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Full Length Research Paper

Effect of cellulase-glucanase-xylanase combination on the nutritive value of *Telfairia occidentalis* leaf meal in broiler diets

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Telfairia occidentalis **leaves were purchased fresh from local farmers, sun dried and milled into *T. occidentalis* leaf meal (TOLM) . The proximate analyses revealed that TOLM contained 35.14 ± 0.44% crude protein; 9.61 ± 0.01% fat; 12.68 ± 0.02% crude fibre; 10.87 ± 0.02% ash; 9.04 ± 0.13% dry matter and 3.25 ± 0.01 kcal/ g gross energy. Eight broiler starter diets were formulated to be isocaloric and isonitrogenous. Diet 1 was duplicated such that the enzyme combination of cellulase, glucanase and xylanase (Roxazyme G2) was incorporated into one while Roxazyme G2 was lacking in the other (control diets 1a & 1b, with and without enzyme supplementation). Diets 2 and 3 were duplicate diets at 10% TOLM inclusion levels with the only difference being the Roxazyme G2 supplementation in diet 3. Diets 4 and 5 were also duplicates at 20% TOLM inclusion level with Roxazyme G2 supplementation in diet 5. Diets 6 and 7 at 30% TOLM inclusion were also in duplicate with diet 7 having Roxazyme G2 supplementation. The weight gain (WG) value for chickens on diet 1b with enzyme supplementation was highest at 19.51 ± 2.10g/chick although similar (P > 0.05) to WG values for birds on diets 1a (without enzyme supplementation), 2 and 3 while diet 7 with 30% TOLM inclusion with Roxazyme G2 supplementation had the lowest WG of 11.09 ± 0.39g/chick. Feed consumption (FC) was similar and higher in birds on diets 1a & b, 2 and 3. FC was also similar and lower in birds on diets 4, 5, 6 and 7. Feed conversion ratios did not differ significantly among the experimental diets except Diet 7. The operative protein efficiency ratio (PER) was higher and similar (P > 0.05) for birds on diets 1a & b, 2 and 3 on one hand and lower and similar (P>0.05) for birds on diets 4, 5, 6 and 7 on the other hand. The nitrogen retention (NR) value was highest for diet 1b (with enzyme supplementation) at 72.37 ± 0.10 but similar (P > 0.05) to birds on diets 1a, 4 and 5. There were no significant differences (P > 0.05) among means of the heamatological parameters tested. The mortality during the experimental period was insignificant and less than 1% of the total number of birds used.**

Key words: Enzyme supplementation, leaf meal, Roxazyme G2, chicks, poultry feed.

INTRODUCTION

Protein from plant leaves sources is perhaps the most naturally abundant and the cheapest potential source of protein. Natural resources are available for the synthesis and polymerization of amino acids into less mobile forms and stored as such in plant leaves. However, the build-up of the amino acids in plant leaves is also accompanied with other anti-nutritional factors that render them less nutritious for consumptive purpose in man and animal. Such factors limiting the nutritive value of leaf protein are the high fibre content and other anti-nutrients (Aletor and Adeogun, 1995; Fasuyi, 2007).

Benefits can be realized by supplementing poultry diets with exogenous enzymes like carbohydrases and phytases (Bi and Chung, 2004), and other cellulases and proteolytic enzymes (Kocher et al., 2003). These benefits include improvement in nutrient digestibility, reduction in excretion of nitrogen and phosphorous, increased use of alternative feed ingredients, reduction in variation of nutrient quality of feed ingredients, and reduction of the incidence of wet litter when feeding diets rich in viscous grains (Bedford, 2000).

Previous studies carried out by the author Fasuyi (2007)

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and Fasuyi et al. (2007) on the use of vegetable leaves and the knowledge gained from the application of minimum values of 1,600 U/g of cellulase, 3,600 U/g of endo-1,3(4)- -glucanase, and 5,200 U/g of endo-1, 4- -xylanase contained in Roxazyme G2 suggest that the development of substrate-specific enzyme and subsequent introduction into vegetable-based poultry diets is justified and feasible. The intent of this study is to investigate the additive effects of Roxazyme G2 in *Telfairia occidentalis* leaf meal (TOLM) supplemented diets on the growth performance, nitrogen utilization and heamatology of broiler chickens.

