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Further utilisation of vegetable (*Amaranthus cruentus)* leaf meal using prebiotics and fibre degrading enzymes in broiler diets

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Abstract

The additive value of a fibre degrading enzyme (Roxazyme G2G) and a prebiotic (Turbotox) in high fibrous Amaranthus cruentus leaf meal (ACLM) as alternative protein supplemented diets were investigated in broiler birds. The Amaranthus cruentus leaf meal contained crude protein (23.0%0.55); fat (5.4%0.01) and nitrogen free extract (43.5%0.52); phytic acid and oxalate at 680 and 620mg100g^{-1}, respectively. The Ca, Mg and Fe and to lesser extent K, Na and Zn were present. The average weight gain of 45.9g/b/d and 46.0g/b/d obtained for birds on 10% and 20% enzyme and prebiotic supplemented ACLM diets, respectively were similar (p>0.05) to the control diet. The feed conversion ratio of 2.20 obtained for broilers on 10% ACLM with enzymes and prebiotics supplementation was also similar (p>0.05) to control and literature. The highest nitrogen retention value of 72.3% was obtained for broilers on the 10% ACLM diet with enzymes and prebiotic supplementation. Most carcass cuts were similar (p>0.05). The weights of some organs associated with digestion (intestine, proventriculus, and crop) were consistently and significantly higher in broilers on 10, 20 and 30% ACLMwith enzyme and prebiotics supplementation than their corresponding weights in broilers on the control diets (p<0.05). Combination of appropriate fibre degrading enzymes such as Roxazyme G2G and Turbotox prebiotic facilitated a better utilisation of ACLM for enhanced growth performance, carcass characteristics and weights of organ.

Keywords: *Amaranthus cruentus* leaf meal, enzymes and prebiotics fortification

Utilisation ultérieure du végétal (AmaranthеCruentus) Feuille de feuilles à l'aide de prébiotiques et d'enzymes dégradantes en fibres dans les régimes de poulet à griller

Résumé

La valeur additive d'une enzyme dégradante de la fibre (Roxazyme G2G) et d'un prébiotique (Turbotox) dans un repas de feuilles de cruentus amaranthus fibreux élevé (CAFE) à mesure que des régimes alternatifs en protéines complétés ont été étudiés dans des oiseaux de gril. L'amaranthus cruentus feuille de feuilles contenait des protéines brutes (23,0% 0.55); graisse (5,4% 0,01) et extrait sans azote (43,5% 0.52); acide phytique et oxalate à 680 et 620 mg100g-1, respectivement. Le Ca, Mg et Fe et à moindre mesure K, Na et Zn étaient présents. Le gain de poids moyen de 45,9 g / b / j et 46.0g / B / D obtenu pour les oiseaux sur 10% et 20% d'enzyme et de régimes CAFE supplémentés prébiotiques, respectivement étaient similaires (p> 0,05) au régime de contrôle. Le rapport de conversion d'aliment de 2,20 obtenu pour les poulets-grils sur 10% de CAFE avec des enzymes et une supplémentation prébiotique était également similaire (p> 0,05) au contrôle et à la littérature. La valeur de rétention d'azote la plus élevée de 72,3% a été obtenue pour les poulets de chair sur le régime CAFE à 10% avec des enzymes et une supplémentation prébiotique. La plupart des coupures de carcasse étaient similaires (p> 0,05). Les poids de certains organes associés à la digestion (intestin, proventuriculus et cultures) étaient toujours et significativement plus élevés dans les poulets-grils sur 10, 20 et 30% de supplémentation en enzyme et prébiotique que leurs poids correspondants dans les poulets de chair sur les régimes de commande (P <0,05). La

combinaison d'enzymes dégradantes de fibres appropriées telles que Roxazyme G2G et Turbotox Prebiotic a facilité une meilleure utilisation de le CAFE pour une performance de croissance améliorée, des caractéristiques de la carcasse et des poids de l'organe.

