823 which is larger than 0.5 and the p - value of the Bartlett's test is lower than 0.05. Therefore it can be concluded that the data collected is eligible for EFA.

Table 1. KMO and Bartlett's Test

KMO and Bartlett's Test		
Kaiser - Meyer - Olkin Measure of Sampling Adequacy		0.823
Bartlett's Test of Sphericity	Approx. Chi - Square	4843.639
	Df	300
	Sig.	0.000

Source: Survey data 2016

Table 2. EFA results of the factor Development

Item	Factor loading
There is development in vegetable supply chain.	0.850
Linkages between producers and distributors are increasingly tighter in the new vegetables supply chain.	0.812
Modern retailers will be the dominant stakeholders in the whole supply chain of vegetables	0.759
Eigenvalues = 1.959	
Sums of Squared Loadings: 65.290%	

Table 3. Cronbach Alpha's coefficients of the factors influencing vegetable supply chain Development

Group of variables	Cronbach's Alpha	% of Variance	Number of variables
Consumption tendency	0.907	16.715	6
Marketing tendency	0.942	16.416	5
Production tendency	0.944	16.405	5
Globalization	0.918	15.404	5
Policies of Government	0.909	12.711	4
Total variance extracted: 64.949%			

Table 4. Cronbach Alpha's coefficients of the factor Development of vegetable supply chain

Item	Scale Mean if Item Deleted	Scale Variance if Item Deleted	Corrected Item - Total Correlation	Cronbach's Alpha if Item Deleted
Development = 0.734 (65.290%)				
There is development in vegetable supply chain	6.500	0.815	0.561	0.643
Linkages between producers and distributors are increasingly tighter in the new vegetable supply chain	6.467	0.770	0.619	0.570
Modern retailers will be the dominant stakeholders in the whole vegetable supply chain	6.252	0.897	0.495	0.718

Source: Survey data 2016

b. Extraction of the factor Development

The factor Development is considered as the result of the factors Consumption tendency, Marketing tendency, Production tendency, Globalization, and Policies of Government. After applying the EFA for the items measuring the factor Development, the research result shows that eigenvalues is equal to 1.959 > 1 and total variance extracted is 65.290% > 50% (Table 2), proving all assumptions of EFA are suitable

3.3. Reliability analysis

Cronbach's alpha coefficients of all elements after extracted from the observed variables by EFA are greater than 0.7. Within each group, the variable correlation coefficients of total variation of the observed variables are greater than 0.3 (Table 3 and 4). These confirm the scales extracted from the observed variables are consistent and reliable. So we can use these 6 factors in the next steps.

3.4. Regression analysis of the factors influencing Development of vegetable supply chain in Thua Thien Hue province

In regression analysis model, the dependent variable is "Development". The independent variables are factors extracted from the observed variables in the EFA.

Regression model:

$$TD = \beta_0 + \beta_1 CS + \beta_2 MK + \beta_3 PR + \beta_4 TR + \beta_5 PL$$

Hypotheses:

H_0: There is no correlation between independent factors and "Development of vegetable SC"

H_1, H_2, H_3, H_4, H_5: There is correlation between five independent variables and "Development vegetable SC"

a. Model test

- Testing the relevance value

The result of building multivariate regression models with SPSS software gives results in table 5.

The relevance of the model is expressed through adjusted R^2 value. The results in the table above shows that 5 - independent - variable model has the highest adjusted R^2 value being equal to 0.559. In other words, 55.9% of change in development of vegetable supply chain is explained by these 5 observable variables. The next steps will use regression model consists of 5 independent variables for analysis.

- F test

The next step in the regression analyzes was performing the F test (Table

6) on the relevance of the overall linear regression model, considering the dependent variable associated with the entire linear independent variables or not.

Hypothesis H_0: $\beta_1 = \beta_2 = \beta_3 = \beta_4 = \beta_5 = 0$,

ANOVA analysis results shows that the value of Sig. is 0.000 < 0.05, allowing rejection of the hypothesis H0. Thus, the obtained regression model is quite good, because the total estimated squared error is very small compared to the total volatility of the data. The combination of the independent variables better explain the change of the dependent variable in the Development of vegetable supply chain.

b. Multivariate regression analysis results and the importance of each factor

Table 7 shows that the t - test analysis regression coefficient shows: the Sig. value of all the independent variables are less than 0.05. Therefore it can be said that all independent variables have an impact on Development of vegetable supply chain. All these factors are significant in the model, and the impact in the same way to Development of vegetable supply chain because the regression coefficients are positive.

General regression equations of the model are rewritten as follows:

Development of vegetable supply chain = - 0.136 + 0,187 * Consumption Tendency + 0.154 * Marketing Tendency + 0.305* Production Tendency + 0.102* Globalisation + 0.286* Policies of Government.

Through the standardized regression coefficients, the important level of the factors was involved in the equation. Specifically, the factors "Production tendency" has the highest influence (β = 0.305) to the Development of the vegetable supply chain. Factor of Globalization has standardized regression coefficients lowest (β = 0.102) indicates that development of vegetable supply chain are also somewhat influenced by the trend of globalization and foreign investment is increasing in Vietnam, but the impact is still unclear due to priority sectors of FDI inflows is industry and services rather than agriculture.

4. DISCUSSION

In order to enhance the development of the vegetable supply chain as well as improve the efficiency of this development process, we propose some solutions as below.

4.1. Solutions on "Production Tendency" factor

Help farmers learn and apply the new and modern technologies in vegetable producing by organizing field trips to the local big areas of vegetable production such as Da Lat, Lam Dong or Hung Yen as well as to the neighbor countries such as: Thailand, Indonesia or the Philippines. These field trips are very useful in persuading farmers to believe in the efficiency of new technologies.

New technologies in vegetable production lead the decreasing demand of labor but require the skilled employees. Hence, farmers should improve themselves in terms of knowledge and skills to meet the demand of the labor market. In addition, the local authorities should organize campaigns to encourage farmers to keep farming vegetables.

Demand of consumers on vegetable products has become higher and more diversified due to the increase of income and the impacts of cross - cultural. To meet the changes of consumers' taste, farmers should try to plant new varieties with technical supports from the local Agricultural Department.

Table 5. Model summary

Model	R	R^2	Adjusted R^2	Std. Error of the Estimate	Durbin - Watson
4	0.756	0.571	0.559	0.28380	1.938

Source: Survey data 2016

Table 6. Test the relevance of the model

ANOVA					
Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	18.858	5	3.772	46.828	0.000b
Residual	14.175	176	0.081		
Total	33.034	181			

Source: Survey data 2016

Table 7. Multivariate regression analysis results

Model	Unstandardized Coefficients		Standardized Coefficients	t	Sig.
	B	Std. Error			
(Constant)	- 0.136	0.283		- 0.480	0.632
Consumption tendency	0.187	0.038	0.246	4.916	0.000
Marketing tendency	0.154	0.031	0.279	4.914	0.000
Production tendency	0.305	0.037	0.472	8.229	0.000
Globalization	0.102	0.033	0.156	3.066	0.003
Policies of Government	0.286	0.053	0.269	5.426	0.000

Source: Survey data 2016

In the recent years, dry seasons come sooner and tend to last longer in the central region of Viet Nam, due to climate change. It has caused many problems related to irrigation for vegetable production and leads a higher payment of power fee. In addition, pesticides and fertilizers that are the important inputs of vegetable production are mainly imported from foreign companies. To actively control price of all inputs and to decrease this kind of cost, farmers should adapt to new ways of production that avoid pesticides and fertilizers.

4.2. Solutions of "Policies of Government" factor

To take advantages the supports from the Government and avoid as much as possible law violation, all stakeholders of the vegetable supply chain should often update new laws and policies delivered by the Government. Especially, the authorities of all levels should implement more the trading promotion campaigns in abroad markets to present Vietnamese agricultural products in general as well as for vegetable products in particular. Finally, the current burning situation of food safety requires both the Government and the related Ministries to issue more detailed and clear regulations in this concern and have the strict punishments for the violation cases.

4.3. Solutions on "Consumption tendency" factor

Organize more campaigns to propagate and improve awareness of consumers on food safety. High demand on quality of

vegetables will create high pressure on producers and orient vegetable supply chain developing in a sustainable way.

Maintain the economic growth to ensure the gradual increase of income per capita, foster the speed of urbanization and create more chances for the appearance of middle class. As a result, there will be more driving forces that speed up the development of the vegetable supply chain in TTH province, in the context that TTH province still belongs to the average group of per capita income.

4.4. Solutions on "Marketing Tendency" factor

Government should issue the policies that encourage development of modern retailers such as: supermarkets and convenience stores. These policies should focus on simplifying the procedure of applying for business' license, on tax incentive and on business premises.

Propagate the importance of contract farming through leaflets, typical channels on television, radio and the conferences.

Establish non - profit organizations with purposes of helping the stakeholders of the vegetable supply chain in setting up business, drafting contract, finding business partners, or studying laws and regulations related to agricultural business.

4.5. Solutions on " Globalization" factor

It is predicted that Viet Nam will became the " garden of the world", and the agricultural sector will be considerably invested by Vietnamese Government and by the foreign companies. To well prepare for receiving FDI flows, from now all stakeholders should change the old habits in producing and exchanging. Moving from fragmental production to big scale production is required in order to meet the high demand from abroad markets in terms of quality and quantity. In addition, the local authorities should invest in upgrading rural infrastructure, improving knowledge and skills for all stakeholders of the vegetable supply chain, editing regulations of food safety and adjusting favor policies in calling foreign investors.

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NEW IDEAS FOR MODEL OF AGRICULTURE ECONOMY DEVELOPMENT IN NORTHERN MOUNTAINOUS AREAS IN VIETNAM

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ABSTRACT

After the success of "Contract 10 - The Resolution 10-NQ/TW in 1988 by Poliburo Committee VI", the agricultural economy has actually created a major breakthrough, and made fundamental changes in the organization of production and liberation of labor power, which has returned Vietnam to its rightful place alongside the largest agriculture producing nations in the world. Over time, international economic integration as a storm has swept across all countries and in all fields. This storm has exposed such weaknesses of Vietnam's agriculture as fragmentation, small scale and weak competitiveness, especially in the northern mountainous region. On that basis, this paper serves as a guide to some models of economic organization for the northern mountainous region of Vietnam in the context of the present integration into the global economy.

Keywords: Agricultural economics, northern mountainous areas, models, public-private partnerships.

1. INTRODUCTION

Since 1988, after nearly 30 years of implementing "Contract 10", agricultural and rural economy in Vietnam has actually created the biggest breakthrough, changing the basic organization of agricultural production. From one country that relies on aid and food imports, Vietnam has rapidly joined with other "largest agriculture producing countries" in the world. "Contract 10" not only helps improve the productivity and quality of labor constantly, but it also frees up plenty of labor in the agricultural and rural sector. However, in the context of deep economic integration into the world, the competition in the agricultural sector is increasingly complex and intense. Household economic model, especially in the Northern mountainous region, has shown a number of drawbacks such as production fragmentation, weak competitiveness, high product costs and difficulties in consumption. What do we need to do to overcome these existing weaknesses of household economic model, and how can Vietnam avoid the risk of being defeated on our own turf? The reality demands for the application of a new model to overcome the current problems in the way of investment for current agricultural and rural development in Vietnam, and disadvantages of cooperation model, while promoting the role of farmers in the product value chain, especially the coordination mechanism between the "households" in agricultural production. Based on the operational experience of the public-private partnerships model of agricultural development, which has been done in Bac Kan province, funded by

Agribussiness Promotion and Investment Fund (APIF) and some relevant research by the author, this paper proposes some new model applications in the agricultural and rural development, studying the northern mountainous region of our country.

2. MATERIAL AND METHODS

2.1. Data collection

The data was obtained from the operational results which the APIF implemented in Bac Kan province, and the actual findings in scientific research at provincial level "A research into the innovative solutions and developing effective production and business models to improve farm households' income in the new rural establishment in Bach Thong district, Bac Kan province for period 2015-2020" by the author as the main researcher. The information was done by direct interviews and not using questionnaires. Respondents included residents and business owners, cooperatives, private sector establishments directly involved in partnership projects for the poor in the development of agriculture and forestry.

Selection of research sites: The author chose two of the three districts in the project area which were Ba Be district and Pac Nam district in Bac Kan province to examine. The number of selected communes was 4 including: My Phuong commune in Ba Be district participating in the Arrowroot Planting Project, Chu Huong commune in Ba Be district involved in Chicken Raising Project, Co Linh commune in Pac Nam district participating in the Arrowroot Planting Project, Dia Linh commune in Ba Be district participating in the Potato Planting Project.

Selection of households: The author selected 15 households per each commune including the head of the village/the head of the interest group and 14 randomly selected households. The total number of residents interviewed was 60. For the group meeting interview, the following interview contents were: evaluating the benefits, the advantages and disadvantages of each household when participating the project; assessing the roles of local authorities and businesses in the process of implementing the contracts between residents and businesses; problems that need solving in the process of implementing the contracts.

