Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X Oct - Dec 2015 / Special Issue
Nutrition - Poultry Feeding Additives / 001-006
http://dx.doi.org/10.1590/1516-635xSpecialIssue
Nutrition-PoultryFeedingAdditives001-006

Review

The Effect of Jatropha Curcas Seed Meal on Growth Performance and Internal Organs Development and Lesions in Broiler Chickens

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Keywords

Broiler, *Jatropha curcas*, phorbol ester, toxicity.

Submitted: September/2014
Approved: November/2015

ABSTRACT

The meal of *Jatropha curcas* (JCM) seed is a by-product of the biofuel industry and may potentially to be used as animal feed. However, its toxicity has prevented its utilization in animal nutrition mainly due to its high concentration of phorbol esters. This study was conducted to evaluate the effects of the dietary inclusion of JCM on the growth performance, feed digestibility and internal organs development of broilers. Thirty two 48-d-old Ross 308 broiler chickens housed in 16 pens (2 birds/pen) were used in this study. Birds were randomly allocated to dietary treatments comprising four JCM levels (negative control, 25, 50, or 100 g JCM/kg of diet) for four weeks. Results showed that increasing levels of JCM had a negative impact on broiler performance, reducing live weight, weight gain, and feed intake. Treatments led to a decrease of the relative weight of testis and spleen, and to an increase in heart relative weight. In broilers fed diets containing JCM, the testis were atrophic, presenting reduced size of the seminiferous tubule, which were small and lined within active sertoli cells and rare spermatogonia. This study illustrates the negative impact of diets containing JCM on broiler performance and JCM pathological effects on several organs.

INTRODUCTION

The animal feed industry has faced serious challenges as the demand for feeds and raw materials is expected to escalate in order to cope with increasing demand for animal food products. Furthermore, there is also a growing awareness that due to the competition between humans and the animal industry, the existing resources should be drastically reduced. These and other potential drawbacks may hamper the development of poultry production industry. In this global scenario, research aiming at studying new alternative feedstuffs is particularly important. Nevertheless, an information gap is clearly identified for studies where the impact of such feedstuffs is evaluated, especially those containing toxic compounds.

Jatropha curcas meal (JCM), obtained after oil extraction, has been characterized as a potential animal feedstuff due to its high crude protein (CP) content (Makkar & Becker, 1999; Makkar *et al.*, 2008) and high levels of essential amino acids, except for lysine (Makkar *et al.*, 1998; Rakshit & Bhagya, 2008). However, its toxicity, mainly attributed to phorbol esters (PE), has hindered its use as animal feed (Makkar *et al.*, 1997). Studies evaluating its utilization in animal nutrition have been performed using different animal models and plant materials. Negative effects have been observed in goats (Gadir *et al.*, 2003; Katole *et al.*, 2013), sheep (Katole *et al.*, 2011), pigs (Chivandi *et al.*, 2006), and fish (Becker & Makkar 1998; Kumar *et al.*, 2010). El-Badwi & Adam (1992) and El-Badwi *et al.* (1995) reported mortality and severe pathological changes in Brown Hisex chicks. These results indicate that PE may have a wide range of toxicological effects at different levels of intake and that animal species may also respond differently. Thus, it is important to characterize the effects of adding different levels of these compounds in broilers diets to evaluate their growth performance and health.

In this study, different levels of JCM (25, 50 and 100 g/kg) were incorporated in broiler diets in order to evaluate their effects on growth performance and on internal organs development and lesions.

MATERIALS AND METHODS

Animals and diets

A total of 32 Ross male broilers chickens with 48 days of age were randomly allocated in sixteen floor pens (2 birds per pen). The pens were located in a temperature-controlled room, which was daily adjusted to the recommended values, according to standard management practices. Birds were acquired from a local hatchery and were fed a commercial pre-starter diet until the beginning of the trial.

