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REGULAR ARTICLES

Effects of partial dietary substitution of groundnut meal by defatted, *Aspergillus niger*–fermented and heated *Jatropha curcas* kernel meal on feed intake and growth performance of broiler chicks

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Abstract

This study was conducted to determine intake and growth performance of broiler chicks fed with *Jatropha curcas* kernel meal physico-chemically and biologically processed. The feed experiment lasted for 7 days with 20-day-old Ross 308 strain unsexed broiler chicks. Two dietary treatments were given each to ten animals, according to a complete randomized design. Kernels, manually obtained from *J. curcas* seed, were defatted, heated, and fermented with a strain of *Aspergillus niger* and oven-dried, in order to obtain the treated jatropha kernel meal. This latter was used to replace one third of a groundnut meal premix which was then incorporated in a commercial diet to warrant iso-nitrogenous and iso-caloric characteristics of the diets. Data collected were analyzed according to ANOVA procedure. The results revealed that the animals that received the diet incorporating jatropha kernel meal had numerically higher live weight (156.1 vs. 152.7 g/animal) ($P > 0.05$) and average daily weight gain (12.3 vs. 11.7 g/day/animal) ($P > 0.05$) than the control ones, at the end of experiment. The average daily feed intake was the same for the two groups of animals (23.2 g/day/animal) ($P > 0.05$) with a similar feed conversion ratio (2.0 vs. 2.1 respectively for the jatropha group and the control group). The survival rate, at the end of the experiment, was 100% for the two groups of animals. Physico-chemically and biologically processed *Jatropha curcas* kernel could be an interesting by-product for poultry feeding.

Keywords Broiler chicks · *Jatropha curcas* · Animal performance · Detoxification

Introduction

Jatropha curcas L. belongs to the Euphorbiaceae family. It is distributed all over the tropics and subtropics and can grow on degraded soils (Heller 1996). Fruit produces a seed which contains oil, 25 to 47% of which is extractable (Üllenberg 2007) by various methods (Beerens 2007), and can be used as fuel directly or as substitute to diesel after transesterification (Lu et al. 2009).

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The meal obtained after oil extraction contains approximately 60% crude protein (Devappa & Swamylingappa 2008) and is an excellent source of nutrients the presence of anti-nutrients and toxic components (Makkar et al. 2008).

Investigations demonstrated that phorbol esters were the most important toxic molecules (Becker & Makkar 1998; Makkar et al. 1997; Makkar et al. 1998; Roach et al. 2012). These are diterpenoid esters tigliane polyunsaturated represented by the 12-deoxy-16 hydroxyphorbol (Haas et al. 2002) which activate protein kinase C resulting in cytotoxicity (Oskoueian et al. 2011) and most concentrated in the kernel of the seed (He et al. 2011). Outside phorbol esters, jatropha meal contains not only curcin which is capable inhibit protein synthesis (Lin et al. 2003), but also anti-nutrients including trypsin inhibitor, phytate, and saponins (Francis et al. 2002). Curcin and trypsin inhibitor could interfere with physiological process of monogastrics causing severe growth depression (Palacios et al. 2004), but they may be removed by heat and biological treatment (Aderibigbe et al. 1997; Aregheore et al. 1998; Abou-Arab & Abou-Salem 2010; Sumiati et al. 2012). A reduction of more than half the content of tannins, saponins, and phytates, which are heat resistant, was additionally obtained after a fermentation of jatropha meal with different combinations of fungi (Belewu et al. 2011a; Oseni and Akindahunsi 2011).

Efforts are under way to detoxify jatropha seed by removing phorbol esters or develop varieties that are deprived of this molecule so that the meal could be used as an ingredient in livestock diet (Makkar and Becker 2009) and without risk to human health associated with phorbol esters (King et al. 2009).

Many processing methods have been explored to detoxify meal of *J. curcas* with different levels of success. These include physical (Aregheore et al. 1998) and chemical (Haas and Mittlebach 2000; Aregheore et al. 2003) methods, the combination of these two (Martinez-Herrera et al. 2006), and biological methods (Belewu and Sam 2010; Joshi et al. 2011). But chemical de-oiling of jatropha kernel, followed by a physico-chemical treatment, did not cause a complete removal of phorbol esters (Kumar et al. 2010).

