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BIOLOGICAL AND IMMUNOLOGICAL EFFECTS OF BOVINE COLOSTRUM ON THE NEWLY-WEANED PIGLET

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Résumé

Le sevrage est une des périodes les plus critiques en production porcine à cause d'une plus forte sensibilité des animaux aux problèmes intestinaux et aux infections suite aux stress psychologique, social, environnemental et alimentaire interférant avec le développement du tube digestif. Cette période délicate a été maîtrisée durant des décennies par l'incorporation d'antibiotiques dans l'aliment. Cependant, depuis le 1er Janvier 2006, cette pratique est totalement interdite dans l'Union Européenne. Dans ce contexte, de nombreuses alternatives sont étudiées. Nous avons choisi le colostrum bovin pour sa richesse en éléments essentiels et surtout en peptides bioactifs connus pour leurs propriétés promotrices de croissance et antimicrobiennes chez le bovin mais également chez d'autres espèces (poulet, porc, homme). Il a également été retenu pour sa grande disponibilité (banque de colostrum, CER, Marloie, Belgique).

L'objectif de cette thèse est d'évaluer l'intérêt d'utiliser du colostrum bovin dans l'alimentation du porcelet au sevrage et d'étudier son mécanisme d'action.

La thèse se compose de deux parties :

Dans la première partie, les effets d'une supplémentation en colostrum bovin sur les performances, l'ingestion et certains paramètres physiologiques ont été étudiés au cours de deux expériences.

Au cours d'une première expérience, un aliment supplémenté quotidiennement avec 0, 1 ou 5 g de colostrum bovin dégraissé a été testé. Au niveau immunitaire, nos mesures ont montré une influence du colostrum bovin sur le développement de la réponse en IgA en induisant une réponse de type Th2 au niveau de la plaque de Peyer iléale. Dans le tube digestif, aucun effet n'a été observé sur la morphologie de la paroi intestinale, mais une immunisation locale anti-colostrale a été mise en évidence.

Une seconde expérience a démontré l'efficacité d'une supplémentation de 2 % de sérum de colostrum dans l'aliment pour réduire la perte de poids et la sous-alimentation provoquées par le sevrage. Les paramètres sanguins ont montré une augmentation des IgA, confirmant nos résultats précédents, et un taux en IGF-I plus important chez les porcelets recevant le colostrum. Par contre, aucun effet n'a été observé sur la population d'*Escherichia coli* fécale.

Dans la seconde partie de la thèse, différents moyens de réduire le coût de la supplémentation en colostrum bovin ont été étudiés. Il a été démontré qu'il était possible de maintenir l'efficacité du colostrum tout en réduisant la dose (de 2 % à 1 %) et la durée de supplémentation (de 28 à 10 jours) et en remplaçant le sérum de colostrum par du colostrum dégraissé, un produit 50 % moins cher.

Boudry Christelle (2009). Biological and immunological effects of bovine colostrum on the newly-weaned piglet (thèse de doctorat). Gembloux, Belgium: Gembloux Agricultural University, 141 p., 21 tabl., 7 fig.

Summary

Weaning is one of the most critical periods in pig production due to a high susceptibility to gut disorders and infections induced by psychological, social, environmental and dietary stresses interfering with gut development and adaptation. This period was managed for decades by incorporating antibiotics in the diet. However, the European Union implemented a full ban on in-feed antibiotics since 1 January 2006. In this context, many alternatives are studied. We chose to study bovine colostrum for its richness in essential nutrients but also in bioactive peptides known for their growth promoting and antimicrobial properties in the calf but also in other species (poultry, pig, human). It was also selected for its high disponibility (Banque de colostrum, CER, Marloie, Belgium).

The objective of this thesis is to investigate the potential of the use of bovine colostrum in the newly-weaned piglet diet and its mechanism of action.

This thesis is composed of two parts:

In the first part, the effects of bovine colostrum on growth performances, feed intake and physiological parameters were studied in two experiments.

In the first experiment, 24 newly weaned piglets were fed daily a diet supplemented with 0, 1 or 5 g of defatted bovine colostrum. Our measures on the immune system showed that bovine colostrum could influence the development of the IgA response by potentiating a Th2 response in the ileal Peyer patch. In the digestive tract, no effects were shown on the morphology of the intestinal wall but a local anti-colostral immunisation was observed.

In a second experiment, we demonstrated the efficiency of a 2 % bovine colostrum whey supplementation in weaning piglet diet to reduce the post-weaning growth check and undernutrition. The blood parameters showed a systemic IgA response, confirming previous results, and a higher IGF-I level in the colostrum-fed piglets the first week post-weaning. No effects on the faecal *Escherichia coli* population were recorded.

In the second part of this thesis, different ways to make the use of bovine colostrum more cost-effective for pig production were studied. It was shown that it was possible to maintain the same efficiency while reducing the dose of supplementation from 2 to 1 % and the period of administration from 28 to 10 days and replacing bovine colostrum whey by defatted bovine colostrum, a product 50 % less expensive to produce.

I dedicate this thesis in memory of my Promoter Prof André Buldgen. He was one of the instigators of this work. His determination to collaborate with other researchers, his thirst of knowledge and his optimism were the driving forces of this work. He participated to each step of this thesis, the elaboration of the studies, the writing and correction of the articles; he even participated actively to the reception of our first great group of piglets. For this all, I thank you, Dear Professor, with all my Heart.

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ABBREVIATIONS

ADFI: average daily feed intake

ADG: average daily gain

BW: body weight

EGF: epidermal growth factor

FCR: feed conversion ratio

FITC: fluorescein isothiocyanate

GALT: gut associated lymphoid tissue

Ig: immunoglobulin

IGF: insulin-like growth factor

IGFBP: IGF binding protein

IL: interleukine

iPP: ileal Peyer's patch

LPS: lipopolysaccharide

ME: metabolisable energy

MLN: menseteric lymph node

PE: phycoerythrin

PW: post-weaning

SI: stimulation index

T_3: 5.3'-triiodothyronine

T_4: thyroxine

TGF: transforming growth factor.

INTRODUCTION

INTRODUCTION

The objective of this thesis is to investigate the potential of the use of bovine colostrum in the newly-weaned piglet diet and its mechanism of action.

Weaning is one of the most critical periods in pig production due to a high susceptibility to gut disorders and infections induced by psychological, social, environmental and dietary stresses interfering with gut development and adaptation. This period was managed for decades by incorporating antibiotics in the diet. However, the European Union implemented a full ban on in-feed antibiotics since 1 January 2006. In this context, many alternatives are studied. We chose to study bovine colostrum for its richness in essential nutrients but also in bioactive peptides known for their growth promoting and antimicrobial properties in the calf but also in other species (poultry, pig, human). It was also selected for its high disponibility (Banque de colostrum, CER, Marloie, Belgium).

This work was subsidized by the "Direction générale des Technologies de la Recherche et de l'Energie" (DGTRE) of the Walloon Region Ministry (Namur, Belgium) (Research project "Valorisation du colostrum bovin", VACOBO, 2002-2005) and by the "Direction générale de l'Agriculture" (DGA) of the Walloon Region Ministry (Research project "Valorisation du colostrum bovin en production porcine comme alternative aux additifs alimentaires antibiotiques", 2002-2008).
The "Banque de Colostrum" from the Animal Immunology Department of the "Centre d'Economie Rurale" (Marloie, Belgium) was associated to both projects to collect and prepare the bovine colostrum fractions needed for the research.

The interest of the use of bovine colostrum whey in weaned piglet diet to reduce the "growth check" post-weaning was first highlighted. Then we investigated the mechanism of action of the colostrum on the digestive and immune system of the piglets in relation to its richness in growth factors, immuno-modulatory components and antimicrobial factors. Finally, considering the high price of the bovine colostrum powders used in our studies, we tested different ways to reduce the price of its use (dose and duration of administration, other fractions less expensive).

This manuscript is a compilation of published and submitted articles and is structured as follows: after a review of the literature on the use of bovine colostrum as a natural growth factor in the newly weaned piglet (Chapter I, review), the research strategy followed in this thesis is described (Chapter II). The third chapter (Chapter III) presents two studies on the effects of bovine colostrum on the growth performance, the faecal microflora and physiological parameters (articles 1 and 2) of newly weaned piglets. Two studies carried out to reduce the costs related to the use of bovine colostrum in the weaning diet are described in the fourth chapter (Chapter IV, article 3). Finally, a general conclusion and future prospects are drawn (Chapter V).

CHAPTER I

REVIEW OF THE LITERATURE

REVIEW OF THE LITERATURE

BOVINE COLOSTRUM AS A NATURAL GROWTH PROMOTER FOR NEWLY WEANED PIGLETS: A REVIEW

The aim of this review is to present the potential of the supplementation with bovine colostrum in the piglet-weaner diet.

In the first part of this review, the consequences of weaning on the piglet are described: the main consequence of weaning is a critical period of underfeeding of which results in the so-called "weaning growth check". Near this reduction in feed intake and growth performance, the gastro-intestinal tract is also negatively affected (morphological, digestive and absorptive capacity, microflora and immunological modifications) and metabolic and endocrinal changes are related mainly during the first week post-weaning.

The second part is dedicated to the bovine colostrum, with a description of the actions of to its main bioactive peptides. They may be classified in two classes: the growth factors, which promote the growth and development of the newborn, and the antimicrobial factors, which provide passive immunity and protects against infections. The growth promoters are mainly represented by IGF-I and II and their binding proteins, the epidermal growth factor (EGF) and the transforming growth factor βeta (TGF-β). The antimicrobial factors group contains principally the lactoferrin, the lactoperoxidase, the lysozyme, the immunoglobulins and the cytokines.

In the third and final part of this review, the reported effects of bovine specific components or colostrum fractions on the growth performance and on the structure and function of the gastro-intestinal tract of piglets in the early postweaning period are presented. It appears clearly from the collected informations that bovine colostrum supplementation improves the growth performance and the sanitary status of piglets during the early postweaning period. But they show also the lack of information about the mechanism of action of bovine colostrum.

BOVINE COLOSTRUM AS A NATURAL GROWTH PROMOTER FOR NEWLY WEANED PIGLETS: A REVIEW

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Running head: Bovine colostrum for newly weaned piglets

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Abstract

The aim of this review is to present the potential of bovine colostrum as growth promoter in piglet-weaner diet. The consequences of weaning on the growth performance, on the gastro-intestinal tract and on the metabolic and endocrine systems of the piglet are described in the first part of this review. The second part is dedicated to the bovine colostrum, with a description of the actions due to its main growth promoters and antimicrobial factors. Finally, the reported effects of bovine specific components or colostrum fractions on the growth performance and on the structure and function of the gastro-intestinal tract of piglets in the early postweaning period are presented. They show clearly the potential of bovine colostrum to reduce the growth-check related to the weaning of the piglet.

Keywords: weaned pig, bovine colostrum, growth promoter, antimicrobial factor

Abbreviations: ADFI: average daily feed intake; ADG: average daily gain; BW: body weight; EGF: epidermal growth factor; FCR: feed conversion ratio; GALT: gut associated lymphoid tissue; Ig: immunoglobulin; IGF: insulin-like growth factor; IGFBP: IGF binding protein; IL: interleukine; LPS: lipopolysaccharide; ME: metabolisable energy; PW: post-weaning; T_3: 5.3'-triiodothyronine; T_4: thyroxine; TGF: transforming growth factor.

1. Introduction

Weaning can be regarded as one of the most critical periods in the modern-day pork production cycle. In addition to mother-young separation, weaning involves abrupt and profound modifications of the environment, feeding habits and social interactions when litters of piglets are mixed. These changes contribute to the post-weaning (PW) "growth check", intimately associated with a range of intestinal and immunological alterations (Pluske *et al.*, 1997).

Over the last decades, antibiotic growth promoters have been used in weaner diets to reduce the production penalty associated to weaning. However, increased bacterial resistance to antibiotics led the European Union to implement a full ban on in-feed antibiotics from January 2006. Efficient alternatives, therefore, have to be found to conform to this policy change. One possible alternative could be bovine colostrum powder.

Bovine colostrum is a commercially available co-product of the dairy industry. More than a source of nutrients, colostrum also contains several biologically active molecules which are essential for specific functions (Pakkanen and Aalto, 1997). The most important bioactive components in colostrum include growth and antimicrobial factors. Growth factors promote the growth and development of the newborn, while antimicrobial factors provide passive immunity and protect against infections during the first weeks of life. Growth factors include insulin-like growth factors-I and -II (IGF-I and IGF-II), transforming growth factor-β1 and -β2 (TGF-β1 and TGF-β2) and epidermal growth factor (EGF). Antimicrobial factors include lactoferrin, lysozyme, lactoperoxidase, immunoglobulins (Igs) and cytokines (IL-1β, IL-6, TNF-α, IFN-γ and IL-1ra).

The aim of this review is to present the potential of bovine colostrum as growth promoter in piglet-weaner diet. The review is divided into three sections. A first section describes the consequences of weaning on the growth performance, on the *gastro-intestinal* tract and on the metabolic and endocrine systems of the piglet. A second section is dedicated to the bovine colostrum, with a description of the actions due to its

main growth promoters and antimicrobial factors. A third section presents the reported effects of bovine specific components or bovine fractions on the growth performance and on the structure and function of the *gastro-intestinal* tract of piglets in the early PW period.

2. Piglet weaning

In natural or semi-natural conditions weaning is a progressive process taking place around 12 to 17 weeks of age in pig (Stolba and Wood-Gush, 1989 ; Boe, 1991). In modern pig husbandry, weaning occurs abruptly at the age of 3 to 4 weeks (Mormède and Hay, 2003), inducing numerous stressors to piglets. All these factors contribute to a range of intestinal and immunological alterations.

2.1. Consequences of weaning on growth

Behavioural studies reported by Brooks (1999) indicate that, although the majority of pigs starts to eat solid feed within 5 h after weaning, some of them take up to 54 h before eating their first meal. Thereafter, metabolisable energy (ME) intake gradually increases at a rate of 100-120 kJ ME.$kg^{-0,75}$ per day. Regardless of the age of weaning, the level of ME intake attained at the end of the 1^{st} PW week ranges between 700 and 800 kJ.$kg^{-0,75}$, which accounts for 60-70 % of the pre-weaning milk ME intake. This reduction of ME intake is due to the transition from milk to a less digestible solid feed, resulting in a critical period of underfeeding (Le Dividich and Sève, 2000). This abrupt reduction in voluntary feed intake immediately PW results in the so-called "weaning growth check" and its severity has a major impact on subsequent performance (Tockach *et al.*, 1992 ; Azain, 1993). On the first day PW, piglets loose from 100 to 250 g of body weight (BW) (Le Dividich and Sève, 2000). This loss of BW is dependent of the age of weaning. Carroll *et al.* (1998) measured a reduction of average daily BW gain (ADG) of 0.09 kg.d^{-1} for piglets weaned at 2 weeks of age while the reduction of the ADG for piglets weaned at 3 weeks was of 0.06 kg.d^{-1}. Growth rate subsequently returned to pre-weaning levels within 9 and 6 days for the piglets weaned at 2 and 3 weeks of age, respectively.

2.2. Gastro-intestinal modifications induced by weaning

2.2.1. Morphological changes

The small intestine and its mucosa lose 20-30 % of their relative weight during the first 2 days PW (Lallès *et al.*, 2004). This reduction is associated with a villous atrophy followed by a crypt hyperplasia described by a lot of authors (figure 1) (see Pluske *et al.*, 1997 for a review). These changes are more conspicuous when weaning occurs earlier at 14 days rather than later at 28 days of age. Cera *et al.* (1988) reported additionally to those changes, a reduction in the length of microvilli 3 to 7 days after weaning. Villous atrophy at weaning is caused by an increased rate of cell loss which induces an increase in crypt-cell production and, hence, an increase in crypt depth. As a result of these changes in villous height and crypt depth after weaning, the villous height:crypt depth ratio in weaned pig is markedly reduced compared to unweaned animals. These structural changes are variable along the small intestine. Hampson (1986) reported that the change in villous height was greater at the proximal small intestine in piglets weaned on day 21, but the change in crypt depth was greatest at the distal small intestine. Gu *et al.* (2002) confirmed a greater change in duodenum morphology compared to the other parts of the small intestine.

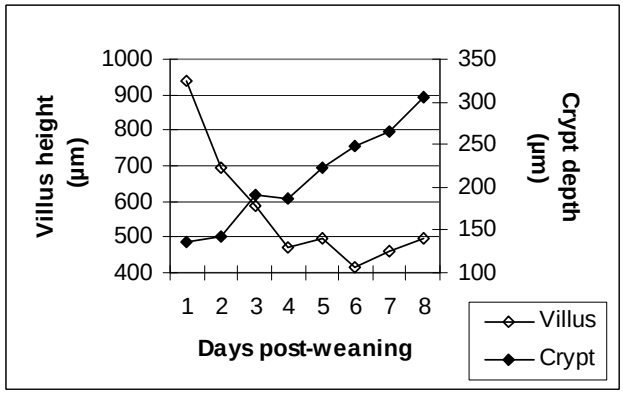

Figure 1. Villus height and crypt depth (µm) evolution at a site of 25 % along the small intestine in pigs weaned at 21 days (adapted from Hampson, 1986).

Associated with the reduction in villous height and the increase in crypt depth, the morphology of the villi also changes from long finger-shaped before weaning to leaf- or tongue-like structures after weaning (Cera *et al.*, 1988 ; Wiese *et al.*, 2003) (Figure 2).

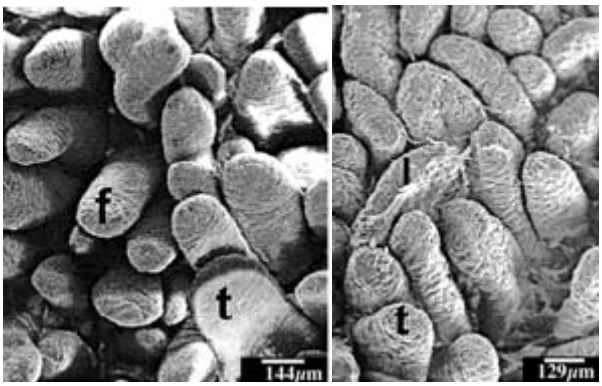

Figure 2. Duodenal villi morphology in pigs: finger-shaped (f), tongue-shape (t) and leaf-like (l) villi (adapted from Wiese *et al.*, 2003).

2.2.2. Digestive and absorptive capacity modifications

- *Enzymatic activity*

Concomitant to the structural changes, there are marked alterations in intestinal functions following weaning. According to Gu *et al.* (2002), the villous atrophy and the crypt hyperplasia reduce the number of mature enterocytes and lead to a reduction in the activity of the brush-border enzymes, which is related to the limited digestive and absorptive area or specific digestive and absorptive capacity of the small intestine, respectively. Hampson and Kidder (1986) and Pié *et al.* (2004) reported rapid reductions in the specific activities of lactase and sucrase during the first 4-5 days after weaning, with a greater loss in lactase than in sucrase activity, probably due to the more apical distribution of lactase activity along the villous (Tsuboi *et al.*, 1981 and 1985, cited by Pluske *et al.*, 1997). Miller *et al.* (1986) reported that the specific activities of sucrase, lactase and isomaltase fell by at least 50 % of during the first 5 days after weaning in pigs weaned at 28 or 42 days of age. On the other hand, the activities of maltase and glucoamylase doubled from the day 3 to the day 7 PW (**Figure 3**) (Kelly *et al.*, 1991). Increases in these polysaccharidases are likely the result of substrate induction.

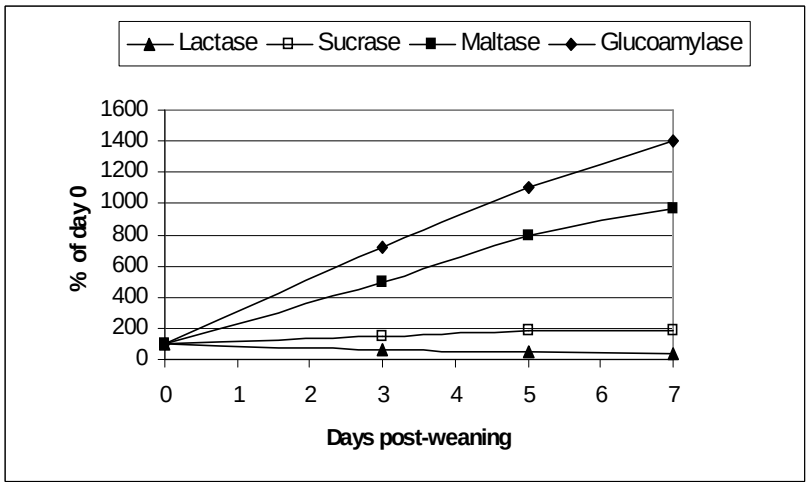

Figure 3. Evolution of lactase, sucrase, maltase and glucoamylase (µmol.min^{-1}.g of mucosa^{-1}) activities of piglets after weaning (% of activity measured the day of weaning) (adapted from Kelly *et al.*, 1991).

- *Absorptive activity*

In several studies, the decrease in villous height, the increase in crypt depth, and the loss of digestive enzyme activity after weaning, coincided with a reduced ability in intestinal absorption of sugar (D-xylose: Hampson and Kidder, 1986), amino acids (alanine: Miller *et al.*, 1986) and glucose and electrolytes (Nabuurs *et al.*, 1994). Nevertheless, some other studies failed to detect a significant reduction in the ability of sugar absorption (Kelly *et al.*, 1991).

2.2.3. Microflora modifications

Whilst the piglet is suckling, the dominant bacteria within the stomach and small intestine tend to be *Lactobacilli* and *Streptococci*, which are well adapted to utilise substrate from the milk diet. Following weaning, the period of starvation and then the consumption of the new solid diet result in altered availability of specific microbial substrate all along the digestive tract. The intestinal structural and functional changes may also be responsible for modifications to the mass, composition and complexity of the intestinal microflora, leaving the pig more susceptible to overgrowth with potentially disease-causing pathogenic bacteria (Hopwood and Hampson, 2003). But these modifications may also be responsible for structural and functional changes. For example, Mroz *et al.* (2003) showed that enteric infections after weaning further depress intestinal enzyme activities.

Jensen (1998) quantified changes in bacterial populations that occur in the small and large intestine of piglets weaned at 28 d of age. In the small intestine, the previously predominant *Lactobacilli* decreased in number during the first week after weaning, whilst the total number of bacteria and the proportion of coliforms, *Escherichia coli* in particular, increased. Following this period of perturbation of the intestinal microflora, it subsequently re-stabilises. Franklin *et al.* (2002) observed also an effect of the age of weaning of the piglets on the microflora modifications, younger piglets having a higher variation of their microflora than older (17 d *vs.* 24 d of age).

2.2.4. Immunological modifications

The intestinal epithelium provides an extensive and complex interface between the piglet's immune system and its environment, which must function simultaneously to absorb digested nutrients and provide a barrier against a vast array of ingested antigens. In addition to its barrier function, the epithelium also functions in surveillance. It can signal the onset of the host innate and acquired immune response through the production of cytokines and chemokines that are crucial for the recruitment and activation of neutrophils, macrophages, T and B cells, and dendritic cells (King *et al.*, 2003 ; Pié *et al.*, 2004).

- *Immune cells*

McCracken *et al.* (1999) observed that piglets weaned at 21 days of age had an increase in jejunal lamina propria $CD4^+$ and $CD8^+$ T lymphocytes within 2 and 7 days, respectively, after weaning. They reported also an increased expression of the active form of the matrix metalloproteinase stromelysin in jejunal explants during the initial 7 days after weaning and a decrease in jejunal expression of MHC class I and II mRNA. Vega-Lopez *et al.* (1995) measured, 4 days after weaning at 21 days of age, an increase in the $CD2^+$ and macrophage cells population in proximal small intestinal villi and only in the $CD2^+$ cells population in crypts, but no changes were observed in cells populating the distal small intestine. Similar results were reported by Solano-Aguilar *et al.* (2001), who observed gradual changes in $CD4^+$ and $CD8^+$ T cells, monocytes, granulocytes and macrophages in the month after weaning.

- *Cytokines*

The first observations on cytokine responses to weaning were reported by McCracken *et al.* (1995). They observed a transient increase of plasma IL-1 during the first 2 days PW.

More recently, with the development of the RT-PCR analysis, it was demonstrated that weaning in piglets is associated with an early regulation of inflammatory cytokines in the gut. Pié *et al.* (2004) reported an intestinal up-regulation of the pro-inflammatory cytokines IL-1β, IL-6 and TNF-α during the first 2 days after weaning. While between day 2 and day 8 PW, gene expression returned to pre-weaning levels, except for TNF-α in the colon. This early gene up-regulation of pro-inflammatory cytokines probably contributes to early functional disorders favouring diarrhoea (Lallès *et al.*, 2004).

2.3. Metabolic changes around weaning

Due to the weaning stressors and the low feed intake, newly weaned piglets are often in negative energy balance until 3 to 5 days PW (Le Dividich and Sève, 2000). However, the nitrogen balance remains positive (Bruininx *et al.*, 2002). It follows that the energy required for maintenance, physical activity and protein deposition implies necessary a loss of body fat. The body fat catabolism conduce to a transient increase in plasma free fatty acids concentrations, whereas lipogenesis is marginal (Fenton *et al.*, 1985). This catabolism continues until an ADG of 200g.d^{-1} is reached and it takes 3 to 6 weeks until the initial body fat content is recovered, depending on body weight and age at weaning. This decrease in body fat induces a reduced body thermal insulation which, associated to low feed intake, results in a transient increase in the lower critical temperature of the piglets from 22-23°C at weaning to 26-28°C during the first week PW (Le Dividich and Herpin, 1994).

2.4. Effects of weaning on the hormonal status

The onset of weaning results in some quite profound hormonal changes, although it is difficult to separate causes and effects. Many of the changes are in response to the social and nutritional stress associated to weaning, but there are also overlying developmental changes that likely occur independently of the weaning process (Dunshea, 2003).

2.4.1. Somatotropic hormones

Weaning results in an increase in the level of blood GH and a decrease of blood IGF-I concentrations (Carroll *et al.*, 1998 ; Matteri *et al.*, 2000 ; White *et al.*, 1991). These observations may be related to the transient underfeeding period at weaning as fasting for 36 h resulted in the same response of the somatotropic axis (Kasser *et al.*, 1981). Moreover, Carroll *et al.* (1998) showed that circulating IGF-I did not recover to pre-weaning value until 7 to 10 days PW, a period roughly corresponding with the time required by the piglets to attain their pre-weaning ME intake.

2.4.2. Hypothalamic-pituitary axis hormones

Regardless of the age of weaning, a transient increase in plasma cortical concentration and in urinary cortisol excretion is observed during the 2 first days PW (Carroll *et al.*, 1998 ; Colson *et al.*, 2006). This increased cortisol secretion could reflect both the weaning stress and stimulation of gluconeogenesis associated with the low PW feed intake (Le Dividich and Sève, 2000).

The actual role of the catecholamine system in the regulation of the weaned pig metabolism is still unclear Colson *et al.* (2006) and Hay *et al.* (2001) studied the effect of weaning on catecholamine production and observed decreased levels of urinary adrenaline and noradrenaline. Hay *et al.* (2001) interpreted this as a consequence of food intake deficit. However, according to Dunshea (2003), underfeeding would lead to an increase in circulating catecholamine to favour the mobilisation of energy stores during the early PW period.

2.4.3. Thyroid hormones

Plasma concentration of thyroid hormones (3.5.3'-triiodothyronine, T_3; and thyroxine, T_4) are reported to respond to feeding level, decreasing with the reduction in feed intake or with malnourishment (Le Dividich and Sève, 2000). Carroll *et al.* (1998) observed unaltered levels of T_3 and T_4 after weaning, but measured a decline of those two hormones after a change in the diets.

3. Bovine colostrum

3.1. Definition and composition

Colostrum is the lacteal secretion directly after parturition common to all mammals and essential for development and immune status for newborn (Scammell, 2001). More than a source of nutrients such as proteins, carbohydrates, fat, vitamins and minerals, colostrum also contains several biologically active molecules which are essential for specific functions (Pakkanen and Aalto, 1997). The most important bioactive components in colostrum include growth factors and antimicrobial factors. Some of these factors are also present in regular milk, but in much lower concentrations (e.g., typically 1:100 to 1:1000 of what is found in colostrum (Maher, 2000)). Table 1 compares the concentrations of the main components of bovine colostrum and regular milk.

Table 1. Main components of bovine colostrum and bovine milk.

	Bovine colostrum (/litre)	**Bovine milk (/litre)**	**References**
DM	153-245 g	122 g	Blum and Hammon, 2000
Crude protein	41-140 g	34 g	Gopal and Gill, 2000
Lactose	27-46 g	46 g	Gopal and Gill, 2000
Crude fat	39-44 g	37 g	Gopal and Gill, 2000
Crude ash	5-20 g	7 g	Gopal and Gill, 2000
IgG1	50-90 g	0.30-0.40 g	Elfstrand *et al.*, 2002
IgG2	1,5-2 g	0.03-0.08 g	Elfstrand *et al.*, 2002
IgA	3.0-6.5 g	0.04-0.06 g	Elfstrand *et al.*, 2002
IgM	3.8-6 g	0.03-0.06 g	Elfstrand *et al.*, 2002
Lactoferrin	1.5-5 g	0.1-0.3 g	Korhonen, 1977
Lactoperoxydase	30 mg	20 mg	Korhonen, 1977
Lysozyme	0.14-0.7 mg	0.07-0.6 mg	Korhonen, 1977
IL-1β	840 µg	3 µg	Hagiwara *et al.*, 2000
IL-1ra	5.2 mg	27 µg	Hagiwara *et al.*, 2000
IL-6	77 µg	0.15 µg	Hagiwara *et al.*, 2000
TNF-α	926 µg	3.3 µg	Hagiwara *et al.*, 2000
IFN-γ	260 µg	0.21 µg	Hagiwara *et al.*, 2000
IGF-1	100-2000 µg	<25 µg	Elfstrand *et al.*, 2002
IGF-2	200-600 µg	<10 µg	Pakkanen and Aalto, 1997
GH	<1 µg	<0.03 µg	Scammell, 2001
EGF	4-8 mg	2 µg	Scammell, 2001
TGF-β2	100-300 µg	1-2 µg	Elfstrand *et al.*, 2002

DM = dry matter ; Ig = immunoglobulin ; IL = interleukin ; TNF = tumor necrosis factor ; INF = interferon ; IGF = insulin-like growth factor ; GH = growth hormone ; EGF = epidermal growth factor ; TGF = transforming growth factor.