Mots-clés: AmaranthusCruentus Feuille de feuilles, Enzymes et Fortification Prebiotiques

Introduction

Malnutrition (undernutrition) continues to be a major problem in developing countries particularly in Southern Asia and sub-Saharan Africa (WHO, 2002; Brabin and Coulter, 2003; WHO, 2004; FAO, 2004; Global Nutrition Report, 2020). Diets in populations there are frequently deficient in macronutrients (protein, carbohydrates and fat), micronutrients particularly iron, vitamin A and iodine leading to specific micronutrient deficiencies or both (Pinstrup-Andersen *et al*., 1993; Levin *et al*., 1993; Brabin and Coulter, 2003; Millward and Jackson, 2004). A particular study showed that malnutrition among children in developing countries is mainly due to the consumption of cereal based porridge which is bulky, low in energy and density and high in antinutrients (Michaelsen, 1998). The world shortage of animal protein, particularly in developing countries of Africa, has necessitated investigations of several novel alternative feeding materials for possible incorporation into human/animal diets (Fasuyi, 2005). A growing interest in the use of unconventional sources of protein and energy in animal feed to significantly reduce the cost of producing animal protein has gained prominence especially in poultry (Fasuyi, 2007a; Fasuyi, 2007b; Fasuyi, 2007c; Fasuyi *et al*., 2007; Fasuyi, 2010). Making animal protein available and affordable especially in the underdeveloped regions with acute food insecurity of the world is of essence. *Amaranthus cruentus* is a well-known vegetable crop grown in tropical regions of the world including Africa, India, Bangladesh, Sri Lanka and the Caribbean (Fasuyi, 2010). It is also grown as leaf vegetables through South-East Asia and Latin America. The economic and nutritional advantages of this vegetable crop are further accentuated by their agronomic superiority over many other plant protein sources. For instance, harvesting is done 20–30 days after planting. Thereafter, shoots can be harvested at 1–4 weeks intervals for a period of two months depending on the vegetable type. On the average, farmers can harvest four times from a plant before its growth starts to decline for Amaranthus plants (Akachuku and Fawusi, 1995). Another potential nutritional advantage is the chemical composition of the vegetable leaf meals which is highly skewed in favour of the leaves as rich sources of plant protein (Aletor and Adeogun, 1995; Fasuyi, 2006), vitamins (Kachiguma *et al*., 2015) and minerals (Fasuyi, 2007a; Makobo *et al*., 2010). However, such factors limiting the nutritive value of leaf protein are the high fibre content (Oke, 1973; Fasuyi, 2010). The build-up of the amino acids in plant leaves is also accompanied with other toxic factors and antinutritional components (Aletor and Adeogun, 1995). The vegetable leaves are known to be considerably high in oxalates, phytins, tannins and to lesser extent saponins (Kohda and Yamaoka, 1992). Processing and biotechnological techniques such as shredding, sundrying, steeping (Fasuyi,2005; Fasuyi and Aletor, 2005), solid state fermentation (Dairo *et al*., 2008; Fasuyi and Akinboyowa, 2018; *Aturamu et al.,* 2018; Fasuyi *et al*., 2018) and enzyme fortification (Fasuyi and Kehinde, 2009; Fasuyi, 2010; Fasuyi *et al*., 2014; Fasuyi and Okeke, 2014) have been

employed to significantly degrade the fibre and reduce/eliminate some of the antinutritional factors inherent in plant leaves. Studies conducted by the same authors in conjunction with other researchers had looked at various biotechnological processing methods of improving the utilisation of *Amaranthus cruentus* leaf meals and leaf concentrates in monogastric livestock animals. The present study is therefore, aimed at investigating the utilization of *Amaranthus cruentus* leaf meal when further processed with the addition of a combination of cellulase-glucanase-xylanase enzymes (Roxazyme G2G) and a functional growth promoting prebiotic (Turbotox) in broiler diets.

Materials and methods

Preparation of Amaranthus cruentus leaf meal (ACLM) and experimental rations formulation