The objective of this study was to evaluate the impact of groundnut cake partial substitution with *J. curcas* kernel meal from Senegal, which was subjected to combined chemical, biological, and thermal treatments in order to remove phorbol esters and anti-nutritional compounds out of the product in a diet, on ingestion, and on growth performance of broiler chicks.

Materials and methods

The experiment was conducted in *Ecole Nationale Supérieure d'Agriculture* (*ENSA*), University of Thies (Senegal), after the rainy season (November) with a temperature ranging from 25.7 to 35.1 °C and a relative humidity ranging from 36.0 to 39.8%.

Five hundred grams of mature and dry seeds of *Jatropha curcas* was collected from Dialacoto, geographical coordinates 13° 19′ 0″ N and 13° 18′ 0″ W, in the Tambacounda region (Senegal). The seeds were weighed and cracked individually to remove the kernel, which was milled using WARING®-type speed blender with timer grinder and then defatted for 6 h, by 20-g amounts each in a series Soxhlet-type extractor, using diethyl ether (boiling point, 60–80 °C) as solvent.

Three strains of *Aspergillus niger* van Thieghem were obtained from Belgian Coordinated Collections of *Microorganismes/Mycothèque de l'Université Catholique de Louvain* (Agro) Industrial Fungi and Yeast Collection, Louvain-la-Neuve, Belgium (BCCM™/MUCL 19001, 29039, and 19002) and maintained on potato-dextrose-agar (PDA) medium. Ten milliliters of spore suspension concentrated to 1.10^6 in water in 0.05% Tween 80 was used as inoculum.

The substrate, consisting of jatropha kernel meal, was spread into a dish, moistened with distilled water in the ratio of 1:1.5 *w/v* (62% initial moisture content) and sterilized in a vertical stand autoclave at 121 °C for 30 mn so as to get rid of any microbes that could be present in the meal. The crystallizer, before sealed with a film paper, was inoculated with spore suspension (1.10^6 spores/ml); the content were mixed and incubated at 39 °C for ten days. The fermented substrate was left in a universal-type oven with horizontal ventilation at 70 °C for 48 h to terminate the fungi growth. The spent substrate, fermented jatropha kernel meal (FJKM), was later used in the formulation of diet.

Two broiler starter diets were formulated. The control diet (0FJKM) and the experimental diet contained 2/3 of a complete starter commercial feed (SEDIMA S.A., Dakar, Senegal) for broiler chicks. This commercial feed was mainly composed of maize, cereal issues, soybean meal, peanut meal, fish meal, calcium carbonate, and vitamin-mineral complex. To formulate the control diet, 1/3 of a mixture of groundnut meal (160 g), corn (480 g), disodium phosphate (32 g), and calcium carbonate (32 g) was added to the commercial feed and the experimental diet (8FJKM) was formulated similarly but the groundnut meal of the previous mixture was replaced with FJKM (Table 1). These final mixtures were iso-nutrients for ME, CP, Ca, P, and Na, and in agreement with the recommendations of N.R.C. (1977).

Twenty unsexed 1-day-old broiler chicks Ross 308 strain were used for the study. The birds were kept in a well-ventilated broiler chicken barn and divided into two groups of ten chicks (control group (CG) and jatropha group (JG)). Each group was placed in an area of 2.25 m^2 and randomly assigned to one of the two diets.

Table 1 Composition of diets incorporating *J. curcas* kernel meal

Raw materials (%)	0FJKM	8FJKM
Complete starter commercial feed	68.0	68.0
Groundnut meal	8.0	–
FJKM	–	8.0
Maize	20.0	20.0
Phosphate disodium	2.0	2.0
Calcium carbonate	2.0	2.0
Total	100.0	100.0

Complete starter commercial feed (SEDIMA) composed of maize, cereals issues, soybean meal, peanut meal, fish meal, calcium carbonate, and vitamin-mineral complex

FJKM fermented jatropha kernel meal, *0FJKM* control diet, *8FJKM* diet incorporating 8% of fermented jatropha kernel meal

During the test, animals were heated by electric light ensuring thermal comfort and water was available ad libitum. Feed was weighted early in the morning and provided once a day. Refusals of feed were collected and weighed the day after the distribution.

