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HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF BROILER CHICKENS FED DOUM PALM (*HYPHAENE THEBAICA*) SEED MEAL BASED DIET

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An 8week study was conducted to evaluate the effects of *hyphaene thebaica* seed meal (HTSM) as substitute for maize on haematological and serum biochemical parameters of broiler chickens.
Five experimental diets were formulated to meet nutrient requirement standards of broilers (NRC, 1994). Diet 1 (0 % HTSM) served as the control while diets 2, 3, 4 and 5 contained 5, 10, 15 and 20 % HTSM respectively replacing maize in the diets of birds. A total of two hundred and twenty five (225) day-old broiler chicks were randomly allotted into five treatment groups with three replicates of fifteen birds each. Each group was assigned to the five experimental diets in completely randomized design (CRD). Throughout the experimental period, feed and water were provided *ad libitum* for all treatment groups. There were significant differences (P<0.05) in the haematological parameters measured except the packed cell volume (29.08- 31.89 %) and mean corpuscular haemoglobin (14.69-15.67 pg). The white blood cell (11.45-18.14 x 10^9/l), red blood cell (4.71-6.99 x 10^{12}/l), haemoglobin (6.92-10.05 g/dl) and mean corpuscular haemoglobin concentration (23.80-34.34 %) increased (p<0.05) in response to the increased level of HTSM in the diet. The results of the serum biochemical indices of broiler chickens fed HTSM diet revealed that the glucose level (6.82-9.00 mmol/l), globulin (6.78-10.10 g/l), aspartate transaminase (72.95-90.16 iu/l), alanine aminotransaminase (62.84-79.50 iu/l) and alkaline phosphatase (100.26-108.77 iu/l) increased (p<0.05) as the dietary levels of HTSM increased across the treatments while the total protein (31.83-29.11 g/l) and albumin (25.05-19.01 g/l) significantly decreased (P<0.05) as the level of HTSM increased in the diets. The serum cholesterol and urea were however not significant (P>0.05). It was concluded that HTSM has a high potential as feed ingredient in broiler diets and adequate processing of the seed to reduce the anti-nutritional factors to a tolerable level before being used in broiler diet was recommended.
Keywords: broiler, haematology, serum indices, *hyphaene thebaica* seed meal

The largest dietary requirements for poultry are energy and protein (Kanyinji and Moonga, 2014). These are predominantly supplied by maize grain and soybean meal in poultry diets, respectively. The high cost of these feed ingredients resulting from diverse usage in human diets as well as industrial applications makes it necessary to search for alternative replacements that are cost effective. It is this line of thought that has generated this research interest in Doum palm (*Hyphaene thebaica*), which is an African palm tree, common in Upper Egypt, originally native to the Nile valley, bearing an edible fruit which is glubose quandrangular, about 6 x 5 cm with a shinny orange-brown to deep chestnut skin (epicarp). It belongs to the family *Arecaceae* commonly known as doum palm, Dum Nut or gingerbread tree. It also grows in the Sahel in the hot Savanah between 12-18 N from Senegal to Northern Nigeria, Chad, Zaire and North East Africa. The young leaves are readily eaten by livestock while the old leaves are bitter and unpalatable. The rind (mesocarp) in some palm is inedible but of other, it is very palatable, highly aromatic and sweet with a taste like ginger bread hence the English name. When eaten, it serves as vermifuges and parasite expellant (Burkill, 1997). The chloroform extract of the fruits improve spermatic count of male rats at low concentration (Hetta and Yassin, 2006) but decrease it at high concentration (Hetta *et al.*, 2005). The fruit has nutritional and pharmacologic properties. Doum extracts are being used in the treatment of bilharziasis, haematuria, bleeding especially after child birth (Adaya *et al*., 1977) and also as hypolipidemic and hematinic suspension (Kamis *et al*., 2003). Research on the fruit pulp has shown that it contains nutritional trace minerals, proteins and fatty acids, particular the nutritionally essential linoleic acid (Cook *et al.*, 2000). Nwosu *et al*. (2008) reported that the mesocarp of Doum palm fruits contains 8.10 % ash, 0.95 %ether extract, 0.01 %protein, 89.25 %carbohydrate, 3655.9 Kcal/kg Metabolizable energy while Eissa *et al.* (2008) showed that the fruit pulp contains 4.91% proteins, 5.26% fat, 4.5% ash and 85.33% total carbohydrate. Doum was reported to lower the blood pressure, when its biological activity was evaluated in rat feeding experiments (Betty *et al*., 2006). There is paucity of information on the nutritional properties of Doum palm (*Hyphaene thebaica*) and its use in poultry feed. The objective of this study was therefore to evaluate the effect of different replacement levels of maize with HTSM on the haematological parameters and serum biochemistry of broiler chickens.