Amaranthus cruentus plantation was purchased from a local farmer at Iworoko-Ekiti near Ekiti State University, Ado-Ekiti located at 7°712'N and 5°251'E coordinates. *Amaranthus cruentus* leaves were locally 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 composition, amino acid profile and mineral contents were determined in triplicates to chemically evaluate the nutritional potentials of the *Amaranthus cruentus* leaf meal (ACLM) in Tables 1, 2 and 3.Thereafter, antinutrients such as phytic acid, oxalate, tannin and cyanide were determined (Table 4). The ACLM was used to formulate diets at both broiler starter and finisher phases of growth along with other locally purchased ingredients (Tables 5 and 6). The feed ingredients used in ration formulation were purchased locally from a reputable commercial feed miller in Ado-Ekiti, Ekiti State, Nigeria. The ACLM was sourced as earlier discussed and the enzymes (Roxazyme G2G) and prebiotics (*Turbotox*) were sourced as earlier discussed. The results of the proximate analyses on ACLM were used as guides in the manual ration formulation of the experimental diets. The experimental diets were prepared at the Poultry Unit of the Teaching and Research Farm, Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. All diets were compounded to contain identical crude protein content (isonitrogenous) and metabolisable energy (isocaloric) at both starter and finisher phases of broiler experiments. Control diet contained neither the enzyme nor the prebiotics. Other experimental diets were formulated by including ACLM at 10% (diet 2), 20% (diet 3) and 30% (diet 4) levels. The enzyme fortification and prebiotics addition were done by mixing the manufacturer specified quantities (1kg per ton of feed) with the ACLM protein 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. The four compounded experimental feeds were stored in plastic containers with airtight lids to preserve the nutrients and prevent the attack by pests.

Experimental animals, management, nitrogen digestibility, design of experiment and statistical analyses of data collected

A total of 480 day-old broiler chicks of the *Arbor acre* heavy strain purchased from Zartech hatchery, a division of Zartech Farms, Ibadan, Oyo-State, Nigeria, were used for the experiment. All chicks were

brooded using a combination of charcoal heat and 100W electric bulbs powered by 2.5KVA generating plant at the Teaching & Research Farm of Ekiti State University for a period of 3 weeks until the feathers were adequately spread on the bodies of the chickens for enough conservation of body heat. The chicks were allowed an acclimatization period of 5 days before the commencement of the feeding trials during which they were separated into males and females chickens using the method described by Laseinde and Oluyemi (1997). The chickens were later randomized into four treatments with each treatment replicated six times. All replicates were ensured to have uniform average weight before the commencement of the feeding trial. Each replicate had 20 chickens (10 males and 10 females). The feeding trial commenced on the sixth day with fresh experimental diets fed appropriately and *ad libitum* to the chickens in randomized positions within the experimental pens. Clean drinkable water was also provided *ad libitum*. Routine veterinary care and vaccinations against Newcastle disease, coccidiosis and chronic respiratory diseases were administered at appropriate intervals during the experiment. The experiment was conducted as a completely randomised design with a total of 24 experimental units (replicates) with each treatment having six replicates After the four weeks starter phase, the experimental finisher diets were administered to the broiler birds at the finisher phase of production. Records of daily feed consumption were taken daily and 5-day periodic weight changes were recorded. Six birds were randomly selected (three males and three females) such that at least a bird emerged from each replicate for the nitrogen digestibility experiment after the termination of the experiment on the 56^{th} day and transferred into metabolic cages for easy monitoring of feed intake and collection of droppings (feaces+urine). Records during the nitrogen digestibility trial were taken for five days by monitoring the feed intake and droppings to determine the nitrogen retention of chickens on each diet. Total droppings voided during the five day nitrogen digestibility experiment 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 (AOAC, 1995). Nitrogen retained was calculated as the algebraic difference between nitrogen intake and nitrogen in the droppings (on dry matter basis) for the period. Apparent nitrogen digestibility was computed by expressing the nitrogen retained as a fraction of the nitrogen intake multiplied by 100. Data were analysed using Minitab Statistical Package (Version 16) in one-way analysis of variance and for mean separation.

Proximate, gross energy, amino acids and mineral content determination

The proximate compositions of the ACLM were adapted from previous studies by same author. These compositions were determined according to the methods described by Association of Official Analysis Chemist (AOAC, 1995) and the amino acids composition was determined using Amino Acid Analyzer Model 80-2107-07 Auto Loader (Fasuyi, 2007d). The mineral elements as determined were quoted (Fasuyi, 2007d). Gross energy of the ACLM samples and the four formulated diets were determined against thermocouple grade benzoic acid using a Gallenkamp ballistic bomb calorimeter (Model CBB-330-0104L). The results and other results from other determinations for comparison are presented in Tables 1 and 2.