The study was carried out for 7 days.

Data were collected on feed intake, weight gain, feed conversion ratio, and mortality.

The daily feed intake was estimated as the difference between the feed supplied and the feed rejected over 24 h period. The average daily feed intake (ADFI) was then calculated.

Birds in each replicate were individually weighed at the beginning and the end of experiment and the average daily weight gain (ADWG) obtained.

The feed conversion ratio (FCR) was determined as the feed intake per unit weight gain.

Mortality was recorded in each replicate and expressed as a percentage of the total number of birds in the replicate at the beginning of the experiment.

Samples of jatropha kernel meal, fermented jatropha kernel meal, control diet, and diet incorporating the fermented jatropha kernel meal were analyzed. Dry matter (DM) was determined by oven-drying at 70 °C for 15 h, 90 °C for 5 h, and 102 °C for 5 h consecutively. Diets were analyzed for crude protein (CP; Method 954.01, AOAC 1990), ether extract (EE; Method 920.39, AOAC 1990) with petroleum ether solvent, ash (Method 942.05, AOAC 1990), and crude fiber (CF; Method 962.09, AOAC 1990).

The following values were calculated from those measured (Sibbald 1976):

$$\text{Organic matter (OM)} = 100 - \text{Ash}$$
$$\text{Non-Nitrogen Extract (NNE)} = \text{OM} - \text{EE} - \text{CP} - \text{CF}$$
$$\text{ME} = \text{metabolic energy (kcal/kg DM)} = 3951 + (54.4 \times \text{EE}) - (88.7 \times \text{CF}) - (40.8 \times \text{Ash})$$

All data generated were subjected to analysis of variance for complete randomized design with Statistix 8.1 software package.

The model was

$$Yij = \mu + \alpha i + \varepsilon ij = \mu i + \varepsilon ij.$$

where

Yij jth observation in the sample from the ith population.
μ overall mean.
μi mean in the ith population (with $(\mu i = \mu + \alpha i)$).
εij random error.

Significant means were separated using Tukey HSD all-pairwise comparisons test of the same package.

Results

Chemical composition of feed

Table 2 shows the proximate composition of the experimental diets. Both 0FJKM and 8FJKM showed almost a similar value, with regard to the DM, CP, EE, ash, and CF. The true metabolic energy of each diet was 3551 and 3328 kcal/kg DM for respectively 0FJKM and 8FJKM. Analytical results showed that diets were almost iso-proteic and iso-energetic.

Concerning the jatropha kernel meal, the treatment with *Aspergillus niger* resulted in a decrease of organic matter, crude protein, ether extract, and crude fiber, respectively from 901, 618, 50, and 82 g/kg DM to 874, 598, 40, and 40 g/kg DM.

Feed intake

Figure 2 shows the daily individual feed intake of broiler chicks during the experimental sequence. No significant differences were observed in the feed intake of the two groups of animals (23.2 ± 6.1 g/d/animal in CG vs. 23.2 ± 5.5 g/d/animal in JG).

For both groups of animals, a similar feed intake was noted, with a decrease on the sixth day, which was related to an insufficient amount of diet.

Growth performance

Figure 1 shows synthetic body weight changes over the experiment. During the 7 days, it was found that the control group showed a linear weight growth, evolving from 74.4 ± 8.7 g on day 1 (d1) to 152.7 ± 26.3 g on day 7 (d7). For the same period, animals that received the fermented jatropha kernel meal diet had the same profile, from 76.2 ± 6.2 to 156.0 ± 9.7 g. Thus, ADWG per animal did not change significantly for both groups regardless of the rate of fermented

Table 2 Proximate analytical composition of the diets used during the experimentation

	DM (%)	Chemical composition (% in DM)						ME (kcal/kg DM)
		OM	CP	EE	CF	Ash	NNE	
JKM	91.6	90.1	61.8	5.0	8.2	9.9	15.1	3091.7
FJKM	93.5	87.4	59.8	4.0	4.0	12.6	19.6	3299.7
0FJKM	91.9	89.5	25.0	5.9	3.3	10.5	55.3	3550.9
8FJKM	90.3	87.3	27.4	4.1	3.7	12.7	42.1	3327.7

JKM jatropha kernel meal, *DM* dry matter, *OM* organic matter, *CP* crude protein, *EE* ether extract, *CF* crude fiber, *NNE* non-nitrogen extract, *0FJKM* control diet, *8FJKM* diet incorporating 8% of fermented jatropha kernel meal

jatropha kernel meal incorporation, from 11.7 g/d/animal for CG to 12.3 g/d/animal for JG (Fig. 2).

