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Nutrient retention, haematology and serum biochemistry of cockerels fed graded levels of cassava (*Manihot esculenta*) grit supplemented with moringa (*Moringa oleifera*) leaf meal

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Abstract

A sixteen week trial was conducted to assess the effect of replacing cassava grit supplemented with Moringa leaf meal (MLM) for maize on the nutrient retention, haematology and serum biochemistry of 120 "day old" Harco cockerel chickens. Four experimental cockerel starter and finisher diets were formulated with diet 1 formulated to contain 0% cassava grit while diet 2, 3 and 4 were formulated to contain cassava grit at 33.3, 66.6 and 100% replacement for maize with 5% of moringa leaf meal inclusion in diets 2, 3 and 4 respectively. Chicks were randomly assigned to the four treatment diets in a completely randomized designed (CRD). Result on nutrient digestibility show that crude fibre was significantly (P<0.05) affected by the treatment diets with highest values of 68.21 recorded in birds fed diet 4. Ash and NFE were significantly (P<0.05) influenced by the treatment diets with highest mean values (50.81 and 55.15) observed in birds placed on 100% CGM. Haematological indices revealed that Packed Cell Volume was significantly (P<0.05) highest 32.17% among birds fed the control diet. However, neutrophil was significantly (P<0.05) affected by the treatment diets with highest value 17.67% recorded in birds fed the control diet. Serological study revealed that Albumin value was significantly (P<0.05) influenced by the dietary treatments with highest value (1.60mg/dl) recorded among birds fed 33.3% CGM based diet. Therefore, Cassava grit can replace maize up to 66.6% inclusion level with 5% appropriate supplementation with moringa leaf meal in cockerel diet for optimum nutrient utilization and blood quality.

Keywords: Cockerel, cassava grit, Moringa leaf meal, digestibility, blood indices

Introduction

Cockerel as a class of chicken has being neglected for too long in the pullet operation enterprise and this raises a great deal of concern to its survivability and acceptability. It has the ability to thrive on Agro waste and high fibrous feed stuff (Okosun and Ehebha, 2015) and this has really stimulated a lot of interest on them. Poultry diet consist mostly of cereals and legumes, and these feed sources like maize and sorghum, soya bean meal and groundnut meal are expensive and constitute about 50-55% of the formulated poultry diet; their productivity is low in Nigeria which means it does not meet national demand (Agbede *et al,* 2002; Hamzat *et al,* 2003; Okereke *et al,* 2006) so the need for intensification of search for cheaper alternatives that would support commensurate performance of this class of bird is inevitable (Egbewande, 2008). Effort has also been geared towards the utilization of relatively cheaper and available root and tubers in recent years.

Cassava (*Manihot Spp*) which contain a fibrous peel (10-15% of tuber weight) and a core, the main region for starch (IITA, 1990) has a higher metabolizable energy of (3,870kca/kg ME) compare to maize with (3430kcal/kg ME), (Tion and Adeka, 2000). Cassava products have been used in feeding