Selection of Businesses: The author selected 5 out of 11 businesses involved in the project to investigate. The selected enterprises including Lan Thieu Poultry establishment, Minh Be Export Private agro-processing enterprise, Dong Tam Cooperative of producing and processing of agricultural products, Nhat Thien Arrowroot Noodles Enterprise, Hong Ha Joint Stock Company. The direct interviews to business owners was used, including: the benefits, the advantages and disadvantages together with problems that need solving in the process of participating in the project, proposals of enterprises for authorities in solving dilemmas in the process of implementing the contracts among residents.

2.2. Data analysis

From the results of the interviews, the data will be aggregated, coded and analyzed, then the conclusions were drawn. Besides, the author used descriptive statistical method to indicate the current status of the process of implementing public-private partnership model in the development of agriculture in Bac Kan Province; comparison method was used to show the differences which public-private partnership model had brought in comparison with the traditional household economy.

3. RESULTS AND DISCUSSION

Forming a public-private partnership model of agricultural development in Vietnam

In the agricultural sector, although there is no specific regulations on investment in the form of public-private partnerships, since the 2000s, the government has realized the difficulties if farmers are supposed to find their paths themselves. Furthermore, the situation "good crops-bad value & good value-bad crops" occurs frequently. In 2002, the Prime Minister issued Decision No. 80/2002/QD-TTg to encourage consumption of agricultural products via contracts, also known as the "four-house link" in agricultural development. This is the premise of creating a partnership between farmers, businesses and scientists in the development of agricultural production. Decision 80/2002 / QD-TTg is the basis for the people, businesses and scientists to sign a contract in the production and consumption of products.This is an open door to remove difficulties for farmers, and to help them have better conditions for the development of production and consumption of their products. However, even with Decision 80/2002/QD-TTg, farmers still face many difficulties in production, farming and animal husbandry. The "four-house link" is proved to be lax and ineffective, and the main reason given is the lack of mechanisms and sanctions in collaboration between businesses and residents. This relationship is easily broken by the intervention of a third party. These "knots" in the implementation of policies has limited the effects of Decision 80/2002/QD-TTg, therefore, the difficulties faced by citizens and businesses in the production and consumption of agricultural products remain unsolved.

To solve these problems, as well as to build commodity value chain from input supply, production and consumption of goods and products, it is important to bring people and businesses together in the same production chain while the government builds and improves policies on supporting "four-house link". On the part of businesses, there is a necessity to develop strategies and plans to produce in line with domestic and foreign markets, to build and protect brand names, expand consumption markets and sign contracts with the residents On the part of the residents, it is necessary to strengthen cooperation to create material areas which are larger in scale and better in quality for businesses. The government creates a favourable legal framework for development projects which are potential to develop sustainably for the residents and businesses.

From the requirements of the reality, the Government published Decree No. 210/2013/NQ-CP on policies to encourage investment in agriculture and rural areas on December 19, 2013, also known as a form of public-private partnership in agricultural development. The Decree provides a number of incentives and supports from the Government for additional investment into agriculture and rural areas made by enterprises. Subjects receiving preferential incentives are enterprises established and operated under Vietnamese law; agencies, organizations and individuals involved in the implementation of policies in this Decree (Government, 2013). With this policy, the public-private partnership program is expected to open up a new path, promote the development of agriculture and rural areas in Vietnam.

Introduction to public-private partnership model in agricultural development in Bac Kan province.

Investments in the form of public-private partnership initially deployed in Bac Kan province was a program of the APIF. The total budget of the fund was two million dollars (about 42 billion VND) and it was designed with the principle of operation towards the poor, implementing sustainable poverty reduction in the project area. This project began in March 2011 and ended in December 2015. The funds were seen as tools to attract businesses to invest in agriculture and forestry in order to connect the farmers' products to the markets in a sustainable way, consequently create more job opportunities, increase incomes, and improve the residents' livelihoods.

The objective of this budget was to create investment opportunities and employment for the rural beneficiary households in the region, through the co-financing grants for business so as to create the market for raw materials.

Regarding the approach, different from the traditional approach which involves supporting funds directly for the residents with a variety of risks, this budget approached via enterprises by co-investment with them (Public-Private Partnership) for the poor through grants to build factories, material areas and support techinical training.

Enterprises directly invested their capital in agricultural development and supported the residents, while the Government invested indirectly for its citizens through grants for businesses. This form of investment benefited not only the enterprises, allowing businesses to expand production scale and seek for markets, but it also helped farmers stabilize production, generate more job opportunities, increase incomes and reduce poverty. Concerning the government budget, this type of investment was more sustainable than the previous direct grants.

Export performance of the program: With this loan, 11 companies were selected to be funded in the form of public-private Partnership to implement their projects in three poor districts in Bac Kan province including Ba Be, Pac Nam and Na Ri. Total capital invested in the project implementation period was 88 billion VND, in which: 55.168 billion VND for reciprocal enterprises; 1,407 billion VND for people reciprocal residents; APIF granted 31.430 billion VND (APIF, 2013).

Results from beneficiary residents

This was not a program which directly supported the residents, but enterprises implemented those activities. Through enterprises, households were supported with breeds of animals and plants, science and technology training, product underwriting, and other support. In particular, 3315 households, 3683 households were supported with breeds of animals and plants, and science & technology training respectively; 7017 households signed contracts and sold their products. In some projects, farmers even got support for fertilizers, plant protection drugs and animal feeds with a total of 2199 households receiving funds.

Public-private partnership program brought more practical results than the previous form of direct support for the residents. All activitiy that enterprises deployed was included in the content of the project and directly supported the production needs of the residents. Therefore, the effectiveness of this support would be higher, and it helped avoid the situation in which farmers received support but did not apply it into actual production.

Table 1. Summary of the number of beneficiary households in the program

Names of the activities	Actual beneficiary households		
	Total households	Poor households	Near-poor households
Breeds of animals and plants	3315	976	522
Technical & Technology transfer	3683	707	431
Product underwriting	7017	1203	1023
Other support (fertilizers, plant protection drugs, animal feeds…)	2199	587	206
Total	16214	3473	2182

Source: APIF (2014)

Table 2. Summary of increased incomes of beneficiary households

Name of the project	Increased average income per household (VND)	Number of extra job offering (jobs)
Developing models of strains and potato production (KCT Thai Binh)	2231341 (2014 - 2015)	3680
Growing and consuming ginger (Minh Be Private Enterprise)	28850174 (2014)	1208
Developing material zones, investing in Nhat Thien starch producing & clear noodles factory	6531069 (2013)	3230
Growing, processing cassava & oriental canna (Dong Tam Cooperative)	22453846 (2012)	900
Lan Thieu poultry raising enterprise (chicken project)	8620000 (2014)	150

Source: APIF (2014)

Overall, all project generated high economic efficiency, typically model of ginger cultivation and consumption by Minh Be private enterprise deploying in Pac Nam district. According to the results of specialists' independent evaluation, increased income per each household reached an average of 28.8 million VND, and the number of jobs generated reached 1,208 in 2014. Potato planting model being implemented in the Ba Be district, increased value reached an average of 2.2 million VND per a household in the crop 2014-2015, and created 3860 jobs. Potatoes are grown in third crop for a short growing period. This investment not only generated economic benefits but it also changed the farming practices of local people, because the residents had not grown the third crop, or just grew corn which had less economic value.

Regarding public-private partnership model, the production of the farmers was based on signed contracts, products produced were underwritten by the enterprises. The production under contract really helped stablize incomes for the farmers, and reassure the residents of the production expansion.

Results from enterprises

Firstly, investment in expansion of facilities, equipment and machinery: Participating in the program was a good opportunity for businesses to be able to expand production and business operations, infrastructure investment, as

well as machinery and equipment. Only 5 out of 11 enterprises received the grants; total investment capital for the enterprises reached more than 20.5 billion, in which more than 5.6 billion VND for reciprocal businesses, nearly 15 billion VND for grants. For small and medium enterprises, and enterprises operating in agriculture, this was a significant investment which helped enterprises to overcome difficulties due to lack of funds.

Secondly, expansion in the scale of production: Thanks to the public-private partnership program, many enterprises had the opportunity to sign contracts with the residents, consequently expanded and stablized the market for the output for the products. Previously, businesses were almost passive in building material sources, so the production was small and fragmented. With support from the public-private partnership program, the size and turnover of the business has increased significantly. Minh Be Enterprise, which had an average consumption from 1400 to 1600 tons per year, has increased to 3000 tons per year after joining the program. Lan Thieu poultry raising Enterprise, which was a small business with only 6-7 tons of chicken per year, has increased the scale of up to 30 tons a year after developing the material areas.

Table 3. Investment results (CO) of the enterprises supported by the program
(× 1000VND)

Enterprises	Investment Value	Captial Structure	
		Reciprocity	Grant
Mien dong Nhat Thien Company	1870000	350000	1520000
Minh Be Private Enterprise	3200000	182495	2017784
Dong Tam Cooperative	1222403	633752	588651
Lan Thieu poultry raising enterprise	127288	288	127000
Thai Binh Center for consultancy, farm science and environment development	14163495	3450646	10712813
Total	20583429	5617181	14966248

Source: APIF (2014)

Table 4. Sales Volume and average revenues of the enterprises involved in the project

Project	Sales volume and revenues	
	Before the project	After the project
Building potato production (x10^6VND /year)	3899,6	5347,8
Planting & consuming ginger (ton/ year)	1400-1600	3000
Developing & producing Mien dong (x10^6VND /year)	8500	22000
Poultry Raising (ton/ year)	6-7	30

Source: APIF (2014)

It cannot be denied that public-private partnership program in agricultural development, and poverty reduction in Bac Kan province has created great benefits. This would be the basis to expand the model to other regions in the whole country as this would benefit the citizens, enterprises and the government.

The strengths and weaknesses of public-private partnership model

Strengths were included: *first*, the efficiency of investment capital has increased with the participation of a group of enterprises; incomes of the residents and businesses have increased dramatically; *second*, a stable market for some agricultural products in the program was created; the pressure of the comsumption of agricultural products has decreased; *third,* farming customs and practices of the residents initially changed in a positive way with the objective of meeting the demand of the market; *fourth,* enterprises were more active in signing contracts with farmers; *fifth*, forms of cooperation such as interest (Nguyen Quang Hop, 2015).

Besides the strengths above, public-private partnership model has revealed some weaknesses which need to be solved in order to enhance its effectiveness as well as sustainability such as: *first*, the binding of the contracts between businesses and households remained low, regulatory role of the government agencies was faint; there is no mechanism to effectively coordinate among the Government, enterprises and households; *second*, the habbit of relying on the government or the enterprises was still common, the initiative to perform the tasks in production was not high; and *third*, the capacity of management and administration in the business activities of agricultural enterprises and cooperatives was still unqualified *(Nguyen Quang Hop, 2015)*.

Propose a new model for agricultural development in the Northern mountainous region in Vietnam

It can be seen from practical research in Bac Kan province that public-private partnership model based on value chain development in agricultural production is appropriate in the present context in the northern mountainous region of Vietnam (Tran Quang Huy, 2016).

The content of the model: Forming the link between businesses, agricultural cooperatives and farm households; Constructing value chain of agricultural products in which enterprises now serve as the motive of the chain, farm households act as satellites responsible for production and supply of agricultural goods for businesses.

Model of the link is as follows:

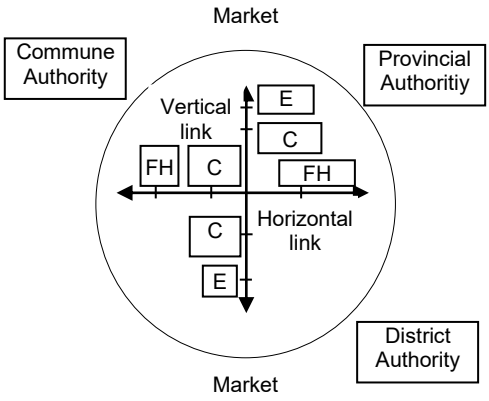

Diagram: Model of the link in agricultural development

Note: E: Enterprise; FH: Farmer Households, C: Cooperatives

The value chain in agricultural production is often built vertically or horizontally. However, the reality shows that in order to make this link work effectively, the chain must be built in the form of "a network", including vertical and horizontal links. Only when farm households conduct associated manufacturing (horizontal link) to create

new production volume, having pressures involving in greater need to cooperate with businesses to provide input to the production and consumption of the products; enterprises only cooperate with the farm households (vertical link) when there is an input market that is large and stable enough. Horizontal link is a form of connection among the farm households involved in the production of one or a number of agricultural commodities. This link will form interest groups, cooperative groups and cooperatives to produce the product volumes that are large enough. Vertical link is a form of association between enterprises, cooperatives and farm households to provide input of production and consumption of agricultural products for vertical-link chains. In this network, enterprises and cooperatives will serve as the motive of the chain leading the farm households to produce, and ensure sufficient and sufficiently qualified products that meet the demand of the market.