3.2. Growth promoters

3.2.1. IGF-I and -II and their binding proteins (BP)

The most abundant growth factors of bovine colostrum are IGF-I and -II. Both IGF-I and -II are single chain polypeptides with 70 and 67 amino acid residues and molecular weights of about 7.6 and 7.5 kDa, respectively. The primary structures of IGF-I and -II are highly conserved across species and have identical sequences in pigs, humans and cattle (Xu *et al.*, 2000). They stimulate cell growth and differentiation and are proposed to act both as endocrine hormones through the blood and, locally, as paracrine and autocrine growth factors. IGF-I is biologically more potent than IGF-II (Jones and Clemmons, 1995).

Six IGFBP have been identified and cloned. The detectable level and rank of specific IGFBP in bovine mammary secretions are IGFBP-3 > IGFBP-2 ≈ IGFBP-4 > IGFBP-5 (Blum and Baumrucker, 2002). Those binding proteins are involved in the regulation and the coordination of biological activities of the IGF-I and -II (Hwa *et al.*, 1999).

In biological fluids, IGF-I is usually bound to its binding proteins (IGFBP), which have also been detected in bovine milk. IGF-I appears in mature milk mainly in the bound form (85-90 %), but in the first milkings postpartum the free form of IGF-I predominates (73 %). The slightly acidic pH (6.3) of the colostral secretion is correlated with an increased proportion of the free IGF-I (Einspanier and Schams, 1991).

3.2.2. Epidermal Growth Factor (EGF) receptor ligand family

The polypeptides of this family have the common property of binding to the EGF receptor (a 175 kDa cell surface glycoprotein with tyrosine kinase activity). The most important members of this family are EGF itself and TGF-α (Barnard *et al.*, 1995).

EGF is a 6 kDa peptide, composed of 53 amino acids. The peptide is highly homologous among species and elicits similar effects across species (Odle *et al.*, 1996). Colostral EGF may play a role (i) in the prevention of bacterial translocation and (ii) in the stimulation of gut growth in suckling neonates by playing an important role in cell differentiation rather than cell proliferation and in stimulating mucus secretion (Schweiger *et al.*, 2003).

TGF-α is a 6 kDa peptide, composed of 50 amino acids and shares about 30 % sequence identity with EGF. It may play a complementary role to that of TGF-β (see below) in controlling the balance between cell proliferation and differentiation in the intestinal epithelium (Playford *et al.*, 2000).

3.2.3. Transforming growth factor βeta (TGF- β)

Three isoforms of TGF-β (TGF-β1, β2 and β3) are known. Those forma are homodimeric proteins with a molecular weight of approximately 25 kDa (Jin *et al.*, 1991). TGF-β1 and β2 have been isolated from bovine colostrum, with a predominance of the β2 form (85-95 %) (Elfstrand *et al.*, 2002).
TGF-β is a highly pleiotropic growth factor with several different types of function. It stimulates proliferation of some cells, especially in connective tissue, whereas it acts as a growth inhibitor of some other cells, such as lymphocytes and epithelial cells. TGF-β plays an important role in embryogenesis, tissue repair, formation of bone cartilage, and in the control of the immune system (Tripathi and Vashishtha, 2006). During injury or disease, it acts in concert with EGF to stimulate cell proliferation (Border and Noble, 1995).

3.3. Antimicrobial factors

The colostral antimicrobial factors contribute to the protection of the neonate against infectious diseases, which is crucial for its survival. These factors may be classified in three groups according to their action: (i) specific antimicrobial factors (immunoglobulins), (ii) non-specific antimicrobial factors (lactoferrin, lactoperoxidase and lysozyme) and finally (iii) factors which have both specific and non-specific activities (cytokines).

3.3.1. Lactoferrin

Lactoferrin is an 80 kDa iron-binding glycoprotein present in colostrum, milk, and to a lesser extent in other exocrine fluids such as tears. It is a member of the transferring family of non-heme iron-binding protein, characterised by their unique anion requirement for binding of iron (Viljoen, 1995).

Lactoferrin has been shown to inhibit the growth of several microbes, including *Escherichia coli, Salmonella typhimurium, Shigella dysenteria, Listeria monocytogenes, Streptococcus mutans, Bacillus subtilis* (Pakkanen and Aalto, 1997). In fact, lactoferrin exhibits both bacteriostatic and bactericidal activity against a range of microorganisms. The bacteriostatic activity is related to the high iron binding affinity of the protein that deprives iron-requiring bacteria of this essential growth nutrient. The bactericidal mechanism is related to its ability to cause release of lipopolysaccharide (LPS) molecules from the outer membrane of the Gram-negative bacteria. Recent results suggest that a cationic domain at the N-terminus of lactoferrin is responsible for its bactericidal properties. This domain is distinct from the amino acids involved in iron-binding, indicating that the bactericidal activity of lactoferrin is distinct from metal chelation (Conneely, 2001).

In addition to its antimicrobial activity, it has been proposed that lactoferrin plays an important role in iron uptake in the intestine and in the activation of phagocytes and immune responses. Receptors for lactoferrin are found on intestinal tissues, monocytes, macrophages, neutrophils, lymphocytes, platelets and on some bacteria (Viljoen, 1995; Hoek *et al.*, 1997). By its function of binding free iron, lactoferrin may act as an antioxidant, protecting the immune cells against free radicals produced by themselves in areas of inflammation or infection (Britigan *et al.*, 1994). Moreover, by its ability to bind and neutralize LPS, lactoferrin will reduce production of cytokines in response to inflammation or infection (Cohen *et al.*, 1992).

3.3.2. Lactoperoxidase

Lactoperoxidase is a basic glycoprotein of 78 kDa containing a heme-group with Fe^{3+}. It catalyses the oxidation of thiocyanate (SCN^-) in the presence of hydrogen peroxide (H_2O_2), producing a toxic intermediary oxidation product. This product inhibit bacterial metabolism via the oxidation of essential sulphydryl groups in microbial enzymes and other proteins (Pruitt and Reiter, 1985).

According to Kussendrager and van Hooijdonk (2000), lactoperoxidase has a bactericidal activity against Gram-negative and a bacteriostatic activity against Gram-positive bacteria. This difference in sensitivity can probably be explained by the difference in cell wall structure and their different properties. Additionally, some viruses, including polio viruses, appear to be sensitive to the toxic effects of

lactoperoxidase. Next to that antimicrobial and antiviral activity, degradation of various carcinogens and protection of animal cells against peroxidative effects have been reported (Kussendrager and van Hooijdonk, 2000).

3.3.3. Lysozyme

Lysozyme, originally described by Alexander Fleming in 1922, is a lytic enzyme of 14.3 kDa. The natural substrate of this enzyme is the peptidoglycan layer of the bacterial cell wall and its degradation results in lysis of the bacteria (Reiter, 1978). Due to the difference of the outer membrane structure between Gram-negative and Gram-positive bacteria, the lysozyme action is more intensive on Gram-positive bacteria, leading to the death of the bacteria, while it does not adversely affect the viability of Gram-negative bacteria (Ibrahim *et al.*, 1994). Over the last 15 years, several authors have proposed a novel antibacterial mechanism of action of lysozyme, independent of its enzymatic activity. The precise mechanism remains unknown, but it seems evident that bactericidal activity depends on the passage through the outer membrane (Masschalck *et al.*, 2001).

A particularity of the lysozyme is its interaction with other factors present in the colostrum. It activates partly lactoperoxidase by forming a complex with it (Hulea *et al.*, 1989). In presence of lactoferrin, the antimicrobial activity of lysozyme against *E. coli* is also enhanced as lactoferrin damages the outer membrane of Gram-negative bacteria and the organism becomes susceptible to lysozyme (Yamauchi *et al.*, 1993). Finally, it works also in synergy with IgA and complement factors against *E. coli* (Hill and Porter, 1974).

3.3.4. Immunoglobulins (Igs)

Igs are glycoproteins constituted by four amino acid chains: two identical light chains (23 kD) and two identical heavy chains (50-70 kD). According to the structure of their heavy chains, they can be divided into five classes: IgG, IgA, IgM, IgE and IgD.

Igs are present in very high concentrations in colostrum. They represent 70-80 % of the total protein contents in colostrum (up to 100 gL^{-1} in bovine colostrum, Elfstrand *et al.*, 2002), whereas in mature milk Igs account for only 1-2 % of the protein (see table 1).

Three classes of Igs are present in bovine colostrum: IgG, IgA and IgM. The major Igs present in bovine colostrum are IgG, among which 95 % belong to the subclasse IgG1 and 5 % to the IgG2. Table 2 shows the evolution of the Igs concentrations during the 80 first hours postpartum in colostrum and in mature milk. It shows that the concentrations of the individual Igs decline at different rates over the time it takes to reach the concentration in mature milk (Elfstrand *et al.*, 2002).

Table 2. Mean concentrations (standard deviation) of IgG1, IgG2, IgA and IgM in bovine colostrum at various time intervals *postpartum* (Elfstrand *et al.*, 2002), compared to bovine mature milk (Lindmark-Mansson *et al.*, 2000).

Time interval *postpartum* (h)	IgG1 (g/l)	IgG2 (g/l)	IgA (g/l)	IgM (g/l)
0-6	90 (*7.1*)	2.8 (*0.85*)	1.6 (*0.12*)	4.5 (*0.20*)
7-10	79 (*28*)	1.9 (*0.08*)	1.7 (*0.01*)	4.0 (*0.50*)
11-20	65 (*12*)	1.8 (*0.50*)	0.9 (*0.06*)	2.3 (*0.08*)
21-30	24 (*1.2*)	1.1 (*0.14*)	0.7 (*0.02*)	1.8 (*0.01*)
31-40	31 (*4.2*)	0.5 (*0.03*)	0.3 (*0.02*)	1.0 (*0.01*)
41-50	17 (*0.9*)	0.4 (*0.03*)	0.2 (*0.01*)	0.8 (*0.12*)
51-80	12 (*0.2*)	0.2 (*0.01*)	0.1 (*0.01*)	0.7 (*0.08*)
Mature milk	0.51 (*0.2*)	0.03 (*0.02*)	0.02 (*0.01*)	0.10 (*0.07*)

In the neonate the colostral Igs are transferred from the lumen of the intestine into the circulation through a non-selective macromolecular transport system across the small intestinal epithelium. This non-selective absorption occurs only within about 24-36 h after birth and provides the transmission of passive immunity from the cow to its calf (Pakkanen and Aalto, 1997). However, it has been shown that older animals can absorb Igs, but larger quantities of these antibodies are required for an effective transport (Maher, 2000).

All Igs exhibit one or more effector function in addition to antigen binding. Whereas one part of an antibody binds to antigen, other parts interact with other elements. The immunological function mediated by the Igs depends on the Ig class. The most important action of IgG antibodies is the activation of complement-mediated

bacteriolytic reactions. Another vital function is their ability to increase the recognition and phagocytosis of bacteria by leucocytes. IgM antibodies are considerably more efficient than IgG in regards of the above activities, especially complement-mediated lysis. IgA, in contrast, does not fix complement or opposing bacteria, but agglutinates antigens, neutralises viruses and bacterial toxins, and prevents the adhesion of enteropathogenic bacteria to mucosal epithelial cells. Moreover, this Ig is present in bovine colostrum under a secretory form (sIgA) which makes it resistant to the activities of proteolytic digestive enzymes (Korhonen *et al.*, 2000).

3.3.5. Cytokines

Colostrum and milk contain many cytokines, including IL-1β, IL-6, TNF-α, IFN-γ and IL-1ra (Hagiwara *et al.*, 2000). The concentrations of these cytokines are really low, but they are active from picomolar to nanomolar concentrations.

Cytokines are small peptide molecules that are important mediators in the regulation of the immune and inflammatory responses. In general, cytokines do not regulate normal cellular homeostasis, but alter cellular metabolism during times of perturbation, e.g. in response to inflammation. In the newborn, these factors play an important role in combination with the ingested maternal Ig and the non-specific antibacterial components in colostrum (Playford *et al.*, 2000). Of particular interest is the role of cytokines as major regulators of epithelial cell growth and development, including intestinal inflammation and epithelial restitution following mucosal damage (Elson and Beagley, 1994).

4. Use of bovine colostrum in pig production at weaning

Bovine colostrum and its main components have already been largely studied on neonates and *in vitro*. In this review, we will focus on the results obtained on weaned or adult pigs when they are existent. Their particularity, compared to neonates, is the gut closure, suggesting that absorption of molecules of bovine colostrum is no more possible. Nevertheless, according to Jensen *et al.* (2001), the presence of bioreactive components in colostrum may be responsible for an enhanced uptake of molecules after closure by inducing changes in brush border enzyme activities. Moreover, in the case of

Igs, Stirling *et al.* (2005) demonstrated that the absorption of bovine or porcine IgG colostrum is possible in adult animals (more than 4-weeks-old animals).

4.1. Effects of specific components

4.1.1. Growth promoters

According to Pluske *et al.* (1997), an opportunity may exist to enhance growth and development through supplementation of the newly-weaned pig with exogenous growth factors. As argued by Dunshea and Walton (1995), this is particularly pertinent in the case of the weaned piglet since (a) its gut is relatively "immature" at weaning, (b) the pig suffers a growth check, and (c) the gut of the newly-weaned pig is often colonised by enteropathogenic bacteria.

- *IGF-I*

Oral IGF-I and IGF-II at pharmacologic doses can stimulate cellular proliferation in the gastro-intestinal tract in newborn pigs (Burrin *et al.*, 1996 ; Xu *et al.*, 1996). Intestinal tissues in porcine neonates apparently do not have the capacity to absorb significant amounts of ingested IGF-I and transport this peptide intact to the circulation (Xu and Wang, 1996; Donovan *et al.*, 1997). Insulin-like growth factors are anabolic and possible differentiation-inducing factors for intestinal epithelium of newborns, suggesting possible applications of recombinant IGF and IGF analogues for repair of damaged *gastro-intestinal* tissues (Simmen *et al.*, 1998). However, Marion *et al.* (2002) showed that a weaning diet supplemented with IGF-I induced an increase in plasma concentrations in IGF-I, but had no effect on the intestinal structure of 7 days old weaned piglets. These controversial observations need further investigations.

- *EGF*

Exogenous EGF administered either orally or systemically stimulates *gastro-intestinal* tissue growth in weaned animals. Read *et al.* (1986) reported that inclusion of EGF (200 $\mu g.kg^{-1}$) in the diet significantly accelerated intestinal growth in weaning rats following 50 % removal of the small intestine. However, no studies on the effects of EGF on the growth of the intestinal tissues of weaned piglets were found.

In addition to its growth-promoting effect, EGF appears to be able to modulate the enterocyte differentiation during the transition phase from maternal milk to solid food in weaned piglets (Schweiger *et al.*, 2003). In 21-day old newly-weaned piglets, orally administered EGF (372 $\mu g.d^{-1}$) increased jejunal lactase (by 77 %) and sucrase (by 97 %) specific activities measured after 3 days of feeding (Jaeger *et al.*, 1990). Moreover, Kingsnorth *et al.* (1990) also reported increased tensile strength of gastric wounds in 20-kg pigs after 5 days of *intra-peritoneal* infusion of EGF (0.5 $\mu g.kg^{-1}.d^{-1}$). These results suggest that supplementation with EGF may aid in the recovery of traumatised gastric and intestinal tissues. Playford *et al.* (2000) proposed that EGF acts as a "luminal surveillance peptide" in the adult gut, readily available to stimulate the repair process at sites of injury.

4.1.2. Antimicrobial factors

- *Immunoglobulins*

Effects of bovine milk Igs on weaned piglets' performance have been demonstrated. Leibbrant *et al.* (1987) showed that milk-derived Igs added to milk replacers increase growth performance following gut closure in early weaned pigs. Pierce *et al.* (2005) attributed this improvement to the continuous source of Igs until the pig is capable of synthesising its own Igs. Moreover, Stirling *et al.* (2005) demonstrated the presence of porcine Fc receptors that transferred orally delivered (bovine or porcine) IgG into the blood supply and, concluded that this receptor has the potential to deliver protein antigens to the pig immune system.

In the gastro-intestinal tract, immuno-supplementation with bovine immunoglobulin in the form of specific antibody has been shown to be effective against various enteric diseases. Although bovine colostrum contains Igs to neutralise enteric pathogens, the titer of Igs present is considered by some researchers too low to afford protection against specific infectious organisms. This limitation is overcome by the production of hyperimmune bovine colostrum. In trials on infants, it has been successfully shown that specific antibodies in bovine milk are effective against both enteropathogenic and enterotoxigenic *Escherichia coli, cryptosporidium, Helicobacter pylori, Rotavirus* and *Shigella flexneri* (See Tripathi and Vashishtha, 2006 for a review).

No studies with hyperimmune colostrum in weaning piglets have been found. However Schaller *et al.* (1992) demonstrated in a gnotobiotic piglet model that both viral shedding and diarrhoea were effectively reduced or eliminated in a dose-dependent manner, as a result of feeding Igs preparations containing antibodies specific for human rotavirus strains. Moreover, Mroz *et al.* (1999) showed that passive protection of piglets against PW collibacillosis can be provided by egg IgG immunised against enterotoxigenic strains of *Escherichia coli*.

- *Lactoferrin*

Wang *et al.* (2007) studied the effects of lactoferrin ($1 g.kg^{-1}$ basal diet) on growth performance, intestinal microflora and morphology of weaning pigs. They observed an increase in growth performance (increase in ADG by 34 % and in ADFI by 17 %), a better feed efficiency (decrease in F/G by 12.8 %). Effects on the intestinal microflora were a reduction of the total viable counts of *Escherichia coli* and *Salmonella*, and an enrichment of the *Lactobacillus* and *Bifidobacterium*. The villus height was increased and crypt depth decreased at the small intestinal mucosa, as compared with the basal diet. They concluded that the use of lactoferrin as an additive would be a good method of defending weaned pigs from infections and weanling stress.

4.2. Effects of colostrum supplementation

The administration of bovine colostrum presents two main advantages due to its specific components: (i) it allows the synergic effects of different component such as described above for the lysozyme for example and (ii) it has been reported that porcine milk contains potent inhibitory activity against trypsin and chymotrypsin, and prevents growth factors, such as EGF and IGF-I, hydrolysis in neonatal and newly-weaned pig intestinal fluids (Shen and Xu, 1996, 2000) suggesting that colostrum, the natural carrier of milk-borne growth factors, may protect the peptides from gastro-intestinal luminal digestion.

Studies of the effects of bovine colostrum fractions on growth performance, feed ingestion and feed conversion ratio of newly-weaned piglets are presented in Table 3.

Table 3. Effects of bovine colostrum (BC) supplementation on growth performance, feed ingestion and feed conversion ratio of newly-weaned piglets.

References	BC supplementation		Piglets		Effects of BC *vs.* control treatment
	Description	g.kg^{-1} feed	n	Weaning age	
Pluske *et al.*, 1999	BC powder rich in IgG	0, 50 and 100 during 10 d	131	28 d	↗ ADG Week 1 and 2 PW ≈ ADFI and FCR ↘ Days to slaughter
King *et al.*, 2001	Spray-dried BC	0 and 60 during 7 d	110	28 d	≈ ADG and FCR ↗ ADFI Week 1 PW
Dunshea *et al.*, 2002	Freeze-dried BC	0 and 60 during 7d	24	14 d	≈ ADG, ADFI and FCR
Le Huërou-Luron *et al.*, 2004	Freeze-dried BC	0 and 40 during 11 d In uncleaned pens	150	28 d	↗ ADG Week 1 and 2 PW ↗ ADFI Week 1 PW ↘ FCR Week 1 PW
		0, 20 and 40 during 14 d In clean pens	12	21 d	↗ ADG d5-d7 PW ≈ ADFI and FCR

↗ increase, ↘ reduction, ≈ no effect

BC = bovine colostrum, ADG = average daily gain, ADFI = average daily feed intake, FCR = feed conversion ratio, PW = post-weaning.

Except for Dunshea *et al.* (2002), the three other studies presented in the table 3 showed an increase in ADG during the first week PW. Pluske *et al.* (1999) observed an increase of 40 % and 80 % of the ADG with a starter diet supplemented with 50 and 100 g of a bovine colostrum extract.kg^{-1} of feed, respectively. King *et al.* (2001) observed an increase of 20 % ($P > 0.05$) with 60 g of bovine colostrum.kg^{-1} feed and Le Huërou-Luron *et al.* (2004) showed an increase of the ADG up to 110 % with a starter diet supplemented with 40 g of bovine colostrum.kg^{-1} of feed. In all these studies, the authors explained the increase in ADG by an increase in the ADFI (+ 10 and + 25 % for Pluske *et al.* 1999, + 25 % for King *et al.*, 2001 and Le Huërou-Luron *et al.*, 2004).

Interestingly, Le Huërou-Luron *et al.* (2004) observed, in an "unclean" environment a reduction of 10 % of the FCR, which was not observed in the other studies performed in cleaned rooms. They concluded that bovine colostrum had more effects in bad environmental conditions (uncleaned pens) than in a clean environment, as it was observed with plasmatic proteins (van Dijk *et al.*, 2001). This suggests that the positive response of bovine colostrum might result from an improvement in the sanitary status of the colostrum-treated piglets by a direct effect of the colostrum on gut health.

Effects of bovine colostrum observed on the *gastro-intestinal* tract of newly weaned piglets are presented in Table 4. Bovine colostrum helped to preserve intestinal integrity after weaning, especially in the duodenum where bovine colostrum maintained higher villi (+ 10 to 20 %) (Le Huërou-Luron *et al.*, 2003 ; Huguet *et al.*, 2007 and King *et al.*, 2007) and reduced crypt depth (- 6 %) (King *et al.*, 2007) the first week PW compared to a control treatment. Moreover, Le Huërou-Luron *et al.*, 2003 showed also an increase in the duodenal protein synthesis. All these observations show the beneficial effect of bovine colostrum to preserve the intestinal mucosa integrity.

Table 4. Effects of bovine colostrum (BC) supplementation on the gastro-intestinal tract of newly-weaned piglets.

References	BC supplementation		Piglets		Effects of BC *vs.* control treatment
	Description	g.kg^{-1} feed	n	Weaning age	
Le Huërou-Luron *et al.*, 2003	Defatted BC	BC provided 5 µg IGF-I and 21 mU insulin kg^{-1} BW d^{-1} during 5 d	6	7 d	↗ plasma IGF-I ↗ duodenal VH ↗ duodenal protein synthesis ≈ SI mucosa weight and protein content ≈ SI lactase and aminopeptidase N activities
Huguet *et al.*, 2006	Freeze-dried defatted BC	50 during 14 d	12	21 d	≈ plasma insulin and IGF-I ↘ gastric pH on d7 and d14 ↗ duodenal *lactobacilli:coliform* ≈ duodenal mucosal structure, crypt cell proliferation, migration index, digestive enzyme activities
Huguet *et al.*, 2007	Freeze-dried defatted BC	50 during 14 d	12	21 d	≈ duodenal mucosa/muscularis ratio ↗ duodenal villi perimeter ≈ duodenal crypt size and crypt cell proliferation
King *et al.*, 2007	Spray-dried BC	75 during 19 d	7	21 d	↗ proximal and mid jejunal VH ↘ proximal and mid jejunal and distal ileal CD ↗ VH:CD in distal ileum ↗ epithelial cell height in mid jejunum

↗ increase, ↘ reduction, ≈ no effect

BC = bovine colostrum, BW = body weight, SI = small intestine, VH = villi height, CD = crypts depth.

An effect of bovine colostrum-supplemented diets was also observed on the intestinal microflora by Huguet *et al.* (2006), who showed an improved *Lactobacilli:Coliform* ratio in bovine colostrum treated weaned piglets, compared to weaned piglets receiving a control diet without colostrum (1.63 *vs*. 1.19). This effect was mainly caused by lower *coliforms* counts whereas *Lactobacilli* counts were identical between colostrum treated and control piglets. This observation confirms the higher effects of bovine colostrum reported by Le Huërou-Luron *et al.* (2004) in an "unclean" environment and indicates the positive contribution of bovine colostrum in the improvement of gut health around weaning in piglets.

Another effect of importance observed by Huguet *et al.* (2006) in the gastro-intestinal tract is the lower gastric pH in bovine colostrum treated piglets. This strengthened the effects of colostral antibacterial components and may also be at the origin of the *Coliform* population reduction, the later being less resistant to low pH than *Lactobacilli*. Finaly, Le Huërou-Luron *et al.* (2003) reported an increase in plasma IGF-I (+ 33 %) in weaned piglets receiving bovine colostrum. They explained this observation by an increase of the synthesis and secretion of IGF-I under the action of growth promoters contained in bovine colostrum, but they couldn't determine which one.

5. Conclusion and perspectives

According to the results presented on the use of bovine colostrum in piglets diet PW, it appears clearly that bovine colostrum supplementation improves the growth performance and the sanitary status of piglets during the early PW period. These beneficial effects are explained by both an increase in feed intake level the first days and a likely direct effect on gut health (preservation of gut integrity and reduction of the coliform population).

Those results are really encouraging but much work is still necessary to understand the mechanism of action of the bovine colostrum and to make this natural product technically and economically competitive compared to other alternatives (probiotics, prebiotics, enzymes, organic acids, plant extracts…) in the weaner diet.

Firstly, the action of bovine colostrum on the structure of the gut has been studied but no information is available on its local action on the gut associated lymphoid tissue (GALT) of newly-weaning piglets. This is of first importance to evaluate its effect on the local immune system of the piglet.

Secondly, the effects on the systemic immune system should also be studied to evaluate the effect of bovine colostrum to extend our knowledge about the action of bovine colostrum to the other organs of the weaned pig.

Finally, future investigations on lower doses of bovine colostrum supplementation in the weaner diet are necessary to reduce the costs of its use. Indeed, except the study of Le Huërou-Luron *et al.* (2004) where 20 and 40 g of bovine colostrum.kg^{-1} of feed were used, all the other cited authors incorporated from 5 to 10 % of colostrum in their starter diets, thereby increasing to excessively the price of the diet in our modern pork production systems.

References

Azain MJ. (1993). Impact of starter diet on nursery performance. *University of Georgia, Swine Report* **86** p. 49-54.

Barnard JA., Beauchamp RD., Russell WE., Dubois RN., Coffey RJ. (1995). Epidermal growth factor-related peptides and their relevance to *gastro-intestinal* pathophysiology. *Gastroenterology* **108** p. 564-580.

Blum JW., Hammon H. (2000). Colostrum effects on the *gastro-intestinal* tract, and on nutritional, endocrine and metabolic parameters in neonatal calves. *Livest. Prod. Sci.* **66** p. 151-159.

Blum JW., Baumrucker CR. (2002). Colostral and milk insulin-like growth factors and related substances: Mammary gland and neonatal (intestinal and systemic) targets. *Domest. Anim. Endocrin.* **23** p. 101-110.

Boe K. (1991). The process of weaning in pigs: when the sow decides. *Appl. Anim. Behav. Sci.* **30** p. 47-59.

Border WA., Noble NA. (1995). Targeting TGF-β for treatment of disease. *Nat. Med.* **1** p. 1000-1001.

Britigan BE., Serody JS., Cohen MS. (1994). The role of lactoferrin as an anti-inflammatory molecule. *In Hutchens TW., Rumball SV., Lonnerdal B. Lactoferrin: Structure and Function, Advances in Experimental Medicine and Biology,* New York, Plenum Press, p. 143-156.

Brooks PH. (1999). Strategies and methods for the allocation of food and water in the post-weaning period. In: *EAAP, 50th Annual Meeting of the European Association for Animal Production, 22-26 August 1999*. Zurich, Switzerland.

Bruininx EMAM., Heetkamp MJW., van den Bogaart D., van der Peet-Schwering CMC., Beynen AC., Everts H., den Hartog LA., Schrama JW. (2002). A prolonged photoperiod improves feed intake and energy metabolism of weanling pigs. *J. Anim. Sci.* **80** p. 1736-1745.

Burrin DG., Wester TJ., Davis TA., Amick S., Heath JP. (1996). Orally administered IGF-I increases intestinal mucosal growth in formula-fed neonatal pigs. *Am. J. Physiol.* **270** p. R1085-R1091.

Carroll JA., Veum TL., Matteri RL. (1998). Endocrine responses to weaning and changes in post-weaning diet in the young pig. *Domest. Anim. Endocrin.* **15** p. 183-194.

Cera KR., Mahan DC., Cross RF., Reinhart GA., Whitmoyer RE. (1988). Effect of age, weaning and postweaning diet on small intestinal growth and jejunal morphology in young swine. *J. Anim. Sci.* **66** p. 574-584.

Cohen MS., Mao J., Rasmussen GT., Serody JS., Britigan BB. (1992). Interaction of lactoferrin and lipopolysaccharide (LPS): effects on the antioxidant property of lactoferrin and the ability of LPS to prime human neutrophils for enhanced superoxide formation. *J. Infect. Dis.* **166** p. 1375-1378.

Colson V., Orgeur P., Foury A., Mormède P. (2006). Consequences of weaning piglets at 21 and 28 days on growth, behaviour and hormonal responses. *Appl. Anim. Behav. Sci.* **98** p. 70-88.

Conneely OM. (2001). Antiinflammatory activities of lactoferrin: Review. *J. Am. Coll. Nutr.* **20** p. 389S-395S.

Donovan SM., Chao JC., Zijlstra RT., Odle J. (1997). Orally administered iodinated recombinant human insulin-like growth factor-I (^{125}I-rhIGF-I) is poorly absorbed by the newborn piglet. *J. Pediatr. Gastroenterol. Nutr.* **24** p. 174-182.

Dunshea FR. (2003). Metabolic and endocrine changes around weaning. *In: Pluske JR., Le Dividich J., Verstegen MWA. Weaning the pig. Concepts and consequences.* Wageningen Academic Publishers, p. 61-80.

Dunshea FR., Walton PE. (1995). Potential of exogenous metabolic modifiers for the pig industry. *In: Hennessy DP., Cranwell PD. Manipulating pig production V*, Australasian Pig Science Association, Werribee, Victoria, Australia, p. 42-51.

Dunshea FR., Kerton DK., Eason PJ., Pluske JR., Moyes T. (2002). Diets containing high-quality animal proteins increase growth of early-weaned pigs. *Aust. J. Agric. Res.* **53** p. 779-784.

