American Journal of Food Technology

ISSN 1557-4571

Academic Journals Inc.

www.academicjournals.com

American Journal of Food Technology, 4 (3): 108-118, 2009
ISSN 1557-4571

Nutritional Evaluation of *Amaranthus cruentus* Leaf Meal Based Broiler Diets Supplemented with Cellulase/Glucanase/Xylanase Enzymes

A.O. Fasuyi and A.O. Akindahunsi
Department of Animal Production and Health Sciences,
University of Ado-Ekiti, P.M.B. 5363, Ekiti State, Nigeria

Abstract: Sundried leaves of *Amaranthus cruentus* (*Amaranthus cruentus* leaf meal, ACLM) were milled and analyzed for their proximate composition. Crude protein was 23.0±0.55%; crude fat, 5.4±0.1%; crude fibre, 8.8±0.02%; ash, 19.3±0.01% and gross energy, 3.3±0.01 kcal g^{-1}; metabolizable energy, 2.8±0.21 kcal g^{-1} all on dry matter basis. Minerals, amino acids and antinutrients were also determined. Methionine and to a lesser extent, lysine, arginine, leucine and aspartate were high. The ACLM was incorporated into formulated broiler starter diets at varying inclusion levels of 0, 5, 15 and 25%. The diets were duplicated with a set supplemented with Roxazyme G2 in a 2×4 factorial experiment. All the 8 diets including the control diets were formulated isocaloric and isonitrogenous and fed to the experimental chicks (n = 288) from day 3 to day 24. Statistical main effects indicated that broiler chicks in which ACLM was incorporated at 5% inclusion level in their diet with Roxazyme G2 supplementation was found to have the highest weight gain. Feed consumption value is found to be highest in chicks fed diet 8 at 25% inclusion level of ACLM with Roxazyme G2 supplementation. The feed conversion value obtained for birds on diet 4 with Roxazyme G2 supplementation was the best. Broiler chicks on diet 4 also had the best value for Protein Efficiency Ratio (PER). There were no significant differences ($p>0.05$) in all the hematological parameters investigated. The additive inclusion of Roxazyme G2 in broiler diets can further increase the use of ACLM as a protein source effectively at 5%. There were no deleterious effects even up to 25% ACLM inclusion level with enzyme supplementation.

Key words: Nutritional evaluation, cellulase, protease activities

INTRODUCTION

Earlier studies on the utilization of leaf meals indicated a considerable utilization in poultry diets (Fasuyi and Aletor, 2005; Fasuyi, 2006, 2007a-c; Fasuyi and Nonyerem, 2007; Fasuyi *et al*., 2007). There is no doubt that green leaf is still the cheapest available protein source and the initial point of ecological food chain ostensibly because of the free natural materials (water, CO_2 and solar energy) used in the photosynthetic manufacture of its food. Substantial variations exist among the amino acid profiles of the proteins from leaf sources and as such dietary leaf proteins are not completely utilized by chickens. It is necessary to further exploit the nutritional potential of the leaf meals by enzymatic supplementation in poultry diets using established cellulase/glucanase/xylanase combination as found in Roxazyme G2.

The economic and nutritional advantage of the amaranth as a leaf vegetable is primarily based on its agronomic superiority over many plant leaf protein sources. For instance, harvesting is done 20-30 days after transplanting and then every 2-3 weeks for a period of 1 to 2 months (Leung *et al*., 1968). Another potential nutritional advantage of the amaranthus plant is the chemical

Corresponding Author: A.O. Fasuyi, Department of Animal Production and Health Sciences, University of Ado-Ekiti, P.M.B. 5363, Ekiti State, Nigeria

composition which is highly in favor of the plant leaves as a veritable source of plant protein (Leung *et al*., 1968; Aletor and Adeogun, 1995) and its rich source of vitamins and minerals.

A growing interest in the use of unconventional sources of protein and energy in poultry feed has gained prominence (Eggum, 1970; Ravindran and Ravindran, 1988; Siddhuraju *et al*., 2001) as a result of the world shortage of animal protein particularly in developing countries in Africa. This study was therefore designed to determine the nutritional benefit of the additive role of Roxazyme G2 in enhancing the utilization of leafy vegetable meals (*Amaranthus cruentus* leaf meal, ACLM) in broiler rations.