MATERIALS AND METHODS

The research was carried out at the Poultry unit of the Teaching and Research Farm of the Department of Animal Science, Federal University, Gashua, Yobe State, Nigeria. It has an area of 772km^2 and Coordinates: 12°52′5″N 11°2′47″E. The hottest months are March and April with temperature ranges of 38-40^{o}C. In the rainy season, June-September, temperatures fall to 23-28^{o}C, with rainfall of 500 to 1000 mm (Climatemp, 2018).

Preparation of *Hyphaene thebaica* seed meal (HTSM)

The dried *H. thebaica* fruits were purchased from Gashua market, Yobe state, in the North Eastern part of Nigeria. The fruits were crushed with machine and the kernels were discarded. The mesocarps were then milled to particle sizes that would pass through a 3mm sieve, using a hammer mill. Other feed ingredients such as maize, soyabean, wheat offal, maize bran etc were purchased from Gashua market.

Proximate Analyses and Metabolisable Energy Determination of HTSM

Samples of the meal were subjected to proximate analysis using the methods described by

A.O.A.C (2006) to determine the following proximate fractions: Moisture content, dry matter (DM), Crude protein (CP), Crude fibre (CF), Ether extract (EE), Ash, Nitrogen free extract (NFE). The metabolisable energy (ME) of the seed meal was calculated, using the methods of Pauzenga (1985).

Phytochemical Analysis

The following phytochemicals were determined using the standard procedures (AOAC, 2006): Flavonoids, tannin, oxalate and saponin.

Experimental Diets

The experiment was in two phases (starter and finisher), five diets were formulated to meet nutrient requirement standards of broilers (NRC, 1994). Diet 1 (0 % HTSM) served as the control while diets 2, 3, 4 and 5 contained 5, 10, 15 and 20 %HTSM respectively replacing maize in the diets of birds. The gross composition of the experimental diets and their analysed nutrient contents are presented in Table 1.

Experimental Birds and Design

A total of two hundred and twenty five (225) day-old broiler chicks were purchased from a reputable distributor. They were raised on commercial broiler starter diet for one week, after which they were randomly allotted into five treatment groups with three replicates of fifteen birds each. Each group was assigned to the five experimental diets in completely randomized design (CRD). Throughout the experimental period, feed and water were provided *ad libitum* for all treatment groups. This was accompanied by necessary prophylactic medication and vaccination. The experiment lasted for a total of eight weeks (56 days).

Blood collection

At the end of the study period, 2 ml of blood was collected from two birds per replicate

