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Evaluation of Differently Processed Karanj (*Pongamia Pinnata*) Seed Cake on Haematological and Serum Biochemical Indices of Broiler Chickens

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Abstract: *A study was conducted to determine the effects of differently processed karanj (Pongamia pinnata) seed cake (KSC) on blood profile broiler chickens. The karanj seed were processed using three different methods (soaking for 24 hours; boiling for 60 minutes; and toasting). Diets were formulated and designated as: T1 (0% KSC), T2 (5% of raw KSC), T3 (5% soaked KSC), T4 (5% KSC boiled for 60 minute) and T5 (5% toasted KSC). The birds were allocated to the five treatments in groups of 30 birds, each treatment replicated three times with 10 birds per replicate in a completely randomised design (CRD). Data collected were RBC, Hb, WBC, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration, (MCHC) with the exception of white blood cells count, MCH, MCHC, neutrophils, and lymphocytes, all other haematological parameters measured were not affected (P>0.05) by the treatments. The results of serum biochemical indices showed that total protein, globulin, glucose, conjugated bilirubin, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were significantly (P<0.05) affected by the dietary treatments. Broiler chickens fed 0 % KSC recorded significantly (P<0.05) lower values for both ASAT and ALAT than the other treatments groups. The outcome of this study therefore indicated that inclusion of 5 % toasted KSC has no adverse effects on the blood profile of broiler chickens.*

Keywords: *Karanj Seeds Cake (KSC), Haematology, Serum Biochemical, Pongamia pinnata*

Introduction

The development of poultry industry has been described as the fastest way of ameliorating the animal protein deficiency in third world countries, due to the high turn-over rate associated with poultry production and economic efficiency. (Dipeolu *et al*., 2004). However, the ever-increasing cost of poultry product (meat and eggs) make it necessary to explore the use of alternative feed ingredients that are cheaper, locally available and low human preference. In realization of this, there is the need to evaluate the nutritional adequacy of such feed stuff. One of such alternative feed ingredients is karanj (*Pongamia pinnata*) seed cake.

Pongamia pinnata popularly known as karanj, belongs to the family *Leguminosae,* and is a medium-sized glabrous tree capable of growing under wide range of agro-climatic conditions. The tree is abundantly found in Andhra Pradesh, Bihar, Karnataka, Maharastra, Tamil Nadu and West Bengal. It naturalized from India, Pakistan and Sri Lanka throughout south-east Asia to north-eastern Australia, Fiji and Japan. It is planted in the humid tropical lowlands around

the world, and has been introduced into Egypt and the United States (Orwa *et al.,* 2009); It is also available in Nigeria as ornamental plant.

Oil is the most important product of the pongamia seed and vast amounts of seeds are collected in India for commercial processing for industrial uses. In India, the availability of karanj seed has been estimated to be 2, 000,000 metric tons per year (Anon., 2006). The production of bio-fuel from karanj oil has already commenced in some areas and considerable quantity of karanj seed cake is produced as a by-product (Anon., 2006). Besides, karanj oil has been traditionally used for different industrial purposes like leather dressing, soap making, lubrication, and illumination among others. Currently, karanj seed has limited demand and is going for low value applications like manure especially in India (Anon., 2006).

MATERIALS AND METHOD

The study was conducted at the Livestock Unit of the Teaching and Research Farm, Department of Animal Production Technology, Ramat Polytechnic, Maiduguri. Maiduguri is located between latitude 11°5' and 12^0 North, longitude 13°09' and 14^0 East at an altitude of 354 m above sea level
(DNMA, 2013).