Cellulase-glucanase-xylanase combination (Roxazyme G2G)

Roxazyme G2G was obtained from Nutrivitas Ltd., Plot 33 Mobolaji Johnson Road, Eleganza Industrial Building, Oregun, Lagos, Nigeria. Roxazyme G2G contains a minimum of 1,600 U/g of cellulase, 3,600 U/g of endo-1,3(4)-β-glucanase, and 5,200 U/g of endo-1,4-β-xylanase. The recombinant enzymes used in this experiment were the single domain cellulase 5a (Cel5a) from *cellvibriomixtus* (Fontes *et al.,* 1997) and a truncated derivative of xylanase 11a (Xyn11a) from *Clostridium thermocellum* termed GH11-CBM6 (Fernandes, 1999). The bacterial xylanase is a modular enzyme containing a catalytic domain and a noncatalytic xylan-binding module separated by a short linker sequence (Fernandes, 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 (Fernandes, 1999). Extracts were incubated at 50°C for 20min and centrifuged for 30min 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, 0.1g/kg of Roxazyme G2G; recombinant xylanase, 4,000 U/kg of GH11-CBM6; and recombinant cellulase 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°C).

Prebiotics (Turbotox) and composition

The prebiotics, *Turbotox*, used in the present study was obtained from Afrimash, a reputable agroallied company located in Akobo, Ibadan, Oyo State, Nigeria. *Turbotox* is a multi-action prebiotic feed supplement (*Saccharomyces cerevisiae* extract) which confers improved efficiency, optimal performance to ensure responsible rearing of farm animals. It is a useful tool for non-antibiotic growth enhancement. *Turbotox* is especially indicated to improve the integrity of the digestive system. It is a carefully prepared in feed formulation proven to promote an ideal gut health and multiplication of beneficial flora. Its major features include inactive yeast, MOS, diatomaceous earth and organic acid combination, non-antibiotic growth promoter, control of mycotoxins, bacteria, molds and vectors. The composition of *Turbotox* are mannanoligosaccharides which are known for adsorption of pathogenic bacteria, improvement of intestinal function, modulation of the immune system and binding of mycotoxins; inactivated *Saccharomyces cerevisiae* extract, a natural source of vitamins, amino acids, minerals, and enzymes; organic acids and their salts (propionic acid, formic acid, citric acid) known for their ability in improving feed digestibility with acidifying effect while also stimulating the pancreatic enzymes and providing antifungal and antibacterial control in the feed and digestive tract; diatomaceous earth which is a mycotoxin adsorbent and mechanical insect repellent. The experimentally established activities of *Turbotox* prebiotics as performance enhancer include lowering pH in the stomach improving digestion, supporting normal gut flora, improving breakdown of feed and protection against pathogens. Other performance enhancing activities include extra supply of highly bioavailable amino acids, vitamins, and minerals. *Turbotox* is also a natural energy source. Absorption and binding of toxins have been detected in poultry diets where Turbotox were used as additives protecting against

the negative effects on the hepatic and renal functions (Afrimash, https://www.afrimash.com). It is also known to prevents molds and reduces bacterial colonization in the feed. There is evidence of improved intestinal humoral nonspecific immunity giving protection and saving energy. The overall result of Turbox as additive in poultry rations is better FCR (Afrimash, https://www.afrimash.com). The recommended additive inclusion rate for performing enhancing for poultry is 1kg/ton.

Results and discussion

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

The results of the proximate composition, gross/metabolisable energy, amino acid contents and some noticeable antinutrients were adapted from previous works by the same author (Fasuyi, 2007a; Fasuyi, 2007b; Fasuyi, 2007c; Fasuyi *et al*., 2007; Fasuyi, 2010) and presented in Tables 1, 2 and 3. The 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. 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 (Table 3) were relatively higher than most other plant protein origins at 680 and 620mg100g^{-1}, respectively. The phytin-P was also high at 160mg100g^{-1}. The protein level and amino acid composition of ACLM suggest that ACLM can be harnessed as a plant protein source if well processed to reduce/eliminate the noticeable antinutrients inherent in its composition. It has been established that about 75% of the total nitrogen in most vegetables is protein-nitrogen although this proportion varied with vegetable species (Schmidt, 1971). The ash content was remarkably high and further investigation revealed ACLM as a rich source of Ca, Mg and Fe and to lesser extent K, Na and Zn (Aletor and Adeogun, 1995). The notable antinutritional factors (ANFs) found in ACLM are phytins and oxalates (Leung *et al*., 1968; Aletor and Adeogun, 1995). The inhibition of leaf meals as a potential protein source in monogatric diets especially poultry has been adduced to their high fibre levels and some antinutritional factors present in most green leaves (Aletor and Adeogun, 1995; Fasuyi, 2007d). It is therefore conceived in this present study that the supplementary addition of the combination of Roxazyme G2G (cellulase-glucanase-xylanase) and a growth promoting prebiotic (*Turbotox)* would further facilitate the breakdown of cellulose and other nonstarch polysaccharides which are mainly found in the cell wall of plant leaves, and which are bound together in a complex matrix.