MATERIALS AND METHODS

Experimental Site and Preparation of *Amaranthus cruentus* Leaf Meal (ACLM)

The experimental diets were prepared at the Poultry Unit of the Teaching and Research Farm of the University of Ado Ekiti, Ekiti State, Nigeria. *Amaranthus cruentus* leaves 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 ACLM. Thereafter, the ACLM was used to formulate diets along with other ingredients purchased locally.

Proximate Gross Energy, Amino Acids and Mineral Content Determination

Proximate composition of the ACLM was determined by AOAC (1995) method while the amino acids were determined using amino acid analyzer model 80-2107-07 Auto Loader. The sodium and potassium contents were determined by flame photometry while phosphorus was determined by the Vanado-molybdate method (AOAC, 1995). The other mineral elements were determined after wet digestion with a mixture of nitric, sulfuric and hydrochloric acid using Atomic Absorption Spectrophotometer (AAS model SP9). Gross energy of the ACLM sample and the 8 formulated diets were determined against thermocouple grade benzoic acid using a Gallenkamp ballistic bomb calorimeter (model CBB-330-0104L). The results showing the above determinations are presented in Table 1 and 2.

Experimental Rations Formulation

The feed ingredients used in ration formulation were purchased locally from a reputable commercial feed mill in Ado-Ekiti, Ekiti State, Nigeria. The ACLM was source as earlier discussed. The results of the proximate compositions earlier determined were used as guides in the manual ration formulation of the 8 experimental diets. All diets were compounded to contain identical crude protein content (isonitrogenous) and metabolizable energy (isocaloric). The eight diets formed the eight dietary treatments as follows:

- **Diet 1 (Ref. diet 1):** Reference diet 1 in which there was no ACLM inclusion and no enzymes (Roxazyme G2) supplementation
- **Diet 2 (Ref. diet 2):** Reference diet 2 in which there was no ACLM inclusion but with enzymes (Roxazyme G2) supplementation
- **Diet 3 (5NE):** 5% ACLM inclusion without enzymes (Roxazyme G2) supplementation
- **Diet 4 (5ENZ):** 5% ACLM inclusion with enzymes (Roxazyme G2) supplementation
- **Diet 5 (15NE):** 15% ACLM inclusion without enzymes (Roxazyme G2) supplementation
- **Diet 6 (15ENZ):** 15% ACLM inclusion with enzymes (Roxazyme G2) supplementation
- **Diet 7 (25NE):** 25% ACLM inclusion without enzymes (Roxazyme G2) supplementation
- **Diet 8 (25ENZ):** 25% ACLM inclusion with enzymes (Roxazyme G2) supplementation

Table 1: Proximate composition (g/100 g), gross energy (kcal g^{-1}) and amino acid profile (g kg^{-1}) of *Amaranthus cruentus* leaf meal (ACLM) (Mean values, n = 2)

Composition (g/100 g)	ACLM	Amino acids	*Amaranthus cruentus*	FAO/WHO (1973) recommended pattern	Whole egg
Dry matter	88.60±0.01	Alanine	396.3		
Crude protein	23.00±0.55	Aspartic acid	320.0		
Ether extracts	5.40±0.01	Arginine	375.6	381.3	
Crude fibre	8.80±0.01	Glycine	251.3		
Ash	19.30±0.01	Glutamic acid	644.4		
Nitrogen free extracts	43.50±0.52	Histidine	131.9		150.0
Gross energy (kcal g^{-1})	3.25±0.01	Isoleucine	300.6	250.0	350.0
		Lysine	111.9	343.7	393.8
		Methionine	86.3	218.8	200.0
		Cystine	81.9	437.5	112.5
		Meth.+Cys.	168.2		312.5
		Leucine	529.4		518.8
		Serine	273.1		
		Threonine	196.9	250.0	318.8
		Phenylalanine	363.8		318.8
		Valine	326.9	312.5	475.0
		Tyrosine	312.5	375.0	250.0
		Tryptophan	147.5	62.5	112.5

Source: Fasuyi (2007d)

Table 2: Mineral composition of *Amaranthus cruentus* leaf meal (ACLM g/100 g) (Mean values, n = 2)

Mineral composition	Values (ppm)
Ca	2.4
P	1.8
K	5.8
Na	7.2
Mg	3.1
Fe	1175.0
Mn	198.0
Cu	36.0
Zn	890.0

Other conventional ingredients of 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 days 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 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. The following medications were administered:

- Intraocular vaccination against Newcastle disease at day one
- Neoceryl (antibiotics) for a period of 4 days from 3 days of age
- Coccidiostat for the treatment/control of coccidiosis
- Gumboro vaccine at 2 weeks of age
- Lasota vaccine (New castle booster) administered in a day at the age of 3 weeks

The experiment was a 2×4 factorial experiment in a completely randomized design. A total of 288 chicks were used for the experiment. 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 assigned at the rate of 36 chicks per diet in 2 replications of 18 chicks per replicate such that the mean group weights were similar at the beginning of the experiment. 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.