Seed Collection and processing

The karanj seeds were obtained from pongamia plant (karanj) *(Pongamia pinnata)* across Maiduguri Metropolitan Councile. The seeds were divided into four (4) batches. The first batch was left raw, while the other three (3) batches were classified according to the following processing methods;

1. Process 1: The second batch was soaked in tap water for 24 hours at room temperature in a plastic container. At the end of soaking, the water was decanted and the seeds later sun-dried for three days or more depending on the weather.
2. Process 3: The seeds was boiled for 60 minutes. Timing was commenced few minutes after adding the karanj seeds in boiling water. The boiled seeds were drained and sundried.
3. Process 4: The karanj seeds was toasted on open frying pan containing sand; it was stirred continuously until the seeds are crispy and acquired a characteristic aroma of roasted beans. The processed seeds were milled and oil was extracted and stored in bags until needed for feed formulation.

Experimental Stock and their Management

A total of 150 day- old broiler chicks were purchased from Zatex hatcheries for the study. The chicks were brooded for two weeks during which they were fed commercial broiler starter diet and then fed the formulated/ experimental starter diet from three to four weeks and experimental finisher diet from 5^{th} to the 9^{th} weeks.

Experimental Diets and Experimental Design

Experimental diets at the starter and finisher phases were formulated using locally procured feed ingredients which include maize, wheat offals, full-fat soya bean, karanj seed cake, fish cake, bone cake, limestone, premix, methionine, common salt and lysine.

five starter and finisher diets were formulated with the same inclusion levels of 5% of karanj seed cake. The diet was designated as: T1
(control) (0% karanj seed cake), T2 (5% cake from raw karanj), T3 (5% cake from karanj seed soaked in water), T4 (5% cake from boiled karanj seed), and T5 (5% cake from roasted karanj seed) as shown in Tables1 and 2. The experimental birds were allocated to the experimental diets in groups of 30 birds each and each treatment group were replicated three times with 10 birds per replicate in a completely randomized design (CRD). The study lasted for 7 weeks.

Table 1: Ingredient Composition and Calculated Analysis of the Experimental Broiler Starter Diets

Ingredient	Level of karanj seed cake included				
	T_1	T_2	T_3	T_4	T_5
	(0% KSC)	(5% RKS)	(5% SSKM)	(5%BKSC)	(5% RKSC)
Maize	47.93	44.95	45.27	44.73	46.14
Full-fat Soya bean cake	28.37	25.85	25.53	26.07	24.66
GNC	05.00	05.00	05.00	05.00	05.00
KSC	00.00	05.00	05.00	05.00	05.00
Wheat offal	10.00	10.00	10.00	10.00	10.00
Fish cake	05.00	05.00	05.00	05.00	05.00
Limestone	01.00	01.00	01.00	01.00	01.00
Bone cake	02.00	02.50	02.50	02.50	02.50
Min-vit-premix*	00.25	00.25	00.25	00.25	00.25
Methionine	00.10	00.10	00.10	00.10	00.10
Lysine	00.10	00.10	00.10	00.10	00.10
Table salt (NaCl)	00.25	00.25	00.25	00.25	00.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Crude protein (%)	23.88	23.35	22.59	23.69	22.96
Crude fibre (%)	04.00	04.06	04.10	04.07	04.10
Ether extract (%)	03.89	03.87	03.65	0385	03.77
Methionine (%)	00.48	00.47	00.46	00.44	00.44
Lysine (%)	01.49	01.48	01.46	01.44	01.39
Calcium (%)	01.00	01.00	01.00	01.00	01.00
Phosphorus (%)	00.65	00.65	00.65	00.65	00.65
ME (kcal/kg)	2848.15	2841.65	2854.49	2850.54	2921.44

ME= Metabolizable energy; GNC= groundnut cake, KSC= karanj seed Cake, RKS= Raw karanj seed Cake, SSKM= Cake from seeds soaked in water for 24 hours, BKSC A= Boiled for 60 minutes karanj seed Cake, RKSC= Roasted karanj seed Cake.