Table 1: Proximate composition (g/100g dry matter), gross energy (kcal/g) and amino acid profile (%) of *Amaranthus cruentus* leaf meal (ACLM) (means, n = 2)

Composition (g/100g)	ACLM
Dry matter	88.6?0.01
Crude protein	23.0?0.55
Ether extracts	5.4?0.01
Crude fibre	8.8?0.01
Ash	19.3?0.01
Nitrogen free extract	43.5?0.52
Gross energy (kcalg^{-1})	3.25?0.01

Means are determinations in triplicate with standard deviation (SD)

Table 2: Amino acid Profile of the *Amaranthus cruentus* leaf meal (ACLM) (means, n = 2)

Amino acids	gKg^{-1}	FAO/WHO (gKg^{-1})	Whole egg (gKg^{-1})
Alanine	396.3		
Aspartic acid	320.0		
Arginine	375.6		381.3
Glycine	251.3		
Glutamic acid	644.4		
Histidine	131.9		150.0
Isoleucine	300.6	250.0	350.0
Lysine	111.9	343.7	393.8
Methionine	86.3		200.0
Cystine	81.9		112.5
Methionine + Cysteine.	275.6	218.8	312.5
Leucine	529.4	437.5	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
Trytophan	147.5	62.5	112.5

FAO/WHO (1973) is incorporated on Table 1 to illustrate the recommended amino acid pattern and required standard in human nutrition while the profile of whole egg with a biological value (BV) of 100% is used as standard comparison
Source: Fasuyi (2007a)

Table 3: Mineral composition of air -dried *Amaranthus cru entus* leaf meal (ACLM) (means, n = 2)

TOLM	Ca	P	K	Na	Mg	Fe	Mn	Cu	Zn
	g/100g					(ppm)			
	2.0	0.9	4.8	7.1	2.5	1.1	198	36	0.9

Source: (Fasuyi, 2007a; Fasuyi et al., 2007)

Table 4: Phytic acid, phytin-P, oxalic acid, tannic acid and cyanide contents of *Amaranthus cruentus* leaf meal (ACLM) (means, n = 2)

	Noticeable antinutrients present in *Amaranthus cruentus* leaf meal					
TOLM	Phytic acid (mg/100g)	Phytin-P (mg/100g)	Phytin-P (% of total P)	Oxalate (mg/100g)	Tannin (mg/100g)	HCN (mg/100g)
	680	160	12.2	620	43.0	61.2

Source: (Fasuyi, 2007a; Fasuyi et al., 2007)

These supplements and additives are envisaged to enhance the utilization of the ACLM in substantial inclusion levels while replacing the conventional protein sources in broiler diets.

The addition of *Turbotox* prebiotic would probably **enhance the utilization of ACLM by** lowering pH in the stomach while improving digestion, supporting normal gut flora, improving breakdown of feed and protection against pathogens. *Turbotox* prebiotic has also been identified to enhance other activities during digestion such as extra supply of highly bioavailable amino acids, vitamins, and minerals (Afrimash, https://www.afrimash.com). Previous studies have corroborated the efficiency of enzyme supplementation in poultry diets and the significant positive responses of growth parameters (Wyatt *et al.,* 1997; Pack *et al*., 1998).