Data Collection and Statistical Analysis

The data were analyzed using ANOVA at factorial design (two-way classification). Significant differences were determined by Duncan's multiple range test.

Estimation of Nitrogen Retention, Nitrogen Digestibility and Protein Efficiency Ratio

Total feces voided during the last 5 days were collected, weighed, dried at 65-70°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. Nitrogen digestibility was computed by expressing the nitrogen retained as a fraction of the nitrogen intake multiplied by 100. The protein efficiency ratio was calculated as the ratio of weight gain to total protein consumed.

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 μL 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 cyanomethemoglobin method while the Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH) and the Mean Corpuscular Volume (MCV) were calculated.

Roxazyme G2

Roxazyme G contains a minimum of 1,600 U g^{-1} of cellulase, 3,600 U g^{-1} of endo-1, 3(4)-β-glucanase and 5,200 U g^{-1} of endo-1,4-β-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). The bacterial xylanase is a modular enzyme containing a catalytic domain and a noncatalytic xylene-binding module separated by a short linker sequence (Fernandes *et al*., 1999). Plasmids containing the DNA encoding regions of both proteins, under the control of a T7 promoter in the prokaryotic expression vector pET21a (Novagen), were transformed in *Escherichia coli* BL21 cells. Recombinant *E. coli* strains were grown on Luria Bertani gene expression induced by adding isopropyl β-D-thiogalactoside to a final concentration of 1mM. Cells were collected after 5h induction at 37°C and protein extracts prepared by ultrasonication as described by Fernandes *et al*. (1999). Extracts were incubated at 50°C for 20 min and centrifuged for 30 min at 10,000. x g to remove much of the *E.coli* proteins (both recombinant enzymes are thermostable at the referred temperature). Total enzyme used in each treatment was commercial polysaccharidase mixture, 0.1 g kg^{-1} of Roxazyme G; recombinant xylanase, 4,000 U kg^{-1} of GH11-CBM6; and recombinant cellulase plus a xylanase, 4,000 U kg^{-1} of GH11-CBM6 plus 4,000 U kg^{-1} of Cel5a (1 U of enzyme activity released 1 mol of product min^{-1} at 37°C).

RESULTS AND DISCUSSION

Energy, Amino Acids, Mineral Content and 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 Table 2 and 3. The *Amaranthus cruentus* Leaf Meal (ACLM) was relatively high in crude protein at 23.0±0.55%; fat at 5.4±0.01% and sugar±starch (NFE) at 43.5±0.52%. The ACLM was remarkably rich in mineral elements such as Ca, K, Na, Mg, Fe and Zn compared to reported levels of these mineral elements in most plant protein sources. The phytic acid and oxalate levels were relatively higher than most other plant protein origins at 680 and 620 mg/100 g, respectively. The phytin-P was also high at 160 mg/100 g.

There was a curious similarity between the amino acid profile of ACLM and that of other plants origins particularly groundnut cake. This is in agreement with the submission of Oke (1972) 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 ACLM would facilitate cell wall degrading activities while exhibiting cellulase and protease activities (Zanella *et al*., 1999), which later explained the improved protein utilization indices.

Performance Characteristics

The average Weight Gain (WG) for the experimental period of 21 days indicated that chicks on reference diets 1 and 2 to diet 6 were similar ($p>0.05$) in WG values of 19.33, 19.51, 19.32, 19.54, 19.19 and 18.40 g/chick/day, respectively. Chicks on diets 7 (25NE) and 8 (25ENZ) also had similar ($p>0.05$) WG values similar of 13.90 g and 14.96 g/chick/day, respectively Table 4.