** = Bio Mix Broiler Premix supplying the following per Kg of feed:*
Vitamin A=4,000,000IU, VitaminD_3=1,000,000IU, Vitamin E = 9,200mg, VitaminK_3 = 800mg, VitaminB_1 = 400mg, Vitamin B_2 = 2200mg, Niacin=1,100mg, Pantothenic acid=3300mg, Vitamin B_6 = 1200mg, Vitamin B_{12} =6mg Folic acid = 300mg, BiotinH_2=24mg, Choline Chloride=1,200,000mg, Cobalt = 800mg, copper = 1200mg, Iodine=400mg, Iron=800mg, Manganese=16,000mg, Selenium=80mg, Zinc=12,000mg and Antioxidant=500mg

Table 2: Ingredients Composition and Calculated Analysis of the Experimental Broiler Finisher Diets.

Ingredient	Level of karanj seed cake included				
	T_1 (5% KSC)	T_2 5% 5%RKSC)	T_3 (5% SSKM)	T_4 (5% BKSC)	T_5 (5% TKSC)
Maize	49.64	46.66	46.98	46.44	47.86
Full fat Soya bean cake	21.16	19.14	18.82	19.36	17.94
GNC	05.00	05.00	05.00	05.00	05.00
KSC	00.00	05.00	05.00	05.00	05.00
Wheat offal	15.00	15.00	15.00	15.00	15.00
Fish cake	05.00	05.00	05.00	05.00	05.00
Limestone	01.00	01.00	01.00	01.00	01.00
Bone cake	02.50	02.50	02.50	02.50	02.50
Min-vit-premix*	00.25	00.25	00.25	00.25	00.25
Methionine	00.10	00.10	00.10	00.10	00.10
Lysine	00.10	00.10	00.10	00.10	00.10
Table salt (NaCl)	00.25	00.25	00.25	00.25	00.25
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Crude protein (%)	21.00	21.00	21.00	21.00	21.00
Crude fibre (%)	04.05	04.05	04.12	04.11	04.14
Ether extract (%)	03.90	03.88	03.67	03.76	03.78
Methionine (%)	00.45	00.44	00.43	00.42	00.41
Lysine (%)	01.39	01.38	01.36	01.34	01.31
Calcium (%)	01.00	01.00	01.00	01.00	01.00
Phosphorus (%)	00.65	00.65	00.65	00.65	00.65
ME (kcal/kg)	2854.16	2854.30	2940.93	2936.97	2947.29

*ME= Metabolizable energy; GNC= groundnut cake, KSC= karanj seed Cake, RKSC= Raw karanj seed Cake, SSKM= karanj Cake from seeds soaked in water for 24 hours, BKSC A= Boiled for 60 minutes karanj seed Cake, BKSC= * = Bio Mix Broiler Premix supplying the following per Kg of feed:*
Vitamin A=4,000,000IU, VitaminD_3=1,000,000IU, Vitamin E = 9,200mg, VitaminK_3 = 800mg, VitaminB_1 = 400mg, Vitamin B_2 = 2200mg, Niacin=1,100mg, Pantothenic acid=3300mg, Vitamin B_6 = 1200mg, Vitamin B_{12} =6mg Folic acid = 300mg, BiotinH_2=24mg, Choline Chloride=1,200,000mg, Cobalt = 800mg, copper = 1200mg, Iodine=400mg, Iron=800mg, Manganese=16,000mg, Selenium=80mg, Zinc=12,000mg and Antioxidant=500mg

RESULTS AND DISCUSSION

Haematological Parameters of Broiler Chickens Fed Raw and Differently Processed Karanj (*Pongamia pinnata*) Seed Cake

The results of haematological parameters of broiler chicken fed differently processed karanj (*Pongamia pinnata*) seed cake (KSC) are presented in Table 3. With the exception of white blood cells (WBC) count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophils, and lymphocytes, all other haematological

parameters measured were not affected (P>0.05) by the treatments. The packed cell volume (PCV) of 23.33 to 25.00 % obtained in this study were slightly lower than the values (25 to 45 %) reported by Banerjee (1998) for healthy chickens. However, they are close to the values of 24.33 - 26.33 % and 22.70 – 26.16 %, reported by Kwari *et al.* (2019) and Aguibe and Kehinde (2019) for broiler chickens fed processed sickle pod *(Senna obtusifolia*) seed cake and differently processed shea butter cake, respectively. Kwari *et al.* (2019) conducted their study in a similar environment. Mitruka and Rawnsleiy (1997) reported that when the PCV values are below the normal range, the chickens may likely be anaemic which invariably could result in the alteration of other physiological processes such as assimilation and utilization of nutrients. In this study, these adverse effects were not observed.