Table 5: Compositions of experimental diets for broiler starter

	Diets			
	1(0%)	**2 (10%)**	**3(20%)**	**4(30%)**
Ingredients				
Maize (9.0% CP)	50.1	45.2	37.5	27.5
Soyabean (45.0 % CP)	33.5	28.4	27.1	19.1
PKC (18.8 % CP)	10.0	10.0	10.0	10.0
Fish meal (72.0 % CP)	2.0	2.0	2.0	2.0
ACLM* (23.0 % CP)	-	10.0	20.0	30.0
Fat (Palm oil)	-	-	1.0	1.0
Bone meal	2. 5	2. 5	2. 5	2. 5
Oyster shell	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
DL-Methionine	0.2	0.2	0.2	0.2
L-Lysine	0.2	0.2	0.2	0.2
Premix**	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0
Calculated composition				
Crude protein, %	22.9	23.0	22.9	23.1
Crude fibre, %	5.2	6.1	6.4	7.6
Ether extract, %	6.7	7.1	6.7	7.1
ME kcal/kg				
Analysed composition				
Crude protein, %	23.1	22.9	23.1	23.0
Crude fibre, %	4.7	7.1	9.8	11.1
Ether extract, %	6.2	5.9	5.2	4.6

**ACLM, Amaranthus cruentus*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]; I_2 [800mg] and other items as Co [400mg]; choline (475,000);Methionine (50,000mg); BHT (5,000MG); and Spiramycin (5,000mg) per 2.5kg.*

Table 6: Composition of experimental diets for broiler finisher

	Diets			
	1(0%)	**2 (10%)**	**3(20%)**	**4(30%)**
Ingredients				
Maize (9.0 % CP)	50.1	45.2	35.5	25.5
Soyabean (45.0 % CP)	26.5	22.4	23.1	9.0
PKC (18.8 % CP)	17.0	16.0	14.0	12.0
Fish meal (72.0 % CP)	2.0	2.0	2.0	2.0
ACLM* (23.0% CP)	-	10.0	20.0	30.0
Fat (Palm oil)	-	-	1.0	1.0
Bone meal	2. 5	2. 5	2. 5	2. 5
Oyster shell	0.5	0.5	0.5	0.5
Salt	0.5	0.5	0.5	0.5
DL-Methionine	0.2	0.2	0.2	0.2
L-Lysine	0.2	0.2	0.2	0.2
Premix**	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0
Calculated composition				
Crude protein, %	20.2	19.8	20.1	20.1
Crude fibre, %	5.2	6.1	6.4	7.6
Ether extract, %	6.7	7.1	6.7	7.1
ME kcal/kg				
Analysed composition				
Crude protein, %	23.1	22.9	23.1	23.0
Crude fibre, %	4.7	7.1	9.8	11.1
Ether extract, %	6.2	5.9	5.2	4.6

**ACLM, Amaranthus cruentus 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]; I_2 [800mg] and other items as Co [400mg]; choline (475,000);Methionine (50,000mg); BHT (5,000MG); and Spiramycin (5,000mg) per 2.5kg.*

Growth performance parameters and nitrogen digestibility

The feed intake was highest in birds on diet 4 (30% ACLM with enzymes + prebiotics) at 110.1g/b/d and significantly different ($p<0.05$) from other feed intake values obtained for birds on other diets. The lowest feed intake value of 97.8g/b/d was obtained for birds on the control diet without enzymes and prebiotics. Although, the average weight gain value was highest at 46.1g/b/d for birds on the control diet without enzymes and prebiotics, it was similar ($p>0.05$) to 45.9g/b/d and 46.0g/b/d obtained for birds on diets 2 (10% ACLM with enzymes and prebiotics) and 3 (20% ACLM with enzymes and prebiotics), respectively. The nitrogen study (Table 7) revealed 72.3% as the highest nitrogen retention (NR) for broilers on diet 2 (10% ACLM with enzymes + prebiotics). The average nitrogen intake values were similar ($p>0.05$) at a range of 2.51 to 2.57g/chick for broilers on diet 4 (30% ACLM with enzymes + prebiotic) and diet 2 (10% ACLM with enzymes + prebiotic), respectively. Nitrogen retention (NR) value of 72.3% was the highest for broilers on diet 2 (10% ACLM with enzymes + prebiotic) but similar ($p>0.05$) to 71.8 and 69.1% obtained for broilers on the control diet (without ACLM, enzymes + prebiotic) and diet 3 (20% ACLM with enzymes + prebiotic), respectively. Reports have corroborated the enhanced broiler growth performance with the use of enzymes (Fasuyi, 2010; Ogunsipe *et al*., 2015) and prebiotics (Patterson and Burkholder, 2003; Sabiha et al., 2005; Ray, 2006; Král *et al*., 2012; Jadhav *et al.,* 2015; *Menconi and Barton, 2017*). The optimum feed conversion ratio value of 2.12 was obtained for broilers on the control diet without enzymes and prebiotics. However, this value was similar ($p>0.05$) to 2.20 obtained for broilers on 10% ACLM with enzymes and prebiotics. However, the highest NR value of 72.3% obtained for broilers on diet 2 (10% ACLM with enzymes + prebiotics) was similar ($p>0.05$) to 71.8% and 69.1% obtained for birds on the control diet (without enzymes and prebiotics) and diet 3 (30% ACLM with enzymes + prebiotics), respectively. It is obvious that enzyme and prebiotic fortified diets facilitated the accessibility to intracellular entrapped nutrients (Kocher *et al*., 2003). The breakdown of the non-starch polysaccharides and the subsequent utilization of the hitherto bound amino acids could have been responsible for the enhanced protein retention in broilers on ACLM supplemented diets with enzyme and prebiotic fortification.

