HAEMATOLOGICAL AND SEROLOGICAL CHARACTERISTICS OF COCKERELS FED DIFFERENT LEVELS OF TIGER NUT (*Cyperus esculentus*) SEED MEAL

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ABSTRACT
A forty-nine days investigation into the haematological characteristics and serum biochemistry of cockerels fed varying levels of tiger nut seed meal diets were conducted. Five different diets were formulated such that tiger nut replaced maize at 0% (T1), 25% (T2), 50% (T3), 75% (T4), and 100% (T5) respectively in a Completely Randomized Design. The control diet (T1) contained no tiger nut. One hundred and twenty (120) four weeks old cockerels procured from Liberation Farm, Umuahia were used for the experiment. The cockerels were arranged into five dietary treatments, each containing three replicates with eight birds per dietary treatment and eight birds per replicate. Result indicated that the White Blood Cell (WBC), Red Blood Cell (RBC), Haemoglobin (Hb), packed cell volume (PCV), heterophil; lymphocyte, monocyte and basinophil except the eosinophil were not affected by the replacement of maize with tiger nut. The test diet recorded lower eosinophil (2.33-3.67 x 103/ul) compared to the control (5.67 x 103/ul) the eosinophil values however were still within the normal acceptable range, 1-6 x 103/ul. Higher eosinophil value of the control suggests that it was immunologically challenged, whereas, the lower values of eosinophil in the test diets may suggest inherent physiochemical properties of the tiger nut that reduced the vulnerability of the animals against worm infections associated with higher values of eosinophil. T5 recorded lymphocyte value (44.67 x 103/ul) slightly lower than normal range values. Therefore inclusion of tiger nut at any level lower than 100% will favour optimum haematological function of the cockerels. The dietary treatment affected the serum bilirubin, creatinine and cholesterol levels but did not affect the serum total protein, globulin, albumin and blood urea. T4 recorded the highest bilirubin (0.28 g/dl) which compared with the control (0.21 g/dl) suggesting more efficient metabolism. Total protein and globulin decreased, whereas, creatinine increased as level of tiger nut increased. Urea and albumin did not portray a definite trend. Higher creatinine values are associated with higher crude protein. While this is true for control diet (0.38 mg/dl), it was not for the T4 and T5 whose higher creatinine values (0.38 mg/dl) are attributed to the presence of antinutrients such as oxalates which binds proteins resulting in high catabolic products, including creatinine. T3 containing 50% of tiger nut is recommended as the most tolerable level to replace maize due to its lowest FCR, high body weight, comparable haematological characteristics, lowest urea and moderate creatinine value.

Keywords: Tiger nut, cockerels, haematology, serum biochemistry

INTRODUCTION
The increasing human population especially in the sub-saharan Africa has necessitated increase in the supply of animal protein to meet with the challenges of food security, Millennium Development Goals (MDGs) and income generation. High cost of feed has been observed as one of the major constraint militating against efficient development of poultry industry (AG U.K. 2013). Feed is one of the most expensive energy feed ingredient item in poultry production, responsible for between 60-80% of total cost of production (Abeke et al., 2013). The use of maize in livestock diets and its use as food by human as well as its use as an alternative fuel ethanol production have increased the demand for maize, thereby necessitating the exploration of other energy sources for livestock diets (Agbabiaka L.A. 2012). This competition that exists between human and animals has brought about limited availability of maize, thus increasing the price and scarcity. This has also necessitated research into non-conventional energy ingredients that would replace maize without compromising the growth efficiency, economic production and carcass quality of the birds. However the present reality in third world countries source for alternative energy sources to cereal ingredients has necessitated the need to investigate the potentials