The haemoglobin concentration and RBC count were within the normal range (Anon, 1980). This is an indication of nutritional adequacy of the diets. Banerjee (1998) reported RBC values of 2 - 4 (x 10^6 / mm^3) and Hb of 7 – 13 g/dl as the ideal ranges for healthy chickens. The white blood cells (WBC) count of the birds fed 5 % RKSC and 5 % B. 30 M. KSC were (P<0.05) higher than those of control and other treatment groups. This shows that the ability of the bird fed KSC to fight against infections/foreign substances was not compromised due to effectiveness of the processing methods/

Table 3: Haematological Indices of Broiler Chickens Fed Raw and Differently Processed Karanj *(Pongamia pinnata)* Seed Cake

Parameter	Experimental Diets					SEM
	TI(0% KSC)	T2(5% RKSC)	T3(5% SKSC)	T4(5% B 60KSC)	T5(5% TKSC)	
Packed Cell Volume (%)	25.00	28.00	23.33	23.00	24.00	2.78^{NS}
Haemoglobin Concentration (g/dl)	10.20	9.300	7.77	7.63	7.93	0.92^{NS}
Red Blood Cell (10^6/ mm^3)	4.25	4.67	3.90	3.83	3.97	0.41^{NS}
White Blood Cell (x 10^3 /mm^3)	10.20^b	13.10^a	11.00^b	10.00^b	11.40^b	0.67^*
Mean Corpuscular Volume (fl)	67.17	71.86	65.00	69.72	73.61	11.00^{NS}
Mean Corpuscular Haemoglobin (Pg)	6.34^a	6.23^{ab}	5.04^{abc}	4.53^c	4.89^{bc}	0.66^*
Mean Corpuscular Haemoglobin Concentration (%)	33.23^{ab}	33.23^{ab}	33.29^a	33.19^{ab}	33.05^b	0.08^*
Differential counts						
Neutrophils (%)	20.33^b	20.67^b	24.67^{ab}	33.00^a	26.33^{ab}	5.12^*
Eosinophils (%)	0.00	0.67	2.00	0.00	1.00	1.02^{NS}
Basophils (%)	0.00	0.00	0.00	0.00	0.00	0.00^{NS}
Lymphocytes (%)	79.67^a	79.67^a	73.33^{ab}	72.33^{ab}	67.00^b	4.70^*

NS = Not Significant (P > 0.05) * = Significant (P < 0.05): SEM = Standard Error of Mean
a, b, c, = Means within the same row bearing different superscripts differ significantly (P < 0.05)
RKSC = Raw karanj seed cake
SKSC = karanj seed cake soaked for 24 hours
B.60 M. KSC = Karanj seed cake boiled for 60 minutes
TKSC = Toasted karanj seed cake

In reducing the level of anti-nutritional factors in the test ingredient. According to Oyawoye and Ogunkunle (1998), haematological components of blood are valuable in monitoring level of toxicity in feed with emphasis on feed constituents that affects blood formation.
Neutrophils values in all the treatment groups were similar except the group fed 5 % B. 60 M. KSC which recorded higher values than T1 (0 % KSC) and T2 (5 % KSC). Neutrophils are component of WBC that are involved in combating viral and bacterial infections. Broiler chickens fed KSC recorded similar lymphocyte values except birds fed 5 % TKSC which recorded the lowest value. The eosinophils and basophils values were within the normal range (0.00 – 5.00 %) as reported by Anon (1980) for domestic chickens. Eosinophils are known to phagocytize particles formed when an antigen and anti-bodies react, a strategy for combating disease infection by chickens (Adeyemo and Longe, 2007).
With respect to this study, the chickens did not suffer from any problems that may result to anaemic condition. Therefore, the processed KSC at 5 % level were well-tolerated by the broiler chickens.

