



ORIGINAL RESEARCH ARTICLE

Performance and organ histopathology of growing Japanese quails (*Coturnix coturnix japonica*) fed heat treated jatropha seed cake substituted for soyabean meal***Agboola, A. F and Adenuga, A. A***Department of Animal Science, University of Ibadan, Ibadan, Nigeria***Correspondence: +234(8)022004830, adebisi.agboola@gmail.com***ABSTRACT**

The 14-day study investigated the effect of heat treated jatropha seed cake (JSC) on the performance characteristics and histopathological parameters of growing Japanese quails. Two hundred 2-week-old Japanese quails were weighed and randomly assigned to 5 treatments with 5 replicates of 8 birds each. Treatment 1 was the basal (corn-soyabean diet) with no *Jatropha* seed cake (JSC). Treatments 2, 3, 4 and 5 contained the basal diet and 5, 10, 15 and 20% JSC respectively. Weekly weight gain, feed intake and percentage mortality of birds were assessed. At day 28, liver and bursa of fabricius of two birds per replicate were harvested for histopathological examinations. The results showed that feed intake (FI) of birds decreased as the levels of JSC increased across the diets. Birds on the control diet had the highest FI (146.65g/b) while the birds fed 20% JSC diet had the least FI value (69.97g/b), though similar to those on 15% JSC diet. Weight gain of birds on the control diet and those on 5% JSC diet were significantly ($P < 0.05$) improved when compared to birds on other diets. A remarkable weight loss was observed in birds on 20% JSC diet. A similar trend was recorded in the feed conversion ratio (FCR) of birds on 20% JSC diet. However, birds fed with the control diet, 5, 10 and 15% JSC diets had identical FCR. There was no mortality recorded for birds on the control diet and those on 5% JSC diets, meanwhile 25% mortality was recorded for birds on 10% JSC while 70% and 90% mortality were observed in birds on 15% and 20% JSC respectively. The results of histopathological indices of birds on the experimental diets showed that higher inclusion levels of heat treated JSC had a negative effect on the liver and bursa of fabricius of the birds as compared to birds fed 5% JSC diet. Increased congestion of the portal vein, vascular degeneration and sinusoidal congestion of hepatocytes were observed on the liver while hyperemia, necrosis and distension of interfollicular septum by oedema, hemorrhage and fibrosis were observed in the bursa. Conclusively, 5% heat treated JSC dietary level was considered the safest level for growing Japanese quails as percentage mortality increased with increasing levels of JSC inclusion.

Keywords: *Jatropha* seed cake, physical treatment, growth, histopathological indices and Japanese quails**INTRODUCTION**

The increasing world human population averaging seven billions (UN, 2012) resulted into concomitant increase in demand and competition for conventional food/feedstuff used both by man and livestock. This is peculiar to underdeveloped and developing countries where food production cannot keep pace with the high population growth. This competition between feed industry and man brings about an increase in the prices of these orthodox feedstuffs. This justifies research efforts on alternative feed ingredients that are not staple food for human consumption. Several plants with high nutritive values remain unexploited in feeding livestock; among which is *Jatropha curcas*. *J. curcas* is a large drought-resistant shrub belonging to the family *Euphorbiaceae*. It is cultivated in central and South America, Southeast Asia, India and Africa (Schmook and Seralta-Peraza, 1997). It has a lifespan of more than

50 years and can grow on marginal soils with low nutrient content (Openshaw, 2000; Saraiva *et al.*, 2004). Two genotypes of *J. curcas* are available in Mexico, toxic and non-toxic. The seeds of the non-toxic genotypes are consumed by humans after roasting (Soerawidjaja, 2010). *Jatropha curcas* seeds contain around 20- 40% oil. Its oil fraction consists of both saturated (14.1% palmitic acid and 6.7% stearic acid) and unsaturated fatty acids (47% oleic acid and 31.6 of linoleic acid (Martinez-Herrera *et al.*, 2006).

The oil can be converted into bio-diesel; as a substitute for diesel fuel. It also has medicinal function for the treatment of; diseases like dysentery, hemorrhoids, coated tongue, infertility, small pox, skin infections etc. However, the residue; the seed cake and kernel could be detoxified and used as animal feed ingredient. The defatted meal of seed/kernel has been found to contain a

high amount of protein, which is ranged between 50% and 62%. With exclusion of lysine, all other essential amino acids in *J. curcas* meal protein have been reported to be in higher concentrations than those of the FAO reference pattern suggested for pre-school children (Makkar *et al.*, 1998a, b). However, the presence of toxic factors (phorbol esters) and anti-nutrients (tannins, lectins and phytate) restricts the use of *Jatropha* seeds in poultry feeding.