Einspanier R., Schams D. (1991). Changes in concentrations of insulin-like growth factors 1 and 2 and their binding proteins in human milk: effect of heat treatment on IGF and IGF binding protein stability. *J. Dairy Res.* **58** p. 171-178.

Elfstrand L., Lindmark-Mansson H., Paulsson M., Nyberg L., Akesson B. (2002). Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing. *Int. Dairy J.* **12** p. 879-887.

Elson CO., Beagley KW. (1994). Cytokines and immune mediators. *In: Johnson LR. Physiology of the Gastro-intestinal Tract*, Third Edition, Raven, New York, USA, p. 243-266.

Fenton JP., Roehrig KL., Mahan DC., Corley JR. (1985). Effect of swine weaning age on body fat and lipogenic activity in liver and adipose tissue. *J. Anim. Sci.* **60** p. 190-199.

Franklin MA., Mathew AG., Vickers JR., Clift RA. (2002). Characterization of microbial populations and volatile fatty acid concentrations in the jejunum, ileum, and cecum of pigs weaned at 17 vs 24 days of age. *J. Anim. Sci.* **80** p. 2904-2910.

Gopal PK., Gill HS. (2000). Oligosaccharides and glycoconjugates in bovine milk and colostrum. *Br. J. Nutr.* **84** (S1) p. 69-74.

Gu X., Li D., She R. (2002). Effect of weaning on small intestinal structure and function in the piglet. *Arch. Anim. Nutr.* **56** p. 275-286.

Hagiwara K., Kataoka S., Yamanaka H., Kirisawa R., Iwai H. (2000). Detection of cytokines in bovine colostrum. *Vet. Immunol. Immunop.* **76** p. 183-190.

Hampson DJ. (1986). Alteration in piglet small intestine structure at weaning. *Res. Vet. Sci.* **40** p. 32-40.

Hampson DJ., Kidder DE. (1986). Influence of creep feeding and weaning on brush border enzyme activities in the piglet small intestine. *Res. Vet. Sci.* **40** p. 24-31.

Hay MP., Orgeur P., Levy F., Le Dividich J., Concordet D., Nowak R., Schaal B., Mormède P. (2001). Neuroendocrine consequences of very early weaning in swine. *Physiol. Behav.* **72** p. 263-269.

Hill IR., Porter P. (1974). Studies of bactericidal activity to Escherichia coli of porcine serum and colostral immunoglobulins and the role of lysozyme with secretory IgA. *Immunology* **26** p. 1239-1250.

Hoek K., Milne JM., Grieve PA., Dionysius DA., Smith R. (1997). Antibacterial activity of bovine lactoferrin-derived peptides. *Antimicrob. Agents Chem.* **41** p. 54-59.

Hopwood DE., Hampson DJ. (2003). Interactions between the intestinal microflora, diet and diarrhoea, and their influences on piglet health in the immediate post-weaning period. *In: Pluske JR., Le Dividich J., Verstegen MWA. Weaning the pig. Concepts and consequences.* Wageningen Academic Publishers, p. 199-218.

Huguet A., Sève B., Le Dividich J., Le Huërou-Luron I. (2006). Effects of a bovine colostrum-supplemented diet on some gut parameters in weaned piglets. *Reprod. Nutr. Dev.* **46** p. 167-178.

Huguet A., Le Normand L., Fauquant J., Kaeffer B., Le Huërou-Luron I. (2007). Influence of bovine colostrum on restoration of intestinal mucosa in weaned piglets. *Livest. Sci.* **108** p. 20-22.

Hulea SA., Mogos S., Matei L. (1989). Interaction of lactoperoxidase with enzymes and immunoglobulins in bovine milk. *Biochemistry International* **19** p. 1173-1181.

Hwa V., Oh Y., Rosenfeld G. (1999). The insulin-like growth factor-binding protein (IGFBP) superfamily. *Endocr. Rev.* **20** p. 761-787.

Ibrahim HR., Yamada M., Matsushita K., Kobayashi K., Kato A. (1994). Enhanced bactericidal action of lysozyme to Escherichia coli by inserting a hydrophobic pentapeptide into its C terminus. *J. Biol. Chem.* **269** p. 5059-5063.

Jaeger LA., Lamar CH., Cline TR., Cardona CJ. (1990). Effect of orally administered epidermal growth factor on the jejunal mucosa of weaned piglets. *Am. J. Vet. Res.* **51** p. 471-474.

Jensen BB. (1998). The impact of feed additives on the microbial ecology of the gut in young pigs. *J. Anim. Feed Sci.* **7** p. 45-64.

Jensen AR., Elnif J., Burrin DG., Sangild PT. (2001). Development of intestinal immunoglobulin absorption and enzyme activities in neonatal pigs is diet dependent. *J. Nutr.* **131** p. 3259-3265.

Jin Y., Cox DA., Knecht R., Raschdorf F., Cerletti N. (1991). Separation, purification, and sequence identification of TGF-β1 and TGF-β2 from bovine milk. *J. Prot. Chem.* **10** p. 3411-3416.

Jones JI., Clemmons ER. (1995). Insulin-like growth factors and their binding proteins: Biological actions. *Endocr. Rev.* **16** p. 3-34.

Kasser TR., Martin RJ., Gahagan JH., Wangness PJ. (1981). Fasting plasma hormones and metabolites in feral and domestic newborn pigs. *J. Anim. Sci.* **53** p. 420-426.

Kelly BYD., Smyth JA., McCracken KJ. (1991). Digestive development of the early-weaned pig. 1. Effect of continuous nutrient supply on the development of the digestive tract and on changes in digestive enzyme activity during the first week post-weaning. *Br. J. Nutr.* **65** p. 169-180.

King MR., Morel PCH., James EAC., Hendriks WH., Pluske JR., Skilton R., Skilton G. (2001). Inclusion of colostrum powder and bovine plasma in starter diets increases voluntary feed intake. *In: Manipulating Pig Production VIII, Australasian Pig Science Association,* Werribee, Australia, p. 213.

King MR., Kelly D., Morel PCH., Pluske JR. (2003). Aspects of intestinal immunity in the pig around weaning. *In: Pluske JR., Le Dividich J., Verstegen MWA. Weaning the pig. Concepts and consequences.* Wageningen Academic Publishers, p. 219-257.

King MR., Wester TJ., Morel PCH. (2007). The effect of dietary spray-dried bovine colostrum and plasma on the response of pigs to enterotoxigenic *E. coli* challenge after weaning. *Livest. Sci.* **108** p. 292-294.

Kingsnorth AN., Vowles R., Nash JRG. (1990). Epidermal growth factor increases tensile strength in intestinal wounds in pigs. *Br. J. Surg.* **77** p. 409-412.

Korhonen H. (1977). Antimicrobial factors in bovine colostrum. *J Sci. Agric. Soc. Finl.* **49** p. 434-447.

Korhonen H., Marnila P., Gill HS. (2000). Milk immunoglobulins and complement factors. *Br. J. Nutr.* **84** (S1) p. S75-S80.

Kussendrager KD., van Hooijdonk ACM. (2000). Lactoperoxidase: physico-chemical properties, occurrence, mechanism of action and applications. *Br. J. Nutr.* **84** (S1) p. S19-S25.

Lallès JP., Boudry G., Favier C., Le Floc'h N., Luron I., Montagne L., Oswald IP., Pié S., Piel C., Sève B. (2004). Gut function and dysfunction in young pigs: physiology. *Anim. Res.* **53** p. 301-316.

Le Dividich J., Herpin P. (1994). Effects of climatic conditions on the performance, metabolism and health status of weaned piglets. *Livest. Prod. Sci.* **38** p. 79-90.

Le Dividich J., Sève B. (2000). Effects of underfeeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. *Domest. Anim. Endocrin.* **19** p. 63-74.

Le Huërou-Luron I., Marion J., Bebin K., Thomas F., Ganier P., Thibault JN., Le Dividich J. (2003). Supplementation of a weaning diet with a bovine colostrum extract minimise post-weaning duodenal structural, but not functional, alterations in 7-d old piglet. *In: 9th International Symposium on Digestive Physiology in Pigs, 14-17 May 2003,* Banff, AB, Canada, **2** p. 238-240.

Le Huërou-Luron I., Huguet A., Callarec J., Leroux T., Le Dividich J. (2004). La supplémentation de l'aliment de sevrage en colostrum bovin améliore l'ingestion et les performances zootechniques chez les porcelets au sevrage. *Journées Rech. Porcine* **36** p. 33-38.

Leibbrant VD., Kemp RA., Crenshaw TD. (1987). Effect of liquid milk replacer supplementation immediately postweaning on nursery pig performance. *J. Anim. Sci.* 65 (S1) p. 129 (Abstr.).

Lindmark-Mansson H., Svensson U., Paulsson M., Alden G., Frank B., Jhonsson G. (2000). Influence of milk components, somatic cells and supplemental zinc on milk processability. *Int. Dairy J.* **10** p. 423-433.

Maher TJ. (2000). Bovine colostrum, continuing education module. *New hope institute of retailing.* 8 p.

Marion J., Bebin K., Thomas F., Piot M., Fauquant J., Louveau I., Ganier P., Thibault JN., Maubois JL. (2002). La supplementation de l'aliment de sevrage en un extrait de colostrum bovin augmente la hauteur des villosités dans le duodenum chez le porcelet sevré. *Journées Rech. Porcine* **34** p. 103-108.

Masschalck B., Van Houdt R., Van Haver EGR., Michiels CW. (2001). Inactivation of Gram-negative bacteria by lysozyme, denaturated lysozyme, and lysozyme-derived peptides under high hydrostatic pressure. *Appl. Environ. Microb.* **67** p. 339-344.

Matteri RL., Dyer CJ., Touchette KJ., Carroll JA., Allee GL. (2000). Effects of weaning on somatotropic gene expression and circulating levels of insulin-like growth factor-1 (IGF-1) and IGF-2 in pigs. *Domest. Anim. Endocrin.* **19** p. 247-259.

McCracken BA., Gaskins HR., Ruwe-Kaiser PJ., Klasing KC., Jewell DE. (1995). Diet-dependent and diet-independent metabolic responses underlie growth stasis of pigs at weaning. *J. Nutr.* **125** p. 2838-2845.

McCracken BA., Spurlock ME., Roos MA., Zuckermann FA., Gaskins HR. (1999). Weaning anorexia may contribute to local inflammation in the piglet small intestine. *J. Nutr.* **129** p. 613-619.

Miller BG., James PS., Smith MW., Bourne FJ. (1986). Effect of weaning on the capacity of pig intestinal villi to digest and absorb nutrients. *J. Agric. Sci. Camb.* **107** p. 579-589.

Mroz Z., Dekker RA., Koopmans SJ., Le Huërou-Luron I. (2003). Performance, functional features on the digestive tract and haematological indices in weaned piglets fed antibiotic-free diets and exposed to a vitro-bacterial infection. *In: Ball RO. Proceedings of the 9th International Symposium on Digestive Physiology in Pigs, 14-17 May 2003,* Banff, AB, Canada, **2** p. 180-182.

Mroz Z, Grela ER., Matras J., Krasucki W., Kichura T., Shipp TE. (1999). Passive protection of piglets against diarrhoea with specialized egg immunoglobulins (Protimax*). In: Cranwell PD. Manipulating Pig Production VII*. Australasian Pig Science Association, Werribee, p. 238.

Mormède P., Hay M. (2003). Behavourial changes and adaptations associated with weaning. *In: Pluske JR., Le Dividich J., Verstegen MWA. Weaning the pig: Concepts and consequences,* Wageningen Academic Publishers, p. 53-60.

Nabuurs MJA., Hoogendoorn A., Van Zijderveld FG. (1994). Effects of weaning and enterotoxigenic Escherichia coli on net absorption in the small intestine of pigs. *Res. Vet. Sci.* **56** p. 379-385.

Odle J., Zijlstra RT., Donovam SM. (1996). Intestinal effects of milkborne growth factors in neonates of agricultural importance. *J. Anim. Sci.* **74** p. 2509-2522.

Pakkanen R., Aalto J. (1997). Growth factors and antimicrobial factors of bovine colostrum. *Int. Dairy J.* **7** p. 285-297.

Pié S., Lallès JP., Blazy F., Laffitte J., Sève B., Oswald IP. (2004). Weaning is associated with an upregulation of expression of inflammatory cytokines in the intestine of piglets. *J. Nutr.* **134** p. 641-647.

Pierce JL., Cromwell GL., Lindemann MD., Russell LE., Weaver EM. (2005). Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned pigs. *J. Anim. Sci.* **83** p. 2876-2885.

Playford RJ., Macdonald CE., Johnson WS. (2000). Colostrum and milk-derived peptide growth factors for the treatment of *gastro-intestinal* disorders. *Am. J. Clin. Nutr.* **72** p. 5-14.

Pluske JR., Hampson DJ., Williams IH. (1997). Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest. Prod. Sci.* **51** p. 215-236.

Pluske JR., Pearson G., Morel PCH., King MR., Skilton G., Skilton R. (1999). A bovine colostrum product in a weaner diet increases growth and reduces days to slaughter. *In: Manipulating Pig Production VII.* Australasian Pig Science Association, Werribee, Australia, p. 256.

Pruitt KM., Reiter B. (1985). Biochemistry of peroxidise system. *In: Pruitt KM., Tenovuo J. The Lactoperoxidase system: Chemistry and Biology Significance*. Marcel Dekker, New York. p. 143-178

Read LC., Ford WDA., Filsell O., McNeil J., Ballard FJ. (1986). Is orally-derived epidermal growth beneficial following premature birth or intestinal resection? *Endocrinologia Experimentalis* **20** p. 199-207.

Reiter B. (1978). Reviews of the progress of dairy science: Antimicrobial systems in milk. *J. Dairy Res.* **45** p. 131-147.

Scammell AW. (2001). Production and uses of colostrum. *Aust. J. Dairy Technol.* **56** (2) p. 74-82.

Schaller JP., Saif LJ., Cordle CT., Candler E. Winship TR., Smith KL. (1992). Prevention of human rotavirus-induced diarrhea in gnotobiotic piglets using bovine antibody. *J. Infect. Dis.* **165** (4) p. 623-630.

Schweiger M., Steffl M., Amselgruber WM. (2003). Differential expression of EGF receptor in the pig duodenum during the transition phase from maternal milk to solid food. *J. Gastroenterol.* **38** p. 636-642.

Shen WH., Xu RJ. (1996). Stability of epidermal growth factor in the *gastro-intestinal* lumen of suckling and weaned pigs. *Life Sci.* **59** p. 197-208.

Shen WH., Xu RJ. (2000). Stability of insulin-like growth factor I in the *gastro-intestinal* lumen in neonatal pigs. *J. Pediatr. Gastroenterol. Nutr.* **30** p. 299-304.

Simmen FA., Badinga L., Green ML., Kwak I., Song S., Simmen RCM. (1998). The porcine insulin-like growth factor system: at the interface of nutrition, growth and reproduction. *J. Nutr.* **128** p. 315S-320S.

Solano-Aguilar GI., Vengroski KG., Beshah E., Douglass LW., Lunney JK. (2001). Characterization of lymphocyte subsets from mucosal tissues in neonatal swine. *Dev. Comp. Immunol.* **25** p. 245-263.

Stirling CMA., Charleston B., Takamatsu H., Claypool S., Lencer W., Blumberg RS., Wileman TE. (2005). Characterization of the porcine neonatal FC receptor-potential use for trans-epithelial protein delivery. *Immunology* **114** p. 542-553.

Stolba A., Wood-Gush DGM. (1989). The behaviour of pigs in a semi-natural environment. *Anim. Prod.* **48** p. 419-425.

Tokach MD., Goodbank RD., Nelssen JL., Kats LJ. (1992). Influence of weaning weight and growth during the first week post-weaning on subsequent pig performance. *Kansas University Swine Day, Report Progress.* **667** p. 15-17.

Tripathi V., Vashishtha B. (2006). Bioactive compounds of bovine colostrum. *Food Rev. Int.* **22** p. 225-244.

Van Dijk AJ., Everts H., Nabuurs MJA., Margry RJCF., Beynen AC. (2001). Growth performance of weanling pigs fed spray-dried animal plasma: a review. *Livest. Prod. Sci.* **68** p. 263-274.

Vega-Lopez MA., Bailey M., Telemo E., Stokes CR. (1995). Effect of early weaning on the development of immune cells in the pig small intestine. *Vet. Immunol. Immunop.* **44** p. 319-327.

Viljoen M. (1995). Lactoferrin: A general review. *Haematologica* **80** p. 252-267.

Wang YZ., Shan TZ., Xu ZR., Feng J., Wang ZQ. (2007). Effects of the lactoferrin (LF) on the growth performance, intestinal microflora and morphology of weanling pigs. *Anim. Feed Sci. Technol.* **135** p. 263-272.

White ME., Ramsay TG., Osborne JM., Kampman KA., Leaman DW. (1991). Effect of weaning at different ages on serum insulin-like growth factor I (IGF-I), IGF binding proteins and serum in vitro mitogenic activity in swine. *J. Anim. Sci.* **69** p. 134-145.

Wiese F., Simon O., Weyrauch KD. (2003). Morphology of the small intestine of weaned piglets and a novel method for morphometric evaluation. *Anat. Histol. Embryol.* **32** p. 102-109.

Xu RJ., Mellor DJ., Birtles MJ., Reynolds GW., Simpson HV., Breier BH., Gluckman PD. (1996). Morphological changes in the oesophagus of newborn pigs: effects of age, diet and oral insulin-like growth factor-I (IGF-I) or IGF-II. *Reprod. Fertil. Dev.* **8** p. 903-909.

Xu RJ., Wang T (1996). *Gastro-intestinal* absorption of insulin-like growth factor-I in neonatal pigs. *J. Pediatr. Gastroenterol. Nutr.* **23** p. 430-437.

Xu RJ., Wang F., Zhang SH. (2000). Postnatal adaptation of the *gastro-intestinal* tract in neonatal pigs: a possible role of milk-borne growth factors. *Livest. Prod. Sci.* **66** p. 95-107.

Yamauchi K., Toimita M., Giehl TJ., Ellison RT. (1993). Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. *Infect. Immun.* **61** p. 719-728.

CHAPTER II

RESEARCH STAGES

RESEARCH STAGES

The aim of this thesis was to investigate the interest of bovine colostrum on the growth performance and on biological and immunological parameters of the newly-weaned piglet.

1. Context of the research

This work was initiated in the context of the European Union decree for a full ban of in-feed antibiotics by 1 January 2006, which intended to preserve the effectiveness of antibiotics for human use. Many studies on alternatives to in-feed antibiotics were already conducted. The most studied alternatives were pre- and probiotics, organic acids, enzymes, etc. but it is often reported that these products produce inconsistent results, or that benefits are inferior to those offered by antibiotics (Mathew, 2002).

The alternative we choose is the bovine colostrum for its richness in bioactive peptides known for their growth promoting or antimicrobial action (Pakkanen and Aalto, 1997). Bovine colostrum presents also the advantage to be a natural product and its collection is already organised in our country by the "Banque de Colostrum" (CER, Marloie, Belgium, 80 000 l/year), the only one in Europe.

We tested it immediately after weaning as it can be regarded as one of the most critical period in the modern-day pig production (Edwards, 2002; Pluske *et al.*, 1997), during which the use of antibiotics is the most effective.

2. Effects of bovine colostrum on the growth performance, feed intake and physiological parameters of newly weaned piglets

A first study was achieved in collaboration with Professor Dehoux (Experimental Chirurgical Unit, Faculty of Medicine, UCL) to investigate the effect of bovine colostrum on the immune system of newly weaned piglets. In this study, 0, 1 g and 5 g of defatted bovine colostrum powder were administered daily for 3 weeks to 3 groups of 5 newly weaned-piglets housed in an off-site facility. As well *in vivo* as *in vitro* measures were performed as follows:

- *in vivo:*
  - the growth performance;
  - the feed intake;
  - the haematological parameters (WBC, RBC, Platelet);
  - blood, splenic and GALT lymphocyte classes (B, T, Th and Tc);
  - seric and local total and anti-colostrum immunoglobulins (IgA, IgM and IgG);
  - and the cytokine expression in the spleen and the GALT tissues.
- *in vitro:*
  - the ability of blood, spleen and GALT tissues lymphocytes to produce cytokines,
  - and their stimulation index (SI) after colostral or mitogenic stimulation.

This study was published in the journal "*Research in Veterinary Science*".

In this experiment, the morphology of the intestinal wall was also studied (villi height and crypt depth). However, due to the numerous measures cited before and the absence of differences between the treatments for these parameters, these results were not presented in the publication.

A second study was achieved to investigate the effect of bovine colostrum whey supplementation in weaned diet (20 g/kg) on the growth performance, the feed intake, the systemic immune and endocrinal responses and the faecal *E. coli* populations of 96 newly weaned piglets housed in a conventional on-farm nursery. In this study, we measured:

- the growth performance;
- the feed intake;
- the haematological parameters (WBC, RBC, Platelet);
- blood lymphocyte classes (B, T, Th and Tc);
- the serum immunoglobulins (IgA, IgM and IgG);
- circulating hormones (IGF-I, IGF binding proteins, T3 and T4);
- and the faecal *E. coli* population.

All the results of this study were published in the journal "*Animal*", except the results of the endocrinal response which were briefly presented at the 20th International Pig Veterinary Society Congress (Durban, South Africa) in June 2008.

3. Reduction of the costs of to the use of bovine colostrum in weaned piglet diet

This last part of our work is essential for the development of the use of bovine colostrum in pig production. Our previous results clearly show the growth promoting activity of bovine colostrum whey the first week PW. However, the price per piglet with 2 % of bovine colostrum whey supplementation for 28 days is about 19 € which is not cost-effective in conventional pig production. To reduce the costs, two studies were achieved; the first studied three levels of bovine colostrum whey supplementation (0, 1 and 2 %) in the diet and the second compared bovine colostrum whey to defatted bovine colostrum, a 50 % less expensive fraction, on newly-weaned piglets.

In the first experiment, the growth performance and feed intake were measured. In the second one, these parameters were completed with blood parameters (haematological analyse, lymphocyte subclasses (B, T, Th and Tc), serum immunoglobulin (IgA, IgM and IgG) concentrations) and faecal bacterial populations (*Lactobacilli spp.* and *E. coli*) to investigate the effect of the withdrawal of casein on the effects of bovine colostrum.

These two experiments are presented in a publication submitted to the journal "*Animal*".

References

Edwards SA. (2002). Perinatal mortality in the pig: environmental or physiological solutions? *Livest. Prod. Sci.* **78** p. 3-12.

Mathew A. (2002). Seeking alternatives to growth promoting antibiotics. Department of Animal Science, The University of Tennessee, Knowville TH, USA. 14 p.

Pakkanen R., Aalto J. (1997). Growth factors and antimicrobial factors of bovine colostrum. *Int. Dairy J.* **7** p. 285-297.

Pluske JR., Hampson DJ., Williams IH. (1997). Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livest. Prod. Sci.* **51** p. 215-236.

CHAPTER III

EFFECTS OF BOVINE COLOSTRUM ON THE GROWTH PERFORMANCE, FEED INTAKE AND PHYSIOLOGICAL PARAMETERS OF WEANED PIGLETS

ARTICLE 1

EFFECTS OF ORAL SUPPLEMENTATION WITH BOVINE COLOSTRUM ON THE IMMUNE SYSTEM OF WEANED PIGLETS

The aim of this first experiment is to investigate the effect of bovine colostrum on the immune system of newly-weaned piglets.

In this study, 0, 1 g and 5 g of defatted bovine colostrum powder were administered daily for 3 weeks to 3 groups of 5 newly weaned-piglets housed in an off-site facility. Haematological parameters and anti-colostrum immunoglobulin levels were examined. Lymphocytes from the blood, spleen and gut-associated lymphoid tissues were analysed for phenotype as well as for their ability to produce cytokines. Finally, the stimulation index (SI) of mononuclear cells from different organs was obtained after colostral or mitogenic stimulation.

The haematological parameters were not significantly affected by colostrum. However, total serum IgA levels were increased after colostrum supplementation, with a transient decrease in total IgG. Local anti-colostrum immunization was observed in colostrum-fed piglets. The CD21+/CD3+ cells populations of the ileal Peyer's patch (iPP) were markedly affected. The SI of lymphocyte populations changed significantly whereas, naive blood lymphocytes were not stimulated *in vitro* in the presence of bovine colostrum, suggesting local anti-colostrum immunisation and an absence of direct mitogenic effects of the colostrum. Both Th1 and Th2 cytokine production was present in the different organs of colostrum-fed piglets. Bovine colostrum especially stimulated iPP cells.

EFFECTS OF ORAL SUPPLEMENTATION WITH BOVINE COLOSTRUM ON THE IMMUNE SYSTEM OF WEANED PIGLETS

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Abstract

The aim of this study was to evaluate the influence of bovine colostrum supplementation on the immune system of weaned piglets in a context of a full ban on in-feed antibiotics. After weaning at 21 days, 24 outbred piglets were fed with a diet supplemented daily for three weeks with 0, 1 or 5 g of colostrum. Feed intake, growth performance, haematological parameters, and serum and local anti-colostrum immunoglobulin levels were examined. Lymphocytes from the blood, spleen and gut-associated lymphoid tissues were analysed for phenotype as well as for their ability to produce cytokines. The stimulation index (SI) of mononuclear cells from different organs was obtained after colostral or mitogenic stimulation.

Feed intake, growth and haematological parameters were not significantly affected by colostrum. Total serum IgA levels were increased after colostrum supplementation, with a transient decrease in total IgG. Local anti-colostrum immunization was observed in colostrum-fed piglets. The CD21+/CD3+ cells populations of the ileal Peyer's patch (iPP) were markedly affected. The SI of lymphocyte populations changed significantly whereas, naive blood lymphocytes were not stimulated *in vitro* in the presence of bovine colostrum, suggesting local anti-colostrum immunization and an absence of direct mitogenic effects of the colostrum. Both Th1 and Th2 cytokine production was present in the different organs of colostrum-fed piglets. Bovine colostrum especially stimulated iPP cells.

Keywords: Piglet, Colostrum, Immune system, Immunoglobulins, Cytokine

Abbreviations: iPP: ileal Peyer's patch, MLN: mesenteric lymph nodes, SI: stimulation index.

1. Introduction

Bovine colostrum is a milk secreted during the first few days after calving, which has a critical role in post-neonatal health as an immune booster. In addition to nutrients such as proteins, carbohydrates, fat, vitamins and minerals, bovine colostrum contains various bioactive components, such as growth and anti-microbial factors. Growth factors promote the growth and development of the newborn calf, while anti-microbial factors provide passive immunity and protect against infections during the first weeks of life (Mero *et al.*, 1997; Pakkanen and Aalto, 1997).

The most abundant and well-characterized growth factors in bovine colostrum are insulin-like growth factors, insulin, transforming growth factors and epidermal growth factors (Pakkanen and Aalto, 1997). Anti-microbial factors in bovine colostrum include immunoglobulins, lactoferrin, lysozyme and lactoperoxidase. Bovine colostrum is also an extremely important source of immunoglobulins; the concentrations of IgG, IgM, and IgA are 100-fold higher than in normal milk (Rooke and Bland, 2002; Uruakpa *et al.*, 2002).

After birth and the neonatal period, weaning is also a critical period of pig growth because of increased susceptibility to gut disorders, infections and diarrhoea due to psychological, social, environmental and dietary stresses interfering with gut development and adaptation. The immediate effects of weaning are gut structural changes, essentially villous atrophy and crypt hyperplasia, with a decrease in the digestive and absorptive capacity of the small intestine and a dramatic reduction in food intake leading to undernutrition, transient growth check and infection sensitivity (Pluske *et al.*, 1997). The weaning period in young pigs is also associated with inflammation of the gut, involving alteration in intestinal immunity and in the intestinal immune responses to dietary and bacterial antigens (Hannant, 2002).

These post-weaning problems are currently managed by incorporating antibiotics and metals into weaning diets. However, increased bacterial resistance to antibiotics and environmental problems led the European Union to implement a full ban on in-feed antibiotics from January 2006 and a drastic reduction in the levels of copper and zinc supplementation; efficient alternatives, therefore, have to be found to conform to this policy change.

Although the classical role of colostrum is providing key nutrients as well as passive protection to the neonate is well known and described, its use in the weaning and post-weaning period is less well defined and, the ability of the newborn animal to absorb these substances for only a limited period of time prior to gut closure must be appreciated. Nevertheless, bovine colostrum could be of interest in view of its protective effects against various infectious diseases (Tacket *et al.*, 1988; Ebina *et al.*, 1992). Bovine colostrum has been used as a health food supplement for humans. It improves diarrhoea in patients suffering from immunodeficiency syndromes, non anti-inflammatory steroidal drugs-induced inflammatory colitis and acute phase-responses to surgery (He *et al.*, 2001; Morein *et al.*, 2002; Yoshioka *et al.*, 2005). The mechanisms by which bovine colostrum protects the host from infections have been attributed to the anti-microbial action of its functional components on infective agents and toxins and/or on host immune responses which may be beneficial for host health. Nevertheless, the data available on the immuno-modulatory effects of bovine colostrum in non-ruminants (as well as in human subjects) are still limited.

The aim of this study was to determine the effects of three weeks oral administration of bovine colostrum (0, 1 and 5 g daily) on the immune system of newly weaned, healthy piglets.

2. Materials and methods

2.1. Animals, diet and experimental design

Twenty-four 21-days-old newly weaned outbred Belgian Landrace male piglets coming from 5 litters (Rattlerow Seghers, Buggenhout, Belgium) and weighing 7.4 ± 0.4 kg were used in this study. Piglets were randomised into three groups (groups 0, 1 and 5) according to the amount of oral bovine colostrum supplementation. Each group was fed *ad libitum* with the same diet (Table 1) and had free access to water. The piglets received orally 0 g (group 0), 1 g (group 1) or 5 g (group 5) daily of bovine colostrum (Centre d'Economie Rurale, Marloie, Belgium) for three weeks, prepared by dissolving colostrum powder (pooled from three batches) in sterilised water at a concentration of 10 % (w/v). Group 0 was considered as the control group. All animals were kept in a temperature and humidity-controlled environment in a 12 h light-dark cycle.