MATERIALS AND METHODS

Preparation of *Telfairia occidentalis* leaf meal (TOLM)

T. occidentalis plants were harvested fresh from maturing stems at about 20-30 days after transplanting to the field from the nursery.

The fresh leaves were immediately subjected to sun drying in an open cleaned concrete floor space until moisture content became constant at 13%. The sun dried leaves were later milled using a commercial feed milling machine (Artec, model 20). The proximate analysis, amino acid profile and mineral content were determined to chemically evaluate the nutritional potentials of the TOLM (Table 1). Thereafter, the TOLM was used to formulate diets along with other ingredients purchased locally.

Proximate gross energy, amino acids and mineral content determination

Proximate composition of the TOLM as determined by AOAC (1995) and the amino acids determined using amino acid analyzer model 80-2107-07 Auto Loader were quoted (Fasuyi, 2007). The mineral elements as determined were quoted (Fasuyi, 2007). Gross energy of the TOLM sample and the 6 formulated diets were determined against thermocouple grade benzoic acid using a Gallenkamp bal-listic bomb calorimeter (Model CBB-330-0104L). The quoted results and the results from other determinations are presented in Tables 1 and 2.

Roxazyme G2

Roxazyme G contains a minimum of 1,600 U/g of cellulase, 3,600 U/g of endo-1,3(4)- -glucanase, and 5,200 U/g of endo-1, 4- -xylanase. The recombinant enzymes used in this experiment were the single domain cellulase 5a (Cel5a) from *cellvibrio mixtus* (Fontes et al 1997) and a truncated derivative of xylanase 11a (Xyn11a) from *Clostridium thermocellum* termed GH11-CBM6 (Fernandes et al., 1999). Total enzyme used in each treatment was commercial polysaccharidase mixture, 0.1g/kg of Roxazyme G; recombinant xylanase, 4,000 U/kg of GH11-CBM6; and recombinant cellulose plus a xylanase, 4,000 U/kg of GH11-CBM6 plus 4,000 U/kg of Cel5a (1 U of enzyme activity released 1 mol of product/min at 37^{o}C).

Experimental rations formulation

The feed ingredients used in ration formulation were purchased locally from a reputable commercial feed miller in Ado-Ekiti, Ekiti State, Nigeria. The TOLM was sourced as earlier discussed. The results of the proximate compositions earlier determined were used

Table 1. Proximate composition (g/100g dry matter), gross energy (kcal/g) and amino acid profile (g/16g N) of *Telfairia occidentalis* leaf meal (TOLM) (means, n = 2).

Composition (g/100g)	TOLM
Dry matter	90.96
Crude protein	35.14
Ether extracts	9.61
Crude fibre	12.68
Ash	10.87
Nitrogen free extract	31.72
Gross energy (kcal/g)	3.21
***Amino acids**	
Alanine	406.9
Aspartic acid	388.1
Arginine	313.8
Glycine	381.3
Glutamic acid	688.1
Histidine	86.3
Isoleucine	318.8
Lysine	131.3
Methionine	155.0
Cystine	67.5
Meth. + Cys.	285.0
Leucine	473.8
Serine	244.4
Threonine	238.1
Phenylalanine	303.1
Valine	387.5
Tyrosine	351.3
Trytophan	195.0

as guides in the manual ration formulation of the eight experimental diets. The experimental diets were prepared at the Poultry Unit of the Teaching and Research Farm, University of Ado Ekiti, Ekiti State, Nigeria. All diets were compounded to contain identical crude protein content (isonitrogenous) and metabolisable energy (isocaloric). Control diet was duplicated as 1a and 1b for without and with R G2 supplementation. Other experimental diets were formulated by including TOLM at 10% (diets 2 and 3), 20% (diets 4 and 5) and 30% (diets 6 and 7) levels. Diets 3, 5 and 7 were supplemented with Roxazyme G2, where as diets 2, 4 and 6 didn't contain Roxazyme G2. The enzyme fortification was done by mixing the manufacturer specified quantities with the enzyme supplemented diets.

Other conventional protein and energy sources were used in the formulation of the diets. All diets were also supplemented with feed grade methionine, lysine and mineral/vitamin premix.

Management of experimental birds and experimental design

A total of 288 day-old broiler chicks of the *anak* heavy strain were purchased from Zartech hatchery, a division of Zartech Farms, Ibadan, Oyo-State (a reputable hatchery in Nigeria). They were fed a 24% crude protein broiler starter commercial ration *ad libitum* for the first 3 days after arrival from the hatchery prior to the commencement of the experiment. The chicks were also sexed on the second day of

Table 2. Mineral composition of *Telfairia occidentalis* leaf meal (TOLM) (means, n = 2).