Birds on AC5R was found to have the highest weight gain and statistically similar ($p>0.05$) to the WG values obtained for chicks fed reference diets 1 and 2, 5NE, 15NE and 15ENZ. It was noteworthy that WG values recorded for broiler chicks fed diets supplemented with Roxazyme G2 were consistently higher than their pair diets in which Roxazyme G2 was absent implying that benefits were inherent in the use of Roxazyme G2 supplementation in the broiler diets Table 5. This finding is in tandem with the report of Bedford (2000) that benefits have been realized by enzyme supplementations 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 Consumption (FC) for chicks on reference diet 1 to 25NE were similar ($p>0.05$) at 53.10, 52.58, 54.85, 53.10, 56.91, 53.10 and 54.85 g/chick, respectively. Interestingly, chicks on diets AC15 and AC25R also had similar ($p>0.05$) FC values of 56.91±0.92 and 58.86±0.07g, respectively. Feed consumption value was highest in chicks fed diet 25ENZ.

Exogenous 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 (Bi and Chung, 2004). As such, more energy is conserved and made available for body weight and reduced feed intake since

Table 3: Phytic acid, phytin-P and oxalic acid content of *Amaranthus cruentus* leaf meal (ACLM) (Mean values, n = 2)

Antinutrients	Values
Phytic acid (mg/100 g)	680.0
Phytin-P (mg/100 g)	160.0
Phytin-P (As % of total P)	12.2
Oxalate (mg/100 g)	620.0

Aletor and Adeogun (1995) and Fasuyi (2007d)

Table 4: Composition of experimental diets (g/100 g)

	Inclusion levels of ACLM (%)							
Ingredient	(Ref. diet 1)	(Ref. diet 2)	5NE	5ENZ	15NE	15ENZ	25NE	25ENZ
Maize (9% CP)	50.10	50.10	46.60	46.60	41.60	41.60	34.60	34.60
Soyabean meal (45.0% CP)	33.50	33.50	32.00	32.00	27.00	27.00	24.00	24.00
PKC (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
ACLM* (35. 14% CP)	-	-	5.00	5.00	15.00	15.00	25.00	25.00
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	0.20	0.20
Premix**	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated								
Crude protein (%)	23.00	23.00	23.00	23.00	23.00	23.00	23.00	23.00
ME (kcal kg^{-1})	2495.10	2495.10	2495.20	2495.20	2501.50	2495.80	2495.80	2495.20
Crude fibre (%)	5.20	5.20	5.80	5.80	5.80	5.80	5.70	5.70
Ether extract (%)	4.10	4.10	4.40	4.40	4.20	4.20	4.30	4.30
Analyzed								
Crude protein (%)	22.76	23.12	22.90	23.01	22.89	22.91	23.10	23.03
Crude fibre (%)	5.02	5.02	6.37	6.37	6.38	6.38	5.02	5.02
Ether extract (%)	7.61	7.61	7.80	7.80	7.83	7.83	7.61	7.61

*ACLM: Amaranthus cruentus leaf meal; **: Contained vitamin A (100,000,000 IU); D(2,000,000 IU); E(35,000 IU); K (1900 mg); B12(19 mg); Riboflavin (7,000 mg); Pyridoxine (3800 mg); Thiamine (2,200 mg); D pantothenic acid (11,000 mg); Nicotinic acid (45,000 mg); Folic acid (1,400 mg); Biotin (113 mg) and Trace element as CU (8000 mg); Mn (64,000 mg; Zn (40,000 mg); Fe (32,000 mg); Se (160 mg); I_2 (800 mg) and other items as Co (400 mg); choline (475,000);Methionine (50,000 mg); BHT (5,000 mg) and Spiramycin (5,000 mg) per 2.5 kg. NFE: Nitrogen free extract; 1(Ref.), diets 1and 2 (with and without Roxazyme G2 supplementation) are the control diets

Table 5: Performance of broiler chicks fed varying dietary levels of ACLM with/without Roxazyme G2 supplementation

	Inclusion Levels of ACLM (%)									
Parameters	(Ref. diet 1)	(Ref. diet 2)	5NE	5ENZ	15NE	15ENZ	25NE	25ENZ	SEM	Prob.
Average weight gain (g/chick)	19.33^{a}	19.51^{a}	19.32^{a}	19.54^{a}	19.19^{a}	18.40^{a}	13.90^{b}	14.96^{b}	3.03	0.08
Feed consumption (g/chick/day)	53.10^{a}	52.58^{a}	54.85^{a}	53.10^{a}	56.9^{ab}	53.10^{a}	54.85^{a}	58.86^{b}	1.12	0.18
Feed conversion	2.74^{a}	2.71^{a}	2.84^{ab}	2.70^{a}	2.97^{b}	2.89^{b}	3.95^{c}	3.93^{c}	1.91	0.10
Protein efficiency (PER)	1.58^{a}	1.60^{a}	1.53^{a}	1.61^{a}	1.47^{b}	1.51^{ab}	1.10^{c}	1.10^{c}	3.51	0.14