Table 1. Composition of the experimental diet (Exp. diet) and the freeze-dried bovine colostrum

Composition (% FM)	Exp. diet	
Wheat	20	
Pea	13.16	
Soybean meal (48 % CP)	30	
Maize	30	
Soybean oil	1.99	
Vitamin/mineral premix[a]	1	
Synthetic amino acids and minerals[b]	3.85	
Chemical composition	**Exp. diet**	**Colostrum**
DM (% FM)	88.13	90.80
Crude protein (% DM)	22.78	67.50
Ether extract (% DM)	4.21	0.16
Crude fibre (% DM)	3.30	/
Starch (% DM)	36.34	/
Ash (% DM)	3.4	5.34
Net energy (kcal/kg DM)	2330	5190

FM = Fresh matter, DM = Dry matter, CP = Crude protein

[a] Trouw Nutrition, Nutreco, Gent, Belgium

[b]Methionine, lysine, threonine, tryptophane, phosphate, $CaCO_3$, NaCl

Dried colostrum powder was obtained by freeze drying at a maximal temperature of 40°C during the vacuum-warming up. The processing thermal effects on the immunoglobulin titers were evaluated by seroneutralisation. The *in vivo* immunoglobulin resorption was also checked by measuring total serum immunoglobulin levels in calves 48 h after oral intake according to the guidelines of the Centre d'Economie Rirale (Marloie, Belgium).

2.2. Surgical procedures

Three naive animals were operated on day 0 and the 21 remaining piglets on day 21. Before surgery, the animals were anaesthetised with tiletamine/zolazepam (Zoletil 100^R, 6 mg/kg, Virbac) and xylazine (Rompun^R 2 %, 2 mg/kg, Bayer) for induction and enflurane (Ethrane^R 0.8 %)/nitrous oxide (0.2 l/min) for maintenance. Mesenteric lymph nodes (MLN); pieces of the jejunum wall (100 cm after pylorus) and biopsies of the ileal Peyer's patch (iPP) were taken by enterotomy, intestinal fluids were collected from

the ileum and, finally, spleen biopsies were taken. After surgery, the animals were sacrificed by lethal iv injection of embutramide/mebenzonium/tetracaine hydrochloride (T61^{R}, Intervet). All procedures were conducted according to the guidelines established by the ethical committee of the Catholic University of Louvain and according to the Belgian legislation on care and use of laboratory animals.

All the samples removed were analysed immediately or frozen in liquid nitrogen and stored at –80°C.

2.3. Haematology analyses

Blood samples were taken from each animal on days 0, 8, 15 and 21 into heparin-treated tubes and analysed with a blood counter (MS9 vet, Melet Scloesing Lab, France). The total numbers of red and white blood cells were obtained as well as haemoglobin concentration and haematocrit percentage. These samples were also prepared for flow cytometry analysis.

At the same time, blood was also collected in dry tubes and the serum, separated by centrifugation at 1000 x *g* for 15 min, was stored at –20°C until analysis.

2.4. Cell preparation

Ten milliliters of heparinised blood was diluted in 25 ml of RPMI (Gibco, Paisley, Scotland, UK) and the peripheral blood mononuclear cells (PBMC) were recovered after centrifugation (400 x g, 30 min) across a density gradient on lymphocyte separation medium (LSM; International Medical, Brussels, Belgium). The isolated PBMC were blended in culture medium (RPMI medium including 20 % foetal calf serum, 2 mM L-glutamine, 100 µg/ml penicillin and 100 µg/ml streptomycin). Mononuclear cells from spleen, MLN, iPP and jejunum wall were obtained by cutting each organ sample into pieces of less than 1 mm and, after gently crushing, passing them through a gauze to remove debris. The passed cells were centrifuged using the same density gradient as previously described for the blood. The number of viable cells was counted after staining with trypan blue and reported per mg of tissue.

2.5. Peripheral blood, lymph node, Peyer's patch, jejunum and spleen lymphocyte monitoring

The phenotypes of lymphocyte subpopulations from peripheral blood, MLN, iPP, jejunum wall and spleen were monitored by a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) using a panel of fluorescein (FITC) or Phycoerythrin (PE)-labeled mAbs including mouse anti-pig CD3 (FITC, Clone B323-8E6-8C8), mouse anti-pig CD4 (FITC or PE, Clone 74-12-4), mouse anti-pig CD8 (PE, Clone 76-2-11) and mouse anti-human CD21 (PE, Clone B-ly4) markers purchased from BD Biosciences (San Jose, USA). A panel of FITC- and PE-labeled mouse IgG (Simultest control, BD, San Jose, USA) was used as negative control. Five hundred thousand cells per tube were incubated for 30 min with 10 µl of each mAb and after three washes, the percentage of single- or double-positive cells was then assessed by flow cytometry and the relative number of CD cell subpopulations was determined in each sample.

2.6. Mitogenic cell stimulation

A total of 2 x 10^6/ml porcine mononuclear cells from blood, MLN, iPP and spleen of piglets fed with 0 or 5 g of bovine colostrum for 3 weeks (no *in vitro* stimulation was done with 1 g of bovine colostrum), were plated in 96-well microtitre plates (Falcon, Nunc Brand Products, Denmark) in a final volume of 200 µl culture medium (RPMI medium including 20 % foetal calf serum, 2 mM L-glutamine, 100 µg/ml penicillin and 100 µg/ml streptomycin) for 96 h at 37°C under 5 % CO_2. Either 20 µl PHA (80 µg/ml), 20 µl of ConA (25 µg/ml), 20 µl of LPS(20 µg/ml) or 20 µl of bovine colostrum (Col; 200 µg/ml) were added on day 0. Ninety six hours after the initiation of the mitogenic stimulation, the plates were incubated for 4 h with tritiated thymidine (1 mCi/ml; Amersham Life Sciences, Belgium), then harvested (Filtermate 196; Packard, The Netherlands) and counted (Top count, microplate scintillator counter; Packard, Australia). A stimulation index (SI) was obtained from the ratio of counts per minute (cpm) from the wells with mitogens to the cpm of the wells with medium alone.

2.7. Immunoglobulin concentrations

2.7.1. Total serum Ig and serum anti-colostrum Ig levels

- *Total serum Ig levels*

Total IgM: The ELISA method was used. Briefly, 96 well microtitre plates (Falcon, U.S.A.) were coated overnight (4°C) with goat anti-pig IgM (10µg/ml) (Bethyl, Inc., Montgomery, Texas, USA) in 0.05 M borate buffer (pH 9.5). After saturation of non-specific antigenic sites (1h, 37°C) with 5 % skimmed milk in PBS 0.1 % Tween 20 (Sigma, U.S.A.), microtitre plates were incubated with 100 µl of twofold serial dilutions of pig serum starting at 1/1000 and IgM were detected by peroxidase-labeled goat anti-pig IgM (100 µl of 1/100,000 recommended dilution to each well; Bethyl, Inc., USA). Peroxidase activity was revealed by Orthophenyldiamin (OPD, Sigma), 0.03 % H_2O_2 (Merck) in citrate buffer (pH 5.5) solution. Optical density was measured at 450 nm using an optical reader (PR 5000, Dynatech). Purified pig IgM (Bethyl, Inc., USA) was used as the control and standard to create a standard curve for the calculation of results.

Total IgG*:* A similar technique was used to determine the total circulating IgG antibodies. Goat anti-pig IgG (Bethyl, Inc., Montgomery, Texas, USA) was used for coating and goat anti-pig IgG-peroxidase-labeled (1/100,000 recommended dilution; Bethyl, Inc., USA) for detection. Purified pig IgG (Bethyl, Inc., USA) was used as control and standard. The serial dilutions of pig serum started at 1/10,000.

Total IgA*:* A similar technique was used to determine the total circulating IgA antibodies. Goat anti-pig IgA (Bethyl, Inc., Montgomery, Texas, USA) was used for coating and goat anti-pig IgA-peroxidase-labeled (1/80,000 recommended starting dilution; Bethyl, Inc., USA) for detection. Purified pig IgA (Bethyl, Inc., USA) was used as control and standard. The serial dilutions of pig serum started at 1/800.

- *Anti-bovine colostrum serum Ig*

The ELISA method was used. Briefly, 96 well microtitre plates (Falcon, U.S.A.) were coated overnight (4°C) with bovine colostrum (25 µg/ml; pooled from three batches, Centre d'Economie Rurale, Marloie, Belgium) in 0.05 M borate buffer (pH 9.5). After saturation of non-specific antigenic sites (1h, 37°C) with 5 % skimmed milk in PBS 0.1 % Tween 20 (Sigma, U.S.A.), microtitre plates were incubated with 100 µl of twofold serial dilutions of pig serum started at 1/100 and peroxidase-labeled goat anti-

IgG, -IgM and -IgA mAb (100 µl of each recommended dilution as described; Bethyl, Inc., Montgomery, Texas, USA) were used for detection. All the samples were tested in the same test and on the same day under identical conditions. Peroxidase activity was revealed by Orthophenyldiamin (OPD, Sigma), 0.03 % H_2O_2 (Merck) in citrate buffer (pH 5.5) solution. Optical density was measured at 450 nm using an optical reader (PR 5000, Dynatech). Serum from three week-old piglets immunized or not with bovine colostrum (three subcutaneous injections of 100 mg of dried bovine colostrum powder suspended in PBS buffer – 10 % (w/v) – at two week intervals) was used as control.

2.7.2. Local total Ig and local anti-colostrum Ig

For local total Ig and local anti-colostrum Ig levels, intestinal fluids (2 ml of mucus) were freshly collected from the ileal part of the intestine on days 0 and 21. These samples were mixed with the same volume of PBS and centrifuged. Twofold serial dilutions of supernatant starting at ¼ were tested for IgM, IgG and IgA (total Ig and anti-colostrum Ig) by ELISA, as described earlier. Goat anti-pig IgM, anti-pig IgG or anti-pig IgA (Bethyl, Inc., Montgomery, Texas, USA) were used for coating and porcine Igs from diluted supernatants were detected by peroxidase-labeled goat anti-pig IgM, anti-pig IgG or anti-pig IgA (Bethyl, Inc. USA). Serum from three week-old piglets immunized or not with bovine colostrum (as described) was used as control.

2.8. RNA extraction and analysis by real-time PCR (RT-PCR)

Biopsies taken from the iPP, jejunum, MLN and spleen on day 21 were analysed in each group. Total RNA was extracted from frozen (-80°C) tissue samples (400 mg) with TriPure Isolation Reagent (Roche Diagnostics Corporation, Indianapolis, IN, USA), according to the instructions of the manufacturer. RNA was quantified by spectrophotometry (λ=260 nm) and its concentration adjusted to 0.25 µg/µl using RNase free water. Reverse transcription (RT) was performed on 1 µg of total RNA in a reaction volume of 20 µl involving 7.5 µM random hexamers, RT buffer 5X, 9 mM dithiothreitol, 220 µM of each deoxyribonucleotide triphosphate (dNTP) and 200 U of M-MLV (Invitrogen, California, USA). The final RT product was adjusted to 50 µl using RNase free water. The cDNA product was amplified on a GeneAmp 5700

Sequence Detection System and software (Applied Biosystems, Den Ijssel, the Netherlands) using SYBR Green detection (Applied Biosystems, Foster City, CA, USA). RT-PCR was carried out using the following cycle parameters: 10 min at 95°C, followed by 50 cycles of 1 min at 60°C and 15 s at 95°C. The following primers for pig cytokines were obtained using software (Applied Biosystems, Den Ijssel, The Netherlands) after editing pig cytokines sequences from the NCBI web site (www.ncbi.nlm.nih.gov):

- for IL-2 sense primer: cagttgcttttgaaggaagttaagaa;
  antisense primer: gcctgcttgggcatgtaaa;
- for IL-12 sense primer: cgtgcctcgggcaattataa;
  antisense primer: caggtgaggtcgctagtttgg;
- for IFN-γ sense primer: ttcaaagataaccaggccattca;
  antisense primer: cactgatggctttgcgctg;
- for IL-4 sense primer: cacagcgagaaagaactcgt;
  antisense primer: gtccgctcaggaggctcttc;
- for IL-10 sense primer: cttgttgctgaccgggtctct;
  antisense primer: tcggcattacgtcttccaggt.

The ribosomal protein L19 (RPL19) RNA was chosen as an internal standard reporter gene with the sense primer, caagcggattctcatggaaca, and the antisense primer, tggtcagccaggagcttctt. Standardisation of the RT-PCR procedure was performed for each primer, on total RNA extracted from peripheral mononuclear cells (coming from a naïve three week-old piglet) stimulated *in* vitro concomitantly with 20 µl PHA (80 µg/ml), LPS (25 µg/ml) and ConA (20 µg/ml) at 4 x 10^6 cells/ml. The primers were tested at different sample concentrations (20 %, 4 %, 0.8 % and 0.16 %). Results were expressed using the comparative cycle threshold (Ct) method as described in the User Bulletin #2 (Applied Biosystems). Briefly, the ΔCt values were calculated in every sample for each gene of interest as follows: $Ct_{\text{gene of interest}} - Ct_{\text{reporter gene}}$, with RPL19 gene as the reporter gene. The calculation of the relative changes in the expression level of one specific gene (ΔΔCt) was performed by subtracting the control group (group 0) ΔCt from the corresponding ΔCts of the treated groups (groups 1 and 5). The values and ranges given in the different figures were determined as follows: $2^{-\Delta\Delta Ct}$ with ΔΔCt + SEM and ΔΔCt – SEM, where SEM is the standard error of the mean of the ΔΔCt value (User Bulletin #2, Applied Biosystems).

2. 9. Statistical analysis

Results are expressed as means ± SEM. The statistical differences between the groups were tested using the one-way analysis of variance (ANOVA), followed by Student‘s *t*-test. Statistical significance was considered for a *P*-value < 0.05.

3. Results

3.1. Growth performances

During the experimental period, the piglet bodyweight increased from 7.4 ± 0.4 kg (day 0) to 10.7 ± 0.7 kg (day 21). There was no significant effect of colostrum on feed intake or piglet growth ($P > 0.05$; data not shown).

3.2. Haematological and flow-cytometric analysis

There were no significant differences among piglet groups within the same day for all the parameters measured, indicating a lack of significant effect of colostrum supplementation ($P > 0.05$, Table 2). However, there was a significant increase in white blood cell count on day 21 days in each group, involving mainly the granulocyte population in all groups and, additionally, the lymphocyte population in the control group ($P < 0.05$).

Table 2. Haematological parameters[a] on days 0 and 21 of piglets supplemented with 0 g (Group 0), 1 g (Group 1) or 5 g (Group 5) of bovine colostrum for three weeks (n = 7 piglets per group).

Parameters	Group 0		Group 1		Group 5	
	Day 0	Day 21	Day 0	Day 21	Day 0	Day 21
RBC[a] (*M*/mm³)	5.2 ± 0.6	5.6 ± 0.7	5.0 ± 0.7	6.0 ± 0.6	5.0 ± 0.5	5.9 ± 0.5
WBC[b] (*m*/mm³)	6.7 ± 1.5a	14.5 ± 3.4b	6.7 ± 0.7a	11.4 ± 3.4b	6.7 ± 1.9a	14.4 ± 4.9b
Monocytes (*m*/mm³)	0.5 ± 0.1	0.3 ± 0.0	0.4 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.3 ± 0.1
Granulocytes (*m*/mm³)	2.9 ± 0.4a	8.8 ± 2.7b	3.1 ± 0.6a	6.8 ± 3.2b	3.1 ± 1.9a	9.7 ± 5.0b
Lymphocytes (*m*/mm³)	3.4 ± 1.0a	5.4 ± 1.7b	3.2 ± 0.6	4.3 ± 0.3	3.3 ± 0.6	4.2 ± 0.4
Haematocrit (%)	35.6 ± 2.9	34.2 ± 2.8	34.2 ± 3.8	36.1 ± 2.5	33.7 ± 2.4	36.2 ± 3.1
Haemoblobin (g/dl)	11.3 ± 0.4	10.5 ± 0.6	11.1 ± 1.0	10.6 ± 0.4	11.0 ± 0.9	10.9 ± 1.0

Means in the same line with different letters, within the same group, differ significantly ($P < 0.05$).

[a] RBC: cells x 10^6/mm³ ± SEM; WBC, Monocytes, Granulocytes and Lymphocytes: cells x 10^3/mm³ ± SEM; Haematocrit: % ± SEM; Haemoglobin: g/dl ± SEM.

[b] RBC: red blood cells; WBC: white blood cells; $M = 10^6$ cells, $m = 10^3$ cells.

After extraction of the tissues cells for analysis by flow cytometry, mononuclear cells were quantified per milligram of tissue. There was a dose-related decrease in the total number of mononuclear cells per milligram of tissue recovered from iPP samples on days 0 and 21: from 625,454 ± 185,000 to 324,645 ± 157,000 cells/mg for group 1 and from 764,000 ± 104,000 to 189,000 ± 78,000 cells/mg for group 5 (both $P < 0.01$); no difference was observed in the control group: 567,000 ± 109,000 to 492,000 ± 87,000 ($P > 0.05$). For the other organs, no differences were observed in the number of mononuclear cells among groups or over time (data not shown; $P > 0.05$).

Phenotyping of the iPP cells (Figure 1) showed a very significant dose-related decrease in the CD21+ cell count on day 21 ($P < 0.01$), concomitant with an important increase in the CD3+ cell population during the same period in the same groups ($P < 0.01$), involving the CD4+ cell subpopulations (from 4.3 ± 1.6 en D0 to 11.6 ± 1.9 on D21 in the group 1 and from 3.7 ± 0.7 % on day 0 to 23.6 ± 11.9 % on day 21 in the group 5, $P < 0.01$, data not shown). There were no changes in the CD21+, CD3+ or CD4+ cells populations in group 0 between days 0 and 21 ($P > 0.05$, data not shown*).*

In the blood, the MLN, the jejunum wall and the spleen, there were no significant differences in any of the mononuclear cell populations among groups or over time ($P < 0.05$), (data not shown).

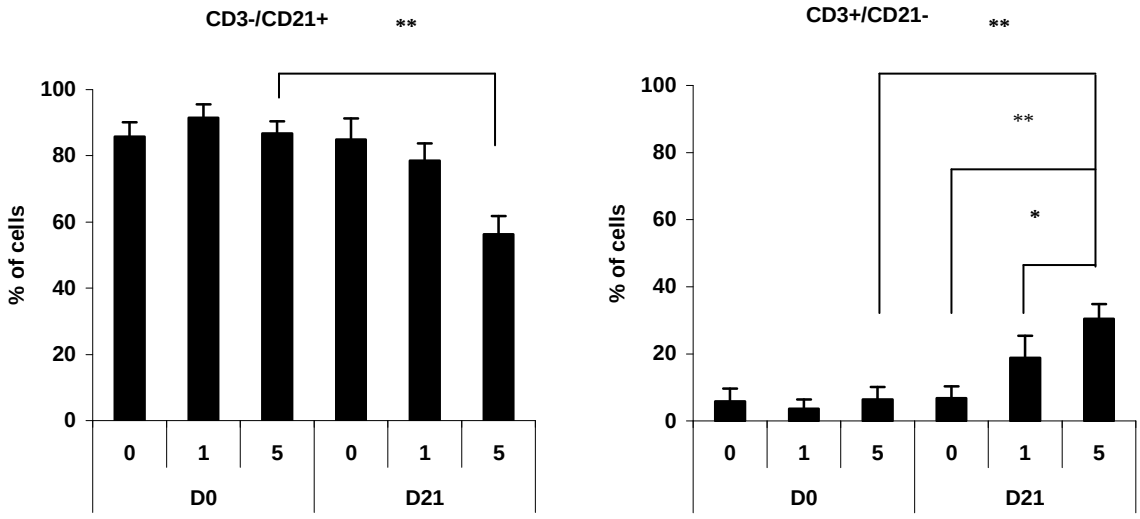

Figure 1. Relative percentage of CD3-/CD21+, CD3+/CD21- after double staining of cells from ileal Peyer's patch, on days 0 and 21 for the different treatments (Groups 0, 1 and 5). Results are expressed in % of cells (Means ± SEM ; *$P < 0.05$; **$P < 0.01$, n = 7 piglets per group).

3.3. Mitogenic stimulation

When stimulating piglet mononuclear cells from the 0 and 5 g colostrum groups (sacrificed on day 21) with PHA, ConA and LPS mitogens (Table 3), we observed a significant increase in the SI of group 5 cells compared to cells from the control group for each tissue. The SI was significantly higher in the iPP compared to the other tissues

($P < 0.01$). When stimulating the cells with bovine colostrum, we observed a significant increase in the SI in iPP cells from colostrum-fed piglets compared to cells from control animals ($P < 0.01$). No significant differences were observed in the SI from other organ cells in the presence of colostrum ($P > 0.05$).

Table 3. Stimulation index (mean ± SEM) of mononuclear cells from different immune organs of 6 week-old piglets supplemented with 0 g (Group 0) or 5 g (Group 5) of bovine colostrum for three weeks. The harvested cells were stimulated for 4 days with PHA, ConA, LPS or colostrum (Col) (n = 3 piglets per group in triplicate).

Mito-gens	Cell origin	Stimulation index			
		Blood	**Mesenteric lymph node**	**Ileal Peyer's patch**	**Spleen**
PHA	**Group 0**	42.6 ± 29.8 a	4.0 ± 0.2 a	3.9 ± 0.2 a	2.8 ± 0.01 a
	Group 5	493 ± 30 b	50.2 ± 14.7 b	113 ± 20.6 b	42.2 ± 6.9 b
ConA	**Group 0**	25.2 ± 2.6 a	2.7 ± 0.3 a	2.3 ± 0.4 a	2.0 ± 0.7 a
	Group 5	142 ±14.6 b	63.1 ± 32.7 b	145 ± 23.6 b	23.2 ± 3.4 b
LPS	**Group 0**	4.7 ± 1.2 a	2.7 ± 0.4 a	9.8 ± 1.7 a	2.4 ± 0.5 a
	Group 5	8.9 ± 0.3 b	7.3 ± 1.5 b	62.6 ± 4.3 b	7.4 ± 0.7 b
Col	**Group 0**	6.4 ± 1.2	4.7 ± 1.3	2.5 ± 0.6 a	3.6 ± 1.4
	Group 5	5.5 ± 0.3	3.2 ± 0.6	632 ± 156 b	4.0 ± 1.3

For each mitogen, means in the same column with different letters, differ significantly ($P < 0.05$)
Cells + medium alone: 179.4 ± 63.9 cpm

3.4. Immunoglobulin concentration

3.4.1. Total serum Ig levels (Table 4)

For total IgM levels, no significant differences were observed among the treatment groups or over time ($P > 0.05$).

For total IgG levels, no significant differences were observed among the different treatment groups at any time ($P > 0.05$). However, in the colostrum-fed groups (groups 1 and 5), a significant decrease ($P < 0.05$) in IgG concentration was observed during the first week (Day 8) following by a significant increase (Day 21) ($P < 0.01$). The profile in group 0 was very similar, with a decrease in IgG levels (Day 8 and 15) followed by an increase on Day 21, although these changes were not statistically significant.

For total IgA levels, there was a significant increase in levels in the colostrum-fed groups from day 0 to day 21, and a significant difference between colostrums-fed groups and the control group on day 21 ($P < 0.01$). In the control group, no significant differences were observed over time ($P > 0.05$).

Table 4. Total serum immunoglobulin concentrations (µg/ml as mean ± SEM) in piglets supplemented with 0 g (group 0), 1 g (group1) or 5 g (group 5) of bovine colostrum for three weeks (n = 7 piglets per group).

	Groups	Day 0	Day 8	Day 15	Day 21
IgM	0	1263 ± 47.2	1457 ± 125	1211 ± 262	1680 ± 91.4
	1	1261 ± 176	1306 ± 256	1463 ± 125	1949 ± 91.8
	5	1302 ± 97.3	1370 ± 138	1965 ± 244	1673 ± 181
IgG	0	6237 ± 602	4588 ± 539	4086 ± 764	5237 ± 339
	1	$5812 \pm 574_{a}$	$3498 \pm 728_{b}$	$4681 \pm 390_{ab}$	$5749 \pm 391_{ab}$
	5	$6525 \pm 319_{a}$	$3793 \pm 720_{b}$	$4926 \pm 190_{ac}$	$5966 \pm 349_{abc}$
IgA	0	215.9 ± 29.2	198.9 ± 18.8	286.7 ± 23.0	$^{x}318.1 \pm 50.4$
	1	$205.5 \pm 11.8_{a}$	$260.4 \pm 51.6_{a}$	$307.2 \pm 21.1_{ab}$	$^{y}430.9 \pm 31.6_{b}$
	5	$206.8 \pm 35.7_{a}$	$206.1 \pm 36.5_{a}$	242.9 ± 53.8a	$^{y}409.1 \pm 24.4$b

For each group, means in the same line with different subscript case letters, differ significantly ($P < 0,05$)
For each Ig class, means in the same column, with different superscript case letters, differ significantly ($P < 0.01$)

3.4.2. Serum anti-bovine colostrum Ig levels

No anti-bovine colostrum Ig was detected in the circulation in any of the groups after three weeks of treatment (data not shown).

3.4.3. Local total Ig levels

No significant differences were observed from day 0 to day 21 within or among groups for local total IgM, IgG and IgA in the intestinal fluid samples (data not shown).

3.4.4. Local anti-colostrum Ig levels

After three weeks of treatment, the levels of the three Ig classes, especially IgG, were higher for the two groups of piglets receiving bovine colostrum compared to the values measured in the control group on day 21 and the values measured in the colostrum groups on day 0 (Table 5). However, for the three Ig classes, the values obtained for groups 1 and 5 after three weeks of treatment were not significantly different.

Table 5. Local intestinal anti-colostrum immunoglobulins (sampled in intestinal fluids) (optical density at dilution 8 as mean ± SEM) in piglets supplemented with 0 g (Group 0), 1 g (Group 1) or 5 g (Group 5) of bovine colostrum for three weeks (n = 7 piglets per group).

	Groups	**Day 0** (n = 3)	**Day 21** (n = 7)
IgM	0	0.109 ± 0.020	0.152 ± 0.085^{x}
	1	$0.115 \pm 0.025_{a}$	$0.391 \pm 0.056_{b}^{y}$
	5	$0.125 \pm 0.019_{a}$	$0.331 \pm 0.048_{b}^{y}$
IgG	0	0.048 ± 0.012	0.111 ± 0.052^{x}
	1	$0.045 \pm 0.015_{a}$	$0.426 \pm 0.097_{b}^{y}$
	5	$0.043 \pm 0.018_{a}$	$0.355 \pm 0.082_{b}^{y}$
IgA	0	0.051 ± 0.008	0.048 ± 0.005^{x}
	1	$0.044 \pm 0.006_{a}$	$0.154 \pm 0.017_{b}^{y}$
	5	$0.050 \pm 0.008_{a}$	$0.167 \pm 0.026_{b}^{y}$

For each group, means in the same line with different subscript case letters, differ significantly ($P < 0.05$).

For each Ig class, means in the same column with different superscript case letters, differ significantly ($P < 0.01$).

3.5. Cytokine mRNA expression

The levels of cytokine expression in tissues from colostrum-fed animals (group 1 and 5) after three weeks of bovine colostrum supplementation were compared (Figure 2) to levels measured in the control group (group 0).

Daily supplementation with 5 g of colostrum was associated with a significant decrease in IFN-γ mRNA expression in iPP ($P < 0.01$), but had no significant effect on IFN-γ mRNA expression in the other organs ($P > 0.05$).

Daily supplementation with 1 and 5 g colostrum was associated with a significant increase in IL-2 mRNA expression in MLN ($P < 0.01$), but had no significant effect on IL-2 mRNA expression in the other organs ($P > 0.05$).

Daily supplementation with 5 g colostrum was associated with a significant increase in IL-12 mRNA expression in MLN, intestine and iPP ($P < 0.05$, $P < 0.01$ and $P < 0.01$, respectively). Daily supplementation with 1 g colostrum induced a significant increase in IL-12 mRNA expression in the intestine and iPP ($P < 0.05$). In the spleen, no significant effect of colostrum supplementation on IL-12 mRNA expression was observed ($P > 0.05$).

Daily colostrum supplementation (1 g and 5 g) was associated with a significant increase in IL-4 mRNA expression in iPP ($P < 0.01$ and $P < 0.001$, respectively) but had no significant effect on IL-4 mRNA expression in the other organs ($P > 0.05$).

Finally, daily supplementation with 1 g colostrum induced a significant increase in IL-10 mRNA expression in MLN and in iPP ($P < 0.01$). No significant effect of colostrum supplementation on IL-10 mRNA expression was observed in the other organs ($P > 0.05$).