TOLM	g/100g					(ppm)			
	Ca	P	K	Na	Mg	Fe	Mn	Cu	Zn
	2.6	1.2	6.2	5.1	4.7	5875	182	136	1036

Source: Fasuyi et al., (2007)

Table 3. Phytic acid, phytin-P, oxalic acid, tannic acid and cyanide contents of *Telfairia occidentalis* leaf meal (TOLM) (means, n = 2)

TOLM	Phytic acid mg/100g	Phytin-P (mg/100g)	Phytin-P As % of total P	Oxalate (mg/100g)	Tannin (mg/100g)	HCN (mg/100g)
	189.2	120.1	8.05	80.7	43.0	61.2

Source: Aletor and Adeogun, 1995; Fasuyi, 2007.

brooding as described by Laseinde and Oluyemi (1997). Water was also provided *ad libitum* with a mixture of appropriate antibiotics and glucose as an anti-stress factor. All routine medications and vaccination were administered viz: intraocular vaccination against Newcastle disease was administered at day one; Neoceryl (antibiotics) was given for a period of 4 days from 3 days of age; coccidiostat for the treatment/control of coccidiosis and chronic respiratory diseases was administered via water intake; Gumboro vaccine was administered at 2 weeks of age and Lasota vaccine (New castle booster) was administered in a day at the age of 3 weeks.

The experimental design was the completely randomized type. A total of 288 chicks were used for the experiment. There were two replicates for each diet. After the uniform brooding of 3 days, the sexed chicks (9 males and 9 females) were randomly distributed into 16 experimental units. The chicks were fed the experimental diet *ad libitum* for 21 days during which records on daily feed consumption and 3 days periodic weight changes were recorded.

Estimation of nitrogen retention and protein efficiency ratio

Total faeces voided during the last 5 days were collected, weighed, dried at 65-70^{o}C in an air circulating oven for 72 h and preserved while the corresponding feed consumed was also recorded for nitrogen studies. The nitrogen contents of the samples were determined by the method of AOAC (1995). Nitrogen retained was calculated as the algebraic difference between feed nitrogen and fecal nitrogen (on dry matter basis) for the period. Protein Efficiency Ratio (PER) was calculated by dividing the gain in body weight by the protein intake of the chicks.

$$PER = \frac{\text{gain in body weight (g)}}{\text{protein intake (g)}}$$

Feed conversion ratio (FCR) was calculated by dividing the feed intake by the body weight gain of the chicks.

$$FCR = \frac{\text{feed consumption (g)}}{\text{weight gain (g)}}$$

Blood collection for analysis

At the end of the feeding trial, a male chick per replicate was randomly selected, weighed and scarified by severing the jugular vein and blood allowed to flow freely into labeled bottles one of which contained a speck of EDTA while the other without EDTA was processed for serum. The serum was kept deep frozen prior to analysis. The packed cell volume (PCV%) was estimated by spinning about 75:l of each blood sample in heparinized capillary tubes in an hematocrit micro centrifuge for 5 min while the total red blood cell (RBC) count was determined using normal saline as the diluting fluid. The hemoglobin concentration (Hbc) was estimated using cyano-methemoglobin method while the mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and the mean corpuscular volume (MCV) were calculated.

Statistical analysis

Data were expressed as means ± standard deviation of two measurements. One way ANOVA (Minitab 11.21 for Windows, 1996) was used to analyse the mean differences between the dietary treat-ments. A significant difference was considered at level of $p < 0.05$.

RESULTS AND DISCUSSIONS

Proximate gross energy, amino acids, mineral content and notable antinutrients

The results of proximate composition, gross energy and amino acids content are presented in Table 1 while the mineral composition and some notable antinutrients are presented in Tables 2 and 3. The *T. occidentalis* leaf meal (TOLM) was relatively high in crude protein at 35.14% and comparable with some conventionally used protein feed ingredients such as brewers dried grains. The ether extracts at 9.61% was also relatively high and higher than the commonly consumed leafy vegetables such as bitter leaf (3.4%); water leaf (1.5%) and *Amaranthus* (2.8%) (Akoroda, 1990). The sugar + starch (NFE) at 31.72% was also comparable with other plant protein sources (Oyenuga, 1968) . The TOLM was remarkably rich in mineral elements such as Ca, K, Na, Mg, Fe and Zn com-pared to reported levels of these mineral elements in most plant protein sources (Leung et al., 1968). There were sig-

Table 4. Composition of experimental diet (g/100g).