Several approaches have been employed for detoxifying defatted seed cake and kernel meal. Makkar and Becker (1997) reported that ethanol (80 percent) or methanol (92 percent) [1:5 w/v] reduced both the saponins and phorbol esters by 95 percent after four extractions. Heat treatment in presence of alkali was also reported to be effective in reducing phorbol esters (Abou-Arab and Abu-Sale (2010). Martinez-Herrera *et al.* (2006) studied the effect of various treatments, such as hydrothermal processing techniques, solvent extraction, solvent extraction plus treatment with NaHCO_3 , and ionizing radiation, to inactivate the antinutritional factors in *jatropha* kernel meal. Trypsin inhibitors were reported to be easily inactivated with moist heating at 121°C for 20 minutes (Makkar and Becker, 1997). Most of the aforementioned processing methods were employed in detoxifying *jatropha* seeds but with limited results and intrinsic limitations. Physical treatment is one of the easiest and simplest method farmers especially the local farmer uses to detoxify the anti-nutrients in *jatropha*. This treatment includes roasting, sun drying, boiling, autoclaving and steam washing or extrusion among many others. It was therefore the objective of this study to determine the effect of heat treated *jatropha* seed cake on the performance and histopathological parameters of growing Japanese quails.

MATERIALS AND METHODS

Experimental site

The research was conducted at the Poultry unit of the Teaching and Research farm, University of Ibadan, Ibadan, Oyo state, Nigeria.

Preparation of the test ingredient

The *jatropha* seeds were sourced from a reliable *jatropha* plantation in Ibadan, Oyo state. The seeds were toasted whole on a frying pan at 80°C for 20 minutes before oil extraction was done with the use of mechanical screw press (a palm oil extraction press). The press residue which is the seed cake was immersed in water and heated to 90 °C for 45 minutes for further oil removal. Cooling was allowed to take place for separation of oil moisture and the residue. The

separated seed cake was further sun dried for 5 days until significant amount of moisture was removed. The cake was milled using hammer mill. Other feed ingredients were purchased and prepared at the University of Ibadan Feed mill. The preparation process was done to simulate the technique carried out by local farmers.

Experimental diets and management of birds

Two hundred (200) one-day-old Japanese quails used for this study were procured from a reputable hatchery in Ibadan. The birds were brooded for 2 weeks, reared and housed in a 25 unit compartment cage placed in a well-ventilated and illuminated poultry house. Birds were randomly allotted to 5 dietary treatments consisting of 5 replicates of 8 birds per replicate. The five diets formulated had varying inclusion levels of heat treated *jatropha* seed cake (JSC) substituting for soyabean meal to meet the requirements of growing quails (NRC, 1994). Treatment 1 which was the control (basal diet) without JSC was a corn – soyabean meal diet. Treatments 2, 3, 4 and 5 had the basal diet and 5, 10, 15, and 20% JSC at the expense of soyabean meal. The diets were offered to the birds from day 14 to 28. Experimental diets (Table 1) were given *ad libitum* and birds had free access to clean water. Feed intake and weight of birds were monitored on a weekly basis. At day 28, two birds from each replicate were sacrificed and organs were harvested for histopathological examination.

Performance parameters

Weight Gain (WG): The initial weight was measured on the first day of the experiment and subsequently on weekly basis. WG was computed as final weight (FW) – initial weight (IW). Feed Intake (FI): This was the measurement of quantity of feed consumed on a weekly basis. FI = Amount of feed consumed – Amount of feed remaining. Feed Conversion Ratio (FCR) = Feed intake (g) /Weight gain (g). Mortality: this was measured in percentage as number of dead birds / total number of birds × 100.

Histopathological examination

Two organs (liver and bursa of fabricius) were harvested from two birds from per replicate and then transferred into specimen bottles containing 10% formalin where normal H and E standard procedures were performed according to the methods of Iji *et al.* (2001). Small specimens of the organs liver were taken from each experimental group, fixed in neutral buffered formalin, dehydrated in ascending concentration of ethanol (70, 80 and 90%), cleared in zylene and

Table 1. Gross composition (g/kg DM) of experimental diets

Ingredients, g/kg	Jatropha seed cake inclusion				
	0	50	100	150	200
Corn	562.0	562.0	562.0	562.0	562.0
Soyabean meal	400.0	380.0	360.0	340.0	320.0
Jatropha seedcake	0.0	20.0	40.0	60.0	80.0
Di-calcium phosphate	15.0	15.0	15.0	15.0	15.0
Limestone (38% Ca)	12.0	12.0	12.0	12.0	12.0
Salt	2.5	2.5	2.5	2.5	2.5
*Vit-Min Premix	2.5	2.5	2.5	2.5	2.5
DL- Methionine	3.5	3.5	3.5	3.5	3.5
L-Lysine, HCL	2.5	2.5	2.5	2.5	2.5
Total	1000	1000	1000	1000	1000
Calculated Nutrient (g/kg)					
Protein	224	225	227	228	229
Metabolisable energy (Kcal/kg)	3010	3020	3030	3039	3049
Calcium	8.4	8.4	8.3	8.3	8.3
Phosphorus	6.8	6.7	6.6	6.5	6.3
Lysine	15.3	15.2	15.2	15.1	15.1
Methionine	7.2	7.4	7.7	7.9	8.2