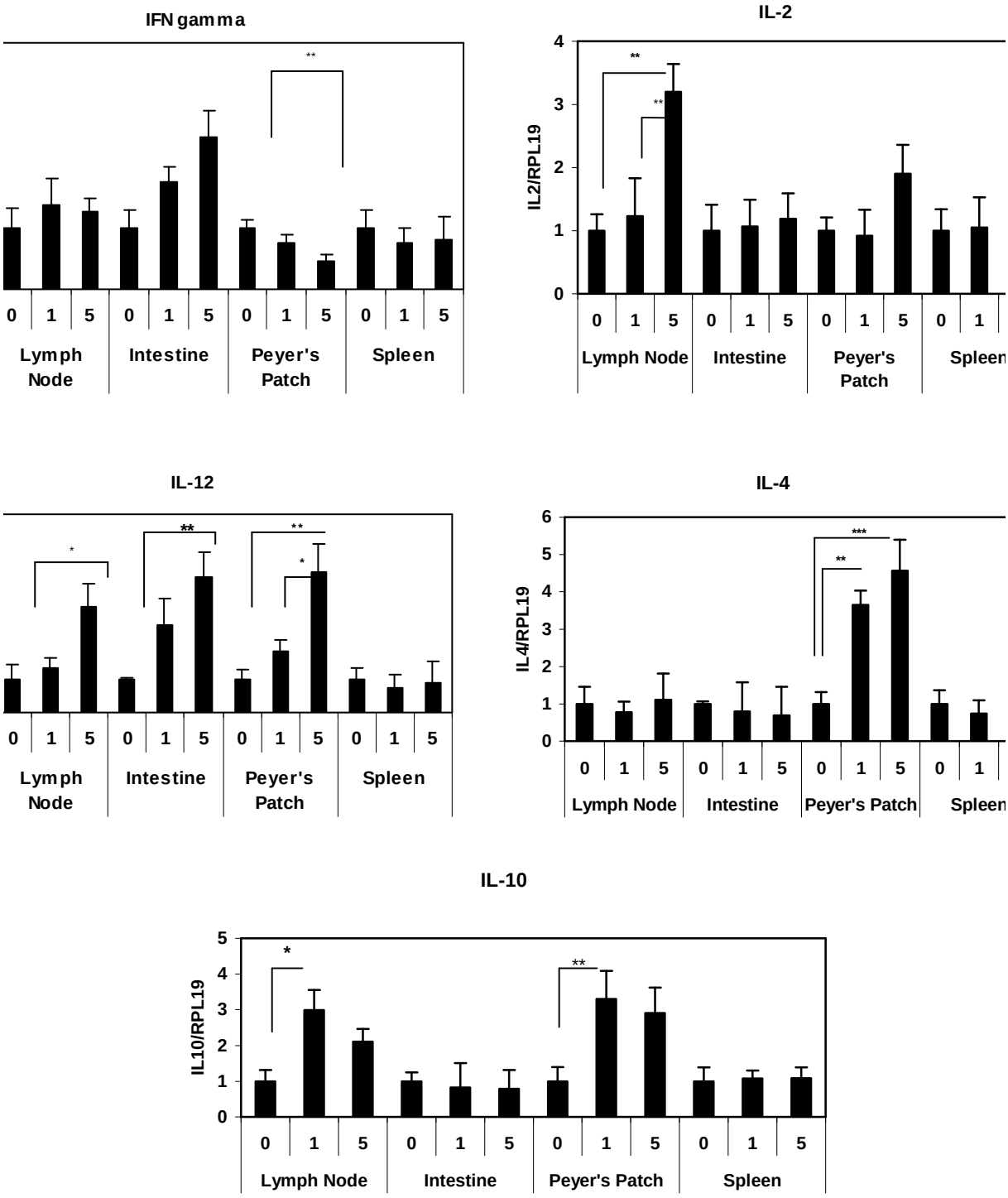

Figure 2. Cytokine gene expression levels in different tissues from animals supplemented with 0 g (group 0), 1 g (group 1) or 5 g (group 5) of bovine colostrum for three weeks. Results are expressed as mRNA relative expression (cytokine of interest/RPL19 reporter gene) (n = 5 piglets per group and per cytokine; Means ± SEM ; **P* < 0.05; ***P* < 0.01; ****P* < 0.001).

4. Discussion

Traditionally, bovine colostrum is known to provide essential food for newborn calves. Since it is relatively easy to collect large amounts of bovine colostrum, it has also been used as a material for other applications. Several issues need to be faced when considering using bovine colostrum as a supplement in piglets feed to alleviate infectious problems during the post-weaning period, in a context of full ban on in-feed antibiotics: (i) the absorption of molecules through the gut three weeks after birth despite gut closure, (ii) the bio-activity of bovine molecules in weaned piglets; and (iii) the dose of bovine colostrum to be administered orally.

Since the ability of the newborn piglet to transfer IgG from the gut to the bloodstream disappears in the first 24-36 h of life, molecules of bovine colostrum may not be taken up during the post-weaning period due to this gut closure. The specificity of absorption of IgG (and other molecules) has been debated because there is no good evidence for the receptor-mediated uptake that occurs in man and in rat (Rooke and Bland, 2002) and because the absorption of various growth factors is limited according to their local actions (Xu *et al.*, 1992). Nevertheless, it is more evident that the absorption of bovine or porcine IgG from colostrum is possible, not only in neonatal pigs but also in adult animals (more than four-week-old animals). Stirling *et al.* (2005) demonstrated the presence of porcine Fc receptors that transferred orally delivered (bovine or porcine) IgG into the blood supply and, concluded that this receptor has the potential to deliver protein antigens to the pig immune system. According to Jensen *et al.* (2001), the presence of bioreactive components in colostrum may also be responsible for the enhanced uptake of molecules from bovine colostrum by 2-20 day-old piglets by inducing changes in brush border enzyme activities.

Many authors (Kiriyama, 1992; Gomez *et al.*, 1998) have shown, by feeding newborn piglets with bovine colostrum that this product can be satisfactorily used as an immunoglobulins source in the artificial rearing of colostrum-deprived neonatal pigs. Although there are some differences between cattle and pigs in the major isotypes present in their colostrum (Salmon, 1999), bovine colostrum, through specific and non-specific factors, retains biological activity in the human gastrointestinal tract (Pakkanen and Aalto, 1997), newborn piglets (Xu *et al.*, 1992), and in newborn calves

(Baumrucker *et al.*, 1994), suggesting a bioactivity of the bovine colostrum through a trans-species barrier.

The dose of bovine colostrum supplementation used is inferior to the estimated colostrum intake of healthy suckling piglets over the first days of life. Bland *et al.* (1999) estimated an intake of 348 ± 46 g/ kg body weight/day of fresh porcine colostrum (with 20 % of dry matter) by newborn piglets. A daily supplement of 5 g of bovine colostrum is a maximum due to the relatively high price of the bovine colostrum powder (70 euros per kg). The aim of this study was by using bovine colostrum during the post-weaning period, to appreciate its immuno-modulatory effect, not only by assessing the concentrations of immunoglobulins but also by evaluating the role of some other bio-active proteins on the immune system of the piglets.

The feed intake and body weight parameters measured in our trial were related, in each group, to the age of the piglets and not to a bovine colostrum effect. However, several authors (Pluske *et al.*, 1999; King *et al.*, 2001; Le Huërou-Luron *et al.*, 2004) have reported an increase in growth from 19 % to 116 % and in feed intake from 12 % to 29 % with a post-weaning diet containing 2 %, 4 % or 10 % bovine colostrum in comparison to a control group. These concentrations of colostrum were far greater than those used in our trial. According to Le Huërou-Luron *et al.* (2004), the piglet response to dietary bovine colostrum supplementation is also clearly far more important when the rearing environment is “unclean”, involving modification of intestinal microflora, not necessarily with pronounced digestive diseases, while in “clean” conditions, as observed in our study, these effects are absent.

Our data show that colostrum feeding mainly reduces the number of cells in the iPP, especially the B-cell population, in an isotype manner, without any impact on local immune responses, since we observed a local anti-bovine colostrum response. These results are consistent with the changes in IgG-positive cells observed by Aldridge *et al.* (1998) in newborn calves separated from their dam at birth, but fed with bovine colostrum within six hours. These authors stated that these modifications were associated with a decrease in some IgG isotypes, with no effect on total IgM and IgA concentrations. The use of rapid freeze-thaw cycle, freeze-drier (colostrum powder) or gamma-irradiation processing, excludes colostral cells and indicates that the colostral

element is an absorbable factor such as immunoglobulins or a cytokine (Sordillo *et al.*, 1991, Sundblad *et al.*, 1994; Sambasivarao *et al.*, 1996). The decrease in total serum IgG could also be related to an age-effect or to a decay in passively acquired maternal antibodies since we observed the same, although not significant, IgG profile in the control group.

If we compare the results of colostrum and different mitogens on mononuclear cells coming from colostrum-fed or naive animals, the SI was clearly higher in cells coming from colostrum treated animals, suggesting an *in vivo* intestinal sensitization to colostral factors leading to a more stimulated or excitable status of the mononuclear cells, even in the presence of classical mitogens such as PHA, Con A or LPS. The increase in the SI was particularly impressive for the iPP cells of colostrum-fed piglets whereas it was weak or absent in the presence of mononuclear cells from the other immune organs. The lack of stimulation of naive PBMC in the presence of colostrum was confirmed by the low levels of IL-2 and IFN-γ production observed after colostrum supplementation, in comparison to the levels of the same cytokines produced after classical mitogenic stimulations (data not published). The anti-colostral immunization was confirmed by the presence of anti-colostrum Igs, especially IgG, in the intestinal fluid of colostrum-fed piglets. These data suggest that bovine colostrum itself has no mitogenic effects on naive mononuclear cells.

Several studies have also investigated the potential of milk proteins to affect lymphocyte function after *in vivo* exposure and have suggested a degree of immuno-enhancement of lymphocyte function (Cross and Gill, 2000) due to various components, including some non-specific products (casein), proinflammatory cytokines (IL-1β, IL-6, TNF-α and IFN-γ) or growth factors (EGF and TGFα) present in bovine colostrum (Carr *et al.*, 1990; Wong *et al.*, 1996; Playford *et al.*, 1999). However, other components, such as lactoferrin, whey proteins or lactoperoxidase, may have the opposite effect (Torre and Oliver, 1989; Otani and Hata, 1995; Wong *et al.*, 1997; Sfeir *et al.*, 2004) by inhibiting the induction of IL-2 (Sambasivarao *et al.*, 1996) or by the presence of the colostrum inhibitory factor, which inhibits the induction of IL-2 in human T lymphocytes (Mandalapu *et al.*, 1995). These reports provide strong evidence that bovine colostrum, with its multiple components, can modulate lymphocyte function in different ways.

The role of cytokines in the regulation and modulation of the immune response has been studied extensively since the Th1/Th2 paradigm was first postulated. IL-2, IL-12 and IFN-γ are considered to be key cytokines in the Th1 response and serve as indicators of a predominance of cell-mediated responses, whereas IL-4 and IL-10 participate in Th2 polarisation and secretion and suggest a predominance of humoral responses (Mosmann and Coffman, 1989; Reiner and Seder, 1995; Raymond and Wilkie, 2004). In the iPP, the increase in Th2 mRNA expression, associated with an impaired Th1 profile, suggests that bovine colostrum supplementation may cause a more marked Th2 immune response. In contrast in the jejunum, an increase in IL-12 (significant) and IFN-γ (not significant) production, with no modification of IL-2, IL-4 and IL-10 production, indicates a Th1 profile. These data confirm results obtained by Yoshioka *et al.* (2005) in four week-old C57Bl/6 mice, where 35 mg of bovine colostrum administered daily for 1-6 months directly stimulated intestinal intraepithelial lymphocytes to polarise Th1 type. In the MLN, IL-2, IL-10 and IL-12 were significantly increased with no modification of IL-4 and IFN-γ expression, suggesting a well balanced Th1/Th2 profile. The spleen was not affected by colostrum supplementation.

Bovine colostrum also contains high levels of cytokines (Il-1β, IL-6, TNF-α and IFN-γ) that can influence the immune response (Hagiwara *et al.*, 2000). Pecquet *et al.* (1999) reported that oral administration of bovine whey protein in adult mice was followed by increased IL-10 production. Taken together, these results suggest an immuno-modulatory effect of bovine colostrum targeting mainly the gut-associated lymphoid tissues, which respond by producing both Th1 and Th2 cytokines. This bipolarity is important in the context of exposure to a wide range of antigens associated with pathogens, with commensal bacteria and with food. It includes the ability to generate tolerance to food and commensal bacterial antigens as well as to activate the immune response to pathogens.

We observed (i) in the local intestinal fluid samples, an increase in anti-colostrum IgM, IgA and especially IgG after colostrum supplementation and (ii) in the serum, an increase only in the total serum IgA in the colostrum-fed group. These data suggest local anti-colostrum immunization. Pathogen-specific and other antibodies, particularly of the IgA isotype, are found commonly in gut secretions of healthy and diseased piglets. This antibody supply is mainly regulated by mucosal CD4+ lymphocytes and

mucosal epithelial cells, which produce many cytokines, including those critical for B cells maturation and IgA secretion, such as IL-4 and IL-10 (Hannant, 2002). Swine Peyer's patch is considered to be involved in the selection of the swine B-cell repertoire and can be considered as a primary immune organ (Bianchi *et al.*, 1999). It can be speculated, from these porcine differences, that bovine colostrum may influence the development of the IgA response by potentiating a Th2 response in the iPP.

References

Aldridge, B.M., McGuirk, S.M., Lunn, D.P., 1998 Effect of colostral ingestion on immunoglobulin-positive cells in calves. Vet. Immunol. Immunopathol. **62**, 51-64.

Baumrucker, C.R., Hadsell, D.L., Blum, J.W., 1994. Effects of dietary insulin-like growth factor I on growth and insulin-like growth factor receptors in neonatal calf intestine. J. Anim. Sci. **72**, 428-433.

Bianchi, A.T., Scholten, J.W., Moonen Leusen, B.H., Boersma, W.J., 1999 Development of the natural response of immunoglobulin secreting cells in the pig as a function of organ, age and housing. Dev. Comp. Immunol. **23**, 511-520.

Bland, I.M., Rooke, J.A., Bland, V.C., Sinclair, A.G., Edwards, S.A., 1999. The acquisition of IgG from colostrum by piglets. Proc. Br. Soc. Anim. Sci. 189.

Carr, R.I., Webster, D., Sadi, D., Williams, H., Walsh, N., 1990 Immunomodulation by opioids from dietary casein. Ann. New York Academy of Sciences. **574**, 374-376.

Cross, M.L., Gill, H.S., 2000 Immunomodulatory properties of milk. Br. J. Nutr., **84** (Suppl. 1), S81-S89.

Ebina, T., Ohta, M., Kanamura, Y., Yamamoto-Osumi, Y., Baba, K., 1992. Passive immunizations of suckling mice and infants with bovine colostrum containing antibodies to human rotavirus. J. Med. Virol. **38**, 117-123.

Gomez, G.G., Phillips, O., Goforth, R.A., 1998. Effect of immunoglobulin source on survival, growth, and haematological and immunological variables in pigs. J. Anim. Sci. **76**, 1-7.

Hagiwara, K., Kataoka, S., Yamanaka, H., Kirisawa, R., Iwai, H., 2000 Detection of cytokines in bovine colostrum . Vet. Immunol. Immunopathol. **76**, 183-190.

Hannant, D., 2002 Mucosal immunology: overview and potential in the veterinary species. Vet. Immunol. Immunopathol. **87**, 265-267.

He, F., Tuomola, E., Arvilommi, H., Salminen, S., 2001 Modulation of human humoral immune response through orally administered bovine colostrum. FEMS Immunol. Med. Mic. **31**, 93-96.

Jensen, A.R., Elnif, J., Burrin, D.G., Sangild, P.T., 2001. Development of intestinal immunoglobulin absorption and enzyme activities in neonatal pigs is diet dependent. J. Nutr. **131**, 3259-3265.

King, M.R., Morel, P.C.H., James, E.A.C., Hendriks, W.H., Pluske J.R., Skilton R., Skilton G. 2001 Inclusion of colostrum powder and bovine plasma in starter diets increase volontary feed intake. Page 213 in: "*Manipulating Pig Production VIII*", Australian Pig Science Association, Werribee, Australia.

Kiriyama, H., 1992. Enzyme-linked immunosorbent of colsotral IgG transported into lymph and plasma in neonatal pigs. Am. J. Physiol. **263**, R976-R980.

Le Huërou-Luron, I., Huguet, A., Callarec, J., Leroux, T., Le Dividich, J., 2004 La supplémentation de l'aliment de sevrage en colostrum bovin améliore l'ingestion et les performances zootechniques chez les porcelets au sevrage. Journ. Rech. Porcine Fr. **36**, 33-38.

Mandalapu, P., Pabst, H.F., Paetkau. V., 1995 A novel immunosuppressive factor in human colostrum. Cell. Immunol. **162,** 178-184.

Mero, A., Mikkulainen, H., Riski, J., Pakkanen, R., Aalto, J., Takala, T., 1997 Effects of bovine colostrum supplementation on serum IGF-I, IgG, hormone, and saliva IgA during training. J. Appl. Physiol. **83**, 1144-1151.

Morein, B., Abusugra, I., Blomqvist, G., 2002 Immunity in neonates**.** Vet. Immunol. Immunopathol. **87**, 207-213.

Mosmann, T.R., Coffman, R.L., 1989 TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu. Rev. Immunol. **7**, 145-173.

Otani, H., Hata, I., 1995 Inhibition of proliferative responses of mouse spleen lymphocytes and rabbit Peyer's patch cells by bovine milk caseins and theirs digests. J. Dairy Res. **62**, 339-348.

Pakkanen, R., Aalto, J., 1997 Growth factors and antimicrobial factors of bovine colostrum. Int. Dairy J. **7**, 285-297.

Pecquet, S., Pfeifer, A., Gauldie, S., Fritsche, R., 1999 Immunoglobulin E suppression and cytokine modulation in mice orally tolerized to beta-lactoglobulin. Immunology. **96**, 278-285.

Playford, R.J., Floyd, D.N., Macdonald, C.E., Calnan, D.P., Adenekan, R.O., Johnson, W., Goodlad, R.A., Marchbank, T., 1999 Bovine colostrum is a health food supplement which prevents NSAID induced gut damage. Gut. **44**, 653-658.

Pluske, J.R., Hampson, D.J., Williams, I.H., 1997 Factors influencing the structure and function of the small intestine in the weaned pig: a review. Livest. Prod. Sci. **51**, 215-236.

Pluske, J.R., Pearson, G., Morel, P.C.H., King, M.R., Skilton G., Skilton R. 1999 A bovine colostrums product in a weaner diet increases growth and reduces days to slaughter. Page 256 in: "Manipulating Pig Production VII", Australian Pig Science Association, Werribee, Australia.

Raymond, C.R., Wilkie, B.N., 2004 Th1/Th2 type cytokine profiles of pig T-cells cultured with antigen-treated monocyte-derived dendritic cells. Vaccine. **22**, 1016-1023.

Reiner, S.L., Seder, R.A., 1995 T helper cell differentiation in immune response. Curr. opin. in immunol. **7**, 360-366.

Rooke, J.A., Bland, I.M., 2002 The acquisition of passive immunity in the new-born piglet. Livest. Prod. Sci. **78**, 13-23.

Salmon, H., 1999. The mammary gland and neonate mucosal immunity. Vet. Immunol. Immunopathol. **72**, 143-155.

Sambasivarao, D., Hooton, J., Dost, A., Paetkau, V., 1996 A novel immunosuppressive factor in bovine colostrum blocks activation of the interleukin 2 gene enhancer at the NFAT site. Biochem. Cell Biol. **74,** 585-593.

Sfeir, R.M., Dubarry, M., Boyaka, P.N., Rautureau, M., Tomé, D., 2004 The mode of oral bovine colostrum lactoferrin administration influences mucosal and systemic immune responses in mice. J. Nutr. **134**, 403-409.

Sordillo, L.M., Redmond, M.J., Campos, M., Warren, L. and Babiuk, L.A., 1991 Cytokine activity in bovine mammary gland secretions during the periparturient period. Can. J. Vet. Res. **55,** 298-301.

Stirling, C.M.A., Charleston, B., Takamatsu, H., Claypool, S., Lencer, W., Blumberg, R.S., Wileman, T.E., 2005. Characterization of the porcine neonatal Fc Receptor-potential use for trans-epithelial protein delivery. Immunology **114**, 542-553.

Sundblad, A., Marcos, M.A., Malanchere, E., Castro, A., Haury, M., Huetz, F., Nobrega, A., Freitas, A., Coutinho, A., 1994 Observations on the mode of action of normal immunoglobulin at high doses. Immunol. Rev. **139**, 125-58.

Tacket, C.O., Losonsky, G., Link, H., Hoang, Y., Guesry, P., Hilpert, H., Levine, M.M., 1998. Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic *Escherichia coli*. N. Engl. J. Med. **318**, 1240-1243.

Torre, P.M., Oliver, S.P., 1989 Suppression of mitogenic response of peripheral blood mononuclear cells by bovine mammary secretions. J. Dairy Sci. **72**, 219-227.

Uruakpa, F.O., Ismond, M.A.H., Akobundu, E.N.T., 2002 Colostrum and its benefits: a review. Nutr. Res. **22**, 755-767.

Wong, C.W., Seow, H.F., Liu, A.H., Husband, A.J., Smithers, G.W., Watson, D.L., 1996 Modulation of immune responses by bovine beta-casein. Immunol.Cell Biol. **74**, 323-329.

Wong, C.W., Seow, H.F., Husband, A.J., Regester, G.O., Watson, D.L., 1997 Effects of purified bovine whey factors on cellular immune functions in ruminants. Vet Immunol. Immunopathol. **56**, 85-96.

Xu, R.J., Mellor, D.J., Tungthanathanich, P., Birtles, M.J., Reynolds, G.W., Simpson, H.V., 1992. Growth and morphological changes in the small and large intestine in piglets during the first three days after birth. J. Dev. Physiol. **18**, 161-172.

Yoshioka,. Y., Kudo, S., Nishimura, H., Yajima, T., Kishihara, K., Saito, K., Suzuki, T., Suzuki, Y., Kuroiwa, S., Yoshikai, Y., 2005 Oral administration of bovine colostrum stimulates intestinal intraepithelial lymphocytes to polarize Th1-type in mice. Int. Immunopharmacol. **5**, 581-590.

ARTICLE 2

EFFECT OF A BOVINE COLOSTRUM WHEY SUPPLEMENTATION ON GROWTH PERFORMANCE, FAECAL *ESCHERICHIA COLI* POPULATION AND SYSTEMIC IMMUNE RESPONSE OF PIGLETS AT WEANING

This second study was achieved to investigate the effect of bovine colostrum whey supplementation in weaned diet (20 g/kg) on the growth performance, the feed intake, the systemic immune response and the faecal *E. coli* populations of 96 newly weaned piglets housed in a conventional on-farm nursery.

During the first week of the trial, the piglets from the colostrum treatment showed improved growth performances, feed intake and a better feed efficiency. Over the same period, the piglets fed the colostrum treatment had also a 25 %-increase in circulating IgA compared to the control treatment.

This shows that bovine colostrum whey may be used in piglet weaning diet at a level of 20 g/kg during the first week post-weaning to reduce under-feeding and weight losses. However, further work is required to confirm the mechanism and minimum level of dietary inclusion of bovine colostrum to obtain performance enhancement.

EFFECT OF A BOVINE COLOSTRUM WHEY SUPPLEMENTATION ON GROWTH PERFORMANCE, FAECAL *ESCHERICHIA COLI* POPULATION AND SYSTEMIC IMMUNE RESPONSE OF PIGLETS AT WEANING

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Bovine colostrum supplementation in weaning piglet diet

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Abstract

This study examined the effect of a bovine colostrum whey supplementation on growth performance, feed intake, faecal *Escherichia coli* population and systemic immune response of piglets at weaning. A total of 96 piglets weaned at 26 ± 2 days of age were assigned for four weeks to one of the two treatments: 1) the control (commercial diet with bovine milk whey powder) and 2) the colostrum (commercial diet with freeze-dried bovine colostrum whey) treatments. The two supplements were incorporated in the diet at a level of 20 g/kg during the first 2 weeks after weaning and lowered to a level of 10 g/kg for the next 2 weeks. BW and feed intake were measured weekly. Faecal *E. coli* counts were determined weekly on specific culture media. Blood samples were collected weekly and submitted to a cell counter analyser for their main components (red and white blood cells, platelets) and flow cytometry was used to determine the lymphocyte population (B, T, Th and Tc). Finally, total seric immunoglobulin (IgM, IgG and IgA) concentrations were determined by the ELISA method. During the first week of the trial, the piglets from the colostrum treatment had improved average daily gain (170 g/day *vs.* 81 g/day, $P < 0.001$), average daily feed intake (346 g/day *vs.* 256 g/day, $P = 0.03$) and feed efficiency (BW gain/feed intake) (0.48 *vs.* 0.31, $P = 0.04$). The pigs fed the colostrum treatment had also a 25 %-increase in circulating IgA ($P = 0.03$) compared to the control treatment the first week. It is concluded that a distribution of bovine colostrum whey (20 g/kg diet) during the first week post-weaning induces a systemic IgA response and has a beneficial action on growth performances and feed efficiency.

Keywords: bovine colostrum, *Escherichia coli*, immunoglobulin, pigs, weaning

1. Introduction

At weaning, the piglet is exposed to nutritional and environmental stressors inducing marked structural and immunological changes in the gut. Structural changes, essentially villi atrophy and crypt hyperplasia, reduce the digestive and absorptive capacity of the small intestine and increase its sensitivity to infections (Pluske *et al.*, 1997). The immunological changes include an alteration of the intestinal immunity and the intestinal immune responses against dietary and bacterial antigens (King *et al.*, 2003). Moreover, composition and stability of the microflora undergo disruption in this period, leaving the piglet more susceptible to overgrowth of potentially disease-causing pathogenic bacteria, principally *E. coli* (Hopwood and Hampson, 2003 ; Melin *et al.*, 2004). This critical period has been controlled over decades by using in-feed antibiotics showing growth promoter properties. However, their total ban in the EU since January 2006 requires alternative solutions.

Active components of bovine colostrum may be of importance in this context. The most interesting include (i) growth promoters which promote the growth and development of the new-born and (ii) antimicrobial factors which provide passive immunity and protection against infections during the first week of life (Pakkanen and Aalto, 1997).
Beneficial effects of high level bovine colostrum supplementations (40 to 100 g/kg of diet) on growth performances and feed intake in piglets at weaning have already been described (Pluske *et al.*, 1999; King *et al.*, 2001; Le Huërou-Luron *et al.*, 2004). Observed effects were explained by both an increase in feed intake level (Le Huërou-Luron *et al.*, 2004) and a direct stimulation of the gut (Huguet *et al.*, 2006 and 2007). Nevertheless, action of bovine colostrum may also be related to its immuno-modulatory effects as some proteins isolated and purified from whey have been shown to be potent modulators of cellular immune functions in ruminant as well as in non ruminant species. Several studies have also shown that *in vivo* administration of bovine milk proteins to heterologous species can affect lymphocyte function and antibody responses (see review of Cross and Gill, 2000). In the weaned piglet, a previous study (Boudry *et al.*, 2007) suggested an influence of bovine colostrum on the development of the systemic IgA response by potentiating a Th2 response in the ileal Peyer's patch.

The objective of this study was to study further the action of bovine colostrum on the immune response in the piglet through the investigation of the effect of bovine colostrum whey supplementation in weaning diet (20 g/kg) on growth performances, feed intake and the systemic immune response of piglets at weaning. Faecal *E. coli* counts were also performed to follow the sanitary status of the piglets.

2. Materials and Methods

The experimental protocol used in this study was approved by the Animal Care and Use Committee (protocol No. 02/05) of Gembloux Agricultural University.

2.1. Animals

Ninety-six Belgian Piétrain × (Large White × Landrace) piglets weaned at 26 ± 2 days of age with an average BW of 8.3 ± 0.8 kg were selected from 15 litters.

2.2. Treatments

Two treatments were compared: i) a control diet (commercial diet with bovine milk whey powder) and ii) a colostrum diet (commercial diet with bovine colostrum whey powder). The commercial diet (SCAR, Herve, Belgium) was a starter diet free of any growth promoters. This diet was distributed the week before weaning to the 15 litters from which the piglets were selected for the trial. The two supplements were mixed with the commercial diet at a rate of 20 g/kg for the first 2 weeks of the trial and 10 g/kg for the next 2 weeks. The compositions of the experimental diets are given in the Table 1. The bovine colostrum whey used in this study was prepared from bovine colostrum standardised at 75 g of Ig per litre (Centre d'Economie Rurale, Marloie, Belgium). This colostrum was defatted by centrifugation. Whey was obtained after rennet coagulation at 37°C for 24 h and separation from curds by a mechanical press. The whey was then freeze-dried. The milk whey used was a commercial spray-dried powder (Euroserum, Port-sur-Soane, France). All pigs had *ad libitum* access to a four-hole feeding trough and a nipple drinker.

Table 1. Composition of the milk and colostrum diets

Ingredients	Control diets		Colostrum diets	
(g/kg feed)	20 g/kg	10 g/kg	20 g/kg	10 g/kg
Barley	247	249.5	247	249.5
Wheat	189	191	189	191
Soybean meal (49 % CP)	175.5	177	175.5	177
Nutribig premix[†]	147	148.5	147	148.5
Maize	98	99	98	99
Heat treated maize	49	49.5	49	49.5
Toasted Soybeans	41.5	42	41.5	42
Chicory pulp	24.5	24.2	24.5	24.2
Soybean oil	5	5	5	5
Synthetic amino acids and minerals[‡]	4.5	4.5	4.5	4.5
Milk whey powder	20	10	0	0
Colostrum whey powder	0	0	20	10
Chemical composition (g/kg DM)				
DM (g/kg feed)	869	871	867	870
Crude protein	182	183	194	189
Ether extract	35	34	34	34
Crude fiber	36	36	36	36
Starch	365	385	377	381
Ash	60	60	60	60
Lysine	9.7	9.3	10.1	9.3

[†] The premix (Roche Vitamins, Deinze, Belgium) is composed by 60 % of milk products, 12 % of oleaginous seeds, 10 % of cereal seeds by-products, 5 % of tuber and roots by-products and 12 % of minerals and vitamins (vitamins, minerals and amino acids supplied per kilogram of premix : Vitamin A, 100,000 IU; vitamin D3, 13,000 IU; vitamin E, 335 mg; vitamin K3, 9 mg; vitamin B1, 13 mg; vitamin B2, 34 mg; vitamin B3, 100 mg; vitamin B6, 20 mg; vitamin C, 302 mg, vitamin PP, 200 mg; folic acid, 2 mg; choline, 2,163 mg; iron (as $FeSO_4$), 1,332 mg; copper (as $CuSO_4$), 1,100 mg; manganese (as $MnSO_4$), 400 mg; cobalt (as $CoSO_4$), 7 mg; Zinc (as $ZnSO_4$), 1,583 mg; Iodine (as CaI_20_6), 14 mg; selenium (as Na_2SeO_4), 3 mg; Ca, 39,586 mg; P, 8,584 mg; Na, 8,100 mg; L-lysine HCl, 16,240 mg; DL-methionine, 6,630 mg; L-threonine, 2,990 mg; L-tryptophan, 260 mg; lysine, 22,740 mg; methionine, 8,994 mg; threonine, 10,217 mg; tryptophan, 2,352 mg).

[‡] Providing the following per kilogram of the complete diet (g): methionine, 0.25; lysine, 0.5; threonine, 0.5; tryptophan, 0.25; monocalcique phosphate, 3 g.

2.3. Experimental design

The animals were blocked according to BW and gender and assigned to one of the two treatments. For each treatment, the piglets were housed in four pens of 12 piglets (6 males, 6 females). Piglets from the same litters were distributed between the two treatments.

BW and feed consumption were evaluated weekly to determine the average daily gain (ADG), the average daily feed intake (ADFI) and the feed efficiency (G/F) which is obtained by the ratio: BW gain/feed intake. Piglets were weighed in the early morning without feed or water restriction.