of some other energy feed stuffs such as tiger-nut that are cheap and readily available with less competition with human and beer brewing industries compared to maize in livestock nutrient. This will reduce the cost of feed and animal products such as meat and egg among others for Nigerian populace. Tiger nut (*Cyperus esculentus*) is one of the underutilized crops of the family *Cyperaceae*, which produces rhizomes from the base and tubers that are somewhat spherical. According to Sanchez-Zapata et al., (2012), tiger nut is an edible perennial grass-like plant native to the 'old world' (consists of Europe, Asia and Africa, regarded collectively as the part of the world known to Europeans before Europe contacts with the Americans), and is a lesser-known vegetable that produces sweet nut-like tubers known as 'earth almond'. It is often cultivated for its nutritive edible nuts and high content of soluble glucose of 21% (Bamishaiye et al., 2010). In spite of the numerous health benefits of tiger nut, its potentials and prospects as energy source for poultry and livestock production has not been delve into extensively (Oladele et al., 2010). This study was therefore designed to investigate the effects of feeding graded levels of tiger-nut diets on the hematological and biochemical characteristics of cockerel birds

MATERIALS AND METHODS

Site and Duration of Study

This experiment was conducted at the Poultry Unit Teaching and Research Farm, College of Animal Science and Animal Production, Michael Okpara University of Agriculture, Umudike. Umudike is located on latitude $5^0 29^1$N and longitude $7^0 321$E in the Rain Forest Zone of Nigeria. This site has a mean daily temperature of between 27c and 35c throughout the year and an average rainfall of 2000mm per annum (NRCRI 2003).

Experimental Diet

The tiger nut seed was sun dried for seven (7) days and pass through a hammer mill to produce tiger nut seed meal. The drying process reduced excess moisture so as to prevent rancidity of the material and growth of moulds. Five experimental diets were formulated such that tiger nut replaced maize at 0%, 25%, 50%, 75% and 100% dietary levels respectively. Other ingredients were similar in all the diets.

Experimental Birds and Design

One hundred and twenty (120) four weeks old cockerel (4 weeks) birds were used in this study which lasted for Forty-Nine (49) days. The birds were reared for two weeks (2 weeks) on commercial grower's diet before the commencement of the trial. The birds were divided into five groups of twenty-four (24) birds each and was randomly assigned to the five diets in a Completely Randomized Design (CRD). Each group was further sub-divided into three (3) replicates of eight (8) birds each and housed in a deep litter compartment. Feed and water were provided ad-libitum and other routine poultry management practices was maintained.

Data Collection and Analysis of Blood Sample

At the end (7^{th} week) of the experiment, three birds, one per replicate were selected from each treatment. The birds were starved for 24 hours before slaughtering by severing the jugular vein and blood samples were collected into sample bottles containing Ethylene Di-amine Tetra Acetic Acid (EDTA) for haematological determinations. The sample bottles were gently shaken to mix up the blood with EDTA to prevent clotting. Blood for clinical chemistry determinations was collected into clean test tubes without anticoagulant. Serum was obtained by allowing the blood sample to clot at room temperature for 30 minutes, after which it was centrifuged for ten minutes at 3,000 revolutions per minute using a table centrifuge to enable a complete separation of the serum from the clotted blood. The clear serum supernatant was then carefully aspirated with syringe and needle and stored in a clean sample bottle. All data collected was subjected to Analysis of Variance technique (ANOVA) as outlined by Steel and Torrie (1980). The mean separation were done using Duncan's new multiple Range Test (1955), where significance occurred.