Ingredient	Diets							
	1a & b(Ref.)		2	3	4	5	6	7
	% inclusion levels of TOLM							
	0		10		20		30	
	-	+	-	+	-	+	-	+
Maize (9% CP)	50.10	50.10	47.60	47.60	44.60	41.60	34.60	34.60
Soyabean meal (45.0%CP)	33.50	33.50	26.00	26.00	19.00	19.00	11.00	11.00
P.K.C (18.8%CP)	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Fish meal (72%CP)	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
TOLM* (35. 14%CP)	-	-	10.00	10.00	20.00	20.00	30.00	30.00
Bone meal	2. 50	2. 50	2. 50	2. 50	2. 50	2. 50	2. 50	2. 50
Oyster shell	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
L-Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.2 0	0.20
Premix**	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Crude protein, %	22.85	23.10	23.00	23.01	22.90	22.91	23.10	23.01
Crude fibre, %	5.02	5.02	6.37	6.37	6.38	6.38	5.02	5.02
Ether extract, %	6.71	6.71	7.10	6.91	6.73	6.83	7.01	7.51

*TOLM, Telfairia occidentalis leaf meal*TOLM, Telfairia occidentalis leaf meal.

** contained vitamin A (100,000,000 iu); D(2,000,000 iu); E[35,000iu]; K (1900mg); B12(19 mg); Riboflavin (7,000mg); Pyridoxine (3800mg]); Thiamine (2,200mg); D pantothenic acid [11,000mg]; Nicotinic acid [45,000mg]; Folic acid [1,400mg]; Biotin [113mg]; and Trace element as CU [8000mg]; Mn [64,000mg; Zn [40,000mg]; Fe [32,000mg]; se [160mg]; I2 [800mg] and other items as Co[400mg choline (475,000);Methionine (50,000mg); BHT (5,000MG); and Spiramycin (5,000mg) per 2.5kg. GEE** [kcal/100g) calculated based on 5.7kcal/g protein; 9.5kcal/g lipid; 4.0kcal 1g carbohydrates (Ng and Wee, 1989).

NFE, Nitrogen Free Extract. 1(Ref.), Diet 1 in duplicate (with/without Roxazyme G2 supplementation) are the control diets.

+, Diet with Rozaxyme G enzyme supplementation; -, without Rozaxyme G enzyme supplementation

nificant traces of phytic acid (189.2mg/100g); phytin phos-phorous (120.1mg/100g), oxalate (80.7%); tannin (43mg/ 100g) and cyanide (61.2mg/100g).

The amino acid profile of TOLM suggested a curious similarity between the protein quality of TOLM and proteins of other plants origins particularly groundnut cake. Of peculiar advantage in TOLM were the contents of lysine (2.10 g/16g N) and methionine (2.48g/16g N) which are always low in most plant protein sources. This is in agreement with earlier submission (Oke, 1973a,b) that the amino acid patterns of some leaves and grasses are as good, if not better than those of the best seed protein, for example soybeans. It was conceivable from this result that Roxazyme G2 when added to diets containing TOLM would facilitate cell wall degrading activities while exhibiting cellulase and protease activities (Zanella et al., 1999), which later explained the improved protein utilization indices on the performance characteristics of the broiler birds. The use of leaf meals in poultry diets is generally inhibited by the high fibre levels and some antinutritional factors present in most green leaves (Aletor and Adeogun 1995; Fasuyi 2007). It is conceivable therefore, that the supplementary addition of Roxazyme G2 would have broken down the cellulose and other nonstarch polysaccharides which are mainly found in the cell wall and are bound together in a complex matrix. In this process, the encapsulated starch molecules were un-locked by solubilizing the cell wall structure and increasing accessibility to digestive enzymes. This process will further enhance nutrient availability for growth. Several studies corroborated the above finding with reports that enzyme supplementation of poultry diets produced signifi-cant positive responses to growth performances (Wyatt et al., 1997; Pack et al., 1998).