Serum Biochemical Indices of Broiler Chicken Fed Raw and Differently Processed Karanj (*Pongamia pinnata*) Seed Cake

The results of serum biochemical indices of broiler chickens fed differently processed karanj **(***Pongamia pinnata***)** seed cake are presented in Table 4. The results showed that total protein, globulin, glucose, conjugated bilirubin, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT) were significantly ($P<0.05$) affected by the dietary treatments. On the other hand, albumin, urea, creatinine, cholesterol, total bilirubin and alkaline phosphate were not significantly ($P>0.05$) affected among the treatment groups. The total protein values fall within the range (5 – 8 g/dl) reported by Jain (1986) for healthy chickens. The normal values for total protein and globulin are indication of adequate protein utilization. The different processing methods used have reduced the level of the anti-nutritional factors which resulted in better protein utilization by the broiler chickens fed the processed KSC. The normal values for total protein and globulin are reflection of better quality and amount of protein in the diets (Omoikhoje *et al.,* 2004). Bush (1991) reported that an increase in total protein may be due to increase in the level of globulin while a decrease in total protein level is always due to a low albumin level.

Table 4: Serum Biochemical Indices of Broiler Chickens Fed Raw and Differently Processed Karanj (*Pongamia pinnata*)
Seed Cake

Parameter	Experimental Diets					
	TI(0% KSC)	T2(5% RKSC)	T3(5% SKSC)	T4(5% B.60 M. KSC)	T5(5% TKSC)	SEM
Total protein (g/dl)	7.00^{a}	6.67^{ab}	6.00^{b}	6.33^{b}	5.67^{c}	1.54^{*}
Albumin (g/dl)	3.6	3.67	3.67	2.67	3.23	4.45^{NS}
Globulin (g/dl)	3.00^{a}	3.00^{ab}	3.33^{a}	2.67^{bc}	1.67^{c}	4.78^{*}

Glucose (g/dl)	3.73^{c}	3.97^{bc}	6.17^{a}	5.93^{a}	5.70^{ab}	0.82^{*}
Urea (mmol/l)	4.36	4.25	4.33	4.46	4.55	0.11^{NS}
Creatinine (mmol/l)	58.43	57.00	58.00	56.43	58.23	6.58^{NS}
Cholesterol (mg/dl)	6.00	6.27	6.57	6.9	6.47	0.43^{NS}
Total bilirubin (mmol/l)	2.23	2.27	1.60	1.33	2.10	0.45^{NS}
Conjugated bilirubin (mg/dl)	3.50^{ab}	3.53^{ab}	4.33^{a}	2.60^{b}	3.90^{ab}	0.63^{*}
Alkaline phosphate (IU/L)	78.67	79.33	73.33	73.67	131.00	28.52^{NS}
Aspartate aminotransferase (ASAT) (IU/L)	139.33^{b}	222.33^{a}	222.33^{a}	217.00^{a}	226.33^{a}	29.82^{*}
Alanine aminotransferase (ALAT) (IU/L)	53.00^{b}	91.00^{a}	110.33^{a}	107.00^{a}	109.00^{a}	11.61^{*}

NS = Not Significant ($P > 0.05$)

* = Significant ($P < 0.05$): SEM = Standard Error of Mean

a, b, c, d = Means within the same row bearing different superscripts differ significantly ($P < 0.05$)

RKSC = Raw karanj seed cake

SKSC = Karanj seed cake soaked for 24 hours

B.60 M. KSC = Karanj seed cake boiled for 60 minutes

TKSC = Toasted karanj seed cake

The albumin values ranged from 2.67 to 3.67 g/dl and these were within the reference value of 2-4 g/dl reported by Jain (1986). Ewulola and Egbunike (2008) reported that values for serum albumin indicates adequacy in quality and quantity of the dietary protein whereas value less than the normal physiological value indicate hypoalbuminemia. Melluzzi *et al*. (1991) reported that changes in nutritional protein status are better shown in the albumin than in the globulin content of the blood.