Vit-Min= Vitamin-Mineral, *Composition of Premix per Kg of diet: vitamin A, 12,500 I.U; vitamin D₃, 2,500 I.U; vitamin E, 40mg; vitamin K₃, 2mg; vitamin B₁, 3mg; vitamin B₂, 5.5mg; niacin, 55mg; calcium pantothenate, 11.5mg; vitamin B₆, 5mg; vitamin B₁₂, 0.025mg; choline chloride, 500mg; folic acid, 1mg; biotin, 0.08mg; manganese, 120mg; iron, 100mg; zinc, 80mg; copper, 8.5mg; iodine, 1.5mg; cobalt, 0.3mg; selenium, 0.12mg; Anti-oxidant, 120mg.

embedded in paraffin. Sections of 4–6µm thickness were prepared and stained with hematoxylin and eosin according to Harris (1988). The slides prepared were viewed under a microscope and photomicrograph captured using a motic camera and the photomicrograph were transferred to the computer for further pathologic reading.

EXPERIMENTAL DESIGN

The experimental design was a completely randomized design.

CHEMICAL ANALYSIS

The proximate compositions of the test ingredient and diets were determined according to the methods of AOAC (2000).

STATISTICAL ANALYSIS

Data obtained were analysed by General Linear Model procedure of SAS (2008). Means differences were separated using Duncan Multiple Range Test (DMRT).

RESULTS

Chemical composition of jatropha seed cake is as shown on Table 2. The dry matter in jatropha seed cake was 95.07%. The crude protein level was 47.97%, ether extract, crude fibre, ash and nitrogen free extract levels

were 2.66%, 6.98%, 5.87% and 36.52% respectively while metabolisable energy of 3193kcal/kg.

Table 2. Chemical composition (g/100gDM) of Jatropha Seed Cake

Parameter	Value (%)
Dry matter	95.07
Crude protein	47.97
Ether extract	2.66
Crude fibre	6.98
Ash	5.87
NFE	36.52
Metabolisable energy (Kcal/kg)	3193

NFE=Nitrogen free extract

Performance of quails on experimental diets

The performance characteristics of growing Japanese quail fed varying levels of heat treated Jatropha Seed Cake (JSC) is shown in Table 3. The Feed Intake (FI) of birds decreased as the levels of JSC increases across the dietary treatments. Birds on the control diet had the highest FI (146.65g/b) while the birds fed 20% JSC diet had the least FI value (69.97g/b), though not significantly different from those on 15% JSC diet. Significant differences ($P < 0.05$) were observed in the weight gain of birds on the experimental diets. Weight gain of birds on the control diet and those on 5% JSC

Table 3. Performance of Japanese quails fed varying levels of heat treated Jatropha seed cake (d 14-28)

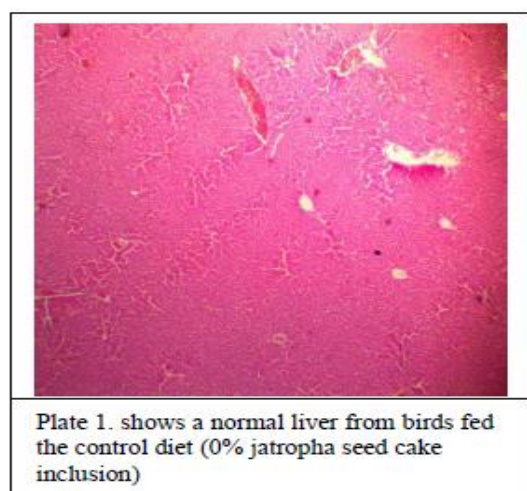
Parameters	Jatropha seed cake inclusion levels (%)					SEM	P value
	0	5	10	15	20		
IW (g/bird)	43.63	40.33	42.48	41.90	43.65	1.05	0.185
FW (g/bird)	108.53 ^a	95.70 ^b	81.75 ^c	68.00 ^d	37.34 ^e	2.86	<.0001
FI (g/bird)	146.65 ^a	139.70 ^a	111.22 ^b	82.46 ^c	69.97 ^c	4.63	<.0001
WC (g/bird)	64.90 ^a	55.38 ^a	39.27 ^b	26.10 ^c	*6.31 ^d	2.96	<.0001
FCR	2.27 ^b	2.54 ^b	2.85 ^b	3.48 ^b	*16.22 ^a	1.35	0.0058
Mortality (%)	0	0	25	70	90		

^{abcde}Means with same superscript are not significantly different ($p > 0.05$), IW= Initial weight, FW= Final weight WC= weight change, FI= feed intake, FCR= feed conversion ratio SEM= standard error of mean, *= Numerical difference as a result of mortality recorded during the duration of the experiment, the weight loss had a negative effect on the feed conversion ratio of the treatment.

diet were significantly ($P < 0.05$) improved as compared to birds on other dietary treatments. A remarkable weight loss was observed in birds on 20% JSC diet. Similar trend was recorded in the Feed Conversion Ratio (FCR) of birds on 20% JSC diet. However, birds fed with the control diet, 5, 10 and 15% JSC diets had identical FCR. There was no mortality recorded for birds on the control diet and those on 5% JSC diets, meanwhile 25% mortality was recorded for birds on 10% JSC while 70% and 90% mortality were observed in birds on 15% and 20% JSC respectively.