During the experiment, no mortality was recorded in any group.

The FCR presented the same mean values, 2.1 and 2.0, respectively for CG and JG without significant difference ($P > 0.05$) (Fig. 2).

Discussion

Oil extraction from *Jatropha curcas* seeds can be done according to mechanical or chemical processes. Mechanical extraction by means of a screw press is the method used generally in developing countries because of the simplicity of the equipment required (Eckart and Henshaw 2012). Depending on the level of adjustment of certain parameters (Pradhan et al. 2011), it was possible to recover more than 80% of the oil (Tambunan et al. 2012). However, solvent extraction could be regarded as the most ideal extraction method since it could recover 95–98% mass fraction of the available oil in the seed (Gubitz et al. 1999).

In our study, because of the non-digestibility of hulls' fibers for monogastrics (Jørgensen et al. 1996), the jatropha seeds were manually shelled before being processed. The kernel obtained was crushed and completely de-oiled by the Soxhlet method. By de-oiling, the aim was to obtain a significant reduction of toxic compounds, allowing animals to ingest the jatropha meal. But previous studies (Makkar et al. 1998; Martinez-Herrera et al. 2006) have shown that de-oiling did not allow this significant reduction. An additional biological fermentation with *Aspergillus niger* was then considered in the light of previous observations that were made (Belewu and Akande, 2010; Belewu and Sam 2010; Rosa et al. 2010; Brand et al. 2000). To complete the fermentation process, jatropha meal was treated by passage in an autoclave (120 °C for 30mn) before inoculation. The fermented substrates were thereafter oven-dried at 70 °C for 48 h to terminate the fungi growth and dry the cake. The aim was to inactivate toxic and anti-nutritional compounds. Martinez-Herrera et al. (2006), by heat treatment in an autoclave (121 °C for 20mn), significantly inactivated trypsin inhibitor activities which are anti-nutritional factors but essentially lectin activity which is considered to be another toxic factor in *J. curcas* seeds. In the same way, Abo El-Fadel et al. (2011) decreased the concentration of trypsin inhibitor and lectin by about 75 and 83% respectively. These results were in agreement with Haas and Mittlebach (2000) and Makkar et al. (2008) who reported also that heat treatment has a positive effect on reducing trypsin inhibitor and lectin concentration in *J. curcas* meal.

Despite of the FJKM incorporation, the daily feed intake per broiler chicks that received this diet did not vary significantly compared to those who received the control diet. Our

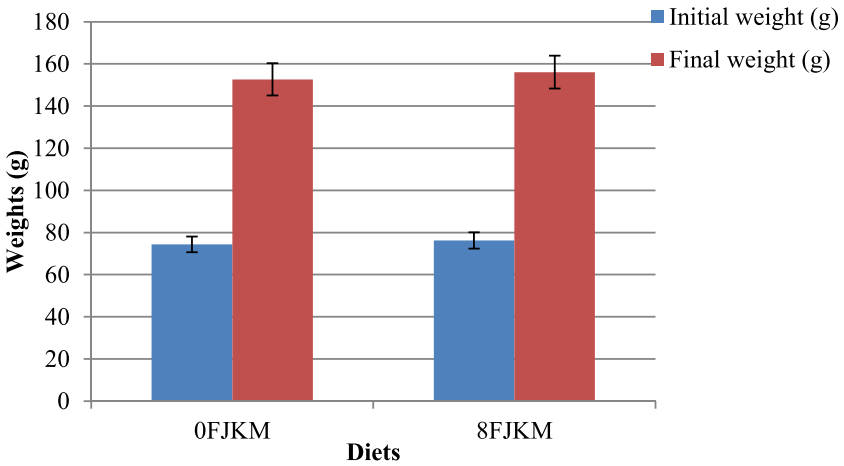

Fig. 1 Weight performances of broiler chicks receiving or not 8% de-oiled, *Aspergillus niger*–treated and heated *J. curcas* kernel meal