The glucose levels of broiler chickens showed significant ($P<0.05$) differences among the treatment groups. The birds fed processed KSC recorded similar values which are significantly ($P<0.05$) superior to the birds fed 0 % KSC and 5 % RKSC diets. Blood glucose, which is an end product of carbohydrate digestion, is directly used to provide energy for the body. However, excess of it is being converted and stored in the form of glycogen in the liver and muscle, and for fat, protein and other biosynthesis. The finding of Melluzzi *et al*. (1991) showed that low blood glucose could be an indication of inadequate intake of energy.

The values for urea are within the acceptable range (4.25 – 4.55 mmo/l) for broiler chickens (Jain, 1986). This could be attributed to the effect of the processing methods. Serum urea originates from the diet and tissue deamination of proteins and it also indicates the quality of dietary protein (Ewulola and Egbunike, 2008). Abiola *et al*. (2001) reported that increase in urea concentration is an indication of poor protein quality. Ani and Omeje (2008) further explained that the values within the normal range imply that the dietary protein is well-utilized by the animal and this tallied with the results obtained in this study.

Serum creatinine values showed non-significant (P>0.05) differences among the treatment groups. Nworgu (2004) reported that the level of creatinine measures the degree of muscle wastage. Creatinine levels have been reported to be used as biochemical marker employed in the diagnosis of renal damage (Ojediran *et al*., 2012). The normal values obtained have further confirmed the nutritional adequacy of the dietary protein.
The levels of cholesterol (6.00 – 6.90 mg/dl) and total bilirubin (1.33 – 2.27 mmol/l) were within the normal range (2.42 – 7.80 mg/dl and (0.3 – 5.1 mg/l) respectively, reported by Zunft *et al.,* 2003. Anti-nutritional factors have been reported to reduce fats and cholesterol levels in tissues (Zunft *et al.,* 2003). Broiler chickens fed 5 % B.60 M. KSC recorded lower values for conjugated bilirubin than T3 (5 % SKSC)

The values for alkaline phosphate of broiler chickens fed the experimental diets fall within the acceptable range (73.33 to 131.00 IU/L). Akinmutimi and Onen (2008) explained that an increase in the level of alkaline phosphate was due to challenge of the liver by toxic components, consequently forcing the liver to produce more of these enzymes in a bid to detoxify the toxic effects. However, this is not the case in the present study.

Broiler chickens fed 0 % KSC recorded significantly (P<0.05) lower values for both ASAT and ALAT than the other treatments. These are liver enzymes that have linkages between the liver and the blood. Mandal and Banerjee (1982) found no adverse effect on ASAT activity in broilers fed diet containing 6 % solvent extracted karanj seed cake (SKC). The slightly higher values of ALAT observed in birds fed KSC diets could be possibly due to the effects of residual tannins and trypsin inhibitors in the diets. Ojediran *et al.* (2012) reported that the appearance of abnormal amounts of certain enzymes of intercellular origin in the blood reflects damage to an organ or tissue and anti-nutritional factors are capable of exerting such effects. The results show that broiler chickens could tolerate up to 5 % processed KSC in their diet without showing adverse effects on some serum biochemical parameters.

Conclusion

The outcome of this study further indicated that inclusion of 5 % toasted KSC has no adverse effects on the blood profile broiler chickens. The performance of the chickens fed processed KSC, especially toasted, showed similar performance to those fed control diet (0 % KSC), an indication that the processing methods used were effective in reducing the adverse effects of Anti-nutritional factors (ANFs) in KSC.

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