Seeds of *Jatropha curcas* acquired in Mozambique were used to obtain the JCM. Whole seeds were pressed with a Komet expeller press CA59G-3 (IBG Monforts Oekotec, Mönchengladbach, Germany) to extract the oil. The obtained JCM was milled to1-mm particle size using a hammer mill. Four dietary treatments were obtained by adding to a basal diet 0, 25, 50 and 100 g of JCM/kg (diets $J_{0,}$ $J_{2.5}$, J_5, J_{10} respectively; Table 1). The basal diet was based on corn and soybean meal and was formulated to meet or exceed the NRC (1994) recommendations for all nutrients. All the diets were in mash form.

Experimental design

In the beginning of the growth trial birds (2557 ± 215 g) were randomly assigned so that each experimental diet (J_0, $J_{2.5}$, J_5 and J_{10}) was randomly distributed to 16 pens, totaling eight birds and four pens per treatment, in a room under natural daylight conditions and temperature of 18 to 23ºC. Birds were allowed ad libitum access to the experimental diets and water during the experimental period (48 to 76 days of age). Body weight, body weight gain and feed intake were recorded weekly per pen.

All trials were carried out in accordance with the Portuguese law (Portaria n° 1005/92) on animal care in experimental research.

Table 1 – Ingredient composition (%, as fed) of the experimental diets

	Diet			
	J_0	$J_{2.5}$	J_5	J_{10}
Ingredients (%)				
Jatropha curcas meal	0.00	2.50	5.00	10.00
Corn	58.90	57.43	55.93	52.93
Soybean meal 47%	24.15	23.45	22.85	21.65
Whole soybean	13.22	12.92	12.52	11.72
Salt	0.23	0.23	0.23	0.23
Calcium carbonate	0.79	0.79	0.79	0.79
Dicalcium phosphate	1.14	1.14	1.14	1.14
Choline 75%	0.10	0.10	0.10	0.10
DL-Methionine	0.25	0.25	0.25	0.25
L-Lysine	0.70	0.70	0.70	0.70
Mineral and vitamin premix[1]	0.50	0.50	0.50	0.50
Analyzed composition (%)				
Crude protein	18.40	18.10	18.30	19.00
Crude fat	3.90	4.20	4.60	5.20
Crude fiber	2.80	3.30	4.30	5.80
Phorbol esters	0.00	0.02	0.03	0.06
Estimated composition (%)				
Starch	36.90	36.00	35.00	33.20
Free sugar	3.50	3.50	3.40	3.30
Metabolizable energy (kcal/kg)	2990	2929	2867	2742

[1]Mineral-vitamin premix provided the following per kilogram of feed: 11,000 IU of vitamin A, 2,150 IU of vitamin D3, 25 mg of vitamin E, 1 mg of vitamin K, 5 mg of riboflavin, 20 mg of niacin, 8 mg of pantothenic acid, 1 mg of folic acid, 0.1 mg of biotin, 200 mg of choline, 0.012 mg of vitamin B12, 50 mg of Mn, 40 mg of Zn, 5 mg of Cu, 0.1 mg of Se.

Organ evaluation

At 80 days of age all birds were euthanized by cervical dislocation for organ evaluation. After euthanasia, liver, kidneys, small and large intestines, testis, spleen, and heart were carefully removed, weighed and examined for gross and microscopic changes. The gross abnormalities observed in proventriculus tissues were submitted to histopathological examination by optical microscopy. The tissues were fixed in 10% neutral formalin. Tissue samples were processed in a Shandon Hipercenter XP automated tissue processor (Thermo Fisher Scientific, Waltham, USA) and embedded in paraffin wax. Three micrometer paraffin sections were cut on a Biocut 2035 microtome (Leica Microsystems, Buffalo Grove, USA) and stained with hematoxylin and eosin.

Chemical Analysis

Ground (1 mm) samples of the experimental diets were analyzed for dry matter (DM), ash, total N as Kjeldahl N and fat contents. The DM content was determined in a forced-ventilation oven at 60 ºC for 72 h. Ash was determined using standard procedures

(AOAC International, 1990; method 942.05). Crude protein (CP) was calculated as Kjeldahl N x 6.25 (AOAC International, 1990; method 954.01). Ether extract (EE) was determined by extracting the sample with petroleum ether using a Soxtec System HT (AOAC International, 1990; method 945.16). Crude fibre (CF) was determined as the insoluble organic residue after digestion (AOAC International, 1990; method 962.09). In addition, diets were also analyzed for phorbol esters (PE) using the methodology described by Makkar *et al.* (1997).