Table 7: Growth performance of broiler chickens on experimental diets

Diets	**1**	**2**	**3**	**4**		
Inclusion of ACLM, %	**0**	**10**	**20**	**30**	**SEM**	***p***
Initial weight, g/bird	50.7	51.1	50.9	50.9	0.14	0.21
Final live body weight, g/bird	2632^{a}	2610^{a}	2628^{a}	2452^{b}	57.4	0.02
Average weight gain, g/bird/day	46.1^{a}	45.9^{a}	46.0^{a}	42.8^{b}	3.01	0.21
Average feed intake, g/bird/day	97.8^{c}	101.2^{bc}	105.2^{b}	110.1^{a}	2.40	0.43
Feed conversion ratio	2.12^{c}	2.20^{bc}	2.28^{b}	2.57^{a}	0.14	0.002

^{ab} *Means without common superscripts along the same row are different at P< 0.05*

Carcass characteristics and weight of internal organs

The average live weight value of 2632g obtained for birds on the control diet (without enzymes and prebiotics) was the highest although similar ($p>0.05$) to 2619g and 2628g obtained for birds on diets 2 (10% ACLM with enzymes + prebiotics) and 3 (20% ACLM with enzymes + prebiotics). The lowest significantly different ($p<0.05$) live weight value of 2452 was obtained for birds on diet 4 (30% ACLM with enzymes + prebiotics). The average dressed and eviscerated weights had similar trend with the live weight. The dressed weight had the highest value of 2368g for broilers on the control diet (without enzymes and prebiotics) but similar ($p>0.05$) the values obtained for broilers on diets 2 (10% ACLM with enzymes and prebiotics) and 3 (20% ACLM with enzymes and prebiotics). The average eviscerated weight of 2052g was also the highest for birds on the control diet without enzymes and prebiotics but also similar ($p>0.05$) to 2036g and 2049g obtained for broilers on diets 2 (10% ACLM with enzymes and prebiotics) and 3 (20% ACLM with enzymes and prebiotics). Most carcass cuts (Table 8) were similar ($p>0.05$) for all experimental broilers birds on all diets including the control diet without enzymes and prebiotics. Only the thigh had a little variation among the treatments as birds on diet 4 (30% ACLM with enzymes and prebiotics) had the lowest average thigh weight of 198.5gkg^{-1}. Most weights of broiler organs on all diets had slight numerical variations and significant differences ($p<0.05$) for gizzard, liver, intestines, proventriculus, bursa of fabricius and crop. The similarity among most carcass cuts for all experimental broilers birds on all diets including the control diet without enzymes and prebiotics was noteworthy. Only the thigh had a little variation among the treatments as birds on diet 4 (30% ACLM with enzymes and prebiotics) had the lowest average thigh weight of 198.5gkg^{-1}. Most weights of broiler organs on all diets had slight numerical variations and significant differences ($p<0.05$) for gizzard, liver, intestines, proventriculus, bursa of fabricius and crop.