Performance characteristics

The composition of the experimental diets is presented in Table 4 while the performance of broiler chicks fed varying dietary levels of TOLM based diets is presented in Table 5.The average weight gain (WG) for the experimental period of 21days indicated that chicks on diets 1 (control diet with/without Roxazyme G2) had similar ($P > 0.05$) WG values of 19.33 ± 0.38 g and 19.51 ± 2.10 g, respectively which were also similar to WG values obtained for chicks on diets 2 (17.65±0.78 g) and 3 (17.70 ± 0.43 g). The average weight gain of broiler birds on diets 4 (20% TOLM inclusion level without Roxazyme G2), 5 (20% TOLM inclusion level with Roxazyme G2), 6 (30% TOLM inclu-

Table 5. Performance of broiler chicks fed varying dietary levels of TOLM.

Parameters	Diets							
	1		2	3	4	5	6	7
	% Inclusion Levels of TOLM							
	0	0	10		20		30	
	-	+	-	+	-	+	-	+
Average weight gain (g/chick)	19.33^{a}±0.38	19.51^{a}±2.10	17.65^{a}±0.78	17.70^{a}±0.43	12.96^{b}±1.36	12.34^{b}±0.93	12.39^{b}±2.07	11.09^{b}±0.39
Feed consumption (g/ chick/day)	53.11^{a}±0.03	52.58^{a}±1.51	52.90^{a}±0.06	51.01^{a}±0.17	40.21^{b}±0.01	40.10^{b}±0.29	40.81^{b}±0.16	37.90^{b}±0.07
Feed Conversion Ratio (FCR)	2.74^{a}±0.01	2.70^{a}±0.17	3.00^{a}±0.01	2.88^{a}±0.07	3.10^{a}±0.01	3.25^{a}±0.02	3.29^{a}±0.01	3.41^{c}±0.01
Protein Efficiency(PER)	1.58^{a}±0.04	1.60^{a}±0.41	1.45^{a}±0.52	1.50^{a}±0.90	1.39^{b}±1.61	1.34^{b}±0.09	1.31^{b}±1.88	1.27^{b}±0.26

TOLM, Telfairia occidentalis leaf meal
Means with different superscripts in the same horizontal row are significantly different (P > 0.05)
+/-, supplementation and non supplementation of the diet with Roxazyme G2

sion level without Roxazyme G2) and 7 (30% TOLM inclusion level with Roxazyme G2) were all similar (P>0.05) and lower than the WG values obtained for birds on diets 1, 2 and 3. It was remark able that WG values recorded for broiler chicks fed diets supplemented with Roxazyme G2 were con-sistently higher than their duplicate diets in which Roxazyme G2 was absent. This observation was in tandem with the submission of Bedford (2000) that benefits have been realized by enzyme sup-plementations in poultry diets and such benefits include improvement in nutrient digestibility, reduction in excretion of nitrogen and phosphorus, increased use of alternative feed ingredients, reduction in the variation of nutrient quality of feed ingredients, and reduction of the incidence of wet litter when feeding diets rich in viscous grains.

The average feed intake values (FI) for chicks on diet 1 (control diet with/without Roxazyme G2) were similar (P > 0.05) at 53.11 ± 0.03 g and 52.58 ± 1.51 g, respectively and also similar to FI values obtained for chicks on diets 2 and 3 at 52.90 ± 0.06 g and 51.01 ± 0.17 g, respectively. All other FI values obtained for broiler birds on diets 4, 5, 6 and 7 were similar (P > 0.05) and lower than the FI values obtained for birds on diets 1, 2 and 3. Feed consumption in Roxazyme G2 supplemented diets were numerically lower than their duplicate diets in which Roxazyme G2 was absent although these values were stastically similar (P > 0.05) . Exoge-nous Roxazyme G2 supplementation to improve dietary nutrient digestibility and utilization might be effective in lowering the energy losses via heat increment and as volatile fatty acids (as a result of energy- inefficient microbial fermentation process) in the excreta (Yu and Chung 2004). As such, more energy is conserved and made available for body weight and reduced feed intake since birds naturally consume feed based on energy require-ment (Zanella et al., 1999). Exogenous enzyme supplementation was also found to be beneficial for reduced- energy diets such as those used in the cool season (Yu and Chung 2004).