Statistical analysis

Statistical analyses were carried out using SAS (2000, SAS Institute Inc., Cary, NC). The effect of JCM inclusion levels on performance, diet digestibility and organ measurements was examined by one-way analysis of variance (ANOVA) using initial body weight as covariate. When the F-test was significant ($p<0.05$), multiple comparisons among adjusted treatment means were examined by the Tukey's test.

RESULTS AND DISCUSSION

The effect of treatments on broiler growth performance is presented in Table 2. Level of JCM inclusion had a negative effect on body weight ($p<0.001$), which was more evident during the last two weeks of the trial. Weight gain ($p<0.001$) was negatively affected during all experimental periods, and these effects were more pronounced when the diets with 50 and 100 g JCM/kg were fed (J_5 and J_{10}). Overall, the birds fed diets $J_{2.5}$ and J_5 presented 31% and 54% lower weight gain compared with the control group, respectively, while animals fed the diet with highest level of JCM inclusion (J_{10}) presented severe weight loss during the trial. Diets with JCM also decreased feed intake ($p<0.001$). Diet $J_{2.5}$ decreased feed intake by 29%, diet J_5 reduced it by 38% and diet J_{10} by 65%. The observed negative effects could be partially explained by the reduction of the metabolizable energy content of the diets containing JCM and the increase in fiber content (Table 1). Even though it is expected that deficient energy diets can negatively affect animal performance, the decrease of the estimated metabolizable energy content of the diets (-2.0%, -4.1% and -8.3%, respectively for $J_{2.5}$, J_5 and J_{10}) could not have influenced growth performance results to the observed extent. Negative effects of fiber on poultry performance have been reported by several authors (Jørgensen *et al.*, 1996; Sklan *et al.*, 2003; Jiménez-Moreno *et al.*, 2011), but it has also been argued that this response depends, amongst other factors, on the type and amount of fiber in the diets and the age of animals (Mateos *et al.*, 2012). In the present experiment, dietary crude fiber levels were below the limit of 70 g/kg maximum incorporation level normally suggested by poultry producers and feed manufacturers, and birds were already 8 weeks old when the trials begun. In this way,

Table 2 – Effect of diets on adjusted treatment means of broiler body weight, weight gain, and feed intake

	Diet				SEM	*P*-value
	J_0	$J_{2.5}$	J_5	J_{10}		
Body weight (g)						
48 days	2470.0	2603.8	2552.5	2618.3	51.37	0.488
55 days	3022.5^{a}	2922.5^{a}	2866.7^{a}	2584.3^{b}	49.08	0.001
62 days	3679.0^{a}	3448.8^{ab}	3258.6^{b}	2593.1^{c}	84.02	<0.001
69 days	4416.2^{a}	3923.1^{ab}	3568.9^{b}	2432.4^{c}	160.23	<0.001
76 days	4923.5^{a}	4179.7^{ab}	3625.9^{b}	2316.1^{c}	193.41	<0.001
Weight gain (g/day)						
48-55 days	66.5^{a}	52.2^{ab}	44.2^{a}	3.9^{b}	7.02	0.001
55-62 days	93.8^{a}	75.2^{ab}	56.0^{b}	1.2^{c}	7.29	<0.001
62-69 days	105.5^{a}	67.8^{ab}	44.3^{b}	-21.6^{c}	13.81	0.001
69-76 days	84.5^{a}	42.8^{b}	9.5^{bc}	-19.4^{c}	9.49	<0.001
48-76 days	87.6^{a}	60.1^{ab}	39.6^{b}	-8.9^{c}	7.14	<0.001
Feed intake (g/day)						
48-55 days	187.9^{a}	182.4^{a}	158.9^{a}	105.6^{b}	9.10	<0.001
55-62 days	254.4^{a}	174.9^{b}	170.8^{b}	114.4^{b}	17.91	0.004
62-69 days	321.2^{a}	202.1^{b}	189.9^{b}	84.9^{c}	21.08	<0.001
69-76 days	300.6^{a}	192.7^{ab}	142.7^{bc}	76.2^{c}	25.51	0.001
48-76 days	266.0^{a}	188.0^{b}	165.6^{b}	95.3^{c}	16.24	<0.001

$^{a-c}$ Means with different superscripts differ at $p<0.05$.

although some effects of fiber might be expected, growth performance data indicate that other factors have contributed to these results.