Table 1: Ingredient Composition of the Experimental Diets

	Dietary levels of *Hyphaene thebaica* seed meal (%)									
	Starter diets					Finisher diets				
Ingredients, Kg	0	5	10	15	20	0	5	10	15	20
Maize	54.00	51.30	48.60	45.90	43.20	56.00	53.20	50.40	47.60	44.80
*HTSM	0.00	2.70	5.40	8.10	10.80	0.00	2.80	5.60	8.40	11.20
Soyabean meal	27.00	27.00	27.00	27.00	27.00	24.00	24.00	24.00	24.00	24.00
Maize offal	5.95	5.95	5.95	5.95	5.95	6.80	6.80	6.80	6.80	6.80
Palm kernel cake	6.00	6.00	6.00	6.00	6.00	5.00	5.00	5.00	5.00	5.00
Fish meal (48%)	2.00	2.00	2.00	2.00	2.00	1.50	1.50	1.50	1.50	1.50
Limestone	0.50	0.50	0.50	0.50	0.50	0.65	0.65	0.65	0.65	0.65
Bonemeal	2.50	2.50	2.50	2.50	2.50	3.00	3.00	3.00	3.00	3.00
Palm oil	1.00	1.00	1.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00
Common salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
L-Lysine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
**Vit/Min Premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100	100	100	100	100	100	100	100	100	100
Calculated analysis (%)										
Crude protein	22.57	22.26	22.35	22.51	22.09	20.04	20.12	20.17	20.08	20.14
Crude fibre	3.76	3.85	3.90	4.11	4.17	3.62	3.75	3.97	4.01	4.05
Energy (Kcal/Kg ME)	2887	2883	2880	2875	2872	3058	3046	3040	3038	3031
Ether extract	5.36	5.33	5.38	5.34	5.37	6.12	6.07	6.11	6.18	6.09
Available P.	0.57	0.58	0.55	0.56	0.54	0.62	0.68	0.64	0.66	0.60
Proximate analysis (%)										
Dry matter	88.24	88.67	87.91	88.34	87.89	89.02	90.15	89.50	88.67	90.00
Crude protein	22.57	21.34	22.09	23.00	22.61	20.04	20.41	20.02	20.11	20.19
Crude fibre	3.76	3.89	3.93	3.98	4.00	3.52	3.70	3.78	3.88	3.97
Ether extract	5.36	4.90	5.21	5.09	4.98	6.12	6.37	6.00	6.04	6.57
Ash	5.62	6.01	6.20	6.09	6.17	5.53	5.86	6.17	6.90	6.14
NFE	62.69	63.86	62.57	61.84	62.24	64.79	63.66	64.03	63.07	63.13

*HTSM=Hyphaene thebiaca seed meal **To provide the following per kilogram of feed: Vit. A, 10,000 iu, Vit. D3, 2 Vit. E, 5iu; Vit.K, 2mg; Riboflavin, 4.20mg; Vit. B12, 0.01mg; Panthotenic acid, 5mg; Nicotnic acid, 20mg; Foli 0.5mg; choline, 3mg; Mg, 56mg; Fe, 20mg; Cu, 10mg; Zn, 50mg; Co.125mg. NFE: Nitrogen Free Extract (%CP+%CF+%EE+%Ash).

through the wing vein and put into bottles containing Ethylene Diaminetetra- acetic Acid (EDTA) to determine the packed cell volume (PVC), red blood cell (RBC), haemoglobin (Hb), and white blood cell (WBC). Blood sample meant for serum biochemical studies were collected into plane bottles (without Anti-coagulant) to enhance serum separation. The blood serum obtained was used to determine total protein (TP), Albumin, Globulin, Glucose and Urea. All the analysis was done at the General Hospital Laboratory, Gashua according to the methods described by Kohn and Allen (1995); Schalm *et al*. (1975) and Peters *et al.* (1982) while the serum enzymes; Alanine aminotransferase (ALT), alkaline phosphase (ALP) and aspartate aminotransferase (AST) were obtained using the Randox Laboratories Ltd, UK test kits. The blood constants such as mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were obtained by calculation according to standard formulae (Jain, 1986) as shown below:

$$MCV = \frac{PCV \times 10}{RBC\ count\ (\times 10^6/mm^3)}$$

$$MCH = \frac{Hb\ (g/dl) \times 10}{RBC\ (\times 10^6/mm^3)}$$

$$MCHC = \frac{Hb\ (g/dl) \times 100}{PCV\ \%}$$

Statistical Analysis

Data generated from the study were subjected to one-way analysis of variance (ANOVA) using software (SAS, 2008). Means were separated with Duncan multiple range test at 5% level of significance.