Some measures to implement the new model of agricultural economy development

To make public-private partnership model associated with the building of a value chain of producing goods in agriculture and rural areas, thereby improve the production efficiency, and increase incomes for farmers, the following measures can be taken: *first,* planning focused production areas and promote the strengths of each region. Each locality should identify their advantages, thereby develop into the area in commodity production. Only when the planning has been done properly, will enterprises be attracted to invest and with farm households to develop production; *second,* institutionalizing the establishment of association in production such as interest groups, cooperative groups and cooperatives. Authorities should make investments to support production through this association, create mechanisms so that this association can mobilize capital from grants or loans; *third*, building a mechanism for coordination among the entities participating the chain including local authorities (districts and communes); businesses and citizens. Responsibilities, duties and roles of local authorities should clearly be identified in promoting the relationship between businesses and residents. These are important issues that determine the success or failure in maintaining and developing the relationship between citizens and businesses; *fourth*, trade promotion organizations need to attract businesses to invest in the agro-forestry field; using funds from the local and central level and other legitimate sources to implement support for enterprises following Decree 210/2013/ND-CP of the Government; and *fifth*, do not support agriculture production directly to farmers but through businesses, cooperatives and cooperative groups, attaching the benefits of businesses with the responsibility of signing product underwriting contracts for farmers.

4. CONCLUSIONS

From practical research in Bac Kan province, it can be seen that public-private partnership model would be an appropriate organizational form for agricultural economic development in Vietnam. This model has many advantages, especially it creates sufficiently large and stable input material areas for enterprises. At the same time, it solves the biggest problem of the farmers in terms of the product output. However, in order for this model to work effectively, the Government needs to investigate, develop and promulgate a mechanism for coordination between the

government, enterprises and farmers, especially prescribe responsibilities of the entities involved in the model, the arbitration regulations of the authorities in securing the contracts between the residents and businesses to be implemented most effectively.

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HOUSEHOLD RISK MANAGEMENT STRATEGIES FOR COASTAL AQUACULTURE RISKS: THE CASE OF CLAM FARMING IN THAIBINH PROVINCE, VIETNAM

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ABSTRACT

Endowed with 3,260 km of coastal line and 112 estuaries, Vietnam has a high potential for aquaculture development. However, long coastal line is also embedded with natural high risks under climate changes and sea level rise. Vietnam is ranked at the 18th in the 2015 world risk index and the vulnerability index of 50.9%. Relying on coastal resources, aquatic farmers have adopted a number of strategies to cope with aquaculture risks. By using the OECD holistic approach, this research used several research tools to identify farmer's performance on clam farming practices and their risk management strategies (RMS) in Thaibinh province (the largest area of the clam production in the north and north central coastal part of Vietnam). RSMs are found of diversification and flexibility among farmers. For production risks, the RSMs are: (1) enlarging clam raising size and (2) actively controlling clam production by experience and technical innovations. For market risks, the RSM is searching for more clam market channels in both input and output market. For financial risks, RSMs are (1) securing family from clam farming loss by diversifying livelihood activities and (2) accessing secure financial sources in term or interest and bond conditions. Although some RSMs had resulted positive impacts but in overall, the clam farming risks have not managed well by those strategies due to the limitation in capacity of households comparing with level of risks. To cope better with different risks in clam sector, besides the adjustment in RSMs of farmers themselves, it is necessary to have the intervention from government (from national to local level) to address the aquaculture risks which the farmers cannot handle by themselves, such as (1) addressing the issue of polluted wastewater to the clam field; and (2) more focusing in supporting farmer in linkages to the both formal financial market and output market. In addition, supports for technical training targeting on improving farmer's skills and knowledge in farming decision making and market information is also of high value to clam farmers in coping with farming risks.

Keywords: Clam, farmer, risk management strategies, Thaibinh province.

1. INTRODUCTION

Risks are often more embedded in agricultural production and business sector which largely depend on external factors. Agricultural risks are basically categorized into five types, such as: production risk, marketing risk, financial risk, legal risk and human one (N.Musser and F.Partrick 2002). Agricultural risks can cause large losses for farmers and traders. However, as driven by commercialization, many farmers are trying to spend more investment for their farms, without adequate agricultural risk management and mitigation strategies. As consequences, many rural households have

been suffered losses in agricultural production (Minot and Hill 2007).

Given a longer production cycle, as well as more initial investments needed, aquatic farms are often faced with a higher risk as compared to farmers of annual crop production (Engle 2010), especially in the context of climate changes and their unpredicted hydrological cycle changes. Handosyde *el al.,* (2006) and Silva and Soto, 2009 (cited in (Barange and Perry 2009), noted that climate changes have caused various impacts on aquaculture in both direct and indirect ways, exaggerating stress and vulnerability of this sector, thus with a higher loss probability. Meanwhile, aquaculture production and its share of the fisheries market are predicted to expand continuously as it's set to play an increasingly important role in meeting increasing global demand on aquatic products (Handisyde, Ross *et al.*, 2006). These two trends will probably exaggerate risks in the aquatic sector which require more active and effective actions and strategies of different actors involved in the sector to help farmers better capable in coping with risks. Ability to survive and/or recover from aquatic farming risks varies among different farms. It could be largely that farmers have various options and strategies in managing and coping with agricultural risks, varying in different farming contexts and risk scope and nature. Household's risk management strategies have thus certain impacts on reducing farmer's vulnerability as well as improving their resilience towards risks (Engle 2010).

Thaibinh province has the largest clam farming area and production among coastal provinces in the north Vietnam (ThaibinhDARD 2014). In the early 1990s, increased market demand for clam coupled with a reduction of wild clam had created a demand for clam production, started with a small area of about 150 ha. Clam production area was slowly expanded in the following years and increased to 500 ha in 2006. In 2009, local governments started paying attention to clam production through zoning and bidding production area with some financial supports to farmers. However, government policies/interventions on clam-farming land-use were officially launched in 2011. Clam production area quickly increased, especially in 2011 and 2012. Given bad hits on clam farming productivity and market demand and price, clam production area expansion was slow down in following years.

The increase in clam production area coupled with higher farming clam density resulted in a sharp increase in clam production, especially in 2009 and 2010. However, increased natural and artificial disasters and low quality of clam breeds (see further below) had resulted in a sharp reduction of clam yield in 2011. Since then, clam yield fluctuated around 18 tons/ha (Figure 1). Clam market price was on increased trend in the period of 2006-2009. In this period, clam was considered as a "golden" farming subsector in Thaibinh as well as in other coastal provinces having clam farming practices in the whole Vietnam. However, shortly enjoying such golden period, farmers were faced with reduced clam market price and increased clam farming risks in the following years. These combining impacts have caused a sharp reduction in clam gross output of the province (Figure 2).

The largely fluctuated trend of clam yield and market price reflects intensity of risks. Different from other aquacultural animals like shrimp, craps, and fishes, clam production cycle is relatively longer, i.e., two to three years, and more vulnerable to risks, both natural and

artificial ones. Clam farming losses have driven thousands of farmers into underemployment and even financial debt traps. After the market shock in 2012, the loan provided to 1,752 clam farmers and enterprises were VND 457.6 billion which has been difficult to retaken by the banks. In Namthinh commune, financial value of unmarketable clam was estimated at about VND 160 billion. In addition, un-harvested clam farms accounted for 70% of total clam farming areas (Tú 2013).

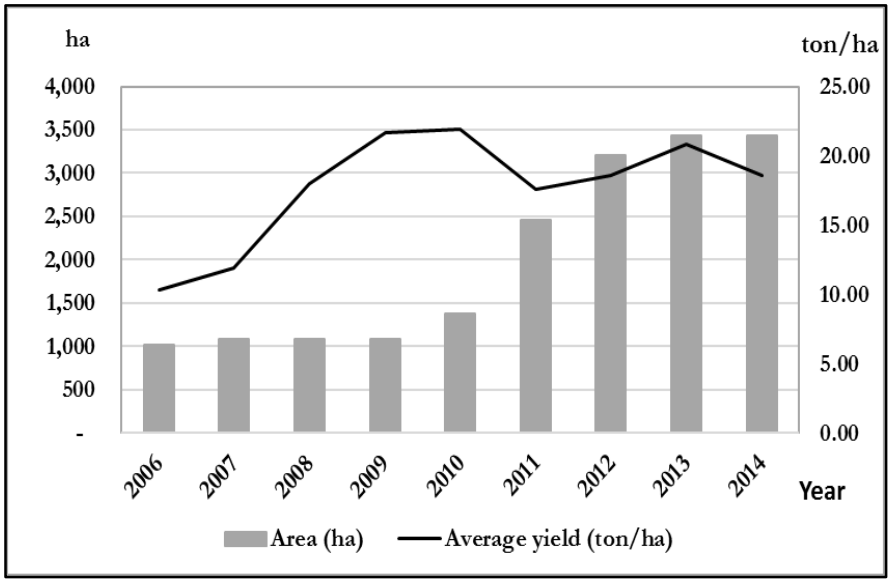

Figure 1. Clam production area and yield (2006-2014)

Source: Thaibinh Statistical Office, 2015

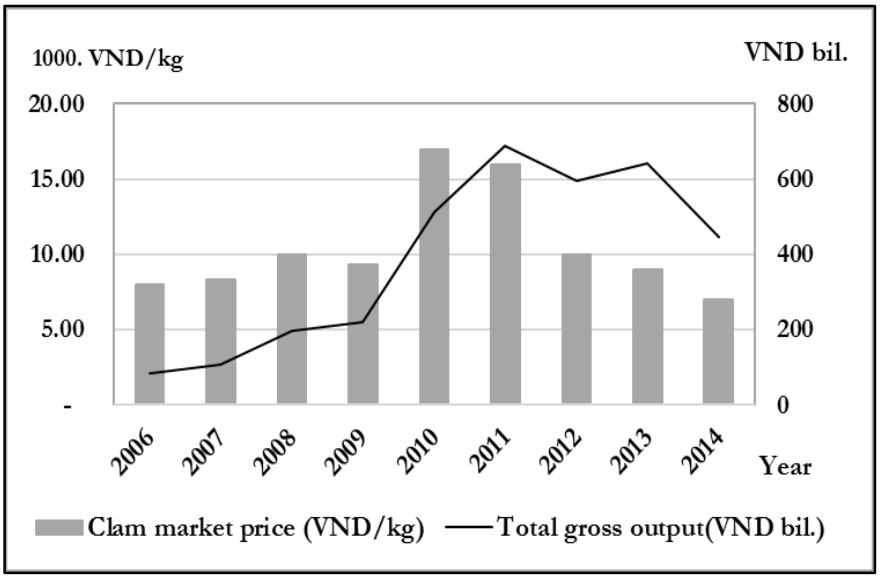

Figure 2. Total gross revenue of clam production (2006-2014)

Source: Thaibinh Statistical Office, 2015

Figure 3. Map of the research sites

Note: ● *Selected communes for the research* ★ *Thaibinh province*

However, there are small number of farmers still survive relatively well despite clam production and market risks. What are risk management strategies that these successful farmers developed and adopted that have survived them from clam farming and marketing risks? This is the major research question of this paper. In specific, the paper aims to provide empirical insights in to: (1) which are the household risk management strategies for clam farming in Thaibinh coastal area? and (2) which are the strategies or tactics significantly contributing to farmer's success in their household risk management strategies?

2. RESEARCH METHODOLOGY

Study site. Among provinces located in the coastal line of north and northern central of Vietnam, Thaibinh has the largest clam farming areas (i.e., 3,430 ha in 2013), followed by Namdinh (1,710 ha), Thanhhoa (1,200 ha) and Quangninh (1,000 ha) (MARD 2014). According to Thaibinh Agriculture and Fishery Extension Center, salinity in estuary areas is just around 1.5-2.5% with a plentiful source of feeds that is very favourable for the development of aquaculture in the province. Total estuary area having potential for aquaculture is around 17,000 ha (Nguyên 2013) of which 15,119 ha (or roughly 89% of the total estuary area) have been brought into aquaculture production. In 2013, total aquaculture production generated a value of VND 723 billion (based on fix-price level in 1994) for the Thaibinh province (ThaiBinhDARD 2014).

Out of 12 communes farming clam along 50 km coastal line of Thaibinh province, three communes were selected for the research because these have the largest area as well as the longest history of clam production in the whole province (see Figure 3). This allows a better trace of clam farming risks and farmer's resilience capacity development and strategies in coping with risks in a relatively long period of time (i.e., 2006-2014). There was a total of 1,310 households raising clam in these three communes at the time this research was conducted.

Data collection. Fieldwork activities were carried out in the study site from 8/2014 to 4/2015. In addition to secondary data obtained from local government offices and published papers/reports, the three major research tools were used to gather information on clam production, marketing, farming and marketing risks, and farmer's capacity and strategies to deal with different clam farming risks in the period of 2006-2014. The main data collection tools are:

(1) Focus Group Discussion (FGDs). One FGD was conducted in each selected commune with a participation of 8-10 farmers having experience with clam farming and marketing. FGDs aimed to explore historical trend of clam production and market, and the name of household risk management strategies which have been applied in research area and the characteristics of households which probably impacted to the applications of those strategies.