As stated before, PE are considered to be the main toxic component of JCM, limiting its use in animal feeding, and the adverse effects on animal performance and health observed in this experiment, due to the level of JCM inclusion in the diets, are consistent with the results presented by other authors. Nevertheless, these deleterious effects depend on the dose and on the vulnerability of the different animal species. Growth depression was observed by El-Badwi & Adam (1992) and El-Badwi *et al.* (1992) when feeding *Jatropha curcas* seeds to Brown Hisex chicks at concentrations of 0.1 and 0.5%. More recently, other authors have also reported reduced feed intake and growth in rats (Aregheore *et al.*, 2003) fed diets containing JCM with final PE concentrations varying from 0.015 to 0.021%. Our results indicate that broilers fed diets containing 0.03 to 0.06% of PE systematically presented lower performances compared with the control diet with no addition of JCM, during all the experimental periods (Table 2). The birds fed the diet with the highest PE concentration (0.06%) immediately showed signs of growth depression in the first week of the trials and lost weight from the second experimental week onward. Although less evident, even the diets containing 0.02% of PE caused a decrease in body weight gain and feed intake. These results show that even at very low concentrations PE can cause severe effects on broilers.

It should be noted that although no deaths were recorded during these trials, signs of morbidity were evident in animals fed the diets J_5 and J_{10}. Nevertheless, clinical symptoms normally described as characteristic signs of intoxication by JCM such as diarrhea, dyspnea and hemorrhages (Makkar & Becker, 1998) were not identified, suggesting that low levels of PE in diets will not cause severe clinical signs. Similar results were reported by Berenchtein *et al.* (2014) in an experiment conducted with pigs. These authors also did not observe typical signs of poisoning when animal were fed diets containing PE concentrations lower than 0.01%. However, the dietary levels of JCM had a negative effect on body weight, average daily gain, and average daily feed intake. Therefore, threshold levels of PE in animals diets must be carefully evaluated as available data on animal toxicity has been basically obtained in trials using force-feeding of feed or its organic solvent/aqueous extracts (Li *et al.*, 2010), and not in growth trials.

The experimental diets had no effect on the weight of liver or kidneys (Table 3). However, a trend ($p=0.0776$) towards an increase in the length of the small intestine as a function of the level of JCM inclusion in the diets was observed. It is well known that intestinal weight and length maybe modified as a response to the diet (Bradford, 1996) and that changes in the amount and type of fiber increase the size of the small intestine (Jorgensen *et al.*, 1996). Performance results of the broilers given diets with different levels of JCM inclusion clearly show a decrease in feed intake, and it seems that the birds had to readjust the development of their digestive tract in order to cope with the reduced amount of nutrients being absorbed. In the same way, the increased metabolic requirements of broilers may have caused increased cardiac output and heart enlargement, leading to the observed increase in heart weight ($p<0.05$).

The detrimental effects of JCM inclusion in the diets, possibly due to the toxic effects of PE, are evidenced by the decrease in spleen and testis relative weight (Table 3). Diet J_{10} decreased the spleen and testis relative weights by 46% and 90%, respectively. A wide range of biological effects, including tumor promotion and inflammation, are attributed to PE, and these adverse effects are highly structure-specific (Baldini *et al.*, 2012). Although lymphoid organ changes should be carefully interpreted (Haley *et al.*, 2005), the observed decrease in spleen weight, associated with other effects such as weight loss and the observed alterations in the testis, may be attributed to severe toxicity. The histological examination of the testis reinforces the

Table 3 – Internal organ relative weight (g/kg live weight) and relative length of small intestine (cm/kg live weight) of broilers