Table 8: Nitrogen utilization of broiler birds fed ACLM based diets

Diets	**1**	**2**	**3**	**4**		
Inclusion of ACLM, %	**0**	**10**	**20**	**30**	**SEM**	***p***
Nitrogen intake, g/chick	2.55	2.57	2.52	2.51	0.44	0.17
Nitrogen in droppings, g/chick	0.72^{b}	0.71^{b}	0.78^{ab}	0.82^{a}	0.13	0.11
Nitrogen Retention, %	71.8^{a}	72.3^{a}	69.1^{ab}	67.9^{b}	0.57	0.07

^{ab} *Means without common superscripts along the same row are different at P< 0.05*

Table 9: Carcass evaluation (% body weight) and relative organ weights (gkg^{-1} body weight) of broiler chickens fed enzyme combination and Turbotox prebiotic additives

	Diets					
Parameters	**0%**	**10%**	**20%**	**30%**	**SEM**	***p***
Live weight	2632^{a}	2610^{a}	2628^{a}	2452^{b}	0.12	0.20
Dress weight	2368^{a}	2349^{a}	2365^{a}	2207^{b}	0.22	0.01
Evisc. weight	2052^{a}	2036^{a}	2049^{a}	1913^{b}	0.15	0.12
Relative carcass weight, gbodyweight^{-1}						
Head(g)	60.2	59.0	59.4	60.1	0.43	0.12
Neck	83.0	83.5	82.2	83.4	0.13	0.11
Wing	149.5	147.0	148.4	142.5	0.57	0.003
Drumstick	201.6^{a}	198.6^{ab}	199.4^{ab}	187.5^{b}	0.57	0.003
Breast	402.4^{a}	390.2^{b}	394.4^{ab}	320.5^{c}	0.43	0.12
Back	352.0^{a}	348.1^{a}	351.2^{a}	301.2^{b}	0.13	0.11
Organs						
Gizzard	39.5^{b}	41.0^{b}	48.5^{a}	48.7^{a}	0.15	0.004
Liver	47.0^{a}	42.2^{b}	48.2^{a}	45.4^{a}	0.71	0.002
Heart	8.1	7.7	8.0	8.0	0.07	0.12
Lungs	6.0	5.8	6.0	6.0	0.43	0.12
Intestine	121.0^{b}	127.5^{b}	139.5^{a}	141.5^{a}	0.13	0.11
Proventiculus	9.0^{b}	11.1^{a}	10.5^{ab}	12.0^{a}	0.57	0.003
Bursa(g)	11.2^{c}	13.4^{b}	16.6^{a}	17.1^{a}	0.15	0.004
Pancreas	6.1	5.6	5.5	5.6	0.71	0.002
Spleen	2.1	2.0	2.0	2.0	0.07	0.12
Crop	16.0^{b}	18.5^{a}	19.0^{a}	19.5^{a}	0.43	0.12

^{ab} *Means without common superscripts along the same row are different at P<0.05*

The organs associated with the digestion process were consistently and significantly higher ($p<0.05$) in weight for broilers on 10, 20 and 30% ACLM dietary inclusions in increasing order. Apart from the identified organs (gizzard, liver, intestines, proventriculus, bursa of fabricius and crop) associated with digestion, most carcass characteristics and organ weights were similar ($P>0.05$) for the experimental birds on the different diets. These findings agreed with previous work of Mutuş *et al*. (2006) but disagreed with some previous works (Mahajan *et al*., 1999; Kabir *et al*., 2004) that reported the occurrence of a significantly higher ($P<0.01$) carcass yield in broiler chicks fed prebiotics on the 2nd, 4th and 6th week of age. The carcass characteristics and organ weights all showed evidence of uniform tissue buildup and muscles development particularly in diets containing 10 and 20% of ACLM dietary levels.

Conclusions

Although the optimum growth parameters were obtained for broiler birds on the control diet without enzymes and prebiotics, the present study also revealed similarities with broilers on 10 and 20% *Amaranthus cruentus* leaf meal inclusions with Roxazyme G2G and Turbotox prebiotic at recommended 1kg per ton of feed. The use of a combination of appropriate fibre degrading enzymes such as Roxazyme G2G and Turbotox prebiotic facilitated higher incorporation of *Amaranthus cruentus* leaf meal (ACLM) to as high as 20% without significant reduction in growth performance, carcass and organ characteristics. Conducting the economic analyses (cost benefit analyses) on the present findings may be necessary to ascertain the commercial viability in different parts of the world particularly,

regions where protein ingredients are very expensive.

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