livestocks (Balogopalan, 2002; Nwokoro and Ekhosueni, 2006; Babatunde, 2013). However, the low protein content (Salcedo *et al.,* 2010) and the dustiness of the feed (Ukachukwu, 2005; Ojewola *et al.,* 2006; Kana *et al.,* 2015) are among the limiting factor in cassava utilization. Many processing methods that have been used to enhance the feeding value of cassava include sundrying (Akinfola *et al.*, 2007), parboiling (Salami and Odunsi, 2003). These methods have however achieved different levels of success. Utilization of cassava mill with its peel at the same time will reduce environmental pollution (Devendra, 1992) as well as eliminate wastages in cassava production and processing. Cassava grit is a product of processing of cassava tuber with its peels. Recently there has been interest in the utilization of Moringa (*Moringa oleifera*) commonly called Horse radish tree or drum stick tree, as a protein source for livestock (Makker and Becker, 1997; Sarwatt *et al.,* 2002). Moringa leaves have quality attributes that makes it a potential replacement for Soyabean meal or fish meal in non-ruminant diet. Supplementing Moringa leaf meal with cassava grit could be a game changer in the poultry industry because apart from augmenting the amino acid profile of cassava, moringa also has some phytobiotic properties that aid in increasing the blood quality of the birds, it also possesses anti oxidants properties which are known to suppress formation of reactive oxygen species (ROS) and free radicals (Sofidiya *et al.,* 2006; Ogbunugafor *et al.,* 2011). *Moringa oleifera* was reported to be a good source of vitamin and amino acid (Olugbemi *et al.*, 2010), it boost the immune system (Jayavardhanan *et al.,* 1994). It also contains some antibacterial properties that make it serve as a phyto-therapeutic agent to combat infectious diseases (Patel, 2011). However, there is paucity of information on utilization of cassava grit with moringa leaf meal in cockerel diet. This study was therefore embarked upon to investigate the Nutrient utilization, haematology and serum biochemical indices of cockerel fed graded levels of cassava grit supplemented with moringa leaf meal.

Materials and methods

The experiment was carried out at the poultry unit of the livestock section, Teaching and Research Farm, Ambrose Alli University, Ekpoma for a period of sixteen (16) weeks. Cassava for the feeding trial was purchased from a reputable farm in Ekpoma Esan West Local Government Area of Edo State. The woody part was chopped off, and the cassava was thoroughly washed to reduce the silica level to near zero. It was then grated without peeling screw pressed for about 48 hours to reduce the hydrogen cyanide level to the barest minimum. It was mixed with palm oil to further encapsulate the cyanide in the milled cassava. It was thereafter fried, air dried and bagged into product known as the Cassava grit which was used in formulating the experimental diets. The moringa leaf meal was air-dried at room temperature for about 6-7 days until it breaks with a crispy feel, it was then stored dried green.

In a complete randomized design, one hundred and twenty day old black harco cockerels were divided into four groups of thirty chicks containing three replicates of ten chicks each. The replicates were housed in floor pens measuring 2.4m^2 with the floor covered with wood shavings as liter material. A plastic trough feeder and drinker were provided in each pen. The birds were vaccinated against Gumboro at 2 and 4 weeks, Newcastle at 3 and 5 weeks, fowl cholera at 6 weeks and fowl pox disease at 9

weeks of age respectively. Four isonitrogenous and isocaloric diets (1, 2, 3 and 4) were formulated to contain 21% and 18% crude protein with 2650 and 2250kcal/kg energy respectively in the chicks and grower mashes respectively (Tables 1 and 2). Cassava grit was included in both the chick and grower mashes at 0.00, 33.30, 66.60 and 100% replacement of maize in diets 1 (control), 2, 3 and 4 respectively while moringa leaf meal (MOLM) after grinding was included at 5% level in all the treatment diets except the control diet. The feed and clean drinking water were provided *ad-libitum* throughout the 16 weeks of the experiment. The chick mash was fed for 8 weeks of age and the finisher mash for the remaining 8 weeks of the experiment.

The proximate composition of Cassava grit was analyzed based on the procedures described by AOAC, (2000). At the end of the 16th week, two birds from each replicate were randomly selected and housed individually in a metabolic cage. A 5-day acclimatization period was allowed prior to a 4-day collection period; during which the birds were fed the specific quantities of the treatment diets. Daily excreta voided per bird was dried over night at 60^{0}C for 12 hours and kept frozen (-20^{0}C) until it's ready for analysis. Prior to analysis, excreta sample was dried at 65^{0}C in an air-tight oven to a constant weight and ground through a 1mm screen for proximate analysis. Ground fed feed and voided faecal samples were analyzed for their respective proximate constituent according to the procedure outlined by AOAC (1990). Apparent digestibility of dry matter, crude protein, crude fibre, ether extract, ash and Nitrogen free extract were estimated respectively.