The feed conversion (FCR) for chicks on diet 1 (with/ without Roxazyme G2 supplementation) at 2.74 ± 0.01 and 2.70 ± 0.17, respectively were statistically similar (P > 0.05) and also similar (P > 0.05) to the FCR values obtained for chicks on diets 2, 3 and 4 at 3.00 ± 0.01, 2.88 ± 0.07 and 3.10 ± 0.01, respectively. However, FCR values obtained for chicks on diets 5, 6 and 7 (3.25 ± 0.02, 3.29 ± 0.01, and 3.41 ± 0.01, respectively) were also similar (P > 0.05) and higher than the other FCR values. The protein efficiency ratio (PER) for chicks on diets 1 (with/without Roxazyme G2 supplementation), 2 and 3 were also similar at 1.58±0.04, 1.60±0.41, 1.45 ± 0.52 and 1.50 ± 0.90, respectively. These PER values were higher than the PER values obtained for broiler birds on diets 4, 5, 6 and 7 at 1.39±1.61, 1.34±0.09, 1.31±1.88 and 1.27±0.26, respectively. The protein efficiency ratio (PER) is an important protein evaluation index which gives an insight into the relationship bet-ween the body weight gain and the actual protein intake.

Roxazyme G2 supplemented diets consistently showed better FCR values than their duplicate diets in which Roxazyme G2 was absent in diets 1 and 2. Roxazyme G2 dietary supplementation had

Table 6. Nitrogen Utilization of broiler birds fed TOLM based diets**.**

Parameters	Diets							
	1		2	3	4	5	6	7
	% Inclusion Levels of TOLM							
	0		10		20		30	
	-	+	-	+	-	+	-	+
Nitrogen intake (NI, g/chick)	2.53^{a}±0.09	2.57^{a}±1.01	2.32^{a}±0.62	2.41^{a}±0.15	2.23^{a}±0.25	2.27^{a}±0.02	2.09^{b}±0.03	2.03^{b}±0.04
Nitrogen in droppings (FN, g/chick)	0.72±0.34	0.71±0.32	0.81±0.32	0.82±0.32	0.71±0.31	0.71±0.42	0.75±0.33	0.71±0.32
Nitrogen Retention (NR, %)	71.54^{a}±1.89	72.37^{a}±0.10	65.09^{b}±1.87	65.98^{b}±1.52	68.16^{ab}±3.36	68.72^{ab}±0.01	64.11^{b}±3.10	65.02^{b}±0.38

TOLM, *Telfairia occidentalis* leaf meal
Means with different superscripts in the same horizontal row are significantly different (P > 0.05).
+/-, supplementation and non supplementation of the diet with Roxazyme G2

the potentials of splitting the complex non-starch polysaccharide molecules, such as cellulose, xylans and beta-glucans. This led to a better and improved utilization of TOLM, while simultaneously improving the digestibility of nutrients and reducing the losses of endogenous amino acids, resulting in the conservation of endogenous utilizable energy that may be otherwise used for protein accretion (Zanella et al., 1999).

Nitrogen utilization

Table 6 presents data on nitrogen utilization. The nitrogen intake (NI) value of chicks on diet 1 (control diet with/without Roxazyme G2 supplementation) at 2.53g/chick/day ± 0.09 and 2.57g/chick/day ± 1.01, respectively were similar (P > 0.05) and also similar to the NI values for the chicks on diets 2, 3, 4 and 5 at 2.32g/chick/day ± 0.62, 2.41g/chick/day ± 0.07, 2.23g/chick/day ± 0.22 and 2.27g/chick/day ± 0.02, respectively. Chicks on diets 6 and 7 had the lowest but similar NI values (P> 0.05) of 2.09g/chick/day ± 0.03 and 2.03g/chick/day ± 0.04, respectively.

The nitrogen retention (NR) values of chicks on diets 1 (control diet with/without Roxazyme G2 supplementation) were similar (P > 0.05) at 71.54% ± 0.60 and 72.37% ± 0.10, respectively. These NR values were also similar to values obtained for birds on diets 4 and 5 at 68.16% ± 3.36 and 68.72% ± 0.01, respectively. However, the NR values obtained for the birds on diets 4 and 5 were also similar (P > 0.05) to NR values obtained for birds on diets 2, 3, 6 and 7.