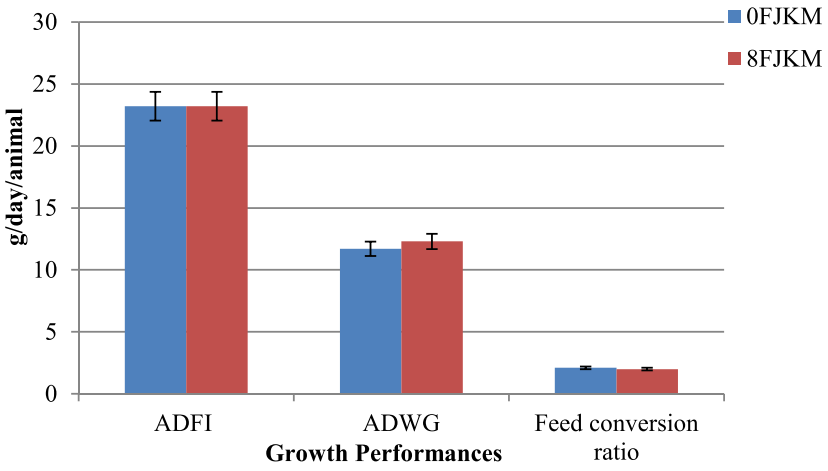

Fig. 2 Growth performances of broiler chicks receiving or not 8% de-oiled, *Aspergillus niger*–treated and heated *J. curcas* kernel meal. ADFI, average daily feed intake; ADWG, average daily weight gain; *0FJKM*, control diet; *8FJKM*, diet incorporating 8% of fermented jatropha kernel meal

ADFI = Average Daily Feed Intake,

ADWG = Average Daily Weight Gain.

0FJKM = control diet,

8FJKM = diet incorporating 8% of fermented jatropha kernel meal.

results confirmed those of Sumiati et al. (2009) who observed that fermented *J. curcas* did not influence feed consumption and body weight gain. These results were contrary to the observations of those obtained by Sumiati et al. (2012) which incorporated fermented *J. curcas* meal using *Rhizopus oligosporus* at 7.5% in the diet of laying hens. They obtained a significant decrease in feed consumption and increase of feed conversion ratio.

Jatropha kernel meals biologically treated are reported to remain toxic, even in ruminants. Belewu and Akande (2010) submitted goats to diets in which jatropha meal, fermented with *Penicillium* sp. or *Aspergillus niger*, was partially or fully incorporated to replace soybean meal. Although dry matter intake was lower than that for the control group tested, animals showed overall a good ingestion without effects on blood parameters. Similar observations were made by Belewu et al. (2010c) with goats receiving diets partially incorporated with jatropha meal fermented with *Rhizopus oligosporus*. These authors showed that despite the low feed intake in the tested group, animals showed higher crude protein intake probably due to the higher crude protein content of the diet. Belewu et al. (2011b) and Belewu et al. (2010a) had yet, with goats also, obtained better feed and nutrient intake and significant higher weight gain with diets that have incorporated fermented jatropha meal successively with *Aspergillus niger*, *Penicillium chrysogenum*, and *Trichoderma harzianum*.

By cons, jatropha meal fermented with, respectively, *A. niger* and *Trichoderma longibrachiatum*, caused persistent diarrhea, poor feed intake, dehydration, and death in goats when it fully substituted to soybean meal in diet (Belewu et al. 2010b).

Despite improved feed intake obtained with diets in which 4 and 6% of jatropha kernel meal fermented with *A. niger* were incorporated, birds did not have a better weight gain and presented, also, smaller internal organs (Oladunjoye et al. 2014). Authors hypothesized that poor feed conversion could be attributed to residues of anti-nutritional and toxic factors. Even at very low levels, phorbol esters may negatively interfere on feed intake (Sumiati et al. 2012). Ojediran et al. (2014) observed also a growth depression due to residual anti-nutritional factors.