For haematology and serum biochemical indices, blood samples were collected through wing veins from the overnight fasted birds per treatment at the 16th week. A set of samples were collected into sterilized tube containing ethylene diamine tetra-acetic acid (EDTA) labeled bottle for Haematological studies while another set of blood samples were collected from the same birds into heparinised tubes for plasma chemistry determination. Packed cell volume (PCV) red blood cell (RBC), while blood cell (WBC) and haemoglobin (Hb) were determined using improved Neubar's haemaetometer after dilution, and cyanomethamoglobin methods respectively as described by Dacie and Lewis (1991). Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) as well as serum metabolites such as total protein, albumin, creatinine, and urea were determined by the method of Hyduke (1975), while globulin was estimated by the subtraction of albumin value from serum total protein value (Dacie and Lewis, 1991). All the data collected were subjected to analysis of variance (ANOVA) and differences between means treatments were determined using Duncan's multiple range test (DMRT) at 5% level of probability. All statistical procedures were according to (Steel and Torrie, 1990) with the aid of SAS (1999) package.

Results

The analyzed nutrient composition of cassava grit (CG) and moringa leaf meal are shown in Table 3. Cassava grit had 88.03% Dry matter (DM), 3,402kcal/kg metabolizable energy ME, 2.05% crude protein (CP), 3.85% crude fibre (CF), 2.32% ether extract (EE), 1.24% ash and 78.57% nitrogen free extract (NFE). While moringa leaf meal had 94.60% Dry matter (DM), 28.00% crude protein (CP), 7.10% crude fibre (CF), 5.90% ether extract (EE), 12.20% ash and 46.80% nitrogen free extract (NFE).

Table 1: Percentage compositions of cockerel starter diets

Ingredients	CGM_1	CGM_2	CGM_3	CGM_4
	Levels			
	0	**33.3**	**66.6**	**100**
Maize	40.71	27.13	13.75	0.00
Cassava grit	0.00	13.75	27.14	40.71
Soya bean meal	29.84	32.84	36.33	36.33
Moringa leaf meal	0.00	5.00	5.00	5.00
Fish meal	0.50	0.50	0.50	0.50
Wheat offal	25.72	19.35	16.66	13.88
DCP	2.00	2.00	2.00	2.00
Limestone	1.20	1.99	1.49	0.96
Premix	0.32	0.32	0.32	0.32
Salt	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein	21.00	21.00	21.00	21.00
ME(Kcal/Kg)	2650	2650	2650	2650

DCP: Dicalcium phosphate

Table 2: Percentage compositions of cockerel finisher diets

Ingredients	CGM_1	CGM_2	CGM_3	CGM_4
	Levels			
	0	**33.3**	**66.6**	**100**
Maize	22.53	15.02	7.51	0.00
Cassava grit	0.00	7.51	15.02	22.53
Soya bean meal	16.45	16.94	16.94	18.06
Moringa leaf meal	5.00	5.00	5.00	5.00
Palm oil	0.00	0.00	0.00	0.09
Wheat offal	50.01	50.05	50.57	50.54
DCP	1.50	1.50	1.50	1.50
Limestone	3.78	3.64	2.85	1.66
Premix	0.30	0.30	0.30	0.30
Salt	0.32	0.32	0.32	0.32
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein	18.00	18.00	18.00	18.00
ME (Kcal/Kg)	2250	2250	2250	2250

Table 3: Analyzed nutrient composition of cassava grit and moringa leaf meal

Nutrients (%)	Cassava grit	Moringa leaf meal
Dry matter	88.03	94.60
Crude protein	2.05	28.00
Crude fibre	3.85	7.10
Ether extract	2.32	5.90
Crude ash	1.24	12.20
Nitrogen free extract	78.57	41.40
ME(Kcal/Kg)	3050	3175

***Metabolizable energy value was calculated using the method 37x %CP + 81x % EE + 35.5 x % NFE for poultry (Fisher and Boorman, 1986)**

Apparent nutrient digestibility of cockerel fed varying levels of cassava grit with moringa leaf meal