2.4. Diet and whey analyses

The diets distributed during the trial were ground to pass a 1-mm screen (Cyclotec 1.093, Foss Tecator AB) before dry matter, ether extract, Kjeldahl N, crude fibre and ash analyses (AOAC, 1990) were conducted. Samples from the 4 diets were also ground to pass a 0.5 mm screen for analyse of lysine (AccQ-Tag, Waters, Milford, MS, USA) and starch (adapted from Faisant *et al.*, 1995). The same analyses were performed on the milk and bovine colostrum wheys. Additional analyses were conducted on both milk and colostrum wheys. IGF-I, IGF-II and Insulin concentrations were determined with sandwich ELISA quantitation kits (Diagnostics Systems Laboratories, Assendelft, The Netherlands) according to the manufacturer's procedure. Total IgG and lactoferrin concentrations were measured by Sandwich ELISA and reverse-phase HPLC (Shodex Asahipak C4P-50 4D column), respectively (Biopole, Les Isnes, Belgium). The results of the analysis on the experimental diets and the wheys are presented in Tables 1 and 2, respectively.

Table 2. Composition of the milk and colostrum wheys

Composition (g/kg DM)	Milk whey	Colostrum whey
DM (g/kg powder)	923	956
Crude protein	83.7	627
Ether extract	15	10
Ash	120	105
Lysine	4.9	43.4
IgG	2	496
Lactoferrin	< 0,1	10,6
IGF-I	33 ng/g	2500 ng/g
IGF-II	12 ng/g	25 ng/g
Insulin	< 1 ng/g	< 1 ng/g

2.5. Faecal *E. coli* counts

Fresh faeces were collected on one piglet in each of the 15 litters the day before weaning (day -1) as Katouli *et al.* (1995) showed a similarity in *E. coli* populations between littermates during suckling. After weaning, five piglets were randomly chosen in each pen and faeces were collected weekly on these piglets from the 4^{th} day after weaning until the end of the study (days 4, 11, 18 and 25). Faeces were collected by rectal massage. On the day of collection, 10 g of faeces were diluted to a concentration of 1/10 (weight/weight) using peptone water, then 10-fold serial dilutions were achieved. Finally, 100 µl of three successive dilutions of each sample were applied in duplicate to plates containing the culture media (Tryptone Bile X-glucuronide, Biokar Diagnostics, Beauvais, France) (6 plates by media and by sample). The dilutions varied from 10^{-3} to 10^{-7} g of faeces/ml, according to the results of the precedent week. Plates were incubated at 44° C for 24 h, in aerobic conditions, according to the manufacturer's procedure to determine the concentrations of *E. coli.* Only the plates containing 10 to 300 colonies were counted.

2.6. Blood collection

Blood samples from the jugular vein were collected into EDTA (Ethylene Diamine Tetra Acetic acid) and dry tubes. The day of weaning (day 0), blood was collected from

one piglet of each litter. These animals were then excluded from the experiment. On days 7 and 21, half of the experimental piglets in each pen were blood sampled. The other half was sampled on the days 14 and 28. This method of sampling was used to minimise the effect of blood sampling on measured parameters. On day 0, blood was taken on pigs that never entered the study to strictly limit the stress to that of weaning, which was the object of the study.

2.7. Blood analysis

Fresh blood collected with EDTA was analysed by a cell counter (MS4.5, MS Laboratoires, Cergy-pontoise, France) for red and white blood cells, hematocrit, haemoglobin and platelet concentrations.

The blood phenotype was analysed by flow cytometry (FACSCalibur flow cytometer, Becton Dickinson, San Jose, CA, US) for lymphocyte subpopulations (B, T, Th and Tc cells). Blood peripheral lymphocytes were isolated from fresh blood collected with EDTA by density centrifugation on Ficoll PM 400 (Sigma-Aldrich, Bornem, Belgium). The cells were then labelled with mouse antibodies directed against porcine leukocyte-differentiation antigens: anti-CD3ε, anti-CD4a, anti-CD8a and anti-CD21 (BD Pharmingen, San Diego, CA, US). The anti-CD3 antibodies were labelled with fluorescein isothiocyanate (FITC) and the three others with phycoerythrin (PE). Relative percentages of lymphocyte subpopulations T (CD3+, CD21-), B (CD3-, CD21+), Th (CD3+, CD4+) and Tc (CD3+, CD8+) were determined. A panel of FITC- and PE-labelled mouse IgG (Simultest control, BD, San Jose, USA) was used as negative control.

2.8. Total immunoglobulins

Blood serum was separated from cells by centrifugation ($1000 \times g$, 10 min) after clotting at 2°C for 24 h. Serum was then frozen at -20°C until use. Total serum IgM, IgG and IgA concentrations were determined with sandwich ELISA quantitation kits (Bethyl Laboratories, Montgomery, TX, US), according to the manufacturer's procedure, except for the solutions used to wash and dilute samples which were the solutions usually used in our lab and tested previously with the kits. Briefly, the analyses were carried out on 96-well ELISA microplates (Nunc 439454, VWR, Leuven, Belgium). Wells were

coated with 1 µg of capture antibody diluted in 100 µl of PBS and incubated for 60 min at room temperature. After three washes with PBST 0.2 % (PBS containing 0.05 % of Tween-20), a blocking solution (PBS/BSA 2 %) was added to block non-specific antigenic sites. Three new washes were performed. Samples and standards were diluted in a PBST 4 % solution, according to the expected concentrations of the studied antibody (serum dilutions 1/800 and 1/1600 for IgA and IgM, and 1/3200 and 1/6400 for IgG), and 100 µl of the preparation were incubated in the assigned wells for 60 min at room temperature. After five washes, the detection antibodies were added in each well for 60 min at room temperature. Wells were then washed five times with the washing solution and 100 µl of the enzyme substrate (TMB) was added for 30 min at room temperature. Finally the reaction was stopped with 100 µl of H_2SO_4 of 2N. The absorbance at 450 nm was determined in a microplate reader PR 5000 (Labsystems Multiskan RC, Helsinki, Finland), and the values for each standard were plotted against the concentration to produce a standard curve for the three antibodies. The concentration of the target samples were extrapolated from those curves.

2.9. Statistical analysis

For all the parameters, there were four repeated measures. However, for the blood parameters, analysis was separated in two groups of piglets with two replicates for each (day 7 and day 21 for the first half of the piglets and day 14 and day 28 for the second half of the piglets). Modelling of repeated records was done using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Analysis of variance tested treatment (control - colostrum) × time (days post-weaning) interactions. Effects were compared using the CONTRAST statement in the repeated MIXED analysis. The pen was used as the experimental unit for ADFI and G/F. For all the other parameters, the pigs were used as experimental unit. To fulfil the requirements of normality, a log10 transformation of the *E. coli* counts was performed. The values presented are lsmeans $\pm$ s.e. The differences were declared significant at $P < 0.05$.

3. Results

3.1. Growth performance

The ADG, ADFI and G/F for the 4-week trial are presented in Table 3. The ADG was higher for the colostrum whey supplemented pigs compared to the control piglets ($P < 0.001$) during the first week. The next 3 weeks, the ADG were similar. Finally, the ADG calculated on the total experimental period was higher for the piglets supplemented with bovine colostrum whey ($P = 0.02$). The ADFI and G/F per pen (n = 4) were greater in the first week of the trial for pigs fed the colostrum treatment compared to pigs fed the control diet (respectively $P = 0.03$ and $P = 0.04$). For the next 3 weeks and the entire 4-week trial, feed consumption and G/F were not affected by the colostrum treatment.

Table 3. BW, average daily gain (ADG), average daily feed intake (ADFI) and feed efficiency (G/F) of piglets fed a commercial diet containing milk (Control) or bovine colostrum whey powder for 4 weeks

Measurements and days		Treatments		s.e.	Significance
		Control	Colostrum		
BW, kg	*(n=48)*				
0		8.34	8.33	0.19	NS[†]
7		8.89	9.51	0.24	*
14		11.0	11.5	0.29	NS
21		14.0	14.5	0.35	NS
28		17.6	18.4	0.45	NS
Significance		*Time*Treatment *** , Time ***, Treatment NS*			
ADG, g/day	*(n=48)*				
0 to 7		81	170	15.6	***
7 to 14		297	280	14.4	NS
14 to 21		430	434	17.6	NS
21 to 28		516	548	18.9	NS
0 to 28		*330*	*361*	*11.3*	*
Significance		*Time*Treatment *** , Time ***, Treatment **			
ADFI, g/day	*(n=4)*				
0 to 7		256	346	38.5	*
7 to 14		497	495	35.8	NS
14 to 21		791	822	46.5	NS
21 to 28		974	992	76.2	NS
0 to 28		*623*	*665*	*43.2*	NS
Significance		*Time*Treatment *** , Time ***, Treatment NS*			
G /F , g/g	*(n=4)*				
0 to 7		0.31	0.48	0.078	*
7 to 14		0.56	0.61	0.023	NS
14 to 21		0.55	0.52	0.038	NS
21 to 28		0.56	0.54	0.032	NS
0 to 28		*0.53*	*0.54*	*0.029*	NS
Significance		*Time*Treatment NS , Time ***, Treatment NS*			

[†] NS: $P > 0.05$

3.2. Faecal microflora

Results of *E. coli* counts are presented in Table 4. No differences between treatments were shown. A high variability was observed between animals and over the time, but no diarrhoea was observed on piglets during the experimental period.

Table 4. Faecal *E. coli sp.* populations (log10 cfu/g of faeces) in piglets fed a commercial diet containing milk (Control) or bovine colostrum whey powder for 4 weeks

Days		Treatments		s.e.	Significance
		Control	Colostrum		
-1	(n=15)	8.12 ± 0.59 †			
4	(n=20)	7.11	6.72	0.29	NS‡
11	(n=20)	6.26	6.02	0.30	NS
18	(n=20)	6.01	5.49	0.35	NS
25	(n=20)	7.44	7.71	0.14	NS
Significance		*Time*Treatment NS , Time ***, Treatment NS*			

† The values on day -1 (mean ± s.d.) were measured on naive piglets coming from the litters in which the piglets were selected for the trial

‡ NS: $P > 0.05$

3.3. Blood parameters

There was no difference ($P > 0.05$) between the dietary treatments in the red and white blood cells, hematocrit and haemoglobin concentrations (data not shown). Phenotyping of the blood lymphocytes, as presented in Table 5, showed a reduction in B cells on day 21 in the control treatment and a reduction of Tc cells on day 7 in the colostrum treated piglets. Moreover, whereas Th population decreased after weaning, Tc increased.

Total IgM, IgG and IgA concentrations are given in Table 6. The total IgM and IgG levels were not influenced by dietary treatment ($P > 0.05$), but IgA concentrations were higher on day 7 (+25 %, $P = 0.03$) for pigs fed the colostrum diet.

Table 5. Relative percent of lymphocyte subpopulations (B, T, Th and Tc) in the blood serum of piglets fed a commercial diet containing milk (Control) or bovine colostrum whey powder for 4 weeks

Measurement and days		Treatments		s.e.	Significance
		Control	Colostrum		
B cells (CD3-,CD21+)					
0	(n=15)	19.5 ± 6.89[†]			
7	(n=24)	12.3	14.6	1.80	NS[‡]
14	(n=24)	16.7	15.8	1.71	NS
21	(n=24)	12.9	15.8	1.27	*
28	(n=24)	14.7	15.4	2.37	NS
Significance		*Time*Treatment NS , Time NS, Treatment NS*			
T cells (CD3+,CD21-)					
0	(n=15)	51.0 ± 7.32[†]			
7	(n=24)	58.4	53.2	3.32	NS
14	(n=24)	57.8	52.8	4.64	NS
21	(n=24)	56.4	52.9	2.23	NS
28	(n=24)	53.2	53.7	4.71	NS
Significance		*Time*Treatment NS , Time NS, Treatment NS*			
Th cells (CD3+,CD4+)					
0	(n=15)	28.2 ± 4.95[†]			
7	(n=24)	33.6	31.1	2.73	NS
14	(n=24)	29.8	27.9	2.28	NS
21	(n=24)	27.0	28.1	1.97	NS
28	(n=24)	24.6	25.0	1.99	NS
Significance		*Time*Treatment NS , Time **, Treatment NS*			
Tc cells (CD3+,CD8+)					
0	(n=15)	19.96 ± 7.58[†]			
7	(n=24)	21.2	16.1	2.01	*
14	(n=24)	21.0	21.6	2.40	NS
21	(n=24)	23.5	21.7	4.67	NS
28	(n=24)	30.4	28.4	4.01	NS
Significance		*Time*Treatment NS , Time **, Treatment NS*			

[†] The values on day 0 (mean ± s.d.) were measured on naive piglets coming from the litters in which the piglets were selected for the trial

[‡] NS: $P > 0.05$

Table 6. Immunoglobulins (IgM, IgG and IgA) concentrations in the blood serum of piglets fed a commercial diet containing milk (Control) or bovine colostrum whey powder for 4 weeks

Measurement and days		Treatments		s.e.	Significance
		Control	Colostrum		
IgM (mg/ml)					
0	(n=15)	1.13 ± 0.21[†]			
7	(n=24)	1.28	1.31	0.069	NS[‡]
14	(n=24)	1.56	1.59	0.097	NS
21	(n=24)	2.65	2.90	0.194	NS
28	(n=24)	2.61	2.56	0.131	NS
Significance		*Time*Treatment NS , Time ***, Treatment NS*			
IgG (mg/ml)					
0	(n=15)	7.07 ± 1.03[†]			
7	(n=24)	8.00	8.22	0.30	NS
14	(n=24)	11.4	11.2	0.76	NS
21	(n=24)	10.3	10.6	0.67	NS
28	(n=24)	12.1	11.2	0.64	NS
Significance		*Time*Treatment NS , Time ***, Treatment NS*			
IgA (mg/ml)					
0	(n=15)	0.18 ± 0.03[†]			
7	(n=24)	0.22	0.28	0.027	*
14	(n=24)	0.38	0.35	0.029	NS
21	(n=24)	0.40	0.45	0.027	NS
28	(n=24)	0.71	0.71	0.035	NS
Significance		*Time*Treatment NS , Time ***, Treatment NS*			

[†] The values on day 0 (mean ± s.d.) were measured on naive piglets coming from the litters in which the piglets were selected for the trial

[‡] NS: $P > 0.05$

4. Discussion

4.1. Composition of both supplements

The analyses of both supplements show an important difference in the concentration of crude proteins between the two wheys (8.37 % for milk whey *vs.* 62.7 % for colostrum whey) which could be mainly explained by the concentration in IgG (2 g/kg in milk whey *vs.* 496 g/kg in colostrum whey). Higher concentrations in lactoferrin and IGF-I were also measured in the colostrum whey. These results indicated that the latter contains higher concentrations in growth promoters and antimicrobial factors than milk whey.

4.2. Growth promoting activity of bovine colostrum

The inclusion of bovine colostrum whey in the weaning diet improved growth performances, feed intake and G/F (respectively by 100, 30 and 50 %) the first week after weaning. These results corroborate observations made by Pluske *et al.* (1999), King *et al.* (2001) and Le Huërou-Luron *et al.* (2004) who measured, on weaning piglets fed with diets containing 40 to 100 g/kg of bovine colostrum extracts, increases in ADG from 20 to 115 % and for ADFI from 10 to 30 % during the first 10 days post-weaning. However in our study, a lower supply of bovine colostrum (20 g/kg feed) increased growth performances and improved feed intake at comparable levels to those reported by the previous authors. These observations suggest that the effects of bovine colostrum on performance and feed intake of piglets at weaning may be obtained with a lower level of supplementation. Nevertheless, the differences in the results may also be explained by the composition of the bovine colostrum used, as little information is given about the preparation and the composition of the colostrum extracts experimented in the above cited studies. Indeed, the action of the colostrum may be related to its composition in growth factors (e.g. epidermal growth factors (EGF), IGF-I, transforming growth factors-β (TGF- β)). Xu *et al.* (2002) reported in newborn piglets a regulatory role for the colostrum growth factors in stimulating intestinal tissue growth. In the newly-weaned piglet, Le Huërou-Luron *et al.* (2003) and Huguet *et al.* (2006 and 2007) showed an effect of bovine colostrum on the digestive and absorptive capacity of

the small intestine which may explain the improved G/F observed in the colostrum treatment.

4.3. Antimicrobial activity of bovine colostrum

Successful use of colostrum in the treatment of diarrhoea caused by *E. coli* has been reported in human patients (Carbonare *et al.*, 1997 ; Honorio-Franca *et al.*, 1997). Colostrum and milk wheys contain antimicrobial components effective against *E. coli* such as lactoferrin (Saito *et al.*, 1991 ; Erdei *et al.*, 1994), lactoperoxidase (Reiter, 1985) and lysozyme (Yamauchi *et al.*, 1993). Despite the higher concentration of lactoferrin in bovine colostrum whey compared to milk whey, no difference between the total *E. coli* populations with the two treatments was observed. This may be due to the absence of post-weaning diseases during this study.

As the IgG may also act as an antimicrobial component by preventing viruses and bacterial from damaging the gut wall, thereby resulting in a more functional intestinal wall (Coffey and Cromwell, 2001), the far higher concentration of bovine colostrum whey in IgG compared to milk whey can also have improved ADG, ADFI and G/F. This is confirmed by Pierce *et al.* (2005) who showed that the IgG fraction of bovine plasma increased growth rate and feed intake of piglets during the early post-weaning period.

4.4. Systemic immune response to bovine colostrum

The main effect of bovine colostrum observed in this study on the systemic immune response of the piglets is an increase in seric IgA concentrations the first week post-weaning. In a previous study (Boudry *et al.*, 2007), an increase in seric IgA was also observed after bovine colostrum distribution to weaned piglets, however this increase occurred 3 weeks after weaning. Many differences between this study and the previous one may be responsible for this early immune response: (i) the sanitary conditions of the experiment (on-farm facility with continuous pig flow *vs.* university facility without other pigs), (ii) the weaning age (28 days *vs.* 21 days) and (iii) the piglet origin (production farm *vs.* selection farm). In our previous study, results indicated an influence of bovine colostrum on the development of the systemic IgA response by

potentiating a Th2 response in the ileal Peyer's patch. IgA is the mostly produced isotype in the intestine, with more than 80 % of the intestinal Ig secreting-cells producing IgA (Bianchi *et al.*, 1999). The increase in blood IgA may be due to an increase in intestinal IgA synthesis, as Vaerman *et al.* (1997) demonstrated that roughly 30 % of the total plasma IgA originated daily from local intestinal synthesis.

Gill and Rutherfurd (1998) reported that oral administration of bovine milk proteins to heterologous species can enhance localised antibody responses to heterologous orally delivered antigens. Therefore, the administration of bovine colostrum which is enriched in proteins can have reduced the sensitivity of the weaned pig to post-weaning infection. Among these proteins, Chun *et al.* (2004) showed that TGF-β2 is the most potent cytokine in the induction of IgA isotype switching in mesenteric lymph node cells of BALB/c mices. Van Vlasselaer *et al.* (1992) showed the same effect of porcine TGF-β1 on the IgA production by human splenic B cells, but no effect on IgG and IgM production. Elfstrand *et al.* (2002) showed that this cytokine is present in higher concentrations in bovine colostrum than in milk (289 ng/ml 0-6 h *post partum* *vs.* 66 ng/ml 51-80 h *post partum*) and that 67 % of it is conserved in freeze-dried whey.

The difference in blood IgA between the two treatments disappeared the second week post-weaning, simultaneously with the difference in feed intake and growth performance. This suggests a relationship between feed intake and the stimulation of systemic and gut IgA production in the first days post-weaning, but no information confirming this postulate was found in the literature.

The phenotyping of the blood lymphocytes showed a reduction of the Tc population on day 7 in the colostrum-fed piglets, suggesting a cytokine-profile related effect. The Tc cells presence is commonly associated to a Th1 immune response whereas a Th2 profile leads to a decrease in this population (McGee and Agrawal, 2006). We previously demonstrated a more marked Th2 immune profile in the ileal Peyer's patch of colostrum-fed piglets with the enhanced production of IL-4 and IL-10 (Boudry *et al.*, 2007), this Th2 profile could be responsible of the decrease of the Tc cells by interfering on IFNγ and IL-12 production (Romagnani, 1991).

Statistical analysis showed also an effect of the colostrum treatment on B cells. However in our study, the sampling protocol did not allow to conclude if this observation is the consequence of the treatments or if they are related to the variability among the piglets. To clarify this point, in further studies, blood samples should be collected on the same animals throughout the experiment.

5. Conclusion

Our study demonstrated that bovine colostrum whey may be used in piglet weaning diet at a level of 20 g/kg during the first week post-weaning to reduce under-feeding and weight losses. The supplementation also induced an increase of the total seric IgA level 7 days post-weaning. The performance response may be in part mediated by an increase in seric IgA. Further work is required to confirm the mechanism and minimum level of dietary inclusion of bovine colostrum to obtain performance enhancement.

References

Association of Official Analytical Chemists 1990. *Official Methods of Analysis.* 15th edition. AOAC, Arlington, VA, USA.

Bianchi ATJ, Scholten JW, Moonen Leusen BHW and Boersma WJA 1999. Development of the natural response of immunoglobulin secreting cells in the pig as a function of organ, age and housing. *Developmental and Comparative Immunology* 23, 511-520.

Boudry C, Buldgen A, Portetelle D, Collard A, Théwis A and Dehoux J-P 2007. Effects of oral supplementation with bovine colostrum on the immune system of weaned piglets. *Research in Veterinary Science* 83, 91-101..

Carbonare SB, Silva ML, Palmeira P and Carneiro-Sampaio MM 1997. Human colostrum IgA antibodies reacting to enteropathogenic Escherichia coli antigens and their persistence in the faeces of a breastfed infant. *Journal of Diarrhoea Disease Research* 15, 53-58.

Chun SK, Nam MS, Goh JS, Kim WS, Han YH and Kim PH 2004. Kinetics and biological function of transforming growth factor-β isoforms in bovine and human colostrum. *Journal of Microbiology and Biotechnology* 14, 1267-1274.

Coffey RD and Cromwell GL 2001. Use of spray-dried animal plasma in diets for weanling pigs. *Pig News Inf.* 22, 39N-38N.

Cross ML and Gill HS 2000. Immunomodulatory properties of milk. *British Journal of Nutrition* 84, S81-S89.

Elfstrand L, Lindmark-Mansson H, Paulsson M, Nyberg L and Akesson B 2002. Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing. *International Dairy Journal* 12, 879-887.

Erdei J, Forsgren A and Naidu AS 1994. Lactoferrin binds to porins Ompf and Ompc in Escherichia coli. *Infection and Immunity* 62, 1236-1240.

Faisant N, Planchot V, Kozlowski F, Pacouret M-P, Colonna P and Champ M 1995. Resistant starch determination adapted to products containing high level of resistant starch. *Sciences des Aliments* 15, 83-89.

Gill HS and Rutherfurd KJ 1998. Immunomodulatory properties of bovine milk. *Bulletin of the International Dairy Federation* 336, 31-35.

Honorio-Franca AC, Carvalho MP, Isaac L, Trabulsi LR and Carneiro-Sampaio MM 1997. Colostral mononuclear phagocytes are able to kill enteropathogenic Escherichia coli opsonised with colostral IgA. *Scandinavian Journal of Immunology* 46, 59-66.

Hopwood DE and Hampson DJ 2003. Interactions between the intestinal microflora, diet and diarrhoea, and their influences on piglet health in the immediate post-weaning period. In *Weaning the pig. Concepts and consequences* (eds JR Pluske, J Le Dividich and MWA Verstegen), pp. 199-218. Wageningen Academic Publishers, Wageningen, The Netherlands.

Huguet A, Sève B, Le Dividich J and Le Huërou-Luron I 2006. Effects of a bovine colostrum-supplemented diet on some gut parameters in weaned piglets. *Reproduction Nutrition Development* 46, 167-178.

Huguet A, Le Normand L, Fauquant J, Kaeffer B and Le Huërou-Luron I 2007. Influence of bovine colostrum on restoration of intestinal mucosa in weaned piglets. *Livestock Science* 108, 20-22.

Katouli M, Lund A, Wallgren P, Kühn I, Soderlind O and Mollby R 1995. Phenotypic characterization of intestinal *Escherichia coli* of pigs during suckling, postweaning, and fattening periods. *Applied and Environmental Microbiology* 61, 778-783.

King MR, Morel PCH, James EAC, Hendriks WH, Pluske JR, Skilton R and Skilton G 2001. Inclusion of colostrum powder and bovine plasma in starter diets increase volontary feed intake. In *Manipulating Pig Production VIII*, pp. 213. Australian Pig Science Association, Werribee, Australia.

King RH, Kelly D, Morel PCH and Pluske JR 2003. Aspects of intestinal immunity in the pig around weaning. In *Weaning the pig. Concepts and consequences* (eds JR Pluske, J Le Dividich and MWA Verstegen), pp. 219-258. Wageningen Academic Publishers, Wageningen, The Netherlands.

Le Huërou-Luron I, Marion J, Bebin K, Thomas F, Ganier P, Thibault JN and Le Dividich J 2003. Supplementation of a weaning diet with a bovine colostrum extract minimise post-weaning duodenal structural, but not functional, alterations in 7-d old piglets. In *the 9th International Symposium on Digestive Physiology in Pigs* (ed RO Ball), Vol 2, pp 238-240. University of Alberta, Edmonton, Alberta, Canada.

Le Huërou-Luron I, Hughet A, Callarec J, Leroux T and Le Dividich J 2004. La supplémentation de l'aliment de sevrage en colostrum bovin améliore l'ingestion et les performances zootechniques chez les porcelets au sevrage. *Journées de la Recherche Porcine en France* 36, 33-38.

McGee HS and Agrawal DK 2006. Th2 cells in the pathogenesis of airway remodelling: regulatory T cells a plausible panacea for asthma. *Immunologic Research* 35, 219-232.

Melin L, Mattson S, Katouli M and Wallgren P 2004. Development of post-weaning diarrhoea in piglets. Relation to presence of *Escherichia coli* strains and Rotavirus. *Journal of Veterinary Medicine Series B* 51, 12-22.

Pakkanen R. and Aalto J 1997. Growth factors and antimicrobial factors of bovine colostrum. *International Dairy Journal* 7, 285-297.

Pierce JL, Cromwell GL, Lindemann MD, Russell LE and Weaver EM 2005. Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned piglets. *Journal of Animal Science* 83, 2876-2885.

Pluske JR, Hampson DJ and Williams IH 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livestock Production Science* 52, 215-236.

Pluske JR, Pearson G, Morel PCH, King MR, Skilton G and Skilton R 1999. A bovine colostrums product in a weaner diet increases growth and reduces day to slaughter. In *Manipulating Pig Production VII*, pp. 256. Australian Pig Science Association, Werribee, Australia.

Reiter B 1985. The biological significance of the non-immunoglobulin protective proteins in milk: lysozyme, lactoferrin, lactoperoxydase. *Development in Dairy Chemistry* 3, 281-336.

Romagnani S 1991. Type 1 T helper and Type 2 T helper cells: functions, regulation and role in protection and disease. *Journal of Clinical Laboratory Research* 21, 152-158.

Saito H, Miyakawa H, Tamura Y, Shimamura S and Tomita M 1991. Potent bactericidal activity of bovine lactoferrin hydrolysate produced by heat treatment at acidic pH. *Journal of Dairy Science* 74, 3724-3730.

Vaerman JP, Langendries A, Pabst R and Rothkötter HJ 1997. Contribution of serum IgA to intestinal lymph IgA, and vice versa, in minipigs. *Veterinary Immunology and Immunopathology* 58, 301-308.

Van Vlasselaer P, Punnonen J and De Vries JE 1992. Transforming growth factors-β directs IgA switching in human B cells. *The Journal of Immunology* 148, 2062-2067.

Xu RJ, Sangild PT, Zhang YQ and Zhang SH 2002. Bioactive compounds in porcine colostrum and milk and their effects on intestinal development in neonatal pigs. In *Biology of the Intestine in growing animals* (eds R Zabielsky, PC Gregory and B Weström), Vol 1, pp. 169-192. Elsevier Science B.V., Amsterdam, The Netherlands.

Yamauchi K, Toimita M, Giehl TJ and Ellison RT 1993. Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. *Infection and Immunity* 61, 719-728.

CHAPTER IV

REDUCTION OF THE COSTS OF THE USE OF BOVINE COLOSTRUM IN THE WEANED PIGLET DIET

ARTICLE 3

WAYS TO LOWER THE COST OF BOVINE COLOSTRUM SUPPLEMENTATION IN WEANED PIGLET DIET

To reduce the costs of the bovine colostrum supplementation in the weaning diet, two studies were achieved; the first studied three levels of bovine colostrum whey supplementation (0, 1 and 2 %) in the diet and the second compared bovine colostrum whey to defatted bovine colostrum, a 50 % less expensive fraction, on newly-weaned piglets.

Results of the first experiment confirmed the effects of bovine colostrum on the post-weaning growth check and showed similar effects between 1 and 2 % of supplementation. In the second experiment, the piglets receiving the defatted bovine colsotrum showed better growth performance and feed intake than the whey-fed piglets.

We may conclude from both experiments that it is possible to reduce the costs of BC use in weaning pig diet by reducing the dose of supplementation in the diet (1 % *vs.* 2 %), by shortening the administration period (from 28 to 10 days) and by using defatted BC in place of BC whey. By this way, the treatment costs were reduced from 19 € per piglet in the first experiment (2 % of bovine colsotrum whey for 28 days) to 1.3 € in the second one (1 % of defatted bovine colostrum for 10 days).

WAYS TO LOWER THE COST OF BOVINE COLOSTRUM SUPPLEMENTATION IN WEANED PIGLET DIET

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Abstract

It has been shown that the use of bovine colostrum (BC) whey in piglet diet reduces the post-weaning (PW) "growth check". Two experiments were conducted to reduce the costs of BC use.

The first experiment evaluated the effect of three doses of BC whey supplementation (0 (Whey 0), 10 (Whey 1) and 20 (Whey 2) $g.kg^{-1}$ of diet) on the growth performance and the feed ingestion of 117 newly weaned piglets (24 days, 7.2 kg) for 28 days. The first week PW, an increase of growth performance, feed intake and feed efficiency in the "Whey 1" and "Whey 2" treatments was observed compared to the "Whey 0" treatment. However, there were no differences between the "Whey 1" and "Whey 2" treatments, suggesting that the supplementation level can be limited to 10 $g.kg^{-1}$ of diet. During the next 3 weeks, no effect of the BC whey supplementation was observed.