ACLM: Amaranthus cruentus leaf meal. Mean values with different superscripts in the same horizontal row are significantly different ($p>0.05$)

birds naturally consume feed based on energy requirement (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 (Bi and Chung, 2004).

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, 2007d). It is conceivable therefore, that the supplementary addition of Roxazyme G2 would have degraded 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 unlocked 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 significant positive responses to growth performances (Wyatt *et al*., 1997; Pack *et al*., 1998).

The feed conversion (FCR) for chicks on the reference diets 1 and 2 at 2.74 and 2.71, respectively were statistically similar ($p>0.05$) to the FCR values obtained for chicks on diets 5NE and 5ENZ at

2.84 and 2.70, respectively. The best FCR was obtained for birds on diet 5ENZ. However, FCR values obtained for chicks on diets 15NE and 15ENZ (2.97 and 2.89, respectively) were also similar ($p>0.05$) to 2.84 obtained for chicks on diet 5NE. FCR values obtained for birds on diets 25NE and 25ENZ were similar ($p>0.05$) and higher than other FCR values at 3.95 and 3.93, respectively. The Protein Efficiency Ratio (PER) for chicks on diet 5ENZ at 1.61 was the highest but similar ($p>0.05$) to PER values obtained for birds on reference diets 1 and 2, 5 and 15 ENZ. The PER is an important protein evaluation index which gives an insight into the relationship between the body weight gain and the actual protein intake (Oluyemi and Robert, 2000).

Roxazyme G2 supplemented diets consistently showed better FCR values than their pair diets in which Roxazyme G2 was absent. This could be explained based on Roxazyme G2 activities. Roxazyme G2 dietary supplementation had the potentials of splitting the complex non-starch polysaccharide molecules, such as cellulose, xylans and beta-glucans. This could have led to a better and improved utilization of ACLM 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). In addition, further report revealed that products of cellulase and hemicellulase activity are more prone to fermentation by the microbial organisms that colonize the last compartments of the gastrointestinal tract, and more energy is consequently absorbed from the hydrolysis of non starch polysaccharide (Bedford and Apajalalahti, 2001). Finally, breakdown of plant cell wall polysaccharides improves the access of the digestive biocatalysts to the endosperm contents that were otherwise trapped (Chesson, 1993). Hesseman and Aman (1986), Campbell *et al*. (1989), Petterson and Aman (1989), Bedford *et al*. (1991), Bedford and Classen (1992) and Choct *et al*. (1996) have buttressed the findings of this present study by reporting that the various impacts of enzyme supplementation in the digestive process are usually reflected by a considerable improvement on growth and feed conversion rates of poultry.

Nitrogen Utilization

Table 6 presents data on nitrogen utilization. The nitrogen intake (NI) value of chicks on diets 1 and 2 (reference diets with and without Roxazyme G2 supplementation) at 8.66±0.01 and 8.66±1.01g, respectively were similar ($p>0.05$) and also similar to the NI values for the chicks on diets 5NE, 5ENZ, 15NE and 15ENZ at 8.67±0.62, 8.85±0.07, 7.79±0.22 and 8.25±0.95 g/chick/day, respectively. Chicks on diets 25NE and 25ENZ had the lowest but similar NI ($p>0.05$) of 5.98±0.50 and 6.02±0.10 g, respectively.

The Nitrogen Retention (NR) values of chicks on diets 1 and 2 (reference diets with and without Roxazyme G2 supplementation) were similar ($p>0.05$) and also similar to NR values obtained for chicks on diets 5NE, 5ENZ, 15NE and 15ENZ of 68.97±0.60, 70.96±0.31, 70.99±0.62 and 71.39±1.08 g/chick/day, respectively. Chicks on diets 5NE and 25ENZ had similar NR values of 68.97±0.60 and 68.11±0.31, respectively. However, the NR value for birds on diets 25NE (67.73±0.29) and 8 were also similar ($p>0.05$).