Table 4 revealed that the dietary treatments has no significant (P>0.05) effect on the apparent digestible dry matter with highest value of 69.00% in birds fed 66% CGM and least in diet 4 with mean value of 67.80%. Digestible crude protein also had similar (P>0.05) values highest 56.54% recorded in birds fed the control diet and least value of 49.60% in birds placed on diet 2. Apparent digestible crude fibre showed a significant (P<0.05) variation among birds fed treatment diets with highest value of 68.21% recorded in birds fed diet 4, similar to 60.04% in control diet and lowest 58.64% in birds fed diet 3. Apparent digestible ether extract was similar (P>0.05) with highest mean value of 50.97% recorded among birds fed diet 2, followed by control (50.77%) and least value of 41.25% in birds fed diet 4. Digestible Ash and Nitrogen Free Extract (NFE) were significantly (P<0.05) highest in birds fed 100% CGM diet with mean values of 50,81 and 55.15% and lowest in diet 3 with values of 42,91 and 43.17% respectively.

Table 4 : Apparent nutrient digestibility of cockerel fed varying levels of cassava grit with moringa leaf meal

	Inclusion levels of CGM (%)				
	0	33.3	66.6	100	
		Diets			SEM±
Parameters	1	2	3	4	
Apparent digestible dry matter	68.10	68.30	69.00	67.80	14.30
Apparent digestible crude protein	56.54^{ab}	49.60	54.57	56.09	4.14
Apparent digestible crude fibre	60.04	59.93^{b}	58.64^{b}	68.21^{a}	3.50
Apparent digestible ether extract	50.77	50.97	46.48	41.15	7.24
Apparent digestible ash	48.55^{a}	48.84^{a}	42.91^{b}	50.81^{a}	1.27
Apparent digestible NFE	49.15^{ab}	43.24^{b}	43.17^{b}	55.15^{a}	4.52

***abc*: means in the same row with varying super script differ significantly (P>0.05)**
SEM±: standard error of mean
CGM. Cassava Grit Moringa supplement

Haemotological indices of cockerel fed varying levels of cassava grit with moringa leaf meal supplementation

Haematological parameters of Cockerel chickens as influenced by the Dietary treatments Table 5 revealed that traits like Haemoglobin (Hb), Red blood cell (RBC), Mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC) and Lymphocyte were not significantly (P>0.05) influenced by the dietary treatment. However, Packed cell volume (PCV), platelet, white blood cell and Neutrophil were significantly (P<0.05) influenced by the treatment diets. Packed cell volume was highest (P<0.05) in birds fed 0% CGM with mean value of 32.17% followed by a similar statistical value 31.03% in birds fed 66.6% CGM and lowest 24.67% in birds fed 25% CGM. Platelet was also significantly (P<0.05) highest in birds fed 66% GCM with a mean value of ($52.33x10^3/_{ml}^3$), followed by ($40.67x10^3/_{ml}^3$) in birds fed 100% CGM and least ($23.67x10^3/_{ml}^3$) in birds fed the control diet. White blood cell was significantly (P<0.05) highest in birds fed the control with value ($65.70\ x10^3/_{ml}^3$), followed by (52.33 and $42.33\ x10^3/_{ml}^3$) in birds placed on diet 2 and 4 respectively while lowest value of ($21.00\ x10^3/_{ml}^3$). Neutrophil was significantly (P<0.05) highest 17.67% in birds fed the

control diet similar to 11.20% in diet2 while least comparable values 8.63 and 7.63% were recorded among birds fed diet 3 and 4 respectively.