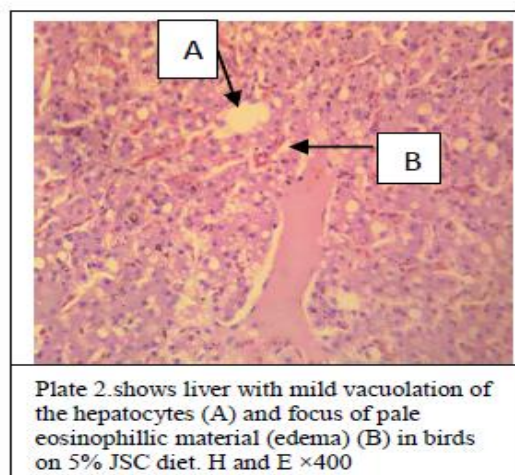
Histopathological readings

Plates 1 and 6 showed the sample sectioning of liver and bursa of fabricius of birds fed 0% JSC based diet. It was observed that there was no visible lesion in the liver (Plate 1). The bursa of fabricius showed primary epithelial fold, bursal lumen and middle submucosal connective tissues and muscle wall.



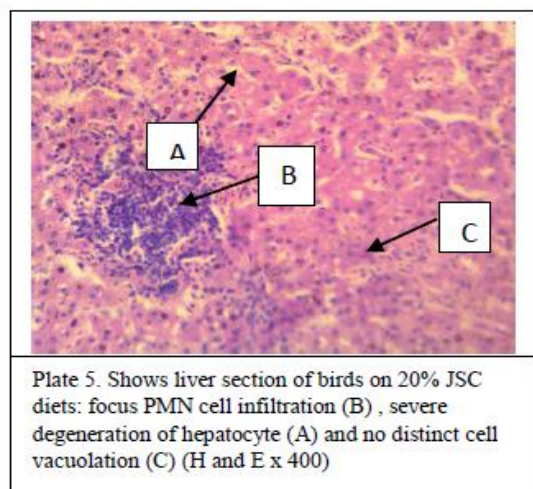
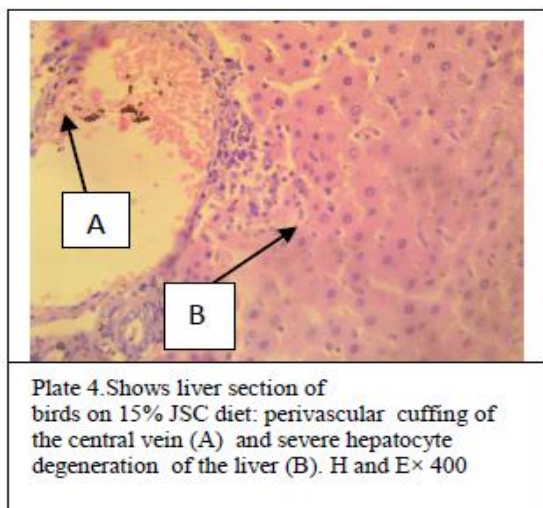
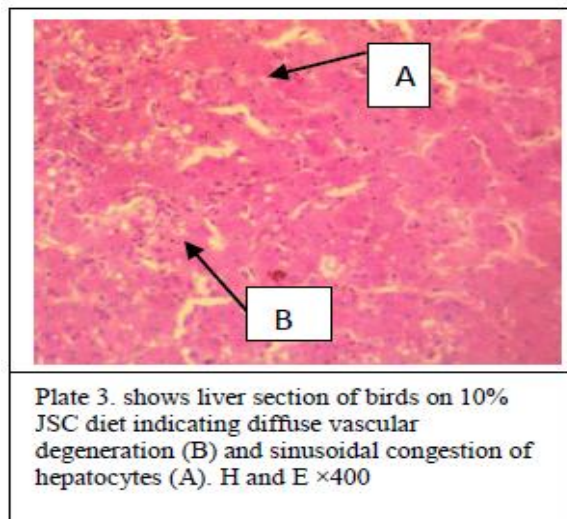
As shown in Plates 2 and 7, observations indicated that the liver of birds fed 5% JSC diet contained moderate congestion of portal veins and mild vascular degeneration of hepatocytes. The bursa showed mild