In the second experiment, 96 piglets weaned at 26 ± 2 days of age (8.1 kg) were assigned for 10 days to three commercial treatments supplemented with 10 $g.kg^{-1}$ of: i) milk ("Milk 1"), ii) defatted colostrum ("Col 1") and iii) whey ("Whey 1"). BW and feed intake were measured two times a week for three weeks. Faecal *Lactobacilli spp.* and *E. coli* counts were determined the day before weaning and on days 2, 5 and 8 PW by real time PCR. Compared to "Whey 1" and "Milk 1" treatments, the "Col 1" treatment induced 25 % average daily gain ($P < 0.10$) and average daily feed intake ($P < 0.05$) increases from day 4 to 7 PW. It is conclude from these experiments that the costs of BC incorporation in weaning diet may be lowered by reducing the period and the dose of supplementation and by using defatted BC (50 % less expensive) in place of BC whey without lowering the effects on the PW growth check.

Keywords: bovine colostrum, cost-efficiency, growth performance, piglet, weaning

Implications

In a previous study (*Animal* **2**, 730-737), we demonstrated the efficiency of freeze-dried bovine colostrum whey to reduce the piglet post-weaning growth check. In this paper, we show that it is possible to reduce the costs of the use of colostrum in weaning piglet diet, without affecting the effects on the post-weaning growth check, by reducing the dose and the duration of supplementation and by replacing whey with defatted colostrum, which is 50 % less expensive to produce.

1. Introduction

At weaning, the piglet is exposed to nutritional and environmental stressors inducing marked structural and immunological changes in the gut (Pluske *et al.*, 1997; King *et al.*, 2003). Composition and stability of the gut microflora undergo disruption during post-weaning (PW), leaving the piglet more susceptible to overgrowth of potentially disease-causing pathogenic bacteria, principally *E. coli* (Hopwood and Hampson, 2003; Melin *et al.*, 2004). This critical period has been managed over decades by using in-feed antibiotics as growth promoter. Increased bacterial resistance to antibiotics led the European Union to implement a full ban on in-feed antibiotics from January 2006. Efficient alternatives are therefore to be found to conform to this policy change.

Active components of bovine colostrum (BC) may be of importance in this context. The most interesting include (i) growth factors which enhance the growth and development of the new-born and (ii) antimicrobial factors which provide passive immunity and protection against infections during the first weeks of life (Pakkanen and Aalto, 1997).

Several studies showed the beneficial effect of a BC supplementation on the performance and feed ingestion of piglets at weaning (reviewed by Boudry *et al.*, 2008a). In most of these studies, high incorporation rates of BC products in the diets were tested (from 4 to 10 %). In a previous study, Boudry *et al.* (2008b) showed that with a 2 % supplementation of freeze-dried BC whey in piglet diet, the PW growth check was lowered. However, the price of BC whey powder is so (100 €/kg, Banque de colostrum, CER, Marloie, Belgium) that it limits its use in pig production.

In this context, two experiments were conducted to reduce the costs of BC incorporation in piglet weaning diet.

2. Materials and Methods

The experimental protocols were approved by the Animal Care and Use Committee (protocols No. 02/05 and 07/01) of the Gembloux Agricultural University.

2.1. Experiment 1

This first experiment aimed to compare the effects of increasing concentrations of BC whey in weaning diet.

2.1.1. Animals

A total of 117 Belgian Piétrain x (Large White x Landrace) piglets, weaned at 24 ± 2 days of age (average BW of 7.2 ± 0.8 kg) were selected from 18 litters from the herd of the Walloon Agricultural Research Centre (Gembloux, Belgium).

2.1.2. Treatments

Three treatments were prepared with a commercial weaning diet and increasing concentrations in BC whey (Table 1): i) 0 ("Whey 0"), ii) 10 ("Whey 1") and iii) 20 $g.kg^{-1}$ ("Whey 2"). The treatments were distributed to the piglets for four weeks and all the litters used for the trial received the commercial diet without BC whey one week before weaning. All pigs had *ad libitum* access to a four-hole feeding trough and a nipple drinker.

2.1.3. Experimental design

The animals were randomised according to BW and gender and assigned to one of the three treatments. For each treatment, the piglets were housed in 3 pens of 13 piglets (7 males, 6 females) in a conventional on-farm nursery with continuous pig flow (Department of Animal Productions and Nutrition, Walloon Agricultural Research Centre, Gembloux, Belgium*)*. Littermates were distributed between the three treatments and the pens.

BW and feed intake were evaluated weekly (on days 0, 7, 14, 21 and 28 PW) to determine the average daily gain (ADG), the average daily feed intake (ADFI) and the feed conversion ratio (FCR) calculated as the ratio between feed intake and body weight

gain. Piglets were weighed in the early morning without feed or water restriction. During the first week of the trial, feed intake measures and visual controls of diarrhoea were performed daily.

2.2. Experiment 2

This second experiment was conducted to evaluate the effect of a less expensive BC fraction than the whey.

2.2.1. Animals

Ninety-six Belgian Piétrain × (Large White × Landrace) piglets weaned at 26 ± 2 days of age (average BW of 8.1 ± 0.8 kg) were selected from 23 litters from the same herd as previously.

2.2.2. Treatments

Three experimental treatments were compared (Table 1): i) a control treatment (commercial diet with 1 % of freeze-dried defatted bovine milk, "Milk 1"), ii) a colostrum treatment (commercial diet with 1 % of defatted BC, "Col 1") and iii) a whey treatment (commercial diet with 1 % of BC whey powder, "Whey 1"). The commercial diet (SCAR, Herve, Belgium) was the same as previously and was distributed the week before weaning to the 23 litters from which the piglets were selected for the trial. The supplements were mixed with the commercial diet at a rate of 10 g/kg for the 10 first days PW. Afterwards the commercial diet was distributed without any supplement. All pigs had *ad libitum* access to a three-hole feeding trough and a nipple drinker.

Table 1. Composition of the diets used in the two experiments

Experiment	1	1 and 2	1	2	2
Ingredients (g/kg feed)	Whey 0	Whey 1	Whey 2	Milk 1	Col 1
Wheat	299	296	293	296	296
Barley	250	247.5	245	247.5	247.5
Toasted Soybeans	150	148.5	147	148.5	148.5
Premix 10 %[†]	100	99	98	99	99
Soybean meal (49 % CP)	80	79	78	79	79
Maize	50	49.5	49	49.5	49.5
Wheat feedmeal	34	33.6	33.3	33.6	33.6
Beat pulp	25	25	24.5	25	25
Soybean oil	8	8	8	8	8
Dicalcium phosphate	3	3	3	3	3
L-Lysine HCl	0.8	0.7	0.7	0.7	0.7
Fumaric acid	1	1	1	1	1
Tryptophan	0.05	0.05	0.05	0.05	0.05
Bovine colostrum whey powder	0	10	20	0	0
Defatted bovine colostrum powder	0	0	0	0	10
Defatted bovine milk powder	0	0	0	10	0
Chemical composition (g/kg DM)					
DM (g/kg feed)	860	862	863	864	860
Crude protein	185	188	190	183	189
Ether extract	55	53	54	54	53
Crude fibre	40	36	37	36	36
Starch	376	382	380	371	370
Ash	62	62	62	64	62
Lysine	12.4	13.1	13.3	12.4	13.1
Tryptophan	3.6	3.6	3.6	3.6	3.6

[†] The premix (INVE België NV, Baasrode, Belgium) is composed by 33 % of milk products, 21 % of cereal seeds by-products, 10 % of tuber and roots by-products and 35 % of minerals and vitamins (vitamins, minerals and amino acids supplied per kilogram of premix : Vitamin A, 150,000 IU; vitamin D3, 20,000 IU; vitamin E, 800 mg; vitamin K3, 20 mg; vitamin B1, 30 mg; vitamin B2, 75 mg; vitamin B5, 250 mg; vitamin B3, 400 mg; vitamin B6, 40 mg; vitamin B12, 0.4 mg; biotine, 2 mg; folic acid, 5 mg; choline, 6,600 mg; iron (as $FeSO_4$ H_2O), 0.11 %; copper (as $CuSO_4(H_2O)_5$), 0.16 %; manganese (as MnO), 0.08 % mg; cobalt (as $CoCO_3$ H_2O), 0.001 %; Zinc (as $ZnSO_4$ H_2O), 0.1 %; Iodine (as CaI_2O_6), 0.002 %; selenium (as Na_2SeO_4), 0/0004 %; lysine (as L-lysine HCl), 3.35 %; methionine (as DL-methionine), 1.43 %; threonine (as L-threonine), 1.61 %; tryptophan (as L-tryptophan), 0.098 %.

2.2.3. Experimental design

The animals were randomised according to BW and gender and assigned to one of the three treatments. For each treatment, the piglets were housed in 8 pens of 4 piglets (2 males, 2 females) in an off-site facility of Gembloux Agricultural University. Littermates were distributed between the two treatments and the pens.

BW and feed consumption were evaluated on days 0, 4, 7, 10, 14, 21 and 28 PW to determine the ADG, the ADFI and the FCR. Piglets were weighed in the early morning without feed or water restriction. Moreover, each day of the first week of the trial, the feed intake was measured and a visual control of diarrhoea was performed

2.2.4. Faecal collection

Fresh faeces were collected by rectal massage on two piglets in each pen the day of weaning and on days 2, 5, 8, 12, 15 and 22 PW (each day, the same piglets were sampled). On the day of collection, 0.5 g of the faeces of each piglet from the same pen were pooled in a centrifuge tube of 15 ml and conserved at -20°C. The rest of the faeces were dried at 60°C to determine their water content.

2.3. Bovine colostrum and milk powders preparation

The BC powders used in these studies were prepared from BC standardised at 75 g of immunoglobulins (Ig) per litre (Centre d'Economie Rurale, Marloie, Belgium). This colostrum was defatted by centrifugation and freeze-dried to obtain defatted BC powder. The BC whey was obtained after centrifugation and rennet coagulation at 37°C for 24 h, separation from curds by a mechanical press, and finally freeze-dried. The bovine milk powder was prepared from fresh milk by the same process as for the defatted BC powder. The processing costs and the composition of the powders are given in Table 2.

Table 2. Composition and processing costs of the defatted bovine milk (DBM) and colostrum (DBC) and the bovine colostrum whey (BCW) powders

Composition (g/kg DM)	DBM	DBC	BCW
DM (g/kg powder)	914	935	899
Crude protein	374	765	688
Ether extract	31	23	11
Ash	78.2	56.5	72
Lactose	43	43	0.1
Lysine	28.6	56.1	43.7
Tryptophan	5.0	14.9	15.5
IgG	6	324	496
Lactoferrin	1.8	14.3	10.6
IGF-I	61 ng/g	2 750 ng/g	2 500 ng/g
IGF-II	16 ng/g	23 ng/g	25 ng/g
Insulin	9.9 ng/g	54 ng/g	< 1 ng/g
Processing costs (€/kg of powder)	50	50	100

2.4. Diet and supplement analyses

The diets distributed during the three experiments were ground to pass a 1-mm screen (Cyclotec 1.093, Foss Tecator AB, Hillerod, Denmark) before dry matter, ether extract, Kjeldahl N, crude fibre and ash analyses (AOAC, 1990) were conducted. The diet samples were also ground to pass a 0.5 mm screen for analyse of lysine (AccQ-Tag, Waters, Milford, MS, USA) and starch (Faisant *et al.*, 1995). The same analyses were performed on the BC and milk supplements. Additional analyses were realised on the latter. IGF-I, IGF-II and insulin concentrations were determined with sandwich ELISA quantitation kits (Diagnostics Systems Laboratories, Assendelft, The Netherlands) according to the manufacturer's procedure. Total IgG and lactoferrin concentrations were measured by Sandwich ELISA (Biopole, Les Isnes, Belgium) and reverse-phase HPLC using a Shodex Asahipak C4P-50 4D column (Showa Denko K.K., Kawasaki, Japan).

2.5. Microbiological analysis of faeces by real-time PCR

2.5.1. DNA extraction

DNA extraction was performed on the faecal samples using the QIAamp DNA stool mini kit (Qiagen, Venlo, The Netherlands) according to the procedure described by Zoetendal *et al.* (2006), including a bead beating step (3 min, 3000 rpm). The DNA was eluted in a final volume of 200 µl and stored at -20°C.

2.5.2. Design of primers and probes

The primers and the probes for *Lactobacilli spp (*Delroisse *et al., 2006)* and *E. coli* (Huijsdens *et al.*, 2002) were based on 16S rDNA sequences, available in the National Center for Biotechnology Information databases (see Table 3). The two Taqman probes were labelled at their 5' end with the reporter dye 6-carboxyfluorescein (FAM). For the *Lactobacilli spp.*, a MGB fluorescent probe with nonfluorescent quencher dyes was used and the *E. coli* probe was labelled with 6-carboxytetramethylrhodamine (TAMRA) as the 3' quencher dye. The real-time quantitative fluorescent Taqman assay used in this study is similar as that described by Heid *et al.* (1996).

Table 3. Forward and Reverse primers and Taqman probes sequences for *Lactobacilli spp.* and *E. coli*

Target bacteria	Primer	Sequence (5'-3')	PCR product
Lactobacilli spp. †	Forward primer	GAGGCAGCAGTAGGGAATCTTC	126 pb
	Reverse primer	GGCCAGTTACTACCTCTATCCTTCTTC	
	Taqman probe	ATGGAGCAACGCCGC	
E. coli ‡	Forward primer	CATGCCGCGTGTATGAAGAA	95 pb
	Reverse primer	CGGTATCGTCAATGAGCAAA	
	Taqman probe	TATTAACTTTACTCCCTTCCTTCCCCGCTGAA	

† Bacterial target species : *L. acidophilus, L. amylolyticus, L. amylovorus, L. crispatus, L. fornicalis, L. gallinarum, L. hamsteri, L. helveticus, L. intestinalis, L. jensenii, L. kefiranofaciens subsp. kefirgranum, L. kitasatonis, L. psittaci, L. suntoryeus, L. ultunensis.* (Delroisse *et al.*, 2006).

‡ Huijsdens *et al.*, 2002.

2.5.3. PCR conditions

The amplification reactions were carried out in a total volume of 25 µl containing 1 x Taqman Gene Expression Master Mix (Applied Biosystems Foster City, Ca, USA) both primers (each at 300 nM concentration), 200 nM Taqman probe and 20 to 100 ng of purified DNA. Amplification (2 min at 50°C, 10 min à 95°C, followed by 45 cycles of 15s at 95°C and 1 min at 60°C) and detection were carried out on an ABI Prism 7900 sequence detection system (Applied Biosystems, Foster City, Ca, USA).

Each essay was performed in duplicate in the same run. The cycle threshold (C_T) was calculated as the cycle number at which the reaction became exponential. The C_T was then compared to a standard curve made by diluting genomic DNA from cultures of *L. acidophilus* and *E. coli*. Cell counts before DNA extraction of the bacterial cultures were determined by culture on specific culture media.

2.6. Statistical analysis

For all the parameters, there were repeated measures. Modelling of repeated records was done using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Analysis of variance tested treatment × time interactions. Effects were compared using the CONTRAST statement in the repeated MIXED analysis. The pen was used as the experimental unit for ADFI, FCR and the faecal bacterial populations. For the BW and the ADG, the pigs were used as experimental unit. To fulfil the requirements of normality, a log10 transformation of the *Lactobacillus spp.* and *E. coli* counts was performed. The values presented are lsmeans $\pm$ s.e. The differences were declared significant at $P < 0.05$.

3. Results

3.1. Experiment 1

In the first experiment, the supplementation with BC whey induced an increase in ADG (+ 85 % for "Whey 1" and + 120 % for "Whey 2") and ADFI (+ 23 % for "Whey 1" and + 27 % for "Whey 2") and a lower FCR (- 42 % for "Whey 1" and – 46 % for "Whey 2") the first week PW compared to the control treatment ("Whey 0", $P < 0.05$) (Table 4). No differences between the two supplementations levels (1 and 2 % of BCW) were observed ($P > 0.05$) for all the weekly records.

Table 4. BW, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of piglets fed for 28 days a commercial diet containing 0, 1 or 2 % of bovine colostrum whey, ("Whey 0", "Whey 1" and "Whey 2" respectively) in the experiment 1.

	Treatments				
Measurements and days	Whey 0	Whey 1	Whey 2	s.e.	*Significance*
BW, kg (n=39)					
0	7.13	7.38	7.17	0.20	NS‡
7	7.44	7.96	7.84	0.21	NS
14	9.47	10.14	9.96	0.28	NS
21	12.61	13.43	13.06	0.34	NS
28	16.38	17.32	16.72	0.42	NS
Significance	*Time*Treatment * , Time ***, Treatment NS*				
ADG, g/day (n=39)					
0 to 7	44 [b†]	82[a]	97[a]	12.7	*
7 to 14	287	312	301	16.0	NS
14 to 21	447	469	442	17.0	NS
21 to 28	541	557	522	21.0	NS
Significance	*Time*Treatment NS , Time ***, Treatment NS*				
ADFI, g/day (n=3)					
0 to 7	157[b]	193[a]	199[a]	8.5	**
7 to 14	395	417	406	20.2	NS
14 to 21	641	681	641	16.8	NS
21 to 28	870	933	805	40.8	NS
Significance	*Time*Treatment NS , Time ***, Treatment NS*				
FCR , g/g (n=3)					
0 to 7	4.16[a]	2.39[b]	2.24[b]	0.61	*
7 to 14	1.35	1.34	1.34	0.04	NS
14 to 21	1.42	1.49	1.45	0.03	NS
21 to 28	1.62	1.60	1.59	0.07	NS
Significance	*Time*Treatment NS , Time ***, Treatment NS*				

† For each parameter, values in the same line with different superscripts are different ($P < 0.05$).

‡ NS: $P > 0.05$

The Figure 1 shows the daily feed intake of the piglets from the three treatments during the first week of the trial. The ADFI of the BC whey-fed piglets ("Whey 1" and "Whey 2") were higher or at least tended to be higher ($P < 0.1$) from day 3 to day 7 compared to the piglets receiving the control diet ("Whey 0"). During this first week, diarrhoea occurred in the three treatments (data not shown).

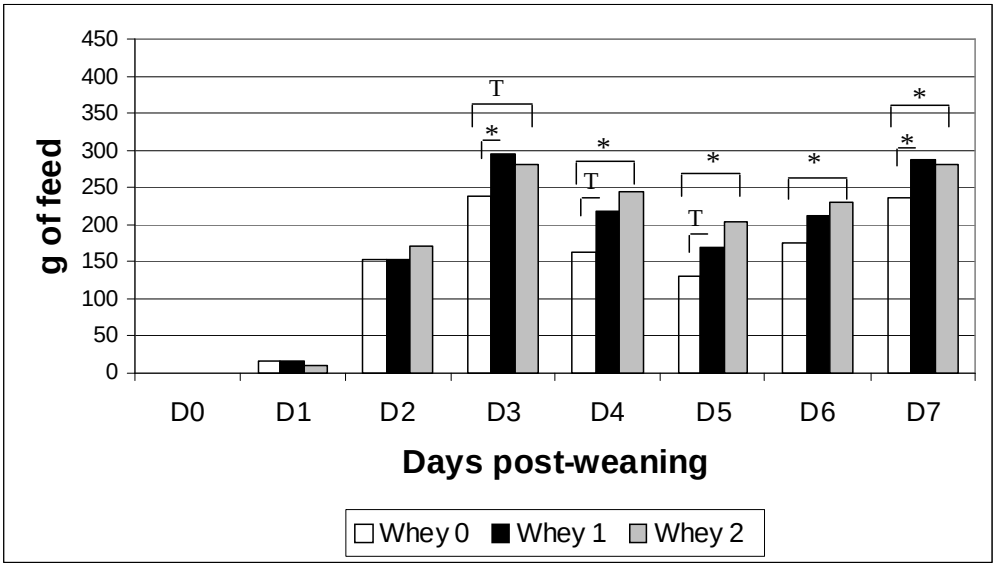

Figure 1. Average daily feed intake (ADFI) of piglets fed a commercial diet containing 0, 1 or 2 % of bovine colostrum whey, ("Whey 0", "Whey 1" and "Whey 2") in the first week of the experiment 1 (* : $P < 0.05$, T : $0.05 < P < 0.10$).

3.2. Experiment 2

3.2.1. Growth performance

The BW, ADG, ADFI and FCR of the second experiment are presented in Table 6. A difference in the ADFI between the "Whey 1" and "Col 1" treatments was observed at the end of the first week PW (from day 4 to 7). During this period, piglets receiving the defatted BC showed a higher feed ingestion compared to piglets from the "Whey 1" treatment (+ 26 %, $P < 0.05$). Concomitantly, the ADG tended also to be higher over this period (+ 23 %, $P < 0.1$) for the piglets receiving the defatted BC. The third week of the trial, the ADG of the "Col 1" treatment was higher than the "Milk 1" and "Whey 1" treatments. No effects of the supplementation were shown on the BW and the FCR at any time of the experiment.

Table 5. BW, average daily gain (ADG), average daily feed intake (ADFI) and feed conversion ratio (FCR) of piglets fed a commercial diet containing 1 % of defatted bovine milk (Milk 1), defatted bovine colostrum (Col 1) or bovine colostrum whey (Whey 1) powder in the experiment 2

Measurements and days	Treatments			s.e.	Significance
	Milk 1	Col 1	Whey 1		
BW, kg (n=32)					
0	8.05	8.01	8.12	0.12	NS‡
4	8.24	8.23	8.32	0.12	NS
7	8.99	9.14	9.06	0.15	NS
11	10.8	11.1	11.0	0.18	NS
14	12.1	12.4	12.4	0.19	NS
21	15.8	16.4	16.2	0.25	NS
Significance	*Time*Treatment NS , Time ***, Treatment NS*				
ADG, g/day (n=32)					
0 to 4	47.7	54.7	48.4	14.8	NS
4 to 7	253_b	305_a	247_b	26.8	*
7 to 11	453	480	508	17.9	NS
11 to 14	423	454	426	16.4	NS
14 to 21	528_b	573_a	550_{ab}	15.3	*
Significance	*Time*Treatment NS , Time ***, Treatment NS*				
ADFI, g/day (n=8)					
0 to 4	114	129	128	10.9	NS
4 to 7	277_{ab}	330_a	262_b	23.1	*
7 to 11	537	564	571	21.2	NS
11 to 14	633_b	693_a	654_{ab}	19.1	*
14 to 21	782	833	820	27.3	NS
Significance	*Time*Treatment NS , Time ***, Treatment NS*				
FCR , g/g (n=8)					
0 to 7	1.53	1.43	1.43	0.16	NS
7 to 14	1.32	1.32	1.29	0.04	NS
14 to 21	1.49	1.46	1.49	0.05	NS
Significance	*Time*Treatment NS , Time ***, Treatment NS*				

† For each period, values in the same line with different subscripts are different ($P < 0.05$)

‡ NS: $P > 0.05$

The Figure 2 illustrates the daily evolution of the ADFI during the first week PW. The feed intake of the piglets receiving the defatted BC was higher ($P < 0.05$) compared to the "Milk 1" treatment on day 4 and the "Whey 1" treatment on day 6 PW.

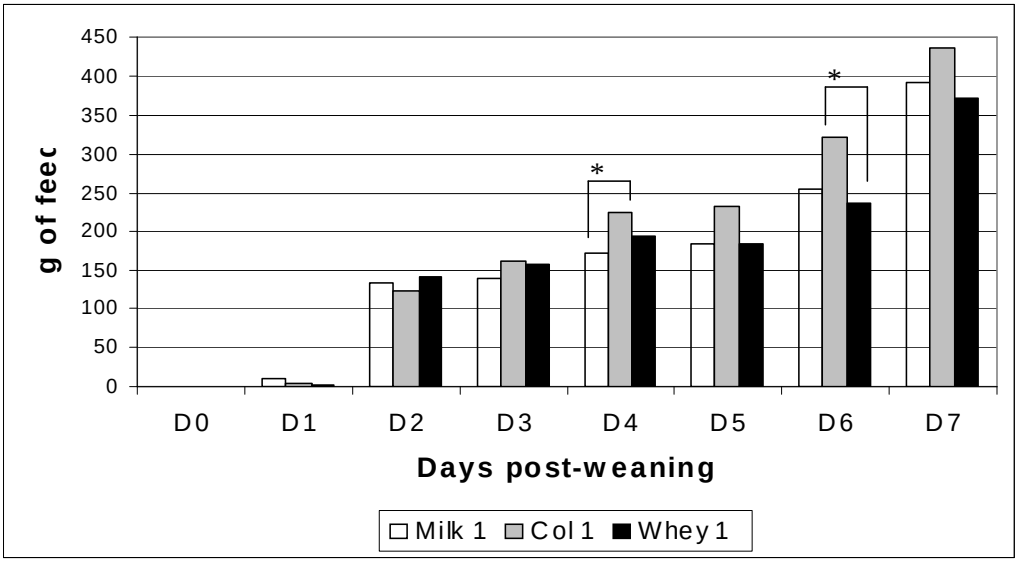

Figure 2. Average daily feed intake (ADFI) of piglets fed a commercial diet containing 1 % of defatted milk ("Milk 1"), defatted bovine colostrum ("Col 1") or bovine colostrum whey ("Whey 1") in the first week of the experiment 2 (* $P < 0.05$).

3.2.2. Faecal analyses

No diarrhoea was observed on piglets during this experiment. The dry matter content of the faeces decreased in the three treatments during the first week PW ($P < 0.001$) but no differences between the treatments ($P > 0.05$, data not shown)) were recorded during the four week-trial.

Results in *E. coli* and *Lactobacilli spp.* counts are presented in Table 6. An evolution in both populations was observed during the first week PW: the *Lactobacilli spp*. counts increased while the *E. coli* populations decreased. An effect of the treatments was observed on day 5, with a lower level of *E. coli* population in the faeces of the BC whey-fed piglets compared to the "Milk 1" treatment. Piglets from the "Col 1" treatment had an intermediary count, similar to the other treatments ($P > 0.05$).

Table 6. Faecal Lactobacilli spp. and E. coli populations (log10 cfu/g faeces) of piglets fed a commercial diet containing 1 % of defatted bovine milk (Milk 1), defatted bovine colostrum (Col 1) or bovine colostrum whey (Whey 1) powder in the experiment 2

	Treatments				
Days	Milk 1	Col 1	Whey 1	s.e.	*Significance*
Lactobacilli spp. *(n=8)*					
-1	$6.85^{y\dagger}$	6.58^{z}	6.86^{y}	0.16	$NS^{\text{Ŧ}}$
2	6.58^{y}	6.65^{yz}	6.63^{y}	0.16	NS
5	7.03^{xy}	7.07^{xy}	7.03^{xy}	0.18	NS
8	7.38^{x}	7.38^{x}	7.38^{x}	0.07	NS
Significance	*Time*Treatment NS , Time ***, Treatment NS*				
E. coli *(n=8)*					
-1	4.65^{xy}	4.54^{xy}	4.60^{x}	0.14	NS
2	5.03^{x}	4.95^{x}	4.96^{x}	0.16	NS
5	$4.42^{y}{}_{a}{}^{\ddagger}$	$4.07^{y}{}_{ab}$	$3.64^{y}{}_{b}$	0.21	*
8	3.35^{z}	3.05^{z}	3.34^{y}	0.26	NS
Significance	*Time*Treatment NS , Time ***, Treatment NS*				

† For the same bacterial population, values in the same column with different superscripts (x, y, z) are different ($P < 0.05$)

† For each day, values in the same line with different subscripts (a, b) are different ($P < 0.05$)

Ŧ NS: $P > 0.05$

4. Discussion

4.1. Cost reduction

In a previous study (Boudry *et al.*, 2008b), we demonstrated the efficiency of a bovine colostrum whey supplementation in piglet weaning diet to reduce the PW growth check. A supplementation of 20 $g.kg^{-1}$ of diet induced significant increases in ADG (170 g/day *vs.* 81 g/day, $P < 0.001$), ADFI (346 g/day *vs.* 256 g/day, $P = 0.03$) and reduced the FCR (2.04 *vs.* 3.16, $P = 0.04$) the first week PW compared to piglets receiving the diet without colostrum. However, the high processing costs of BC whey (100 €/kg) limits its use in pig production. Therefore, we investigated the way to reduce the cost of BC use in the weaning piglet diet without affecting the positive effects of BC on the PW growth check.

In the experiment 1, the important increases in ADG and in ADFI and the reduction of the FCR recorded during the first week PW in the two groups of piglets receiving the BC whey (1 or 2 % of incorporation) corroborate our previous results (Boudry *et al.*, 2008b) and confirm the efficiency of BC whey to reduce the PW growth check. As there were no differences in the performances, the feed intake and the FCR between the "Whey 1" and "Whey 2" groups, we may conclude that 1 % BC whey supplementation in the diet, instead of 2 %, may be used in practice, lowering thereby the costs of supplementation. Moreover as the effects were only observed during the first week PW, like in our previous study (Boudry *et al.*, 2008b), we may recommend stopping the supplementation after 10 days, as done by other authors (Pluske *et al.*; 1999; Le Huërou-Luron *et al.*, 2004).

In the second experiment, we explored the possibility to replace BC whey by defatted BC as the rennet precipitation is an expensive process (50 €.kg^{-1}, Banque de colostrum, CER, Marloie, Belgium). By replacing BC whey by defatted BC, the production costs are reduced by half (100 €/kg for BC whey *vs.* 50 €.kg^{-1} for defatted BC). In this experiment, differences between performances and feed intakes in the "Col 1" and "Whey 1" groups of piglets were not outstanding (+ 23 % of ADG ($P < 0.1$) and + 26 % of ADFI ($P < 0.05$) for "Col 1") and appeared only during the second half of the first week PW. However, they show that we may use defatted BC in place of BC whey without affecting the effect on the PW growth check.

We may conclude from both experiments that it is possible to reduce the costs of BC use in weaning pig diet by reducing the dose of supplementation in the diet (1 % *vs.* 2 %), by shortening the administration period (from 28 to 10 days) and by using defatted BC in place of BC whey. By this way, the treatment costs were reduced from 19 € per piglet in the first experiment ("Whey 2" treatment) to 1.3 € in the second one ("Col 1" treatment).