(2) Household survey: The sample size of households for the survey was calculated by the equation:

$$n = \frac{N * t^2 * S^2}{N * \Delta_x^2 + t^2 * S^2} \qquad (1)$$

In which: "n" (sample size); "N" (total households in research site) =1,310; "t"(confidence interval) = 2.17 (with 97% confidence level). Sample variance and sample errors were estimated based on the trial survey (on the total loss area for each household) of 31 households from the three communes. The statistic results of the survey showed the amount of sample variance (S^2)=194.88 and sample errors (Δ_x^2) = 2.52. The actual sample size was then needed to be increased from 137 to 157 since the sample from Thai Do commune was increased from 11 to 31 in order to have sufficient number of households to be representative of the commune.

Case study: Several cases had been studied with in-depth interviews to explain for the quantitative analysis results from data of household survey.

Data analysis. In this research, factor analysis is applied to define the impact of household's characteristics to the applications of household risk management strategies. It is then followed by a discriminant analysis to measure the level of impacts of each tactic in household risk management strategies to the performance of three clam farmer groups, which have the difference in the results of their risk management strategies.

3. CLAM FARMING RISKS AND HOUSEHOLD RISK MANAGEMENT STRATEGIES

3.1. Clam farming risks

Majority of clam farmers experienced farming risks (i.e., 86% of the survey households underwent at least one massive loss of clam production so far), that is similar to the result in the research on clam farming productivity in some coastal provinces in the North and Northern Central Vietnam (Thuyết and Dũng 2013). Most serious risk has been of high clam mortality rate during farming process because of unsecure quality of juvenile clam; uncontrolled water source; natural disasters such as flood, storms, and drought (for shallow raising areas) (Lebailly, Bui Thi et al. 2015). In addition, market risks caused by reduced market demand and price have been increasingly plagued farmers in recent years. Many farmers have been trapped into financial debts without ability to escape. Rate of loss in clam investment is estimated at

52% in Thaibinh province in the period of 2006-2014.[1]

Results from survey revealed that risks which make clam farmers most worried are the high mortality rate (production risk) and the sudden reduced clam market prices (market risk). In recent years, those risks happened quite frequently and have caused serious losses. Clam farmers have even accepted 30% as the mortality rate for a normal clam farming cycle. However, since 2009, the mortality rate had increased up to 40%-55%, mainly caused by polluted water discharged from inlands or by the extreme weather events (storms or hot weather). In parallel, reduced clam market prices since 2012 led to extreme chaos in clam production sector. The fluctuation in clam farming productivity and price causes a seriously financial impact to farmers. Hundreds of billion VND investment lost each year in the period of 2012 - 2014. Thousands of farmer have been faced with bankrupt. About 457.6 billion VND borrowed from banks couldn't be paid back yet (Long 2013). In group of 157 interviewed households, 16% stopped their clam farming as the result of capital bankrupt; 38 % had to sell their fix assets (like houses, cars, motorbike or even clam fields) to have money to repay borrowed loans.

Different from other farming investments that have a relative definite harvest time, clam harvest can be extended relatively long, up to 1 to 2 years. However, the longer clams stay in the field, the more production risks for farmers caused by bad weather events or polluted water discharges. To cope with these risks, clam farmers have developed some farming strategies, which are going to present in the following sections.

3.2. Farmer's Risk Management Strategies (RMSs)

There are several RMSs were applied with numerous tactics in farmer households in order to manage the clam farming risk and to reduce the vulnerability level probably caused by risks (Table 1). The number of tactics applied in each household were different, as it depended on the household characteristics.

Reducing the mortality rate is crucial purpose in RMSs of clam farmers. For this purpose, there are two main strategies namely enlarging clam plots size (RMS1) and actively controlling clam production by farming experience and innovations (RMS2). According to Decision 11/2012/QD-UBND for clam farming land-use reallocation to farm households, each clam plot should not be bigger than 2 ha. However, according to farmers, small area not only cost farmers more for labor (such as farming practices and protection) and facility investments (such as living shed, boat, protection fences) but also disadvantage for raising clams at different ages.[2] For this strategy, there have been two strategies applied by farmers. The first strategy is to hire land from other farmers nearby. After 2013, a number of farmers have given up their clam farming because

[1] Resulted from Monte Carlo Simulation by application of Crystal Ball software, based on production data's collected from household survey.

[2] Normally, clams of different ages are raised separately, i.e. close to harvest, young, juvenile. However, as revealed by farmers, big raising plot will allow them to combine raising clams at different age, simply separated by a net system. This better allow farmers coping with market risks and production risks (because different clams have different sensitivity to extreme weathers or pollutants discharged from inlands. Moreover, investment on (super) juvenile clams is not much. Once juvenile clams grow and ready for commercial production, mortality rate will reduce because clams have been familiar with local production conditions.

of the previous farming losses. About 50% of these farmers agreed to rent out their land to neighbours and among the surveyed farmers, 70 hire additional lands to enlarge their clam farming plots. As a result, average clam raising plot is 2.46 ha and 2.90 ha in Dongminh and Namthinh commune, respectively while only 1.68 ha in Thaido commune. The second is to form "joint-groups" among farmers who owned the plot close to each other for large raising plots. In 2011, there is 81% of clam farmers in Dongminh and 36% in Namthinh communes decided to group themselves to enlarge farming plot to save production costs and minimize production risks.

RMS2 is adopted by farmers who know which intertidal area is safer and better for clam production as well as dangerous periods in the year for clams. There have been three relevant tactics adopted by farmers. The first tactic (T2.1) was bidding plots that are good for clam raising according to farmer's experience. However, this tactic was applied in Dongminh and Namthinh where the local government allowed farmers to bid with specific land-use renting price (while in Thaido commune, the place of land was assigned by random ballot selecting). For the second tactic (T2.2), farmers try to control starting time of clam cycle and/or juvenile age to minimize impacts of weather shocks on young clams and harvest clams before storm season. This tactic is not too complicated but not easy for all farmers since it requires farmer's ability to purchase juvenile clam and access to market for selling harvested clams in the time they prefer. Besides, it required the careful observations and experiences because the best time for clam production depends on the characteristic of clam raising zones even plots which are corresponding with sea currents and nutrient availability. Among the surveyed households, about 55% are confident on following this tactic. In addition, pressure from risks also helped initiating some innovations associated with clam production at local level to improve clam production as well as to reduce loss rate such as fill-in new sand into clam plots (to reduce pollution and enrich nutrient for clams); better fencing and cleaning practices (for fencing net systems) 71% of surveyed clam farmers in Thaibinh had applied those techniques innovations, but in different levels.

In parallel with coping with production risks, farmers also developed strategies for dealing with market risks. To reduce loss caused by reduced price and/or lack of clam market, farmers tried to search for more clam market channels, for both input and output market (RMS3). For input market, there are two main sources for farmer to buy juvenile clam, and 56% of surveyed households purchase juvenile clams from producers in Namdinh province while 18% directly from wholesalers in commune. The rest (26%) started from juvenile nursery practices (those juvenile clams were in very small size, i.e. 100,000 heads of clam/kg) to reduce cost of purchasing juvenile clam as well as to be more independent for their clam practices. For selling adult clam, while in 2006-2012 there are two type of collectors: local and external ones. In this period, 52 % of farmers sold clams to external collectors because these offered higher price than local ones. Some external collectors did not pay farmers after collecting clams. From middle of 2012 afterward when clam market price getting down, external clam collectors suddenly disappeared.

Table 1. Households Risk Management Strategies in clam farming

Clam farming risks	Strategy	Tactics	Code of tactic	% of HSH applied
Production risk	RMS1: Enlarging clam plots size	Hiring land	T1.1	15%
		Forming up share group	T1.2	40%
	RMS2: Actively controlling clam production by farming experience and innovation	Choosing good place for clam plot (1)	T2.1	50%
		Actively controlling the point for starting & harvesting the clam crop	T2.2	55%
		Applying techniques innovations	T2.3	71%
Market risk:	RMS3: Searching for more clam market channels, for both input and output market	Actively searching for good juvenile clam source	T3.1	84%
		Diversifying in clam selling channel (2)	T3.2	52%
Financial Risk:	RMS4: Diversifying livelihood activities	Carrying out other aquaculture activities	T4.1	52%
		Carrying out rice production	T4.2	64%
		Carrying out livestock activities	T4.3	20%
		Carrying out other activities	T4.4	74%
	RMS5: Accessing to more secured sources of capital	Using family/relatives saving money	T5.1	82%
		Forming up share group	T1.2	40%
		Trying in access the formal credit market	T5.3	79%

Notes:(1): Applied only in Dongminh and Namthinh commune, (2): Applied only before 2014

For financial risk management, having alternative income source is a central theme in RMSs of clam households. There are two strategies contributing to secure households from clam farming loss are: (1) diversifying livelihood activities (RMS4); and (2) accessing to more secured sources of capital (RMS5). RMS4 is considered as a strategy to fulfil household daily spending and contribute to debt payment when clam farming facing with loss. All of clam households have other livelihood activities other than clam production. For instance, households having other aquatic production such as shrimp and fishes account for 52 % of total surveyed households. Households having paddy rice production, livestock raising are 64%, 20% of the total surveyed households, respectively. The reason of RMS5 because the nature of high capital requirement of clam farming and the uncertain of financial market in recent years, farmers had to try to access to more secured financial sources, for instance: (1) T5.1: Using family/relatives saving money: (2): T5.3: Trying to access formal credits with lower interest rates; and (2) T1.2: Forming up "self-credit groups" which can provide members a certain volume of money when necessary. The extent of relying on different financial sources also very much depends on prospects of clam production and marketing. For example, for 94 households who started clam cycle in 2012, 34% used their own capital and/or from self-credit groups, 49% borrowed from formal credit market and 17% borrowed from informal credit market. In 2013, those figures were 27%, 70% and 3%, respectively. In whole period 2006-2014, roughly 70% of farmer's investments and reinvestments (after facing with farming

losses caused by risks) originated from (formal and/or informal) credit systems. Meanwhile, by September 2013, there were 1,752 borrowing applications from farmers and small enterprises for money to invest into clam production, with a total cash amount of 457.6 billion VND from state banking systems (Long 2013). This amount was just equal to one-third of the total cash requirement from farmers for clam production. From 2006-2014, among surveyed households, there were 81 turns of borrowing from informal credit systems (to invest on clam production or to repay overdue debts of the banks or private creditors).

3.3. Evaluation the results of RMSs

In overall, results of RMSs adopted by farmers in clam farming have brought varying results to different farmers in different locations. For instance, among surveyed households, 15% reported that they have been successful in all clam cycles whilst 18% lost in all clam farming efforts, among these households, 8 stopped clam farming after experiencing loss in the first clam cycle. There was even one farmer who joined in 8 farmer's groups with 8 clam raising plots in 2012-2013, and all failed. To understand hidden reasons which caused the difference in performance and resilience of clam households, those 157 households had been classified into 3 groups based on profits/losses in clam production and their recovery from losses (Table 2).

Discriminant analysis test had revealed that among 13 tactics mentioned above, at significant level 5% there were only 7 tactics had critically impacted to the result of RMSs in clam farming households (Table 3), namely (1) T4.1: Carrying out other aquaculture activities; (2) T2.3: Applying techniques innovations; (3) T2.2: Actively controlling the point for starting & harvesting the clam crop: (4) T5.1: Using family/relatives saving money; (5) T4.2: Carrying out rice production; (6): T1.1: Hiring land; (7) T4.3: Carrying out livestock activities.