hyperemia and increased fibrosis of septum. The bursa shows a focus of lymphoid necrosis with associated vacuolation of the follicle. Follicles were reducing in size and also an associated thickening of the interfollicular septae. Plates 3 and 8 shows liver and bursa of fabricius sectioning of birds fed 10% JSC diet: mild and diffuse vascular congestion were observed, there was a severe focal area of vacuolar degeneration of hepatocytes in the liver. In the bursa, it was observed that there was severe hyperemia of the bursa. Mild sinusoidal congestion of hepatocytes was also observed in the liver. Observations from plates 4 and 9 on section from birds fed 15% JSC diets: shows a widespread range of mild vascular congestion and severe degeneration of hepatocytes of the liver. Perivascular cuffing of the portal veins was also seen. Moderate thickening of the septum by fibrosis and oedema were observed in the bursa of fabricius.



Plates 5 and 10 shows liver and bursa of fabricius of birds on 20% JSC diet: Mild, sinusoidal congestion and vacuolation of hepatocytes were observed in the liver. There was no distinct cell vacuolation, also a focus of hepatic necrosis and severe infiltration of

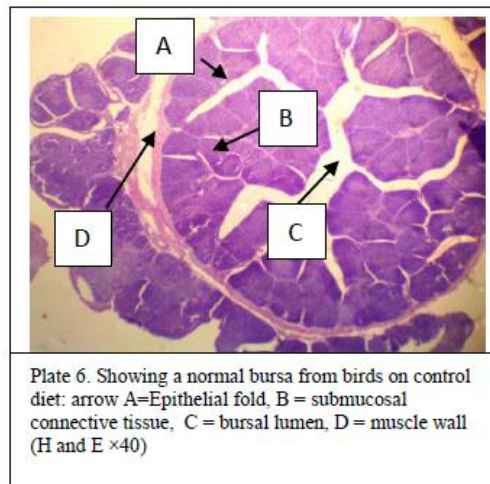
polymorphonuclear cells was observed in the liver. Bursa of fabricius showed degenerating follicle and marked fibrosis. Also degenerating mid submucosal connective tissues was observed.



DISCUSSION

Performance of quails on experimental diets

The results showed that the inclusion of heat treated jatropha seed cake in the diets of growing Japanese quails did not improve the feed intake and the weight gain especially at higher levels of inclusion.



This result tends to be similar to the report of Pasaribu *et al.* (2010) who fed broiler chicks with 4% jatropha seed meal (JSM) diets that were processed using different methods of detoxification. The authors reported that there was significant decrease in the feed intake of birds fed both physically and chemically treated JSM as compared to birds on the control diet. The authors concluded that JSM had a negative effect on palatability and general acceptability even at 4% inclusion level. The reduction in feed intake as the level of heat treated jatropha seed cake increases as observed in the present study resulted in poor weight gain and overall performance and this ultimately led to weaker birds and higher percentage mortality recorded. Similar results was reported for goats in a study by Belewu *et al.* (2010) where the nutritive value of fungi treated *Jatropha curcas* kernel cake (JKC) fed to goats was evaluated, it was reported that dry matter feed intake reduced across the dietary treatments. The highest dry matter intake was recorded for animals on diet devoid of jatropha diet and the least was recorded for animal on diet D (50% *Trichoderma* treated JKC + 50% Soyabean cake) with the highest inclusion level of the cake. They further concluded that decreased dry matter intake with the increased levels of fungi treated JKC in the diet, resulted in reduced weight gain across the treatments. This observation is also in consonance with the work of Chivandi *et al.* (2006) and Belewu (2008) in pigs and rats studies respectively. Kasuya *et al.* (2013) reported that the dry matter intake (DMI) and nutrient digestibility were influenced by the level of detoxified jatropha seed cake in the diet of birds. Although it was

further postulated that the reduction in the DMI of birds may be attributed to a reduction of the energy value in the experimental diets. In which case, the birds consumed more DM to reach their energy requirement, and may not necessarily be as a result of the test ingredient (detoxified jatropha seed cake) in the diets.

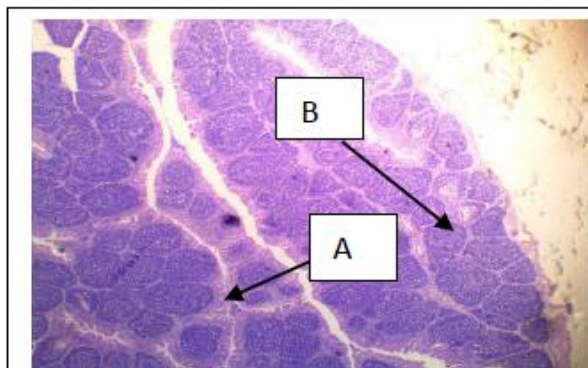


Plate 7. Showing bursa with mild vascular degeneration (B) and reduced follicle size (A) in birds fed 5% JSC diets (H and E $\times 100$).