It is noteworthy that birds on the Roxazyme G2 supplemented diets had remarkably better nitrogen intake than their duplicate diets without Roxazyme G2. It is obvious that Roxazyme G2 had a contributory effect in increasing accessibility to intracellular entrapped nutrients (Kocher et al., 2003). However, the amino acids imbalance in the experimental diets adversely affected the appetite and feed intake (Rostagno et al., 1995) as indicated in the nitrogen intake trend among the experimental birds. It appears that there were some limiting amino acids in TOLM that underscored the digestibility in the growing chicks. It is well documented in various literatures that the limiting amino acids in the diets of chicks depresses feed intake which also affects the growth rate and overall nitrogen utilization (Oluyemi and Robert, 2000).

A close observation at the NR values revealed that in spite of the similarity among the means values, diets supplemented with Roxazyme G2 showed consistently higher and better NR values at least better than the identical diets without Roxazyme G2 supplementation. As discussed earlier, there seemed to be an evidence of increased proteolytic activities in the birds fed diets containing Roxazyme G2. The breakdown of the NSPs and the subsequent utilization of the hitherto bound amino acids could have been responsible for the enhanced protein retention in birds in which the diets were Roxazyme G2 supplemented (Kocher et al., 2003). The benefits of enzyme addition to diets containing high fibre lies in the increased access to intracellular entrapped nutrients as well as in an improved energy utilization (Kocher et al., 2003)

Haematological indices of experimental birds

There were no significant differences (P > 0.05) in all the haematological parameters investigated (Table

Table 7. Haematological parameters of broiler birds fed TOLM based diets.

Parameters	Diets							
		1	2	3	4	5	6	7
	% Inclusion Levels of TOLM							
	0		5		15		25	
	-	+	-	+	-	+	-	+
PCV, %	26.9	27.0	27.0	26.7	26.1	26.3	27.0	26.1
RBC ($X10^6/m^3$	2.1	2.1	2.1	2.2	2.1	2.1	2.1	2.1
Hbc	2.1	2.1	2.1	2.1	2.2	2.1	2.2	2.2
MCHC	7.1	7.2	7.2	7.1	7.1	7.1	7.1	7.2
MCH(pg)	9.1	9.1	9.1	9.1	9.2	9.1	9.1	9.2
MCV	130.1	131.0	131.2	130.1	130.0	131.0	130.2	130.1
ESR	4.1	4.1	4.3	4.2	4.1	4.1	4.3	4.3

TOLM, *Telfairia occidentalis* leaf meal; PCV, Packed Cell Volume; RBC, Red Blood Cell; WBC, White Blood Cell; Hbc, Haemoglobin Concentration; MCHC, Mean Cell Haemoglobin Concentration; MCH, Mean Cell Haemoglobin; MCV, Mean Cell Volume; ESR, Erythrocyte Sedimentation Rate.
No significantly differences among treatment means (P < 0.05)
+/-, supplementation /non- supplementation of diet with Roxazyme G2.

7). The MCV obtained for all birds is in agreement with standard values reported in previous literatures (Aletor and Egberongbe, 1992) who reported 2.2 and $100/mm^3$ respectively.

The blood variables most often affected by dietary influences were identified as PCV, plasma protein, glucose and clotting time (Aletor, 1993; Ologhobo et al., 1986). These values in the experimental birds were found to be consistently higher than most values earlier reported and comparable with the report for chicks fed soybeans in place of fish meal (Aletor and Egberongbe, 1992). On a similar note, the MCHC, MCH and HBC were not significantly affected (P > 0.05) by the dietary treatments suggesting adequate haemoglobin contents. The ESRs of the test diets did not suggest that the birds were predis-posed to any known general infections or malformation of any kind. Frendson (1986) reported that ESRs increased in cases of acute general infection, malignant tumors and pregnancy.

Other physical observations

There was no mortality throughout the 21- day experimental period with the broiler starter chicks. It was also observed that chicks fed varying dietary inclusions of TOLM had pronounced yellow coloration of beaks and shanks which deepened in chicks across the diets from diet 2 to diet 7 (with and without enzyme). Feathering was more pronounced in chicks on diet 2.

Conclusion

For practical poultry feed formulation, inclusion levels between 5% and 15% must not be exceeded with the inclusion of fibre-breaking enzyme and also in consideration of the bulkiness and dustiness of the TOLM. Roxazyme G2 has been established to improve the digestibility of nutrients and also reduce the losses of endogenous amino acids. TOLM may not be appropriate as a sole protein source but a combination of ingredients of different protein sources had a complementary role in enhancing the nutritional benefits of TOLM in broiler diets.

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