Table 2. Clam farming performance in 157 surveyed households (Period: 2006-2014)

Profit/Loss results in clam crops	Number of households			
Gain in all clam crops	23(1)			
		Resilience after clam losses		
		Restarted(a) and Recovered(b)	Restarted but not Recovered yet	Not restarted
Percentage of loss crops < 20%	8	8(1)	0	0
Percentage of loss crops > =20%	98	39(2)	49(3)	10(3)
Lost in all clam crops	28	0	20(3)	8(3)

Notes: (a): Restarted: Household restarted a new clam crop after the loss in previous clam crop;
(b): Recovered: The loss from previous clam crops had been covered by the profit of the clam crops started after that;
(1): Classified in Group A: Households had not been impacted, or had been slightly impacted by the risks and good resilience (31 households);
(2): Classified in Group B: Household had seriously impacted by the clam farming risks but had been able to restart clam production and recover from losses (39 households);
(3): Classified in Group C: Households had been able to restart clam production but had not yet recovered from losses and households had been unable to restart clam production (87 households)

Table 3. Tests of Equality of Group Means

Name and Code of Tactics	RMS	Wilks' Lambda	F	df1	df2	Sig.	
T1.1: Hiring land	RMS1	.88	4.00	2	60	.02	x
T1.2: Forming up share group		.96	1.32	2	60	.28	
T2.1: Choosing good place for clam plot	RMS2	.92	2.62	2	60	.08	
T2.2: Actively controlling the point for starting & harvesting the clam crop		.73	11.38	2	60	.00	x
T2.3: Applying techniques innovations		.61	19.10	2	60	.00	x
T3.1: Actively searching for good juvenile clam source	RMS3	.99	.11	2	60	.90	
T3.2: Diversifying in clam selling channel		.99	.19	2	60	.83	
T4.1: Carrying out other aquaculture activities	RMS4	.42	41.58	2	60	.00	x
T4.2: Carrying out rice production		.83	6.25	2	60	.00	x
T4.3: Carrying out livestock activities		.89	3.88	2	60	.03	x
T4.4: Carrying out other activities		.99	.19	2	60	.83	
T5.1: Using family/relatives saving money	RMS5	.75	10.35	2	60	.00	x
T5.3: Trying in access the formal credit market		.92	2.84	2	60	.07	

Table 4. Impact of the plot size to the Profit/Cost ratio (Period: 2006-2014)

Groups Statistics					Ranks	
Groups	N	Mean	SD	SE	Mean Rank	Sum of Ranks
Group1: Plots ≤ 2 ha	458	0.24	1.12	0.05	304.89	139641.50
Group2: Plots > 2ha	181	0.48	1.06	0.08	358.22	64838.50
Mann-Whitney U: 34530.50; Wilcoxon W: 139641.50; Z:				-3.29; Asymp. Sig. (2-tailed):.001		

Results of RMS1 (with tactic T1.1) was tested by Mann-Whitney U test which revealed the differences of profit per cost ratios between household groups of different clam plot size (maximum of 2 ha – Group1 and larger – Group2) (Table 4). The differences between two groups were caused by three factors, including: (1) Cost: both variable and fix cost is found to be inversely correlated to the field sizes; (2) Clam density: Plots in Group2 has lower density, therefore lower mortality rate as compared to Group1. Lower density allows clam growing faster which helps shortening clam production cycle and reducing production risks; and (3) Farming arrangement: Larger plot size allows Group2 raising clams in combine models, which is less risky than the model raising juvenile clam or adult clam only (according to the experiences of farmers). As revealed by farmer's FGDs, about 10% lower in clam mortality rate in Group2 as compared to Group1.

The tactic T2.2 created good result because the active control over clam production cycle helped to reduce the mortality rate with juvenile clam and having clam harvest before storm season. Parallel with that, the tactic T2.3 with innovation techniques applied such as double net fencing system, fill new sand into clam raising plots, clam catching machine, clam cleaning machine also contribute to not only reduction of risks but

also increase of clam productivity. However, these techniques help farmers to cope relatively good with natural disasters (such as storm, strong wave, lack of food), but not with man-made disasters (i.e. polluted water discharges or clam thief's) since these are still beyond farmer's capacity to cope with.

The group of tactic T4.1; T4.2; T4.3 (RMS4) importantly contributed to the success of household risk management by creating the financial source for farmers to invest in clam farming. This is similar to the findings of Fischerand Buchenrieder (2010) that income diversification is the most common risk management strategies in developing countries, as it has many likenesses to the financial instruments, which consequently reducing the dependence of them to the debts as as well financials risks (Harwood, Heifner et al. 1999).

Last but not least, tactic T5.1 (RMS5) also played an important role in risk management because the farmer's confidence about financial capacity was one of three important factors contributing to household resilience capacity to clam farming risks in Thaibinh province (Hang, Cuong et al. 2016). The reason is accessing to informal credits with high interest rates is easy but risky especially for poor farmers (Nguyen, James et al. 2013). Informal credits of higher interest rate (5-10% higher than formal credits) and higher pressure for repayment brought poor farmers into a dilemma of "easy to borrow money but also easy to fall into debt trap." Certainly, using family/relatives saving prevented them to fall in that trap, as well as protect farmers from uncertainty (Hang and Sheng 2005).

Table 5. The difference in application of the tactics of the RMSs in 3 groups

			Group A	Group B	Group C
T1.1: Hiring land			32%	35%	-
T2.2: Actively controlling the point for starting & harvesting the clam crop			100%	61%	42%
T2.3: Applying techniques innovations	Often		58%	67%	13%
	Sometimes		39%	23%	39%
	Never		3%	10%	48%
T4.1: Carrying out other aquaculture activities	High contribution		65%	23%	-
	Moderate Contribution		29%	19%	-
	Low Contribution		-	-	-
	No contribution		6%	58%	-
T4.2: Carrying out rice production	High contribution		3%	6%	32%
	Moderate Contribution		23%	32%	45%
	Low Contribution		26%	19%	13%
	No contribution		48%	42%	10%
T4.3: Carrying out livestock activities	High contribution		0%	-	6%
	Moderate Contribution		13%	-	23%
	Low Contribution		3%	-	-
	No contribution		84%	-	71%
T5.1: Using family/relatives saving money	Percentage of family/relatives' money in total capital investment to clam farming	Mean	27%	24%	6%
		Max	100%	50%	13%
		Min	0%	0%	0%
		Median	11%	22%	11%

Comparing the RMSs of these 3 groups, several differences were found in the application of the RMSs and related activities (Table 5). Although the RMSs and its tactics were not secret for every farmer, there have been constraints for certain households to follow. For example, to enlarge size of clam raising plot (RMS1), given limitation of financial resource, none of household in Group C hired additional land but 45% of them decided to join farmer's groups. Meanwhile, 32% of Group A hired land and 23% joined farmer's groups. For farmer's groups, initially profits were shared for all members. However, after some crops, different interests and contradictory opinions about clam production and RMSs among members constraining them for keep going as groups or further enlarge ring their clam farming plots. In 2013, many groups have been broken up, mainly caused by different decisions on clam selling times and practices. Similarly, majority of farmers in Group A and B were able to mobilize their own savings (or saving from their relatives) for restarting clam production whilst those sources of farmers in Group C had to finance only 6% (in average) of total capital needed for restarting clam production. Diversification of farming practices has better supported for Group A and B than Group C.

4. CONCLUSIONS AND IMPLICATION

To cope with risks in clam farming practices, farmers have applied several RMSs, separately or in combination. For production risks, the RSMs are: (1) enlarging clam raising size and (2) actively controlling clam production by experience and technical innovations. For market risks, the RSM is searching for more clam market channels in both input and output market. For financial risks, RSMs are (1) securing family from clam farming loss by diversifying livelihood activities and (2) accessing secure financial sources in term or interest and bond conditions. Some tactics had critically impacted to the result of RMSs, namely (1) T4.1: Carrying out other aquaculture activities; (2) T2.3: Applying techniques innovations; (3) T2.2: Actively controlling the point for starting & harvesting the clam crop: (4) T5.1: Using family/relatives saving money; (5) T4.2: Carrying out rice production; (6): T1.1: Hiring land; (7) T4.3: Carrying out livestock activities. However, more farmers have been suffered from clam farming and marketing losses than those with success. The reasons were the difference in the tactics of each RMSs due to the limitation in capacity of households comparing with level of risks.

Apart from the above reasons, the failures in risks management of majority of households (87/159 households in surveyed group) was partly caused by the absence of RMSs for the man-made risks (such as death clam phenomenon because the impacts of waste water from rice production activities or from industrial zone). While the success of aquaculture is greatly dependent on the quality of the cultivating environment, clam farmers in Thaibinh have not got an appropriately strategy for protecting water environment, except to choose the good place for the clam plots which are far enough from polluted water flow. Moreover, the non-correspondence RMS3 with the level of market risks also explained for the inefficiency of this RMS despite the efforts of farmers. According to the holistic approach introduced by OECD (2009), RMSs of households are only efficient in addressing the risks which are in micro level. However, due to the level of the

consequences and likelihoods, the market risks and financial risks in clam farming are at meso/macro level (Hang, Cuong et al. 2015), which really need the interventions/policies of government (from state to local level) to deal with. In fact, clam farmers in Thaibinh had tried to connect with input/output market by themselves, without any supports/protection from government, even when clam farmers working with strange foreigners in local area.

To cope better with different risks in clam sector, besides the adjustment in RSMs of farmers themselves, it is necessary to have further interventions/policies from government (from national to local level) to address the aquaculture risks which the farmers cannot handle by themselves, such as (1) addressing the issue of polluted wastewater to the clam field; and (2) more focusing in supporting farmer in linkages to the both formal financial market and output market. In addition, supports for technical training targeting on improving farmer's skills and knowledge in farming decision making and market information is also of high value to clam farmers in coping with farming risks.

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INFLUENCE OF CONTRACT FARMING ON BLACK TEA VALUE CHAIN: A CASE STUDY IN PHU THO PROVINCE, VIETNAM

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ABSTRACT

Black tea product contributes 80% of exported tea in Phu Tho province. Black tea has been being one of the high valued commodities and brought high income for tea producers. However, the black tea industry has been facing on many difficulties such as low price, perishable cash crop, weak cooperation between stakeholders and low competitiveness. In value chain, contract farming has been viewed as an instrument for improving value chain performance by building the tight integration among actors. Therefore, the study focused on comparing value chains of contract farmers to those of worker farmers and non-contract farmers in order to analyze the influence of contract farming on black tea value chain. The data collection based on semi-structure interviews and 1 1 0 standard questionnaires in Thanh Son and Doan Hung districts. By adopting financial analysis, the paper explored the cost and the benefits of actors and the linkages among the actors of the black tea value chain. The results showed that the value added (VA) was 10.88 million VND/ton of black tea for groups of worker, 10.62 million VND/ton of black tea for contract farmers and 11.40 million VND/ton of black tea for non contract farmers. However, the VA of whole chain of worker farmers and contract farmers (29%) is higher than the non-contract farmers(18%). Therefore, worker farmers and contract farmers chains are more comparative and sustainable than non contract farmers chain. Based on the results, it could indicated that contract farming could promote the black tea value chain in Phu Tho province.

Key words: Contract farming, black tea, Phu Tho province, value chain.

1. INTRODUCTION

The involvement of Vietnam in tea cultivation dates back 3,000 years. Commercialized tea production in Viet Nam has strongly developed in the last decades after the Independence with the establishment of state-owned farms focusing merely on tea production (ADB, 2004). In comparison with 20 years ago, tea production areas have increased 2.5 times higher in which 2.2 times in productivity, 6 times in total output, and 8 times in export value (Nguyen Hoang and Nhat Bac, 2011). Vietnam's tea production and export have shared the 5th place with Indonesia in the world ranking, standing behind India, China, Kenya, and Sri Lanka. Vietnam's tea has been exported to 110 countries and regions in the world, including major markets such as Pakistan, Russia, China, Taiwan, Indonesia (Xuan Hai, 2015).

In 2014, Vietnam exported 130,000 tons of tea which valued approximately 230 million US dollars. Approximately 3 million Vietnamese earn their livelihood from tea through the over 400 businesses involved in production, processing, and export (Nguyen Thanh, 2015). In Vietnam, tea exports account for 75-80 % of total production, 20-25% for domestic consumption (Ha Thu, 2015). In the structure of exported tea, black tea now accounts for about 78%; green tea accounts and the rest are other types (Cao Linh, 2014). Tea area of the northern mountainous province accounts for 80.7% of the country with 90,800 hectares of tea. Several provinces have large tea areas are Thai Nguyen, Ha Giang, Phu Tho, Yen Bai and Nghe An, Tuyen Quang, Son La (Ipsard, 2007).

The total tea production area of Phu Tho 10 years ago was 7.893 ha, accounting for 43.5 thousand tons output. In the recent years, tea production has become the major agricultural programs. At the end of 2011, the total area of the province had risen up to 15.650 ha accounting for more than 12% of the whole country. This brought the province to the 5th place tea production area of the country. Output has reached 115,506 tons of tea leaves (Kim Chi, 2012).

Phu Tho province also has the tea processing industry that typically symbolizes foreign investment in the tea sector. Currently, the province has more than 70 tea processing companies with capacity of 1 ton of fresh tea bud per day (400 companies in Vietnam in total) (Phu Tho DARD, 2015). Tea is considered the top agricultural commodities for exports of the province. In 2010 the province's tea export valued 7.3 million US dollars out of 300 million US dollars of the national tea export. Tea industry has contributed to increasing the rate of employment and income for local people in the province. In recent years, tea production has developed stably with little influence by natural calamities, pests, and fluctuant price. Therefore, tea production guarantees people's lives better than other food crop production (Quoc Vuong, 2012). However, Vietnam' tea export still remains relatively low compared to other countries. The export price is only about $ 1,500/ton which is half of the average world price. The reason is the quality management in tea production and export is not well-considered, especially in food safety issues. The tea quality so far is still keeping Vietnam's tea industry far away from reaching global markets' standards (Tien Anh, 2015).

Contract farming has been defined as an agreement between one or more farmers and a contractor for the production and supply of agricultural products under forward agreements, frequently at predetermined prices (Eaton and Shepherd, 2001). Minot (2007) defined contract farming as agricultural production carried out according to a prior agreement in which the farmer commits to producing a given product in a given manner and the buyer commits to purchasing it. According to Eaton and Shepherd (2001), contract farming can be classified in to 5 types namely centralized model, nucleus estate model, multi-party model, informal model and intermediary model.

The centralized model is the model that a firm directly signs contract with farmers with tight arrangement and strict quality control and quantity determined at the beginning of the production. Firms usually provide inputs, technical supports to contract farmers to have high value agro-products.