Table 6: Nitrogen Utilization of broiler birds fed ACLM based diets with/without Roxazyme G2 supplementation ACLM, *Amaranthus cruentus* leaf meal

	Inclusion levels of ACLM (%)									
Nitrogen utilization	(Ref. diet 1)	(Ref. diet 2)	5NE	5ENZ	15NE	15ENZ	25NE	25ENZ	SEM	Prob.
Intake (NI, g/chick)	80.66^{a}	80.66^{a}	80.67^{a}	80.85^{a}	70.79^{a}	80.25^{a}	50.98^{b}	60.02^{b}	10.55	0.12
Droppings (FN, g/chick)	20.45	20.42	20.69	20.57	20.26	20.36	10.93	10.92	20.01	00.20
Retention	71.71^{a}	72.06^{a}	68.97^{a}	70.96^{a}	70.99^{a}	71.39^{a}	67.73^{b}	68.11^{b}	0.06	0.09

Mean values with different superscripts in the same horizontal row are significantly different ($p>0.05$)

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 ACLM that underscored the digestibility in the growing chicks. It is well documented in various literatures that the most limiting amino acid in the diets of chicks is methionine (Oluyemi and Robert, 2000) and its deficiency not only depresses feed intake but also affects the growth rate and overall nitrogen utilization.

A close observation at the NR values revealed that in spite of the similarity among the mean 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).

Hematological Indices of Experimental Birds

Some hematological indices investigated include Packed Cell Volume (PCV); Red Blood Cell (RBC); White Blood Cell (WBC); Hemoglobin Concentration (HBC); Mean Cell Hemoglobin Concentration (MCHC); Mean Cell Hemoglobin (MCH); Mean Cell Volume (MCV); Erythrocyte Sedimentation Rate (ESR).

There were no significant differences ($p>0.05$) in all the hematological parameters investigated. 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 Table 7.

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 hemoglobin contents. The ESRs of the test diets did not suggest that the birds were predisposed to any known general infections or malformation of any kind.

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 ACLM had pronounced yellow

Table 7: Hematological parameters of broiler birds fed ACLM based diets with/without Roxazyme G2 supplementation

	Inclusion Levels of ACLM (%)									
Parameters	(Ref. diet 1)	(Ref. diet 2)	5NE	5ENZ	15NE	15ENZ	25NE	25ENZ	SEM	Prob.
PCV, (%)	27.6	27.3	25.9	26.6	25.9	26.1	26.2	26.3	5.80NS	0.08
RBC (X106/ m^{-3})	2.1	2.1	2.1	2.3	2.1	2.1	2.1	2.1	2.21NS	0.23
Hbc	2.1	2.1	2.1	2.1	2.2	2.1	2.2	2.3	4.84NS	0.08
MCHC	7.3	7.2	7.2	7.1	7.1	7.1	7.1	7.2	1.56NS	0.19
MCH (pg)	9.1	9.1	9.1	9.1	9.2	9.1	9.1	9.2	2.31NS	0.27
MCV	131.2	131.2	131.1	130.1	129.2	130.1	130.2	130.1	3.20NS	0.12
ESR	4.1	4.1	4.3	4.2	4.1	4.1	4.3	4.3	4.76NS	0.23

ACLM: Amaranthus cruentus leaf meal; PCV: Packed cell volume; RBC: Red blood cell; WBC: White blood cell; Hbc: Hemoglobin concentration; MCHC: Mean cell hemoglobin concentration; MCH: Mean cell hemoglobin; MCV: Mean cell volume; ESR: Erythrocyte sedimentation rate. NS: No significant differences among treatment mean values ($p<0.05$)

coloration of beaks and shanks which deepened in chicks across the diets from diet 3 to diet 8 apparently as a result of the increasing inclusion level of ACLM which like other most protein leaf sources is known to be a precursor of β-carotenes and xanthophylls which have both commercial and nutritional desirability.

CONCLUSION

For practical poultry feed formulation, inclusion levels up to 15% may be used and tolerated by the broiler chickens when fibre-breaking enzymes are used as additives. Other physical factors that must be considered in incorporating ACLM is in large quantities include the bulkiness and dustiness of the diets. There is no doubt that Roxazyme G2 had a complementary role in enhancing the nutritional benefits of ACLM in broiler diets.

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