RESULTS AND DISCUSSION

The result of haematological characteristics of cockerel birds fed varying levels of tiger nut seed meal as dietary replacement for maize is presented in table 3. Significant difference ($p<0.05$) was observed only in eosinophil. The control recorded the most significant ($p<0.05$) value in eosinophil, whereas, T4 recorded the least value. The result showed that tiger nut seed meal inclusion decreases the blood eosinophil. No significant difference ($p>0.05$) was observed in the other parameters including the white blood cell (WBC), red blood cell (RBC), haemoglobin (Hb), packed cell volume (PCV), heterophil, lymphocyte, monocyte and basinophil. The hematological parameters shows that statistically, the inclusion of tiger nut seed meal up to the 100 % level did not affect the hematological values of cockerels except the eosinophil. The control diet recorded the highest value ($p<0.05$) of eosinophil. High numbers of eosinophils may be associated with worm infections or exposure to substances that cause allergic reactions (Aro, S.O and Ojo, B.A 2013). However, the eosinophil value in this study is well within the normal range given by Agbabiaka et al., (2013). The lower values of the trial treatments in comparison to the control may suggest that the tiger nut may contain properties that lower their immunonological agility. The mean values of RBC, Haemoglobin, PCV and WBC obtained from all the experimental birds are perfectly within the range of haematological values of chicken reported by Allain *et al.* (2004). Increasing lymphocytes count in the blood following increase in white blood cells count can play an important role in stimulating the immune system of animals (Al-Kassi, 2009). Increase in white blood cells count represents stimulation of the host's immune system. The White blood cell and lymphocyte in this study did not vary between the treatment diets and the control diets. The stable values of the white blood cell and the lymphocyte between the control and the test treatments suggest that the immune system of the cockerels was stable and was not challenged even by the 100 % inclusion level of the tiger nut seed meal. Heterophil of the control did not differ significantly from the trial diets, suggesting stable condition. The lymphocyte values are well within the normal range except T5 which is slightly below the normal range. This probably suggests that at levels higher than 100%, the lymphocyte values may increase. This might actually be in connection with the anti-nutritional factors in tiger nut which may pose serious antigenic effect. Only T2 and the control fell within the normal range given for monocyte. Monocytes protect the body against bacteria and eat up small particles of foreign matter (Aro, S.O and Ojo, B.A 2013). The lower level of monocyte in T3, T4 and T5 shows their lower ability to fight against antigenic agents. However the result of this study showed statistically, that there was equal effect in the ability of the control and trial treatments to fight against antigenic agents in cockerels. Similarly, this present research has shown that replacing maize with tiger nut has no adverse effect, the difference in eosinophil notwithstanding, since it still falls within the normal range given by some researchers. The serum characteristics of the experimental cockerel birds are presented in table 4. Significant differences ($p<0.05$) were observed in total bilirubin, creatinine and cholesterol among the five treatment diets, whereas, no significant difference ($p>0.05$) was observed in total protein, globulin, albumin and blood urea. In other words, the inclusion of tiger nut up to the 100 % level in the diets of cockerel did not affect the total protein, globulin, albumin and blood urea. T4 recorded the most significant value in total bilirubin, whereas, T5 containing the highest level of tiger nut as used in this study was least; T2 and T3 compared with the control in total bilirubin. This result showed that the bilirubin is increased optimally at the 75% level (T4). The control contained the most significant value in creatinine, whereas, T2, containing the 25% level of inclusion was the least. However, among the trial treatments, an increasing trend was observed in the creatinine values with higher levels of tiger nut. This suggests that the higher the level of inclusion of tiger nut seed meal, the higher the creatinine which may be due to the presence of anti-nutrients such as oxalates which increases with increasing levels of the tiger nut. Oxalates bind with proteins making it difficult to be digested. This process could therefore result in accumulation of creatinine as the body makes effort to break down the oxalate-bound proteins. T3 performed most significantly ($p<0.05$) in cholesterol, followed by T2; whereas, T4 and T5 performed as low as the control in cholesterol. This result shows that the cholesterol is increased optimally at the 50 % level of inclusion and levels higher than the 50% reduces the cholesterol level. The stable values obtained between the control and the test diets with respect to the serum biochemical parameters that were not significantly different among the treatment and control diets including total protein, globulin and albumin, which are components of the total protein, irrespective of the treatment levels suggest that the diets are adequate for the cockerels.

Conclusion

The results obtained from this study amply demonstrated that tiger nut possess relatively nutritional composition, thus making it safe and fitting for animal consumption. Therefore, the utilization of tiger nut based diets will help livestock farmers to produce cheaper feed, making animal protein more readily affordable to Nigerians.