	Diet				SEM	*P*-value
	J_0	$J_{2.5}$	J_5	J_{10}		
Liver	14.78	15.70	15.58	14.27	0.535	0.805
Intestines	40.96	42.78	47.98	54.39	1.907	0.078
Heart	4.22^b	5.80^a	5.59^a	5.23^{ab}	0.211	0.014
Spleen	0.92^a	0.69^{ab}	0.61^b	0.50^b	0.044	0.002
Kidneys	4.51	4.96	4.60	3.98	0.131	0.093
Testis	1.78^a	0.23^b	0.23^b	0.18^b	0.206	0.003

$^{a-b}$ Means with different superscripts differ at $p<0.05$

direct detrimental effect of Jatropha incorporation on the sexual development of the broilers (Figure 1). While the testis from control group were well developed, the testis of birds fed diets $J_{2.5}$, J_5 and J_{10} were atrophic, with a decrease in the size of seminiferous tubules, which were small and lined with inactive Sertoli cells and rare spermatogonia. No significant lesions were observed in the heart, liver, kidney, spleen or lung tissues examined. Rakshit *et al.* (2008) fed different diets containing PE contents similar to the inclusion rates used in our study to rats, and although no statistical histological differences were observed in the vital organs, also identified minor changes in the seminiferous tubules of the testis. Furthermore, these authors also reported negative growth rates in the animals consuming diets with PE.

Therefore, although a wide range of detrimental biological effects are attributed to phorbol esters and their different derivatives, threshold levels should be carefully studied as toxicological studies may not reflect overall animal growth performance. For broiler diets, further studies are necessary to evaluate the inclusion level of *Jatropha curcas* meal, as even very low concentrations of phorbol esters seem to have negative effects on animal performance, as well as to determine the minimum inclusion level of *Jatropha curcas* meal that will require detoxification treatments.

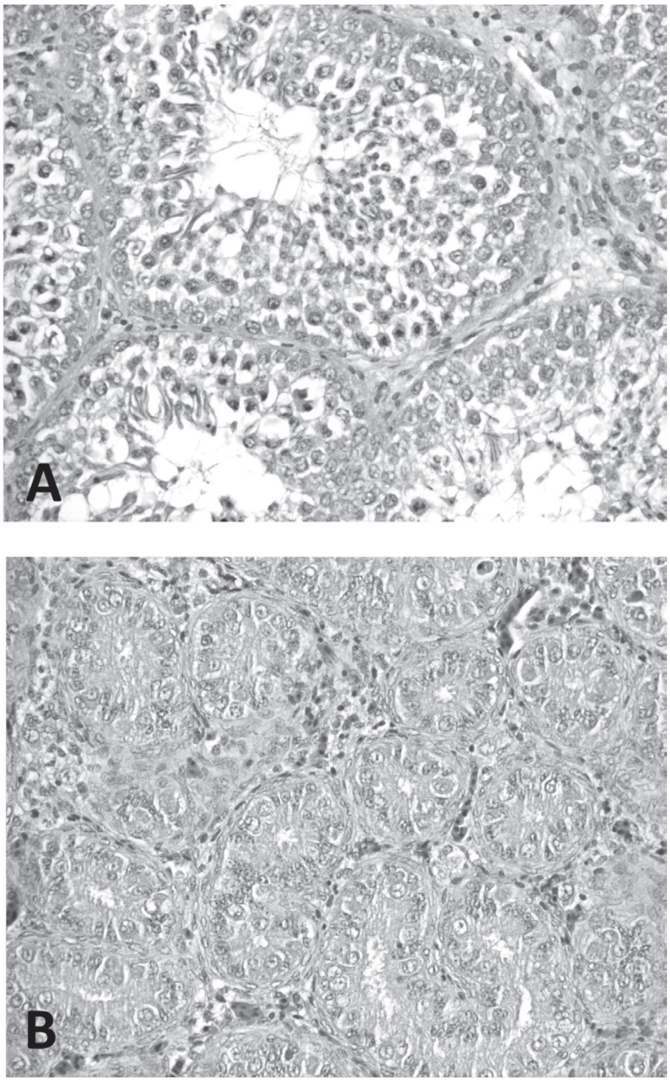

Figure 1 – Testis tissue of broilers fed diets J_0 (A) and J_{10} (B).

ACKNOWLEDGMENTS

This work was supported by a research protocol financed by Galp Energia SGPS, SA, Portugal.

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