Nucleus estate model and centralized model are alike in terms of that company

signs contract directly with farmers, supports inputs, technologies and controls strictly quality of products. However, in the nucleus estate model, firm owns farm assets and facilities and contracted farmers just contribute labors and some inputs to the production processes. The multipartite model normally involves a joint venture (between a public entity and private firm) contracts with farmers. This model might have separate organizations responsible for credit provision, production, management, processing and marketing. Informal model model applies to individual entrepreneurs or small companies who normally make simple, oral contract with farmers on a seasonal basis, particularly for crops such as fresh fruits and vegetables. Crops usually require only a minimal amount of processing, such as sorting, grading and packaging. The intermediary model includes intermediaries (such as representatives of farmer's groups/cooperatives) between firm and farmers. This model, which can be considered as a combination of the centralized and informal models, is common practice throughout Southeast Asia. Given the indirect linkage with farmers, this model has several disadvantages mainly due to the company's losing control over quality, quantity and price.

Contract farming is one specific form of vertical integration (Rehber, 1998). Yoshiko Saigenji (2010) mentioned that contract farming, one of vertical coordination types, encourages small producers to join in tea production. Vertical integration brings benefits for agriculture development in the context of heated competition. It is said that vertical integration within agriculture and the food industry influenced in market structure and competitiveness of agriculture (Grega, 2003). The general aim of vertical integration is creating a larger profit for the participants through linkages. Moreover, it also carries more market share for stakeholders involved in the chain and improves the quality of products (Meulenberg and Kool, 1994). With all above benefits, it can be seen that contract farming influences positively on value chain through mechanism vertical integration.In addition, contract farming brings benefits for firms and farmers (Prowse, 2012).

For firms, the opportunities provided by contract farming are clear and convincing, such as: increased reliability in supply quantity and quality (2) the off-loading of production risk onto farmers (3) greater control over the production process and crop attributes, to meet standards and credence factors; (4) reduced co-ordination costs, as a more regular and stable supply permits greater co-ordination with wider activities. In general, contract farming can increase profits from, and improve governance of, the value chain.

For farmers, contract farming also brings numerous opportunities for farms: access to a reliable market; guaranteed and stable pricing structures; and most importantly, access to credit, inputs, production and marketing services (seed, fertiliser, training, extension, transport, and even land preparation). On a wider note, contract farming can open doors to new markets for a farm's produce, stimulate technology and skill transfer (particularly for higher-risk crops, which resource-poor farmers might typically avoid), and it can support farmers in meeting vital sanitary and phyto-sanitary standards.

With above benefits for firms and farmers when joining in contract farming, they contribute to create higher total value added in the value chain, reduce risks happening along value chain, strengthen close linkage between firm and farmers, thus promoting value chain.

The study therefore aims at comparing value chains between contract farmers, worker farmers and non contract farmers chains in order to analyze the influence of contract farming on black tea value chain in Phu Tho province.

2. METHODOLOGY

2.1. Data Collection

2.1.1. Secondary data

Secondary data were collected from various sources: PTSO (Phu Tho Statistic Office); annual provincial reports of agricultural production; related studies, other scientific materials and the websites of related prestigious organizations.

2.1.2. Primary data

Primary data of the study were collected using a semi-structured questionnaire for the household survey, collectors and companies and structured questionnaires for the household survey. The selection of surveyed households was made using both stratified and random selection in Thanh Son and Doan Hung districts which are large tea cultivation areas and have different chains of black tea in Phu Tho province.

110 farmers were interviewed by survey questionnaires, of which there were 40 worker farmers, 30 contract farmers and 40 non contract farmers. In this study, contract farming includes 2 groups: worker farmers (nucleus estate model) and contract farmers (intermediary model).

Worker farmers: They are now allocated land for up to 30 years on the condition that they produce tea leaf based on company dictates.

Contract farmers: They have their own land but sell a portion or all output to Phu Ben company through Minh Tien Cooperative, Minh Tien commune, Doan Hung strict.

Non-contract farmers: They do not sign contract with company. They sell tea leaf to the open market, either to collectors or processors (Oanh et al., 2016)

In this study, we also surveyed 6 collectors in Dich Qua, Minh Tien and 6 companies in Yen Lap, Thanh Son, Thanh Ba, Doan Hung districts, including 2 large firms with tea cultivation areas - Phu Da joint venture company in Thanh Son district and Phu Ben 100% foreign owned company in Thanh Ba district, and 4 private enterprises in districts of Thanh Son, Yen Lap, and Thanh Ba. The survey contents were mainly tea processing, main markets, the difficulties and the policies of increasing the value of products.

In-depth interviews method was carried out for analyzing advantages and disadvantages of contract farming through discussing with managers of companies and farmers.

2.2. Data processing and analysis

Both quantitative and qualitative methods were used to conduct this survey. Regarding to quantitative method, descriptive statistics was used to describe the tea production among each farmer group. Comparative statistics was used to compare the value added between stakeholders, such as farmer, collector and company in the chain and between farmer groups in different chains.

Cost – benefit analysis was used in order to calculate some indicators: GO, VA and IC. The detailed function are as following the Figure 1.

$VA = GO - IC$

Gross output (GO) is the total value of production outputs.

$GO = P * Q$

P is the market price;

Q is the product quantity;

IC is the the intermediated cost, includes purchasing variable inputs (materials and services)

3. RESULTS AND DISCUSSION

3.1. Black tea value chain in Phu Tho province

There are 3 chains of black tea value chain in Phu Tho province in Figure 2.

First chain: Workers farmers sell total fresh tea output directly to Phu Da, Phu Ben company follow contract signed between worker farmers and these companies.

Second chain: Contract farmers sell a part or total fresh tea output to Minh Tien Cooperative that sells fresh tea to Phu Ben company following the contract signed between Cooperative and Phu Ben.

Third chain: Non contract farmers sell fresh tea to spot the market through the collectors.

In this study, 100% households sell fresh tea to the collectors because they are not near to the processors. Most processors try to export black tea by themselves to seek for higher profit, while others have to export tea through exporters.

3.2. Actors in the black tea value chain in Phu Tho province

3.2.1. The producers

Worker farmers: Farmers received land due to allocated land for up to 30 years from company with the condition that they produce tea leaf based on company requirement. They must strictly follow all the rules of the company in term of tea production process, the quality control rules, using materials, selling fresh tea.

Contract farmers: Farmers who sell a portion or total output of fresh tea to Minh Tien Cooperative whom signed contract with Phu Ben company. The contract along side the company will ensure farmers to consume their fresh tea products. The company also provides them with some technical assistance of technology for growing tea, good fertilizers and financial support by providing loan with preferential interest.

None-contract farmers: Farmers do not have cooperation/linkage with the tea processing company and do not sign contracts with any other actors along the tea chain. They produce and sell their fresh tea on the spot market through collectors (Oanh *et al.,* 2016).

Input		Output value (GO)
IC: seed, fertilizers, pesticide, machine rent, tools, etc.		
VA	Labor remuneration	
	Interest payment	
	Taxes	
	Land rent	
	Depreciation	
	Return to family labor (or net farming income)	

Figure 1. Cost return analysis

Source: Lebailly et al., 2000; Ton and Huyen, 2008

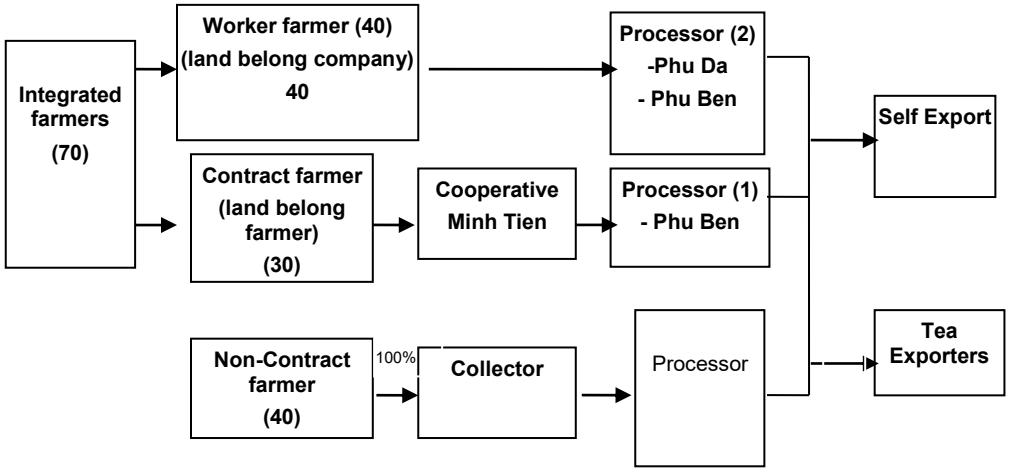

Figure2. Marketing channels of black tea in Phu Tho province

Table 1. Characteristics of 3 farmer groups

Criteria	Worker farmers	Contract farmers	Non-contract farmers
Tea income level	In general, tea income level of worker farmers is relatively good and similar among themselves cultivation	The tea income of contract farmers is less than that of worker farmers. Their tea income is partly from total income, around 20-25% .	The tea income of non-contract farmers is less than that of worker farmers, higher than that of contract farmers due to higher fresh tea price. Their tea income is also partly from total income, around 20-25% .
Areas of tea cultivation	Scale of areas of tea cultiavtion verage 5.000–7000 m^2/household;	Scale of areas of tea cultiavtion is not large and varied, from 1 sao (360 m^2) to 1 – 2 ha.	Scale of areas of tea cultiavtion is not large and varied, from about 1 sao (360 m^2) to 1 – 2 ha.
Ownership of tea areas	Tea areas, which belongs to the company, hires the farmers to manage and make an exploitation. The hiring period follows the Decree No. 135/2005/ND-CP.	Tea areas belonged to the farmers who cultivate and sell a portion or total fresh tea to Minh Tien Cooperative.	Tea areas belonged to the farmers who cultivate and sell tea by themselves.
Types of firms assistances	The company invests fertilizers and pesticides, and trains farmers to use cultivating techniques	The company invests fertilizers and pesticides, and trains members of the Cooperative to use cultivating techniques.	Being trained cultivating techniques by the local agricultural promoting agency; some households that owned new plants had been assisted young tea trees.
Social Policy	Participate in the social insurance system (paying insurance fees), as workers in the agricultural sector.	Do not have social insurance.	Do not have social insurance.
Choosing fresh tea'ssellers	Sell tea to the company following the regulation and certain levels assigned by the company.	Selling a portion or total tea freely to the Minh Tien Cooperative.	Selling tea freely to the spot market through the free negotiation rule.
Standards of fresh tea quality	The quality of the product is strictly managed, following the general standards which are higher than that of other farmers.	Fresh tea quality is not good as of worker farmers, due to undisciplined tea production process.	The quality of fresh tea products is varied based on the quality of inputs and the process of tea production.
Price of fresh tea	Price of fresh tea follows the regulation of the company, usually lower than that of non contract farmers (from 100–500 VND/kg).	Price of fresh tea is usually higher than that of worker farmers and lower than that of non contract farmers.	Price of fresh tea is usually highest among 3 farmer groups.
Consumption market	Stable consumption market.	Stable consumption market.	Unstable consumption market.

Table 2. Results of tea production of farmer groups in 2014 in Phu Tho province

Indicators	Unit	Worker farmer	Contract farmer	Non contract farmer
Gross Output	million VND/ha/year	83.999	65.059	70.715
IC (Intermediate Cost)	million VND/ha/year	31.034	26.258	26.437
1. Fertilizer	million VND/ha/year	19.395	13.696	14.102
2. Pesticide	million VND/ha/year	5.507	6.784	6.789
3. Other costs	million VND/ha/year	6.132	5.778	5.546
VA (Value Added)/ha	million VND/ha/year	52.965	38.801	44.278
Fresh tea price	VND/kg	3922	4041	4237
Productivity	Tons/ha	21.4	16.1	16.7
Tea land	ha	0.64	0.43	0.45
VA/ household/year	Million VND/ha/year	33.873	17.101	19.935
VA /1 ton black tea	Million VND/ 1 tons black tea	10.882	10.622	11.407

Source: Oanh et al., 2016

There is a huge difference between two groups: worker farmers vs. non-contract farmers. The worker farmers group gains a much higher average productivity -21.4 tons per ha – 1.28 times higher than non-contract farmers group with16.7 tons per ha. This difference depends on numerous factors, such as tea ages, tea breeds, land quality for tea cultivation, cultivating method and use of fertilizers (Table 2). These numbers have shown that there is potential for improving the tea productivity, thus increasing the added value for tea products if the tea cultivation areas are well invested based onproper technology processes.