RESULTS AND DISCUSSION

Chemical Composition of HTSM

The proximate/phytochemical composition of HTSM is shown in Table 2. The percentage composition of proximate fractions on dry matter basis was: crude protein, 6.09; ether extract 1.75 and ash, 6.26. Others were crude fibre, 11.49 and nitrogen free extract, 68.39. The results of the phytochemicals analysed were tannin (0.31 %), saponin (1.02 %), oxalate (0.67 %), phytate (1.13 %), and flavonoids (0.78 %).

Table 2: Chemical Composition of *Hyphaene thebaica* seed meal

Nutrients (%)	Concentration
Dry matter	93.08
Crude protein	6.09
Ether extract	1.75
Ash	6.26
Crude fibre	11.49
Nitrogen free extract	68.39
Metabolizable energy (Kcal/kg)	2796.33
Phytochemicals (%)	
Tannin	0.31
Saponin	1.02
Oxalate	0.67
Phytate	1.13
Flavonoids	0.78

NFE: Nitrogen Free Extract =100-(%CP+%CF+%EE+%Ash). *ME**:** Metabolizable Energy ME (Kcal/kg) = 37 x % CP + 81.8 x % EE + 35.5 x %NFE (Pauzenga, 1985).

The results of the haematological parameters of broiler chickens fed HTSM diet is shown in Table 3. There were significant differences ($P<0.05$) in the parameters measured except the packed cell volume and mean corpuscular haemoglobin. The white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb) and MCHC increased in response to the increased level of HTSM in the diet. The non-significant difference ($P>0.05$) in PCV as observed in this study implies that the inclusion of HTSM in broilers diet had little or no effect on the relative quantity of blood cells as compared with the total volume of blood (Health and Olusanya, 1985). The PCV values obtained (29.08-31.89 %) were all within the normal range of 22 to 35% reported by Jain (1993). They were however lower than the values of 25 to 45% reported by Mirtuka and Rawnsley (1977). White blood cells ($x10^9/l$) differed significantly ($P<0.05$) among the treatment groups. Birds fed 20%HTSM diet had the highest value ($18.14\ x10^9/l$). High WBC count is usually associated with microbial infection or the presence of foreign body or antigen in the circulatory system. Also, this might be attributed to the immune system of the birds attempting to detoxify the Anti-nutritional factors (ANFs) in the feed. Anti-nutritional factors have been reported to exert negative effects on some haematological parameters. Saponin is known to cause erythrocyte haemolysis and reduction of blood (Cheeke, 1996). The significant increase in red blood cells and haemoglobin agreed with the report of Annongu *et al*. (2017) and Adeyemo and Sanni (2013) for broiler chickens fed African Star Apple (*Chrysophyllum albidum*) kernel meal and *Aspergillus niger* hydrolyzed cassava peel meal based diet

Table 3: Effects of Different Dietary Levels of HTSM on haematological parameters of Broiler chickens (0-8weeks)

	Dietary levels of *Hyphaene thebaica* seed meal (%)					
Parameters	0	5	10	15	20	SEM
Packed cell volume, %	29.08	29.45	30.62	31.03	31.89	0.70
White blood cell, x 10^9/l	11.45^c	17.25^a	15.90^{ab}	14.63^b	18.14^a	1.54
Red blood cell, x 10^{12}/l	4.71^b	4.98^b	5.56^{ab}	6.01^a	6.99^a	0.57
Haemoglobin, g/dl	6.92^b	7.38^b	7.89^{ab}	9.00^a	10.05^a	0.81
MCV, fl	61.74^a	59.14^a	55.07^{ab}	51.63^b	45.62^b	3.22
MCH, pg	14.69	14.82	14.19	14.98	15.67	0.49
MCHC, %	23.80^b	25.06^b	25.77^b	29.00^a	34.34^a	2.11

[ab]means in the same row with different superscripts are significantly different ($P<0.05$). SEM=Standard error of mean. MCV=Mean cell volume. MCH=Mean cell heamoglobin. MCH=Mean cell heamoglobin concentration

Table 4: Effects of Different Dietary Levels of HTSM on serum biochemical indices of Broiler chickens (0-8weeks)