Table 5: Haemotological Indices of Cockerel Fed varying levels of cassava grit with mo ringa leaf meal supplementation

	Inclusion levels of CGM (%)				
	0	33.3	66.6	100	SEM±
			Diets		
Parameters	1	2	3	4	
Haemoglobin (g/dl)	9.77	8.10	9.70	8.73	0.65
Packed cell volume (%)	32.17^{a}	24.67^{ab}	31.03^{a}	28.97^{b}	2.32
Red blood cell (x 10^6/ml)	2.19	1.96	2.19	1.88	0.16
Mean corpuscular volume (fl)	151.4	144.30	144.70	153.6	3.86
Mean corpuscular Haem. (Pg)	40.17	42.47	43.80	44.83	3.27
Mean corp. Haem. Conc. (Pg)	31.67	30.07	30.73	44.83	3.27
Platelet (x10^3/$_{ml}^3$)	23.67^{c}	30.67^{bc}	52.33^{a}	40.67^{b}	15.15
White blood cell (x10^3/$_{ml}^3$)	65.70^{c}	52.33^{b}	21.00^{c}	42.33^{bc}	15.20
Neutrophil (%)	17.67^{a}	11.20^{ab}	8.63^{b}	7.63^{b}	3.21
Lymphocyte (%)	71.27	76.17	83.80	83.73	4.96

abc: **means in the same row with varying super script differ significantly (P<0.05),**
SEM±: standard error of mean
CGM: Cassava grit Moringa supplement

Serum biochemistry of cockerel fed varying levels of cassava grit with moringa leaf meal supplementation

Serological studies of cockerel finisher as influenced by the dietary treatment (Table 6) revealed that Total protein, Globulin, urea and creatinine were not significantly (P>0.05) influenced by the dietary treatments. However, significant (P<0.05) variation was observed in the albumin values. Total protein was highest at the 33.3% CGM supplementation with mean value of 4.10mg/dl in birds fed 33.3% CGM, followed by 3.47mg/dl among birds placed on 66.6% CGM, while least value of 3.23mg/dl was recorded among birds fed 100% CGM. Albumin was significantly (P<0.05) influenced by the dietary treatment with highest value of 1.60mg/dl was recorded among birds placed on 33.3% CGM, followed by 1.53mg/dl among birds fed the control diet and lowest value of 1.43mg/dl was recorded among birds fed 100% CGM. Globulin value was similar (P>0.05) with highest mean value of 2.50g/dl among birds fed diet 2, followed by 1.97g/dl in diet 3 and least value of 1.80g/dl in birds fed diet 4. Urea was statistically similar (P>O.O5) with highest values of 3.77 and 3.77g/dl recorded among birds fed diets 1 and 4 while least comparable values of 3.33 and 3.33g/dl were recorded among birds placed on diets 2 and 3 respectively. Creatinine value was lowest in birds fed 33.3% CGM with mean value of 0.47g/dl while equal values of 0.53, 0.53 and 0.53g/dl were recorded from birds fed diet 1, 3 and 4 respectively with a mean standard error of 0.07.

Discussion

The highest digestible dry matter value obtained among birds fed 66.6% CGM could be due to the quality of the nutrient composed in this test diet and this is a pointer to the fact that the test diet are of high quality. This negates the report of Ngiki *et al.* (2014) who reported significant (P<0.05) difference when broilers were fed Cassava Root-Leaf meal mixture as replacement for

Table 6: serum biochemical indices of cockerel finisher fed varyi ng levels of cassava grit with moringa leaf meal supplementation

	Inclusion levels oF CGM (%)				
	0	33.3	66.6	100	
	Diets				
Parameters	1	2	3	4	SEM±
Total protein (mg/dl)	3.46	4.10	3.47	3.23	0.36
Albumin (mg/dl)	1.53[b]	1.60[a]	1.50[b]	1.43[c]	0.05
Globulin (g/dl)	1.93	2.50	1.97	1.80	0.35
Urea (g/dl)	3.67	3.33	3.33	3.67	0.36
Creatinine (g/dl)	0.53	0.47	0.53	0.53	0.07