When considering intermediate costs, fertilizers and pesticides account for the main part of around 70- 80%. Worker farmers are provided with high quality fertilizers and pesticides, and cultivate tea following the tea production process so that the quality of tea is good. Although the industrial tea price is lower than that of non-contract farmers, tea consumption of worker farmers is quite stable because of its dominating quality and assistance in output channel by big companies. On the other hand, non-contract farmers sell tea at a higher yet less stable price. For example, during 2014 - the time the research was conducted, the channel from non-contract farmers to retailers was fairly stable, however, in 2015, tea price reached a very low point since retailers also struggled with their product output (Tien Anh, 2015). Moreover, nowadays, most farmers harvest their tea using machines instead of manually harvesting fresh tea. This harvesting method requires a more rigorous and proper tea cultivation technology to prevent tea farms exhaustion, which can severely affect tea quality, tea price and consumption level. The investment cost for a new tea farm is estimated to be about 80-100 million VND/ha, including facility and labor cost. Two crucial factors when considering cost are labor cost while cultivating tea, and the potentially unfavorable effect of weather on tea growth. If taken care properly like industrial tea, a tea tree with a life cycle of 40-50 years can still yield a high level of product with high quality. Meanwhile, non-contract farmers' tea tress usually

have a much shorter life span, thus requiring rapid regrowth of tree and affect the profit of tea farmers. If accounted by VA/household per 1 year, VA/household/year of none contract farmers is 19.9 million VND/household/year and lower than its of worker farmers with 33.8 million VND/household/year whereas that of contract farmer is lowest with 17.1 million VND/household/year. Reasons for this are tea cultivation area and productivity of worker farmers is the highest, leading VA/household of worker farmers the highest.

3.2.2 Collectors

Tea collection stations is a basis establishment of the tea processing company that the company organizes at the tea cultivating area to buy tea leaves from its worker farmers. Each station is usually managed by one team in the tea materials area. Its task is to buy not only tea materials but also other operations such as mornitoring operations between the company and the worker farmers (receiving production materials, fertilizers and pesticides to redistribute to the tea farmers based on the general standardized levels of the company, organizing pumping pesticides to the tea plants…)

Fresh tea collectors collect fresh tea in the local and surrounding areas to sell to the company to process black tea to export. Collector soften have a lot of experience (10-20 years) in buying high quality tea. They usually have a strong financial background within the local area, with working capital of around 200 million VND at all time**.** Some large-scale collectors also buy trucks to transport fresh tea from households in remote areas to the processing company.

Collectors play an important role in the black tea processing chain. Most of tea materials are transported to the factories through the system of collectors. To make a basis for accounting the value added in the chain, the study focus on analyzing enough large-medium scale establishments with financial capacity and ability of transporting products to sell to the processing factories, not counting small collectors. The survey results showed that the buying tea fresh price of 2014 is about 4,200 VND/kg and selling price to the factories is about 4,500 VND/kg.

Collectors often collects around 500 – 1000 tons of fresh tea per year. Tea plants have 5 – 6 times of harvest per year and are collected at the season of harvest. Most harvest periods last 15 days, and each day farmers collect around 10 tons of fresh tea.

Collectors operate around 9 months per year with an average amount of 860 tons of fresh tea. However, many establishments have had 20 – 30 million VND indebted to the tea farmers - money that the tea farmers are paid in advance, and this is the way that the collecting establishment create stable input materials during the harvest season.

Large collectors use trucks to transport tea material. Small collectors use motorcycles, but the number of motorcycles used is fairly low. The collectors divide fresh tea into 2 types: young and old tea leaves. The fresh tea price of 2014 is around 3,800 – 4,400 VND based on the quality of fresh tea. The collectors usually sell to some tea processing companies. They usually choose countable companies which pay in whole and are not indebted because they have to pay immediately to the farmers. The companies transport fresh tea from the collectors or vice versa. The transportation fee per ton of fresh tea is

based on the distance between the collectors and the processing companies.

The buying price of fresh tea of the company is informed to the collectors before they buy tea. This price is determined based on the export price of tea that the company deals with the foreign partners. The price of collected fresh tea is determined based on the promulgated price of the company, the current situation in the tea market and costs that the collectors have to pay. Normally, the collectors estimate a margin of 2-3% compared to the price promulgated by the company to buy the fresh tea from the farmers.

Cooperative: Minh Tien Cooperative buy fresh tea from contract farmers to sell to Phu Ben company. Transportation fees per ton of fresh tea which received from Phu Ben is about 250000 VND/ 1 ton fresh tea.

3.2.3. Processors

There are 2 types of tea processing companies, of which the qualified companies are ussually the large-scale firm, heavilly invested in the technology chain, housing and plants... in orderto access the export markets due to the fact that the processed black tea is mostly for exportation.

However, there is a quite large amount of households which organize small-scale processing establishments that considerably affect the quality and reputation of the tea processing industry.

In general, the tea processors' raw materials in different companies are quite different considering types of ownership, investment level as well as business strategy of each company. For example, the Phu Ben limited company has around 80% oftea materials grown on its self-invested tea areas, meanwhile the Hung Ha limited company fully depends on the tea materials from non-contract farmers and certain collectors.

There are usually 2 type of exportation of black tea: direct export with the large-scale companies and indirect export with the smaller scale companies. However, to reduce the intermediate costs and to increase the value added, the processing companies often make effort to export directy – currently, it is estimated that around 70% black tea export is through the channel of direct export, and only the remaining 30% are exported indirectly.

3.3. Cost and benefit analysis of actors in the black tea value chain in phu tho

To calculate Value added per 1 ha, VA/ha of worker farmers appeared to be by far the highest with nearly 53 million VND which was followed by non-contract farmers and contract farmers. These numbers were over 44 million VND and nearly 39 million VND, respectively. It can be seen that due to the investment of companies in fertilizer, pesticide and strict management in tea production, worker farmers will gain the highest productivity, leading the highest VA/ha among 3 farmer groups. According to estimates, it is necessary to process 4,3 to 4,4 kg of fresh tea in order to have 1 kg of black tea. Therefore, the VA per ton of black tea products is 11.407 million VND for none contract farmers, 10.882 million VND for worker farmers, and 10.622 million VND for contract farmers - the lowest level.

Although the VA per ton black tea of worker farmers (10.882 million VND) and contract farmers (10.622 million VND) are lower than that of non- contract farmers (11.407 million VND), the total VA per ton black tea of non- contract farmers chain is lower than those of contract farmers chain and worker farmer chain, with levels of

20.201 million VND, 23.944million VNDand 26.126 million VND, respectively. Also, the dried tea of enterprises having material areas with high quality leads to the higher price. All of this are due to the advantage of worker farmers that their outputs are guaranteed and stable. Large processors have linkaged with foreign firms, such as Phu Da Tea Company have made joint ventures with Iraq and Phu Ben Tea Company have done similarly with one branch of the famous Indian tea company.

Distribution of the VA among actors per ton of black tea

In the 1st chain: The VA is mainly from company accounting for 58%, the remaining 42 % is from worker farmers. In the 2nd chain: The VA is mainly from company, accounting for 52%, the remaining shares are 45% from contract farmers and 3% from Cooperative. In the 3th chain: The VA is mainly from non-contract farmers, accounting for 57%, from companies of about 39% and from collectors of about 4%.

It can be seen that in the 3 th chain, % VA of non contract farmers is the highest. However, we have to appreciate another sides in the black tea value chain, not only economic benefit.

3.4. Influence Of Contract Farming On Black Tea Value Chain In Phu Tho Province, Vietnam

3.4.1. Influencing of contract farming on whole black tea value chain

a. Increasing the total value added (VA) of chains:

According to the results in Figure 2, total value added of the 1st chain (worker farmers) maximum, attain to 26.126 million VND per 1 ton black tea and total value added of the 2^{sd}chain (contract farmers) attains 23.944 million VND per 1 ton. Minimum is total value added of the 3th (non contract farmers). In the 1st chain, depend on making a contract with the company, fresh tea have higher quality because the supplies are adequate, fertilizers, pesticides quality and complying close up manufacturing process of increasing fresh tea in the company. In addition, companies with contract with farmers have modern technology of manufacturing process for black tea and higher skill level, contributing to producing black tea with high quality, and have the highest prices of black tea in the 1st chain.

In the 2nd chain, although qualification of fresh tea in this chain is not higher than that in the market because the company is only supplying pesticides for contract farmers, but not monitoring closely the manufacturing process of fresh tea of the farmers. However, Phu Ben company made a contract with the farmers for purchasing most of fresh tea to put in the manufacture process so that the black tea quality in this chain is still higher fresh tea quality in the market. Then, the VA of the 2nd chain is standing the second in figure 2. Generally, depending on making contract, the total VA of chains are increasing, and higher than VA of non contract farmers chain.

b. Increasing the close links between the actors in the chain:

In 1st chain, we can see clearly the closed relationship between farmers and processing companies. Farmers must follows all items of contract carefully, from receiving materials to the processes of cultivating and making harvest of tea. The companies has to supply materials in advance and buy all of outputs. That is a closed relationship between factors in the chain.

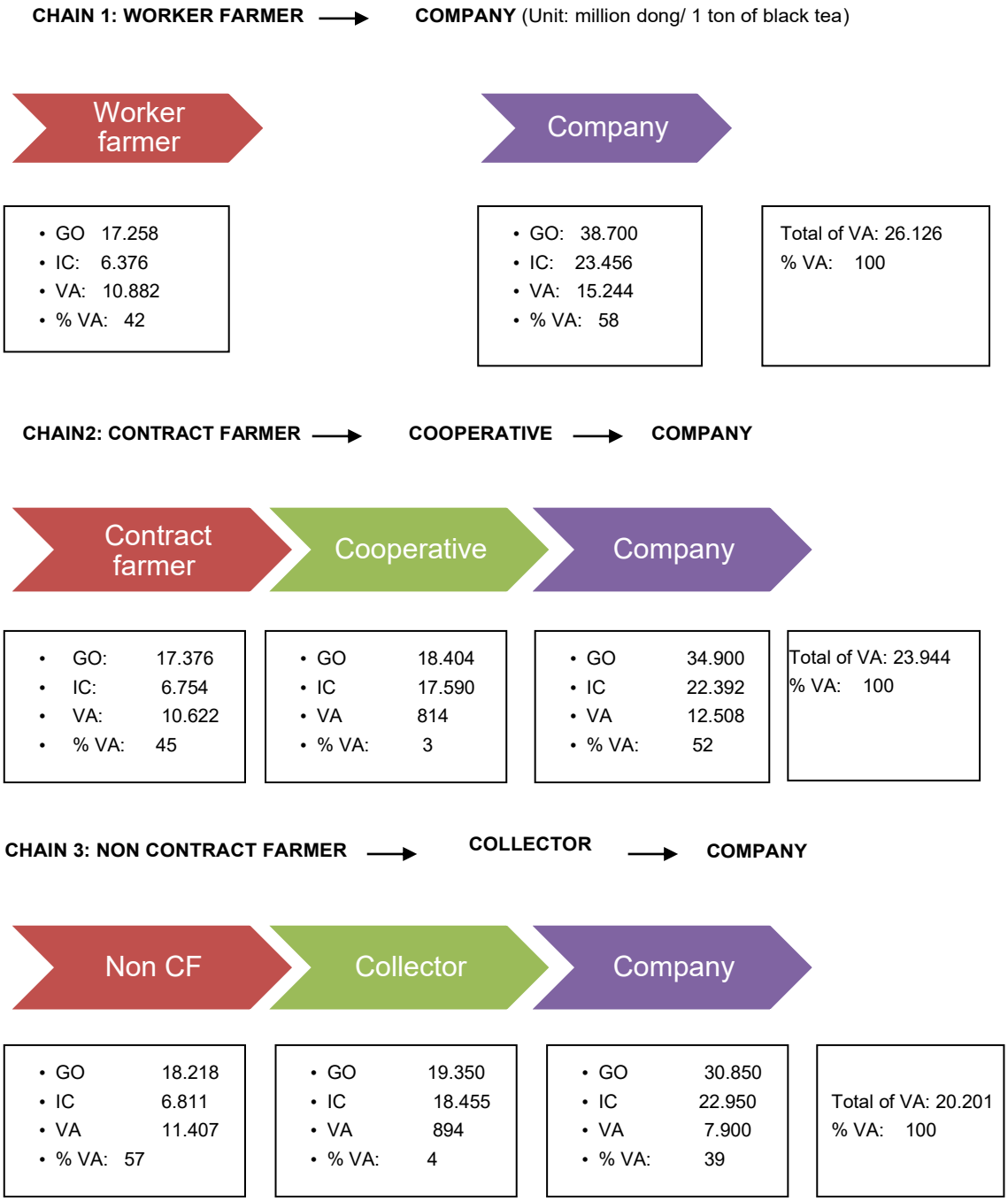

Figure 2. Cost and Benefit of actors in the black tea value chain in Phu Tho province

In the 2nd chain, the relationships between contract farmers and the cooperative and companies are quite sustainable. Contract farmers sell tea to the cooperatives according to oral agreements but quite strong because they are neighbors and even cousins with cooperative managers. Partly they believe

that cooperative managers are dynamic, experienced as longtime collector with reputation, partly because of reverence of knowing each other, such that despite fluctuations in the market price of tea, they sell most of tea to the cooperative. Also, because the cooperative provide fertilizers in advance, they also have certain attachments to the cooperative. However, contract farmers highly appreciated contract benefits of fertilizer provided in advance because fertilizer quality is guaranteed, affordable and comfortable to buy. This is important for the farmers in the context that they have usually to pay many items of family expenses such that there is often shortage of cash. The relation between Cooperative and company is quite close through selling contracts signed by two sides. Thus, in both above chains, linkages between actors is quite sustainable, contributing to strengthening the value chain.