	Dietary levels of *Hyphaene thebaica* seed meal (%)					
Parameters	0	5	10	15	20	SEM
Glucose, mmol/l	6.82^{b}	7.10^{b}	7.94^{ab}	8.09^{a}	9.00^{a}	0.44
Cholesterol, mmol/l	1.85	1.02	0.93	0.51	0.48	0.45
Total protein, g/l	31.83^{a}	32.82^{a}	30.99^{a}	30.72^{ab}	29.11^{b}	0.54
Albumin, g/l	25.05^{a}	24.81^{a}	22.00^{ab}	21.27^{b}	19.01^{b}	1.21
Globulin, g/l	6.78^{b}	8.01^{b}	8.99^{ab}	9.45^{a}	10.10^{a}	0.66
Urea, mmol/l	1.80	1.83	1.88	1.94	1.92	0.31
AST, IU/L	72.95^{d}	77.08^{c}	81.02^{b}	89.71^{a}	90.16^{a}	1.04
ALT, IU/L	62.84^{cd}	65.01^{c}	68.02^{c}	73.09^{b}	79.50^{a}	1.53
ALP, IU/L	100.26^{b}	101.03^{b}	101.58^{b}	102.49^{b}	108.77^{a}	1.06

^{ab}means in the same row with different superscripts are significantly different (P<0.05). SEM=Standard error of mean. ALP=alkaline phosphatase. AST= aspartate transaminase. ALT=Alanine transaminase

respectively. Emenalum *et al*. (2009) and Ogbuewu (2008) had earlier reported that the number erythrocytes of animals in good health varies with species, age, sex, diets and clinical conditions of the animal.

The MCV values (45.62 – 61.74 fl) were below the normal range of 90 to 140 fl reported by Mirtuka and Rawnsley (1977). The reduction in the values of MCV as obtained in this study are supported by the findings of Madubuike and Ekenyem (2006) who observed similar trend among broilers fed varying dietary levels of *Ipomoea asarifolia* Leaf Meal based diet. This observation may be associated with poor intake resulting from the presence of metabolites in these diets (Apata, 2011).

The results of the serum biochemical indices of broiler chickens fed HTSM diet is shown in Table 4. There were significant differences (P<0.05) in the serum indices measured except the serum cholesterol and urea. The glucose level, total protein, albumin, globulin, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) increased (p<0.05) as the dietary levels of HTSM increased across the treatments. The increase in glucose level as the HTSM increased in the diet was in agreement with the report of El-Abasy *et al.* (2002) who observed increased role of sugars in blood and immune response of cockerels. The total protein, albumin and globulin levels obtained in this study compared favorably with the values reported by Annongu *et al*. (2017) for broiler chickens fed African Star Apple (*Chrysophyllum albidum*) kernel meal based diet. The dietary inclusion of HTSM at graded levels did not exert any adverse effect on the cholesterol and urea compositions of the broilers fed the experimental diets. This implies that the test feedstuff contains adequate oil to meet the requirement of the experimental birds. Annongu *et al*. (2017) observed that high blood urea levels in birds are associated with poor utilization of protein in the diets. The increased activity of AST, ALT and ALP as the quantity of HTSM increased in diet (p<0.05) may attest to the presence of phytotoxins in the HTSM which led to high enzyme activities in the blood. However, age of birds is also a factor influencing serum biochemical indices of the birds. Oluyemi *et al* (2002) reported that young chickens (8 - 10weeks) had significantly greater AST and ALT values than adult (50 - 80weeks) birds. The slight increase in ALP values in the study might therefore be in response to age (Arslam *et al.,* 2001). The increased activity of ALT suggests the likelihood of liver damage especially among birds fed 15 and 20%HTSM diets.

CONCLUSION

The findings from this study showed that HTSM has a high potential as feed ingredient in poultry diets and could be included in the diet of broiler birds. However, the haematological parameters and serum biochemical indices revealed that there is need for adequate processing of the seed to reduce the anti-nutritional factors to a tolerable level before being used in broiler diet.

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