In agreement with the present study, Announgu *et al.* (2010) reported that inclusion of jatropha seed meal had no remarkable effect on feed conversion ratio in monogastric rat. This was in agreement with Pasaribu *et al.* (2010) who reported that dietary inclusion of jatropha seed meal had no significant effect on feed conversion ratio in broiler chickens. High mortality recorded in the birds fed higher inclusion level of JSC in the present study was supported by Announgu *et al.* (2010) who indicated that at 20% and 25% dietary inclusion of jatropha seed meal resulted in 100% mortality of animals within one week of the experiment. Rahma *et al.* (2013) also reported higher heart and kidney weights and lower lung weight of rats fed physically treated (roasting, germination, autoclaving) *Jatropha curcas* seeds than those fed casein diet. Improvement in weight gain of quails on the control diet and 5% JSC diet observed in this study agrees with the findings of Belewu *et al.* (2010) and Chivandi *et al.* (2006) where the authors observed a better performance in goats and broilers respectively than those on higher levels of JKC and JSC diets. Meanwhile, Kasuya *et al.* (2013) experiment was not in consonance with the result of the present study but they explained that the low energy of the detoxified jatropha seed cake may have increased the feed intake and in positively affect the performance of the chicks.

Histopathological readings of bird's organs on experimental diet

Microscopic changes as a result of feeding varying levels of heat treated jatropha seed cake (JSC) in the liver and bursa of fabricius of growing Japanese quails reflected the effects of toxic principle (phorbol ester) in jatropha seeds on these birds.

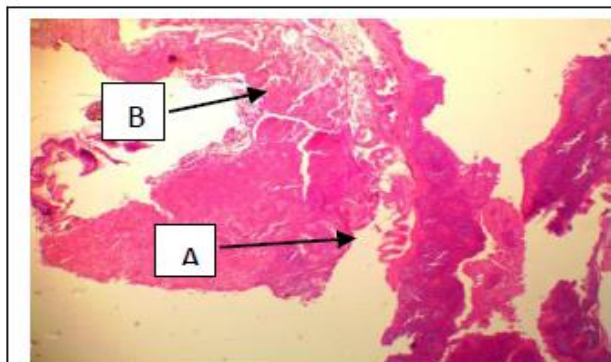


Plate 8. Showing bursa of fabricius section from birds on 10% JSC diet; severe hyperemia of the bursa (A) and loose connective tissue (B) H and E $\times 40$

This effect was directly correlated to increased JSC in the diet of birds. The liver and bursa of fabricius are the main targets of toxin synthesis (Lafi *et al.*, 2010).

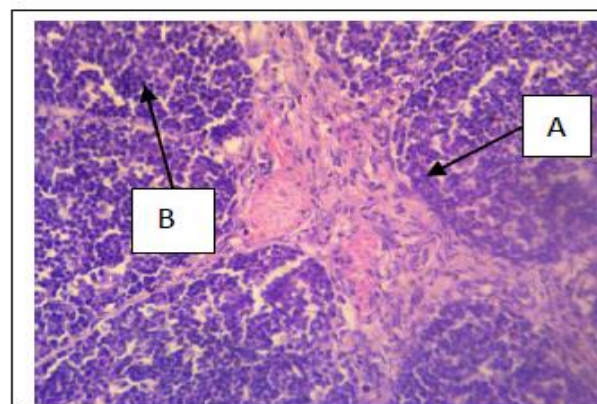
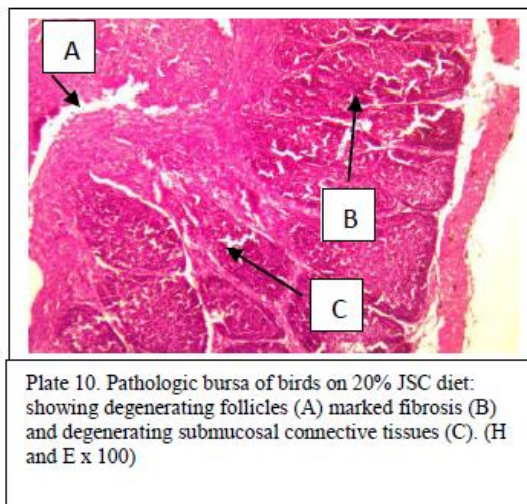


Plate 9. Shows bursa of fabricius of birds on 15% JSC diet: epithelial keratinization (A), thickening of the septum (B) by fibrosis and oedema. (H and E $\times 100$)

Degeneration of vascular tissues were observed, mild focal vacuolar degeneration of hepatocytes were seen in birds on higher levels of JSC diets. Birds on 15 and 20% jatropha seed cake diets showed thickened septum by fibrosis and oedema in the bursa of fabricius. Also perivascular cuffing of the portal veins was also seen. This was supported by pathological changes in animals that consumed jatropha seeds or seed meal as reported (Kingsbury, 1969), Ahmad and Adam (1979),

Seawright (1989), Samia *et al.* (1992) and Burrows and Tyrl (2001).