Because of the presence of phorbol esters, jatropha meal presents, for animals, a very bad palatability. Indeed, Aregheore et al. (2003) reported that a concentration of 0.13 mg/g phorbol esters present in the jatropha meal has a significant adverse effect on feed intake. Moreover, protein isolates produced by papain treatment and associated with *Panicum maximum* induced a low ingestion in guinea pigs (Kouakou et al. 2010).

Veerabhadrappa et al. (2014) have shown that solid-state fermentation of jatropha seed cake using *Aspergillus versicolor* reduced by about 76% phorbol esters and significantly anti-nutrients like phytic acid, tannins, trypsin inhibitors, cyanogenic glucosides, and lectins. Made under optimum conditions, fermentation by *Pseudomonas aeruginosa* PseA, carried out on a substrate consisting of a jatropha seed meal, was allowed to completely degrade phorbol esters in 9 days (Joshi et al. 2011).

Monogastrics generally exhibit a high sensitivity to the presence of phorbol esters and other anti-nutritional factors (Becker and Makkar 1998; Rakshit et al. 2008), but, in our study, their feed behavior was not significantly affected by incorporation of

treated jatropha kernel meal in the diet, on the contrary. In addition, the possible presence of toxic factors did not affect the viability of the animals. This was confirmed by previous studies (Annongu et al. 2010; Belewu and Akande 2010; Oladunjoye et al. 2014). They confirmed that the residual toxic and anti-nutritional components in the jatropha kernel meal biologically treated did not reach the lethal dose for animals.

Fungi could be considered the most suitable organisms to fermentation solid substrate because their hyphae could colonize the substrate by penetrating the interparticle spaces (Pandey et al. 1999). The application of this technology has enabled the development of degradation of hazardous compounds, biological detoxification of crop residues for enrichment of nutrition, and production of value-added products (Dos Santos et al. 2004). Biological processes through use of fungi, bacteria, or enzyme complexes could allow a significant reduction of toxic and anti-nutritional compounds and, in some cases, improve the nutritional value of meal and therefore its use in animal feed (Nesseim et al. 2014). The nitrogen level of jatropha kernel meal increased after treatment with *Aspergillus niger*. This is probably due to the fermentation of non-nitrogen compounds, some volatile end products escaping from the media, thus leaving higher relative amounts of nitrogen. Seed cake and fruit pulp then can be used for biogas production by fermentation (Vyas and Singh 2007).

In our case, the kernel of jatropha was de-oiled, fermented, and heat-treated. These treatments have probably resulted in a significant decrease of phorbol esters but also in most of anti-nutritional compounds and improved the feed consumption and weight gain, contrary to what had been observed in our previous study (Nesseim et al. 2015). To our knowledge, such positive results are reported for the first time in the literature, and anyway in broiler chick production. This suggests that biological and physical treatments presumably allowed the removal of phorbol esters, curcin, and some anti-nutritional factors such as trypsin inhibitors. Considering the reaction of the animals after the intake of tested feed, it can be concluding that a large quantity of toxic compound was probably eliminated in the jatropha kernel meal. Fermentation of jatropha kernel meal with *A. niger* followed by heat treatment was probably an adequate method for suppressing phorbol esters. Moreover, the treatment has even positively affected feed intake and weight and it had no impact on the viability of animals.

This study was the first field experiment on evaluation of jatropha kernel seed fermented with *Aspergillus niger* in broiler chicks feeding in Senegal. The results showed that, after a total dehulling, a chemical de-oiling with diethyl ether as well as a biological and heat treatment, jatropha kernel stop impacting feed intake and growth of chicks. Further studies must be performed in order to confirm the use of fungal fermentation to allow detoxification of *Jatropha curcas* meal.

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Compliance with ethical standards

Statement of animal rights In the absence of proper regulation on the use of animals for research and animal welfare during experiments in Senegal, the protocols were conducted according to the best practices usually accepted by the Ethical Committee of Liège University (Liège, Belgium) when conducting similar experiments.

Conflict of interest The authors declare that they have no conflict of interest.

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