Abc: **means in the same row with varying super script differ significantly (p>0.05),**
Sem±: standard error of mean
Cgm: cassava grit supplemented with moringa

maize. Apparent digestible crude protein values for all the dietary treatment were similar; this might perhaps be due to the cushioning effect of moringa leaf meal as an excellent plant protein source. This corroborates the findings of Ngiki *et al.,* 2014. It also laid credence to the work done by Mukthar (2013) and Okosun and Oyedeji (2015) who reported that Moringa is an excellent plant protein source in Poultry feeding. The significantly lowest value observed for apparent crude fibre digestibility in birds fed 66.6% CGM could be adduced to the balanced ratio of the cassava grit-maize mixture in the formulated diet which in the long run optimizes the crude fibre level of the test diet. This lend support from the report of Ngiki *et al.(*2014) who observed similar value for digestible crude fibre for broilers fed Cassava Root-leaf meal mixture. The comparable values recorded in the apparent digestible ether extract of the birds fed the treatment diets could infer that the fact that the birds on these various treatment diets optimally utilize the fat component of the test diet. The similarity in the apparent digestible ash value could be due to the fact that birds on these diets maximally utilize the nutrient present in their diets. The higher Nitrogen Free Extract value at 100% CGM is obviously due to the high energy content of CGM which agrees with the report of Aderemi, (2007); while its similarities with the control that had no cassava grit clearly proves the calorific value similarity of maize and cassava grit.

The low haemoglobin value recorded in this study could be as a result of the residual cyanide in the cassava grit which must have had greater affinity for metals such as copper and iron and make them unavailable thereby reducing the haemoglobin count and hence effective oxygen transportation This is in agreement with the findings of Tewe,(1999) who reported that 40% whole cassava root meal fed to cockerel resulted in significantly lower haemoglobin value at the finisher phase. The significant variation in the Packed Cell Volume value with highest value in birds placed on 66.6% CGM comparable to the control revealed the fact that these feeds have better nutritional qualities and birds maintained on them had low susceptibility to infections compare to birds placed on diet 2 and 4. The similarity in most of the haematological parameters assayed in this study could be due to the nutritional adequacy and safety of the test diet which agrees with the report of (Olabamiji, *et al.,* 2007). The reduction in the values of neutrophil as inclusion of CGM increases showed that the anti nutrients that may be present in CGM did not affect the blood quality of the birds. The values for all

the parameters falls with the recommended range establish by earlier researchers Maxwell, *et al.,* (1990), (Mitruka and Rawnsley, 1997 and also with the report of Mohammed *et al.,* 2008).

Total proteins are the most abundant compound of serum. The protein make up of an animal is of important diagnostic significance because they are involved in enzyme, hormones and antibodies synthesis and as a reserve source of nutrient for body tissues and muscles. The statistical similarity in the total protein value indicates that the quality of protein in the experimental diets was adequate and the consumption by birds did not result in stress, disease, starvation or malnutrition and this values recorded are within the optimum level reported by Rajurker (2009). The highest albumin and globulin values recorded in birds fed 33.3% CGM are traceable to the highest protein value recorded in the birds fed the same treatment diet. Serum protein, albumin and Globulin synthesis is related to the availability of protein and micronutrients (Hofferberg and Block, 1996). Serum urea is known to be a function of the protein quality ingested by the animal, energy deficiency and disease condition which impair protein utilization. When diet is deficient in essential amino acid, the amino acid present will be deaminated resulting to an increase in urea excretion (Ranyhon, 2001). In other words, the comparable Urea value recorded when Cassava Grit-Moringa leaf meal mixture was included in the diet could be due to the effective protein utilization of the birds placed on the treatment diets. Eggum (1970) reported that creatinine is an indirect measure of protein utilization in poultry birds. The low value and relative non significant differences observed in the Creatinine values of birds fed experimental diets in this study suggest favourable protein utilization as cassava grit/Moringa leaf meal supplement to replaced maize.

Conclusion

The result of the study revealed that Cassava grit at 66.6% replacement level with 5% moringa leaf meal incorporation for maize improves the Nutrient utilization and blood quality of cockerel chickens was best at 33.3% CGM replacement for maize. A practical ration of 33.3 to 66.6% cassava grit as major energy source with as low as 5% MOLM incorporated is therefore advocated for cockerel production.

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