This result was different from the findings of Kumar (2011) where feeding of detoxified jatropha kernel meal (DJKM) as a protein source to rainbow trouts caused no signs of histopathological lesions in the organs. The gastric glands were well developed and the epithelium lining, the luminal surface that consists of highly columnar cells and produces protective mucous was not altered. The case for the branched tubular glands was similar, as they were also well developed. It was however observed that as the level of DJKC increased, lymphocytolysis, cyst formation, marked fibroplasias and incomplete disappearance of the lymphoid follicles were evident in the bursa of fabricius. This is in agreement with the findings of Sellaoui *et al.* (2012) who observed portal and parenchymatous degeneration in the liver of broiler chicken induced with aflatoxin. Fatty liver changes, centro-lobular fatty cytoplasmic vacuolar degeneration and /or necrosis in quails and other types of poultry species reflect the chronic effect of this toxin (Rajesh *et al.*, 2000). However, Rakshit *et al.* (2008) reported that microscopic findings of various tissues/organs of rats fed *Jatropha* flour did not exhibit any related microscopic changes. Meanwhile, Adam (1974) observed histological changes in various tissues/organs in rats fed 5–50% *Jatropha curcas*. However, inclusion of jatropha seed cake at higher inclusion levels produced degenerative changes in the liver and bursa of fabricius of growing Japanese quails in the present study.

CONCLUSION AND RECOMMENDATION

The detoxifying potential of heat treatment employed in this study might not be sufficient enough for the Japanese quails to have responded positively to high levels of heat treated jatropha. Implying that within the limit of this study, 5% heat treated JSC is considered

the safest for growing Japanese quails as percentage mortality increased with higher levels of inclusion. It is therefore recommended that heat treatment detoxification procedure employed in this study should be modified without compromising the proximate profile of the jatropha.

CONFLICT OF INTEREST

Authors have declared that no conflict of interest exists.

REFERENCES

- Abou-Arab, A. A. and Abu-Salem, F. M. 2010. Nutritional quality of *Jatropha curcas* seeds and effect of some physical and chemical treatments on their anti-nutritional factors. *African Journal of Food Science*. 4. 3: 93 – 103.
- Adam, S.E.I. 1974. Toxic effects of *Jatropha curcas* in mice. *Toxicology*; 2: 67-76.
- Ahmed, O.M.M. and Adam, S.E.I. 1979. Effects of *Jatropha curcas* on calves. *Veterinary Pathology*. 16: 476-482.
- Annonu, A. A., Joseph, J. K., Apeta, D. F., Adeyinka, A. O., Yousf, M. B. and Ogunjimi, K. B. 2010. Detoxification of jatropha curcas seeds for use in nutrition of monogastric livestock as alternative feedstuff. *Pakistan journal of nutrition*. 9. 9: 902 – 904.
- AOAC, 2000. Association of Official Analytical Chemists. Official methods of analysis, 16th Ed. Arlington, V.A, USA.
- Belewu, M.A. 2008. Replacement of fungus treated *Jatropha curcas* kernel meal in the diet of rat. *Green Farming J*. 2(3): 154 -157.
- Belewu, M. A., Belewu, K. Y., and Ogunsola, F. O. 2010. Nutritive value of dietary fungi treated jatropha curcas kernel cake: Voluntary intake, growth and digestibility coefficient of goats. *Agricultural and biology journal of North America*. 1. 2: 135 – 138.
- Burrows, G.E. and R.J. Tyrl. 2001. Toxic Plants of North Americas. Iowa State Univ. Press. p. 473-486.
- Chivandi, E., Erlwanger, K.H., Makuza, S.M., Read, J.S and Mtimuni, J.P. 2006. Effect of dietary *Jatropha curcas* meal on percent cell volume, serum glucose, cholesterol and triglyceride concentration and alpha-amylase activity of weaned fattening pigs. *Res. J. Anim. Vet. Sci*. 1:18 24.
- Harris, H. E. 1988. "Cited by Carleton MA, Drury RA, Wallington EA, Cameron H 4th Ed., Oxford Univ. Press, New York, Toronto".
- Iji, P.A., Hughes, R.J., Choct, M. and Tivey, D.R. 2001. Intestinal structure and function of broiler chickens