3.4.2. Influencing of contract farming on actors in the black tea value chain:

a. Producers

- Inputs supply stability for producer

In the 1st and 2nd chains, worker farmer and contract farmer are both provided with high quality fertilizers in advance. For the worker farmers, they are also provided with pesticides in advance. It contributes to increasing productivity, fresh tea quality and ensuring preferred conditions of production for manufacturer (Oanh et al., 2016). If households are free, they can also purchase fertilizers, pesticides in advance in free market but materials quality is not ensured. In addition, purchasing in advance is also not convenient and easy, as worker farmers and contract farmers do.

- Ensuring the stability of the output market

The products of worker farmers and contract farmers are sold by contracts to large companies of Phu Da and Phu Ben. These are companies with vast experience in the market, large capital, modernized production technology, high-level of management and good reputation. They have often signed contracts with major and stable partners. Thus, the selling of products of households provided with contracts is more stable than that of non contract farmers.

- Create higher value added for worker farmers

Worker farmers have the highest tea land area with 0.64 ha due to land allotment from companies. Besides, due to supports from companies, including inputs with late payment, tea production technology advice, strict management, productivity of worker farmers is the highest. As a result, value added of worker farmers/ household/year is the highest, making them feel more securely in tea production (Table 2)

b. Processors:

Due to contract farming, processors have more stable materials zone that satisfy requirement of qualified product. With providing good input (fertilizer, pesticide, technology in tea production for worker farmers and contract farmers), companies receive fresh tea which are higher quality. Moreover, tea material source of these processors is always active because of close integration both sides in contract.

3.5. Limitations Of Worker Farmers And Contract Farmers Paticipating In Black Tea Value Chain in Phu Tho

3.5.1. Low fresh tea price

The price of fresh tea from worker farmers is usually lowest (Table 2). Sometimes, the gap is so high such that the worker farmers are not satisfied, with their efforts in comparison to the results received, leading to the situation that they sell the company's tea outside, illegally.

The price of fresh tea from contract farmers is ranked the second, lower than that of non- contract farmers and higher than that of worker farmers (Table 2). Contract farmers also complain about prices, which are usually lower than the market price, even though the difference is not much. However, this tea price is sometimes significantly lower than the market price. Then, contract farmers sell their tea outside, not to the Cooperative, leading to the situation that the cooperative managers find it very difficult to ensure the quantity of tea, as signed with company.

3.5.2. High quality requirements of fresh tea for worker farmers

The company requires workers to cut young tea leafs to make sure the black tea quality as the increasingly strict requirements of customers. This makes tea production volume of workers greatly decreased. Non contract farmers often wait longer, about 45-50 days to cut tea leafs, coercion long and weighing more. Thus, along with declining volumes of fresh tea, the tea price are also low so that worker farmers argued that they are more disadvantageous than non-contract farmers.

3.5.3. High water discount rate

Moreover, the company imposes the high water discount rate compared with the collectors in the spot market. Worker farmers complain about this because of the high water discount rate, their tea production volumes decline around 10-15%, higher than that of the outside market (In depth interview of worker farmers).This makes their tea production volume actually sold to the companies declining significantly. They look for companies with more transparent procurement system.

3.5.4. The high levels of social insurance and health insurance contributions

Although social insurance contribution is voluntary and worker farmers are entitled to health insurance, receiving pensions when retiring, the insurance cost is a financial burden to the worker farmers. As the average value added of worker household/year is 33.8 million VND (Table 2), the average insurance cost of worker household is 10 million VND annually (In depth interview of worker farmers). Hence, after paying insurance contribution, the remained annual household value added /year is only about 23 million VND, not much higher than the average value added of non- contract farmers (annually 19.9 million VND) (Table 2).

3.6. Discussion

First, under the participation on contract farming, the chains of worker and contract farmers were more competitive and more sustainable due to higher tea quality. Worker farmers receive supports from company with good fertilizer, pesticide and technology. Hence, their tea quality is higher than that of non contract farmers, therefore it is easier to sell. Besides, the closed linkage between worker, contract farmers and companies through contracts will be ensured the sustainability within these chains.

Second, farmers who have signed contract with companies recieving benefit actually from contract farming in terms of

stable outlet. Based on association with big company, they avoided risks from fluctuated tea market which was adverse problems in tea industry. The reason is that black tea was mainly served for exporting purpose (occupied 80%), leading to dependent on tea world market.

Final, the given results were mostly due to the influence of "contract farming". In the first chain, because of having contract with company, worker farmer were provided good inputs (fertilizer, pesticide, technology…), as a result, they achieved higher tea quality compared non contract farmers who did not receive support from company, using inputs from spot market with no commitment. Further more, in the second chain, because of linkage between cooperative and company, fresh tea outlet of cooperatives will be guaranteed to consume more stably than that of non contract farmers. As a result, farmers who selling fresh tea to cooperatives will be ensured outlet in comparison with non contract farmers.

4. CONCLUSIONS AND RECOMMENDATION

Based on value chain approach, the contract farming has crucial roles in the black tea value chain including creating more value added and building good relationship between actors. Thanks to contract farming, worker farmer chain and contract farmers chain have higher VA than non contract farmer chain. Moreover, the link between the actors of two these chains are more sustainable than that in non contract farmers chain. In addition, the input and output of two these chains are stable, creating favorable conditions for these households to produce comfortably, and ensuring a more stable source of income. All such factors help black tea chain of Phu Tho more sustainable and competitive, thus contributing to strengthen and develop the value chain of black tea in particular, and agricultural chain of Vietnam in general. This demonstrates that the important role of contract farming model for promoting black tea value chain is currently facing many challenges, such as small production, fragmentation, poor quality tea, limitation in preservation mode, unclear connection, and instable market (Xuan Hai, 2015).

However, the disadvantage of worker farmers and contract farmers when participating in that chain of black tea is that tea prices are lower than those in the free market. For worker farmers, the requirements for high-quality fresh tea, high water discount rates and high insurance cost from the companies make their real incomes fall considerably. This requires companies to offer appropriate solutions such as strengthening inspection and monitoring purchasing system to create a more transparent procurement system. The company must offer more reasonable prices for tea producers. Tea prices for these households should not be too different than the market price to compensate for the reduced volume of tea because of the above-mentioned requirements of the company. This may ensure a good income for tea producers, helping them to feel secure to promote production, sticking to the company, and consciously developing tea gardens which are also the assets of the company for the period of 40-60 years.

For non-contract farmer chain, farmers should invest in tea crops by shifting to new high yiead and quality varieties, using adequately and sufficiently high quality inputs (fertilizers and pesticides), and developing tea in a techinical way. These

investment would bring about high VA/ha for non-contract farmers as those received by worker farmers.

Households with small areas should be supported in the establishment of groups, cooperatives, and tea growing regions to create a tea area large enough to generate better conditions for them to coordinate with processors to consume tea under the marketing contract; and to access to market, science, technology and credit.

Processing companies need to gradually shift from wide to deep development, by which reducing outputs but increasing qualities in order to get higher export prices. Enterprises should focus on investing in high-quality material areas though contracting with farmers to provide them with materials, technical trainings and tea production control. Besides, enterprises should invest in technology to improve the quality of black tea, increase VA of the value chains and stabilize the markets as the current tendency is the higher requirements of consumers in food safety for agricultural products.

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DETERMINTANTS OF RICE PRODUCTION IN REMOTE AREAS OF VIETNAM: A CASE STUDY IN LAO CAI PROVINCE

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ABSTRACT

Seng Cu rice, a special product in the Northwestern region of Vietnam, provides high economic value and it is one of the main crops for poverty reduction and rural development. Lao Cai, a moutainous province, has various natural advantages for growing Seng Cu rice such as soil, climate, water resource and so on. However, Seng Cu rice production are facing with several challenges including: (i) low investment; (ii) poor farming practices; (iii) weak linkages between farmers and input/output suppliers; (iv) lack of supporting services regarding to agricultural extension and technology; (v) poor economic infrastructure. The main reasons mentioned above caused to low productivity produced at 60% of the potential, low income and high poverty rate at 50%, especially in upland communes the province. Besides, smallholder farmers have high levels of food insecurity and unsustainable livelihood. This paper aims to review the reasons why smallholder farmers in the region fail to plant Seng Cu rice. Then, various recommendations will be proposed to increase productivity and price for smallholders to improve incomes and living standard for the local farmers.

Keywords: Agricultural productivity, Lao Cai, Seng Cu rice, rice production, remote area.

RICE AND SHRIMP FARMING IN THE XUAN THUY NATIONAL PARK: SUSTAINABLE AND UNSUSTAINABLE PRACTICES

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ABSTRACT

Agricultural and aquaculture patterns in Xuan Thuy National Park mainly consists of rice planting and shrimp aquaculture (intensive and semi-intensive). Currently, there are about 11,899 rice households covering 1,873.07 ha, 215 semi-intensive shrimp households occupying 1,730.7 ha and 40 intensive households amounting to 90 ha in the Ramsar site. The local farmers produce 14,411 ton of rice, 450 ton of intensive shrimp and 516 tonof semi-intensive shrimp. Thus, rice and shrimp contribute significantly to local food basket and economy but it is overshadowed by environmental concerns. This paper aims to focus on reviews how farmers practice rice and shrimp at the international important location. It finds some inappropriate management practices regarding to monoculture, use of fertilizer, pesticide, antibiotic and other drugs, and water effluent systems. In addition, almost of respondents aware of water discharge from rice areas after spraying pesticide is the most serious constraint for their shrimp and the effluent from shrimp ponds make pollution for the environment. Lesson learned from review are considered in the context of recommendations to apply effective management measures to mitigate the unsustainable practices.

Keywords: Environment, management, practice, ramsar site, rice planting, shrimp aquaculture, XuanThuy National Park.

THE LOCAL STATE IN THE NORTHWEST HIGHLANDS. LEARNING FROM COLLECTIVE FIELD RESEARCH IN YEN CHAU (SON LA PROVINCE)

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ABSTRACT

From 2011 to 2014, I supervised a field seminar for MA students in the district of Yen Chau. Basing on this experience, I will elaborate on the main research results related to the social changes in that mountainous rural area of Vietnam, and consider briefly some pedagogical issues. About sixteen students of the CUD-VNUA IMARES programme (MA on rural economy and sociology) were yearly involved: they formed small teams and had to investigate a topic linked to rural sociology, using qualitative methods (interviews, observations). Time was limited, with less than one week on the spot, but the research was intensive and turned out to be quite productive. The researches related to four broad categories of topics: infrastructures, land and agriculture; household economy; health and education; ethnicity and culture. They were complementary and can be broached together when considered in relation to the successive policies implemented by the local administration in the area. Beginning with the migration of Kinh villagers in the 1960s (itself the consequence of a national policy), the ways of living of the local population, whose majority are Thái Đen, changed progressively. The subsequent process was one of progressive inclusion into the political economy of Vietnam, in relation to land regulation, work, communication networks, cash-crops, policies against poverty, health and education services, etc. Policies also affected, sometimes directly, sometimes indirectly, "cultural" domains like marriage, language and rituals. Yen Chau eventually turned out to be a good case study for grasping the current transformations of the Vietnamese highlands and their insertion into the national space. Our research showed how policies were instrumental in transforming the social fabric of the region, but also how these policies were practically adopted and adapted (or not) by the local population, whose reaction was never passive. Besides, this pedagogical experience turned out to be useful in making the students more reflexive on the way "data" are produced, and to question the sometimes-abusive use of questionnaires in non-appropriate contexts, as well as the limits of a "problem-driven" approach.

Keywords: Collective research, local State, policies, qualitative methodology, rural changes.

REMITTANCE USING OF MIGRANT'S HOUSEHOLD: A CASE STUDY IN MY LOC COMMUNE, CAN LOC DISTRICT, HA TINH PROVINCE, VIETNAM

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ABSTRACT

Cross-border migration has been on the rise since the 1990s in Vietnam and there is an amount of research has been conducted on the topic of migration and remittances over the last few years. Early studies assumed that migrants leave their countries, settle in a new country, start integrating in their new society, and abandon their ties with their country of origin. Today, however, globalization makes it possible for migrants to remain connected with their native countries while residing abroad through remittance. The objective this research is to review evidence on describing the state of knowledge regarding flows of people and migrant remittances back to their home countries and to analyze the trends and various other aspects of workers' migration and remittances in Ha Tinh province, Vietnam. It further discusses the micro and macro-economic impacts of remittances. While most remittance transfers have been used by migrant-sending households for consumption, there is evidence to show that these transfers have helped reduce poverty in the selected community. The result presented in this paper indicates that these remittances may have significant effects on other macro-economic variables as well.

Keywords: Hatinh province, international remittance, remittance using, Vietnam, women international migration.