4.2. Infectious pressure

The lower effects of BC supplementation in the second experiment compared to the first may be related to a lower infectious pressure. Indeed, the first one was conducted in a

conventional on-farm nursery with continuous pig flow while the second one was carried out in an off-site facility of our university. This induced different infectious pressure which had consequences on the health of the piglets and their ADFI. In the first experiment diarrhoea was observed during the first week PW (days 4 and 5), which coincided with a depression in feed intake. However, the piglets fed with the supplemented diets ("Whey 1" and "Whey 2") maintained a higher feed intake and therefore better performances than the control piglets, suggesting that the colostrum-fed piglets were less affected. In the second experiment, piglets didn't show any problem of diarrhoea or ADFI depression because of a "clean" environment. This may explain why the effects of the BC supplements were low. The environment was probably too clean to allow the BC to express its entire growth-promoter potential as suggested by Le Huërou-Luron *et al.* (2004). Pierce *et al.* (2005) also showed that IgG from bovine spray-dried plasma had increased effects on ADG and ADFI of weaned piglets in unclean conditions.

The low pathogen pressure in the second experiment is confirmed by the results of the faecal bacterial counts. The content in *E. coli* in the faeces is largely inferior to 10^8 cfu/g, reported by Wray and Woodward (1997) during colibacillary diarrhoea. This means also that the difference between the treatments observed on day 5 for the *E. coli* counts cannot explain the differences in ADG and ADFI measured in this experiment.

4.3. BC whey *vs.* defatted BC

The main differences between milk, BC whey and defatted BC powders is their composition in proteins, quantitatively and also qualitatively.

4.3.1. Immunoglobulins

In the defatted milk powder, the content in proteins (37 %) is lower than in both BC powders and its content in IgG is close to 0 (0.6 %). In the BC whey powder, the precipitation of casein induces a lower content in total proteins than in defatted BC (69 % *vs.* 77 %) but it concentrates the IgG in the final powder (50 % *vs.* 32 %). According to Elfstrand *et al.* (2002), the IgG2, IgA and IgM are conserved in the whey after rennet precipitation but IgG1 is reduced by 20 %.

Le Huërou-Luron *et al.* (2008) showed that the Ig fraction of BC is responsible for the enhancement in growth rate and feed intake PW. According to Wilson (1974) these improvements are attributable to the continuous source of Ig after the withdrawal of the mother milk. Moreover, Leibbrant *et al.* (1997) showed that milk-derived Ig added to milk replacers increase growth performance following gut closure in early weaned piglets. However in our case, it seems that other components are responsible for the higher ADFI and ADG measured in the "Col1" treatment compared to the "Whey 1" treatment because defatted BC contains less IgG than the BC whey.

4.3.2. Casein

Caseins counts for about 80 % of the milk proteins (Franke *et al.*, 1988), while it represents 4 to 5 % of BC proteins (Nardone *et al.*, 1997) and we may expect a concentration close to 0 % in the BC whey. Controversial data were found about the effect of casein on the activity of the bioactive peptides in the digestive tract. Several works reported a natural buffering effect of the casein proteins that improve the bioavailability of macromolecules such as IGF-I in the gut of adult rats (Xian *et al.*, 1995) and preserve the molecular size and bioactivity of EGF in the adult human gut (Playford *et al.*, 1993). This suggests that in the BC whey, the action of the colostrum growth factors could be reduced in the intestine compared to the use of defatted BC. However, Shen and Xu (1996, 2000) observed a higher inhibitory activity of the acid-soluble fraction of porcine colostrum than the casein fraction on the EGF and IGF-I degradation in the intestinal fluids of weaned piglets. We can thus not conclude on the effect of the casein on our observations.

4.3.3. Insulin

Our analyses revealed an absence of insulin (< 1 ng/g) in BC whey, which is related to its precipitation with the casein (Aranda *et al.*, 1991). According to Houpt (1984) in many species, insulin injection causes a fall of plasma glucose and initiates hunger. However, no data were found on the effect of an oral administration of insulin.

4.3.4. Other proteins

The differences between the concentrations in lactoferrin and IGF-I and –II between both BC powders seems to be too low to be responsible for differences in their effects.

5. Conclusion

Accordingly to this study, it is possible to reduce the costs of BC use in weaning pig diet by reducing the dose of supplementation in the diet (1 % *vs.* 2 %), by shortening the administration period (from 28 to 10 days) and by using defatted BC in place of BC whey. As the complex composition of BC makes the determination of underlying mechanisms difficult, further research, under a high infectious pressure, is needed to determine the components responsible for the recorded effects and their mode of action.

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References

Aranda P, Sanchez L, Perez MD, Ena JM and Calvo M 1991. Insulin in bovine colostrum and milk: Evolution throughout lactation and binding to caseins. *Journal of Dairy Science* 74, 4320-4325.

Association of Official Analytical Chemists 1990. *Official Methods of Analysis*. 15th edition. AOAC, Arlington, VA, USA.

Boudry C, Dehoux J-P, Portetelle D and Buldgen A 2008a. Bovine colostrum as a natural growth promoter for newly weaned piglets: A review. *Biotechnologie, Agronomie, Société et Environnement* (accepted for publication).

Boudry C, Dehoux J-P, Wavreille J, Portetelle D, Théwis A and Buldgen A 2008b. Effect of bovine colostrum whey supplementation on growth performance, faecal *Escherichia coli* population and systemic immune response of piglets at weaning. *Animal* 2, 730-737.

Delroisse JM, Boulvin AL, Parmentier I, Dubois-Dauphin R, Vandenbol M and Portetelle D 2006. Quantification of *Bifidobacterium spp*. and *Lactobacillus spp*. in rat fecal samples by real-time PCR. *Microbiological Research doi:10.1016/j.micres.2006.09.004*

Elfstrand L, Lindmark-Mansson H, Paulsson M, Nyberg L and Akesson B 2002. Immunoglobulins, growth factors and growth hormone in bovine colostrum and the effects of processing. *International Dairy Journal* 12, 879-887.

Franke AA, Bruhn JC, Lawrence CM 1988. Distribution of protein in California milk in 1983. *Journal of Dairy Science* 71, 2373-2383.

Heid CA, Stevens J, Livak KJ and Williams PM 1996. Real time quantitative PCR. *Genome Research* 6, 986-994.

Hopwood DE and Hampson DJ 2003. Interactions between the intestinal microflora, diet and diarrhoea, and their influences on piglet health in the immediate PW period. In *Weaning the pig. Concepts and consequences* (eds JR Pluske, J Le Dividich and MWA Verstegen), pp. 199-218. Wageningen Academic Publishers, Wageningen, The Netherlands.

Houpt TR 1984. Controls of feeding in pigs. *Journal of Animal Science* 59, 1345-1353.

Huijsdens XW, Linskens RK, Mak M, Meuwissen SGM, Vandenbroucke Grauls CMJE and Savelkoul PHM 2002. Quantification of bacteria adherent to gastrointestinal mucosa by real-time PCR. *Journal of clinical microbiology* 40, 4423-4427.

King RH, Kelly D, Morel PCH and Pluske JR 2003. Aspects of intestinal immunity in the pig around weaning. In *Weaning the pig. Concepts and consequences* (eds JR Pluske, J Le Dividich and MWA Verstegen), pp. 219-258. Wageningen Academic Publishers, Wageningen, The Netherlands.

Le Huërou-Luron I, Drillet M, Fauquant J, Mollé D, Bouhallab S 2008. Effets de différentes fractions colostrales sur les performances de croissance des porcelets au sevrage. *Journées de la Recherche Porcine en France* 40, 221-222.

Le Huërou-Luron I, Huguet A, Callarec J, Leroux T and Le Dividich J 2004. La supplémentation de l'aliment de sevrage en colostrum bovin améliore l'ingestion et les performances zootechniques chez les porcelets au sevrage. *Journées de la Recherche Porcine en France* 36, 33-38.

Leibbrant VD, Kemp RA, Crenshaw TD 1987. Effect of liquid milk replacer supplementation immediately postweaning on nuresery pig performance. *Journal of Animal Science* 65 (Suppl. 1), 129 (Abstr.).

Melin L, Mattson S, Katouli M and Wallgren P 2004. Development of post-weaning diarrhoea in piglets. Relation to presence of *Escherichia coli* strains and Rotavirus. *Journal of Veterinary Medicine Series B* 51, 12-22.

Nardone A, Lacetera N, Bernabucci U and Ronchi B 1997. Composition of colostrum from dairy heifers exposed to high air temperatures during late pregnancy and the early postpartum period. *Journal of Dairy Science* 80, 838-844.

Pakkanen R. and Aalto J 1997. Growth factors and antimicrobial factors of bovine colostrum. *International Dairy Journal* 7, 285-297.

Pierce JL, Cromwell GL, Lindemann MD, Russell LE and Weaver EM 2005. Effects of spray-dried animal plasma and immunoglobulins on performance of early weaned pigs. *Journal of Animal Science* 83, 2876-2885.

Playford RJ, Woodman AC, Clark P, Watanapa P, Vesey D, Deprez PH, Williamson R and Calm J 1993. Effect of luminal growth factor preservation on intestinal growth. *Lancet* 341, 843-848.

Pluske JR, Hampson DJ and Williams IH 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livestock Production Science* 52, 215-236.

Pluske JR, Pearson G, Morel PCH, King MR, Skilton G and Skilton R 1999. A bovine colostrum product in a weaner diet increases growth and reduces days to slaughter. In *Manipulating Pig Production VII*, pp. 256. Australian Pig Science Association, Werribee, Australia.

Shen WH and XU RJ 1996. Stability of epidermal growth factor in the gastrointestinal lumen of suckling pigs. *Life Sciences* 59, 197-208.

Shen WH and Xu RJ 2000. Stability of insulin-like growth factor I in the gastrointestinal lumen in neonatal pigs. *Journal of Pediatric Gastroenterology and Nutrition* 30, 299-304.

Wilson MR 1974. Immunologic development of the neonatal pig. *Journal of Animal Science* 38, 1018-1021.

Wray C and Woodward MJ 1997. Escherichia coli infections in farm animals. In *Escherichia coli. Mechanisms of Virulence* (ed M Sussman), pp. 49-84. Cambridge University Press, Cambridge, United Kingdom.

Xian CJ, Shoubridge CA and Read LC 1995. Degradation of IGF-I in the adult rat gastrointestinal tract is limited by a specific antiserum or the dietary protein casein. *Journal of Endocrinology* 146, 215-225.

Zoetendal EG, Heilig HGHJ, Klaassens ES, Booijink CCGM, Kleerebezem M, Smidt H and de Vos WM 2006. Isolation of DNA from bacterial samples of the human gastrointestinal tract. Nature Protocols 1, 870-873.

CHAPTER V

GENERAL DISCUSSION AND FUTURE PROSPECTS

GENERAL DISCUSSION

At weaning, the piglet undergoes many stresses which will induce a critical period of underfeeding and the so-called growth check during the first week postweaning (Le Dividich and Sève, 2000). This will lead to an alteration of the intestinal structure and function, which may induce diarrhoea and infections (Pluske *et al.*, 1997). Due to its richness in growth promoters and antimicrobial factors (Pakkanen and Aalto, 1997), the bovine colostrum may represent a good feed additive to reduce these consequences of weaning. Moreover, it may constitute a good alternative to in-feed antibiotic, banned since January 2006 over all Europe.

All the colostrum powders used in this work were prepared by the "Banque de Colostrum" (CER, Marloie, Belgique) from bovine colostrum of first milking, standardised at a concentration of 75 g of immunoglobulin/l or 30 % of dry matter. Before treatment, the colostrum was skimmed by centrifugation (0.5 % of fat remaining). The powders of defatted bovine colostrum were then obtained by freeze-drying, even if this is an expensive process (Ratter, 2001) to maintain the biological activity of the growth factors which are sensitive to a temperature higher than 40°C. Whey was obtained, after skimming, by rennet coagulation at 37°C for 24 h and separation from curds by a mechanical press.

In our studies, the bovine colostrum supplementation in the weaning diet induced an increase in feed intake and growth performance the first week PW compared to a control treatment without colostrum, suggesting that bovine colostrum may reduce the postweaning growth check. This corroborates observations made by Pluske *et al.* (1999), King *et al.* (2001) and Le Huërou-Luron *et al.* (2004). However in our studies, lower supplies of bovine colostrum (10 and 20 $g.kg^{-1}$ feed *vs.* 40 to 100 $g.kg^{-1}$ feed) increased growth performances and improved feed intake at comparable levels to those reported by the previous authors (+ 40 to 100 % of ADG and +10 to 25 % of ADFI), suggesting that the action of bovine colostrum is not dose-dependent. Nevertheless, the differences in the results may also be explained by the composition of the bovine colostrum extracts used in the different studies. Their concentrations in bioactive peptides (growth and antimicrobial factors) are perhaps very different, but too little information is given about the preparation and the composition of the colostrum extracts experimented to verify this suggestion.

The effect of bovine colostrum on the feed intake only last for one week but this may be enough to improve subsequent growth performance as the length and severity of the postweaning period of underfeeding has a major impact on subsequent performance (Le Dividich and Sève, 2000).

In our work, near the increased feed intake, bovine colostrum also induced a better feed efficiency the first week PW (+ 50 %). This may be due to an increase in the digestive and absorptive capacity of the newly-weaned piglet small intestine after bovine colostrum intake as described by Le Huërou-Luron *et al.* (2003), Huguet *et al.* (2006 and 2007) and King *et al.* (2008a and b). They showed increases in villus height and reduction of crypt depth in different part of the small intestine, suggesting that colostrum may limit weaning-induced gut structural alterations. However, during our studies, we didn't show any effect of bovine colostrum on the intestinal wall morphology (villi height, crypt depth). This contradiction may be explained by the higher levels of bovine colostrum supplementation used in the other works (20 $g.kg^{-1}$ feed *vs*. 50 to 75 $g.kg^{-1}$ feed) and probably also by the high intra and inter-individual variability of these parameters. Moreover, the infectious pressure during the experiments plays also an important role on the effects of the bovine colostrum. Among the studies cited before, only Le Huërou-Luron *et al.* (2004) showed an increase in feed efficiency (+ 10 %), which was observed in bad sanitary conditions (uncleaned pens). This suggests a higher effect of the bovine colostrum on gut health under a high infectious pressure, which may also explain why we didn't measure effects on the intestinal morphology as these measures were performed during studies realised in off-site facilities.

The only effect of bovine colostrum on the intestinal flora of weaned piglets was reported by Huguet *et al.* (2006) who showed an increase in the duodenal *Lactobacilli:Coliform* ratio. In our work, no effect of the bovine colostrum supplementation on the *lactobacilli* and *E. coli* faecal populations were observed. As for the intestinal morphology, this may be related to a too low infectious pressure during our studies as, for example, successful use of colostrum in the treatment of diarrhoea caused by *E. coli* has been reported in human patients (Carbonare *et al.*, 1997 ; Honorio-Franca *et al.*, 1997).

The different parameters studied from the blood, the spleen and the gut-associated lymphoid tissues indicate a local immunisation to bovine colostrum with a marked Th2 immune response.

In the ileal Peyer patch the bovine colostrum ingestion induces a reduction of the number of cells, especially the B-cell, in an isotype manner and the study of the cytokine expression showed a more marked Th2 immune response. Furthermore, we observed an increase in systemic IgA concentrations and a reduction of the Tc cells, which may both be the consequence of a Th2 immune response (Romagnani, 1991).

The results of the cytokine expression suggest an immuno-modulatory effect of bovine colostrum targeting mainly the gut-associated lymphoid tissues, which respond by producing both Th1 and Th2 cytokines. This bipolarity is important in the context of exposure to a wide range of antigens associated with pathogens, with commensal bacteria and with food. It includes the ability to generate tolerance to food and commensal bacterial antigens as well as to activate the immune response to pathogens.

King *et al.* (2008b) reported a moderate expansion of T lymphocytes subsets in the lamina propria of weaned piglets consuming a bovine colostrum enriched diet. They associated this expansion to an induction of the immunological tolerance to the numerous novel proteins present in bovine colostrum.

The effect of the bovine colostrum supplementation on the IGF-I, IGFBP-2 and -3, and T3 and T4 concentrations in blood serum was also evaluated at weaning. Only the seric IGF-I content was modified by the administration of bovine colostrum, with an increase of 15 % on day 7 post-weaning. This effect was also observed by Le Huërou-Luron *et al.* (2003). They attributed this increase to a stimulation of *de novo* synthesis of IGF-I rather from an intestinal absorption of exogenous IGF-I (Donovan, 1997).

The higher feed intake of the piglets fed the colostrum diet the first week post-weaning may be at the origin of our higher IGF-I circulating levels. Indeed, Clemmons and Underwood (1991) showed that the nutritional status exerts a direct influence on circulating IGF-I, with blood IGF-I being closely related to the energy intake. However, Hathaway *et al.* (1999) demonstrated both effects of food ingestion and antimicrobial factors on IGF-I levels. As bovine colostrum contains many growth factors, a study with restricted consumption would be necessary to evaluate the origin of the increase in circulating IGF-I levels.

Finally, in the last part of this work we investigated possibilities to reduce the costs of the use of bovine colostrum in the weaning piglet diet. In our first studies, freeze-dried bovine colostrum whey (100 €/kg) was added to the weaning diet at a level of 2 % and distributed to the piglets for 28 days. This supplementation generated a cost of 19 € per piglet, which represents nearly the commercial value of a weaned piglet.

Our investigations showed the possibility to reduce this cost to 1.3 € per piglet by limiting the supplementation level to 1 % and the duration of the treatment to 10 days and by using defatted bovine colostrum in place of whey (50 €/kg *vs.* 100 €/kg, Banque de colostrum, Marloie, Belgium) without affecting its efficiency in reducing the PW growth check.

Identification of the bio-active peptides responsible for the observed effects

The mechanism(s) of action of the bovine colostrum on the physiological and bio-immunological parameters remain to be clarified.

Several authors (Dunshea *et al.*, 2002 ; King *et al.*, 2008a and b) compared bovine colostrum to spray-dried plasma to try to explain its mechanism of action because of their similar Ig contents. In both protein supplements, the high molecular weight Ig fraction was shown to be responsible for the improved feed ingestion and growth performance induced in the newly weaned piglet (Gatnau *et al.* (1995) and Weaver *et al.* (1995) for plasma and Le Huërou-Luron *et al.* (2008) for colostrum). Given the resistance of this protein to complete proteolysis in the small intestine of the young pig (Morel *et al.*, 1995), it likely affords some passive protection to the intestinal mucosa through immune exclusion (Schollum *et al.*, 1997).

However, King *et al.* (2008a) showed differences between the effects of spray-dried bovine colostrum and plasma on the intestinal wall morphology. They attributed this to the numerous non-specific antimicrobial and growth factors found in colostrum. Further research is needed to establish the role plaid by these factors. The advances realised in the separation, fractionation and isolation in a purified form of interesting proteins in bovine colostrum (Korhonen and Pihlanto, 2007) will probably be really interesting in studying the effects of these bio-active peptides separately and, of course, also in association as there is much evidence of synergetic effects between the components. This is illustrated, for example, by the interaction between lactoperoxidase, immunoglobulins and lysozyme (Hulea *et al.*, 1989) or between the latter and lactoferrin (Yamauchi *et al.*, 1993).

Experimental conditions

Many works reported that the ability of growth factors and antimicrobial substances to enhance performance is directly affected by husbandry conditions, with benefits being more pronounced when sub optimal conditions occur (Cromwell, 2001). This was confirmed during our investigations. Considering our "clean" off-site facility, the use of

challenges on the animals is indispensable in our installations to study further the effects of growth factors or antimicrobial substances. The best solution is of course the realisation of the experiments in real rearing conditions.

Pig production

Many advances were realised during our works to reduce significantly the cost of the use of bovine colostrum in the weaned piglet diet. However, the defatted bovine colostrum as well as the whey used in our studies is dedicated to calf production. New products have thus to be investigated which will not enter in competition with the use for the calves, such as the colostrum which is refused by the "Banque de Colostrum" for a too low level of Ig (< 50 g IG/l) and accounts for 20 % of the collected volume and the colostrums of the 2nd and 3rd milkings *post-partum* which are collected nor by the dairy, nor by the "Banque de Colostrum". Moreover, the valorisation of these products would bring a new income to the dairy producers.

References

Carbonare SB., Silva ML., Palmeira P., Carneiro-Sampaio MM. (1997). Human colostrum IgA antibodies reacting to enteropathogenic Escherichia coli antigens and their persistence in the faeces of a breastfed infant. *Journal of Diarrhoea Disease Research* **15**, 53-58.

Clemmons DR., Underwood LE. (1991). Nutritional regulation of IGF-I and IGF binding proteins. *Annual Review of Nutrition* **11**, 393-412.

Cromwell GL. (2001). Antimicrobial and promicrobial agents. *In: AJ. Lewis, LL. Southern (Eds) Swine Nutrition. CRC Prss, Boca Raton, FL.*

Donovan SM., Chao JC., Zijlstra RT., Odle J. (1997). Orally administered iodinated recombinant human insulin-like growth factor-I (125I-rhIGF-I) is poorly absorbed by the newborn piglet. *Journal of Pediatric gastroenterology and nutrition* **24**, 174-182.

Dunshea FR., Kerton DK., Eason PJ., Pluske JR., Moyes T. (2002). Diets containing high-quality animal proteins increase growth of early-weaned pigs. *Australian Journal of Agricultural Research* **53**, 779-784.

Gatnau R., Cain C., Drew M., Zimmerman DR. (1995). Mode of action of spray-dried porcine plasma in weanling pigs. *Journal of Animal Science* **73**, 82.

Hathaway MR., Dayton WR., White ME., Henderson TL., Young DA., Doan TN. (1999). Effect of feed intake on antimicrobially increases in porcine serum insulin-like growth factors I. *Journal of Animal Science* **77**, 3208-3214.

Honorio-Franca AC., Carvalho MP., Isaac L., Trabulsi LR., Carneiro-Sampaio MM. (1997). Colostral

mononuclear phagocytes are able to kill enteropathogenic Escherichia coli opsonised with colostral IgA. *Scandinavian Journal of Immunology* **46**, 59-66.

Huguet A., Sève B., Le Dividich J., Le Huërou-Luron I. (2006). Effects of a bovine colostrum-supplemented diet on some gut parameters in weaned piglets. *Reprodroduction Nutrition Development* **46**, 167-178.

Huguet A., Le Normand L., Fauquant J., Kaeffer B., Le Huërou-Luron I. (2007). Influence of bovine colostrum on restoration of intestinal mucosa in weaned piglets. *Livestock Science* **108**, 20-22.

Hulea SA., Mogos S., Matei L. (1989). Interaction of lactoperoxidase with enzymes and immunoglobulins in bovine milk. *Biochemistry International* **19**, 1173-1181.

King MR., Morel PCH., James EAC., Hendriks WH., Pluske JR., Skilton R., Skilton G. (2001). Inclusion of colostrum powder and bovine plasma in starter diets increases voluntary feed intake. *In: Manipulating Pig Production VIII, Australasian Pig Science Association,* Werribee, Australia, p. 213.

King MR., Morel PCH., Pluske JR., Hendriks WH. (2008a). A comparison of the effects of dietary spray-dried bovine colostrum and animal plasma on growth and intestinal histology in weaner pigs. *Livestock Science* doi:10.1016/j.livsci.2008.04.001

King MR., Morel PCH., Revell DK., Pluske JR., Birtles MJ. (2008b). Dietary bovine colostrum increases villus height and decreases small intestine weight in early-weaned pigs. *Asian-Australian Journal of Animal Science* **21**, 567-573.

Korhonen H., Pihlanto A. (2007). Technological options for the production of health-promoting proteins and peptides derived from milk and colostrum. *Current Pharmaceutical Design* **13**, 829-843.

Le Dividich J., Sève B. (2000). Effects of underfeeding during the weaning period on growth, metabolism, and hormonal adjustments in the piglet. *Domestic Animal Endocrinology* **19**, 63-74.

Le Huërou-Luron I., Drillet M., Fauquant J., Molle D., Bouhallab S. (2008). Effets de différentes fractions colostrales sur les performances de croissance des porcelets au sevrage. Journées de la Recherche Porcine **40**, 221-222.

Le Huërou-Luron I., Marion J., Bebin K., Thomas F., Ganier P., Thibault JN., Le Dividich J. (2003). Supplementation of a weaning diet with a bovine colostrum extract minimise post-weaning duodenal structural, but not functional, alterations in 7-d old piglet. *In: 9th International Symposium on Digestive Physiology in Pigs, 14-17 May 2003,* Banff, AB, Canada, **2** p. 238-240.

Le Huërou-Luron I., Huguet A., Callarec J., Leroux T., Le Dividich J. (2004). La supplémentation de l'aliment de sevrage en colostrum bovin améliore l'ingestion et les performances zootechniques chez les porcelets au sevrage. *Journées* de la *Recherche Porcine* **36**, 33-38.

Morel PCH., Schollum LM., Buwalda TR, Pearson G. (1995). Digestibility of bovine immunoglobulin in the piglet. In Manipulating pig production V (Ed. DP. Hennesey, PD. Cranwell). Australian Pig Science Association, Werribee, Australia, p. 181.

Pakkanen R. and Aalto J 1997. Growth factors and antimicrobial factors of bovine colostrum. *International Dairy Journal* 7, 285-297.

Pluske JR, Hampson DJ and Williams IH 1997. Factors influencing the structure and function of the small intestine in the weaned pig: a review. *Livestock Production Science* 52, 215-236.

Pluske JR., Pearson G., Morel PCH., King MR., Skilton G., Skilton R. (1999). A bovine colostrum product in a weaner diet increases growth and reduces days to slaughter. *In: Manipulating Pig Production VII.* Australasian Pig Science Association, Werribee, Australia, p. 256.

Ratti C 2001. Hot air and freeze-drying of high-value foods: a review. *Journal of Food Engineering* 49, 311-319.

Romagnani S. (1991). Type 1 T helper and Type 2 T helper cells: functions, regulation and role in protection and disease. *Journal of Clinical Laboratory Research* **21**, 152-158.

Schollum LM., Buwalda TR., Morel PCH., Pearson G., Gill HS. (1997). Passive protection using bovine milk-derived immunoglobulins in a piglet model. *Immunology Cell Biology* **75**, A53.

Wang YZ., Shan TZ., Xu ZR., Feng J., Wang ZQ. (2007). Effects of the lactoferrin (LF) on the growth performance, intestinal microflora and morphology of weanling pigs. *Animal Feed Science and Technology* **135**, 263-272.

Weaver EM., Russell LE., Drew MD. (1995). The effect of spray-dried animal plasma fractions on performance of newly weaned pigs. *Journal of Animal Science* **73**, 81.

Yamauchi K., Toimita M., Giehl TJ., Ellison RT. (1993). Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. *Infection and Immunity* **61**, 719-728.

AUTHOR'S PUBLICATIONS RELATED TO THIS THESIS

Articles

Boudry C., Buldgen A., Portetelle D., Gianello P., Théwis A., Dehoux J-P, 2007. Effect of bovine colostrum supplementation on cytokine mRNA expression of weaned piglets. Livestock Science 108, 295-298.

Boudry C., Buldgen A., Portetelle D., Collard A.., Théwis A., Dehoux J-P, 2007. Effect of oral supplementation with bovine colostrum on the immune system of weaned piglets. Research in Veterinary Science 83, 91-101.

Boudry C., Dehoux J-P., Portetelle D., Buldgen A., 2008. Bovine colostrum as a natural growth promoter for newly weaned piglets: A review. Biotechnologie, Agronomie, Société et Environnement 12, 157-170.

Boudry C., Dehoux J-P., Wavreille J., Portetelle D., Théwis A., Buldgen A., 2008. Effect of a bovine colostrum whey supplementation on growth performance, faecal *Escherichia coli* population and systemic immune response of piglets at weaning. Animal 2, 730-737.

Boudry C., J-P., Wavreille J., Portetelle D., Théwis A., Buldgen A., 2008. Ways to lower the cost of bovine colostrum supplementation in piglet weaning diet. Submitted to "Animal"

Conferences

Boudry C.*, Dalle M-J., Halleux C., Portetelle D., Collard A., Havaux X., Gianello P., Théwis A., Buldgen A., Dehoux J-P., 2005. Effets du colostrum bovin administré à des porcelets au sevrage sur la morphologie de la paroi intestinale et sur le système immunitaire. Journées de la Recherche Porcine, 37, 219-224.

Boudry C.*, Didderen I., Wavreille J., Portetelle D., Dehoux J-P., Buldgen A., 2005. Effect of a bovine colostrum supplementation on piglet diet at weaning on growth performances, food ingestion and faecal *E. coli* concentrations. BSAS Annual meeting, York University, 95.

Boudry C., Buldgen A., Dehoux J-P.*, 2006. Effects of oral supplementation with bovine colostrum on the immunity of weaned piglets. Proceedings of the 19th IPVS Congress, Copenhagen, Denmark, 1, 300.

Boudry C.*, Dehoux J-P., Wavreille J., Buldgen A., 2008. Bovine colostrum: an efficient and cost-effective growth promoter in piglet weaning diet. EAAP - 59th Annual meeting, Vilnius, Lithuania, 14, 143.

* Speaker

Posters

Boudry.C., J-P Dehoux, J. Wavreille, D. Portetelle, A. Collard, A. Théwis, A. Buldgen. 2006. Effect of a bovine colostrum supplementation on growth performance, faecal microflora and immunological parameters of piglets at weaning. Bioforum Proceedings, 95.

Boudry C., Buldgen A., Portetelle D., Gianello P., Théwis A., Dehoux J-P, 2006. Effect of bovine colostrum supplementation on cytokine mRNA expression of weaned piglets. 10th International Symposium on Digestive Physiology in Pigs, Vejle; Denmark, 42.

Boudry C., Dehoux J-P., Buldgen A., 2008. Effect of a bovine colostrum whey supplementation on growth performance, faecal Escherichia coli population and systemic immune response of piglets at weaning. Proceedings of the 20th IPVS Congress, Durban, South Africa.

Boudry C., Colinet F., Wavreille J., Portetelle D., Dehoux J-P., Buldgen A., 2008. Effect of a bovine colostrum whey supplementation on circulating hormones in weaned piglets. Proceedings of the 20th IPVS Congress, Durban, South Africa.

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