- on wheat based diets supplemented with a microbial enzyme. *Asian-Australian J. Anim. Sci.* 14:54-60.
- Kasuya, M. C., Rodrigues da luz, J. M., Pereira, L. P., Soares, J., Montavani, H. C. and Rodrigues, M. T. 2013. Bio-detoxification of *Jatropha* seed cake and its use in animal feed. Retrieved from <http://dx.doc.org/10.5772/45895>.
- Kingsbury, J.M. 1969. Poisonous Plants of the United States and Canada. Prentice-Hall Inc. p. 190-192.
- Kumar, V. 2011. *Jatropha* meal and protein isolate as a protein source in aquafeed. PhD thesis. University of Hohenheim, Germany.
- Lafi, S. A., Taha N. A., Al-Genabi S. M. H. 2010. Histopathology of the liver affected with Aflatoxins in broiler chicks *Al- Anbar. J. Vet. Sci.*, 3 (1): 1999-6527.
- Makkar, H.P.S. and Becker, K. 1997. Potential of *J. curcas* seed meal as a protein supplement to livestock feed, constraints to its utilization and possible strategies to overcome constraints. *Biofuels and Industrial Products from Jatropha curcas*. G.M. Gubititz, M. Mittelbach & M. Trabi: Graz, Austria. 190-205.
- Makkar, H.P.S., Aderibigbe, A.O. and Becker, K. 1998a. Comparative evaluation of non-toxic and toxic varieties of *Jatropha curcas* for chemical composition, digestibility, protein degradability and toxic factors. *Food Chemistry*. 62. 2: 207-215.
- Makkar, H.P.S., Becker, K. and Schmook, B. 1998b. Edible provenances of *Jatropha curcas* from Quintana Roo State of Mexico and effect of roasting on antinutrient and toxic factors in seeds. *Plant Foods for Human Nutrition*. 52: 31-36.
- Martínez-Herrera, J., P., Siddhuraju, G., Francis, G., Dávila-Ortíz. and K. Becker, K. 2006. Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. *Food Chemistry*. 96:80-89.
- NRC. 1994. *Nutrient Requirements of Poultry*. National Academies Press Washington, D.C
- Openshaw, K. 2000. A review of *Jatropha curcas*: an oil plant of unfilled promise. *Biomass and Bioenergy*. 19: 1-15.
- Pasaribu, T., Wina, E., Tangendjaja, B., Iskandar, S. 2010. Performance of broiler chicken fed physically and chemically treated *Jatropha* seed meal. *Indonesian Journal of Agriculture*. 3:2 121 – 126.
- Rahma, H. E., Mansour, H. E., and Hamonda, S. T. 2013. Biological evaluation of *Jatropha curcas* seed as a new source of protein. *Merit research journal of food science and Technology*. Vol, 1.2: 23-30
- Rajesh, C., Ramesh, C., Katoch, S. P., Singh, S. V., Arvind M. 2000. Concurrent outbreak of chlamydiosis and aflatoxicosis among chickens in Himachal Pradesh, *India veterinarski arhiv.*, 70 (4), 207-213.
- Rakshit, K.D., Darukeshwara, J., Rathina, R. K., Narasimhamurthy, K., Saibaba, P. and Bhagya, S. 2008. "Toxicity studies of Detoxified *Jatropha* meal (*Jatropha curcas*) in Rats," *J. Food Chem. Toxicol.*, Vol. 46, No. 12, pp. 3621-3625.
- Samia, M. A., El Badwi, S. M. A., Adam, S. E. and Hapke, H. J. 1992. Toxic effects of low levels of dietary *Jatropha curcas* seed on Brown Hisex chicks. *Vet. Hum. Toxicol.* 34:112-115.
- Saraiva, L., Fresco, P., Pinto, E. and Gonçalves, J. 2004. Characterization of phorbol esters activity on individual mammalian protein kinase C isoforms, using the yeast phenotypic assay. *European Journal of Pharmacology*. 491: 101– 110.
- SAS, 2008. Statistical Analysis System, SAS users guide: statistics. SAS institute Inc. Cary, N.C. USA.
- Schmook, B. and Seralta-Peraza, L. 1997. *Jatropha curcas*: Distribution and uses in the Yucatan peninsula of Mexico. In: *Biofuels and Industrial Products from Jatropha curcas*. Gubititz GM, Mittelbach M, Trabi M (Eds.). DBV Graz. pp. 53-57.
- Seawright, A.A. 1989. Animal Health in Australia: Chemical and plant poisons. Vol.2. 2nd Ed. Australian Government Publishing Service. p. 53.
- Sellaoui, S., Alloui, N., Mehenaoui, S. and Djaaba, S. 2012. Evaluation of immune status of the Sen, S., Makkar, H. P. S., and Becker, K. 1998. Alfalfa saponins and their implication in animal nutrition. *J. Agric. Food Chem.* 46: 131–140.
- Soerawidjaja, T.H. 2010. Potential of *Jatropha curcas* L. as a non-edible feedstock of biodiesel fuel. 7th Biomass-Asia Workshop; Jakarta, Indonesia. 29 Nov.-1 Dec., 2010.
- UN (United Nations). 2012. World Population Prospects, the 2012 Revision – "Low variant" and "High variant" values". Retrieved June 15, 2013.