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Table 1: Percentage Composition of the Experimental Diets

	Replacement levels of tiger nut seed meal				
Ingredients	T1	T2	T3	T4	T5
Maize	54.00	40.5	27.00	13.5	0.00
Tigernut	0.00	13.5	27.00	40.5	54.00
Soyabean	21.00	21.00	21.00	21.00	21.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Palm kernel cake	10.00	10.00	10.00	10.00	10.00
Bone meal	3.00	3.00	3.00	3.00	3.00
Wheat offal	8.00	8.00	8.00	8.00	8.00
Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25

Lysine	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Crude protein	22.03	22.23	22.41	22.09	22.01
ME (kcal/kg)	3034	3036	3038	3040	3049

Table 2. Proximate Composition of Experimental Diet Containing Different Inclusion Levels of the Tiger Nut Seed Meal

Parameters	T1	T2	T3	T4	T5	SEM
	0%	25%	50%	75%	100%	
Dry matter (%)	94.97	93.50	93.47	95.60	95.57	2.25
Moisture content (%)	5.03	6.50	6.53	4.40	4.43	0.61
Ash (%)	8.90	9.20	9.20	9.03	8.80	0.86
Crude protein (%)	22.05	21.29	20.08	20.58	20.26	2.21
Crude fiber (%)	7.81	8.56	8.79	8.86	9.04	1.24
Crude Fat	5.45	5.98	6.08	6.20	6.40	1.15
Nitrogen free extract	42.20	42.59	42.16	38.86	35.47	2.58

a,b,c Means with different superscripts are significantly different at $p<0.05$; SEM – Standard error of the mean

Table 3: Haematological Values of Cockerels Fed Different Levels of Tiger Nut as Replacement for Maize

Parameters	T1	T2	T3	T4	T5	SEM
	0%	25%	50%	75%	100%	
WBC ($10^6/cm^3$)	11.27	11.70	13.95	14.87	13.62	0.95
RBC ($10^6/cm^3$)	2.33	2.71	2.19	2.38	2.82	0.13
Hb (g/100ml)	7.10	7.56	7.70	7.85	7.67	0.20
PCV (%)	23.83	23.67	24.17	27.17	26.83	0.79
Heterophill	42.67	45.33	47.33	46.67	46.67	1.18
Lymphocyte (x103/μl)	45.00	45.33	45.00	47.33	44.67	0.80
Monocyte (x103/μl)	6.67	5.00	4.33	3.33	4.67	0.56
Eosinophil (x103/μl)	5.67^{a}	3.33^{ab}	3.67^{ab}	2.33^{b}	3.33^{ab}	0.41
Basinophil (x103/μl)	0.67	1.00	0.67	0.33	0.67	0.27

a,b,c Means with different superscripts are significantly different at $p<0.05$; SEM – Standard error of the mean

Table 4: Serum Biochemical Parameters of Cockerels Fed Different Levels of Tiger Nut Seed Meal as Replacement for Maize

Parameter	T1	T2	T3	T4	T5	SEM
	0%	25%	50%	75%	100%	
Total protein (g/dl)	3.41	3.65	3.65	3.57	3.41	0.06
Globulin (g/dl)	1.82	2.22	2.10	2.10	1.86	0.06
Albumin (g/dl)	1.59	1.43	1.55	1.47	1.55	0.05
Total billirubin (g/dl)	0.21^{ab}	0.23^{ab}	0.24^{ab}	0.28^{a}	0.19^{b}	0.01
Creatinine (mg/dl)	0.41^{a}	0.26^{c}	0.29^{bc}	0.38^{ab}	0.38^{ab}	0.02
Cholesterol (mg/dl)	130.35^{b}	158.10^{ab}	177.84^{a}	140.97^{b}	139.67^{b}	5.82
Urea (mg/dl)	4.19	4.29	3.71	5.75	4.49	0.31

a,b,c Means with different superscripts are significantly different at $p<0.05$; SEM – Standard Error of the Mean