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AMARANTHUS CRUENTUS LEAF MEAL AS A PROTEIN SUPPLEMENT IN BROILER FINISHER DIETS
PART 1. PERFORMANCE AND NITROGEN UTILIZATION

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ABSTRACT

Freshly harvested mature leaves of *Amaranthus cruentus* plant were sun dried to a moisture content of between 12-13%. The sun dried leaves (*Amaranthus cruentus* leaf meal, ACLM) were milled and analysed for proximate composition. Some inherent antinutritional factors (ANFs) such as phytic acid and oxalate were determined. The mean and standard deviation (mean $\pm$ SD) for crude protein was 23.0%$\pm$0.55; crude fat, 5.4%$\pm$0.01; crude fibre, 8.8%$\pm$0.02; ash, 19.3%$\pm$0.01 and gross energy, 3.3$\pm$0.01kcal/g all on dry matter basis. Methionine and to a lesser extent, lysine, arginine, leucine and aspartate were high compared with other plant sources. Essential mineral elements such as Ca, P, K, Na, Mg, Fe, Mn and Zn were also appreciably present and comparable with other rich sources as found in vegetable plants. However, significant levels of phytates and oxalates (ANFs) were detected. The ACLM was incorporated at inclusion levels of 5%, 10%, 15% and 20% in broiler finisher diets. All diets were isocaloric and isonitrogenous. The control consistently showed better ($P<0.05$) weight gain (WG), feed efficiency (FE) and protein efficiency ratio (PER) of 52.6$\pm$13.01g/bird/day, 2.13$\pm$0.05 and 3.14$\pm$1.39, respectively. However, the weight gain values recorded for birds on diet 2 (5% ACLM inclusion) and diet 3 (10% ACLM inclusion) were similar ($P>0.05$) at 47.3$\pm$14.87g/bird/day and 48.6$\pm$17.10g/bird/day, respectively. This was also true for the FE values for birds on diets 2 and 3 that had similar values of 2.44$\pm$2.33 and 2.44$\pm$2.14. Birds on diet 3 also had similar ($P>0.05$) PER value with birds on diets 1 and 2. The WG, FE and PER values obtained for birds on diets 4 and 5 (15% and 20% ACLM inclusion, respectively) were similar ($P>0.05$) and consistently lower than values obtained for birds on other diets. The highest nitrogen retention (NR) and apparent nitrogen digestibility (AND) values were recorded for birds on the control diet 1 and the poorest were recorded in diet 5. The apparent nitrogen digestibility values of birds on the control diet and diet 2 were similar at 70.39%$\pm$8.75 and 65.04%$\pm$9.10, respectively. Apparent nitrogen digestibility values for birds on diets 2, 3 and 4 were also similar at 65.04%$\pm$9.10, 64.77%$\pm$10.09 and 59.64%$\pm$8.29, respectively while diets 4 and 5 (55.95%$\pm$9.35) also had similar and lowest values ($P>0.05$) for their birds.

Key words: Plant Protein, Supplements, *Amaranthus cruentus*

INTRODUCTION

The world wide shortage of animal protein sources particularly in developing countries in Africa, has necessitated investigations of several novel protein sources for possible incorporation into animal feeds (particularly poultry) as replacements for the expensive conventional sources such as fish meal, groundnut cake and soybeans. The acute shortage of protein has been attributed to the phenomenal rise in the prices of animal feeds which account for about 75-85% of the recurrent production inputs in intensive monogastric animal production [1].

A growing interest in the use of unconventional sources of protein and energy in poultry feed has gained prominence [2-4]. The recognition of protein from leaf sources is fast gaining prominence because of its availability and perhaps because it is the cheapest and the most abundant potential source of protein. The photosynthetic process of manufacturing amino acids in leaves is a naturally simple process requiring unlimited and readily available primary materials, for example energy from the sun, carbon dioxide, water, inorganic nitrogen (or atmospheric nitrogen in the case of legumes).

The amino acids synthesized are polymerized into a less mobile forms and stored as such in the leaves. However, the build-up of amino acids in leaves is also accompanied with other factors that render the amino acids less nutritious for consumptive purpose in man and animal. Such factors limiting the nutritive value of leaf protein are the high fibre content [5].

Amaranthus plant is a popularly grown leaf vegetable in tropical regions of the world including Africa, India, Bangladesh, Sri Lanka and the Caribbean. The economic and nutritional advantage of the amaranth as a leaf vegetable is accentuated by its agronomic superiority over many plant protein sources. For instance, harvesting is done 20-30days after transplanting and then every 2-3weeks for a period of one to two months [6]. Another potential advantage of the amaranthus is the chemical composition which is highly in favour of the plant leaves as a source of plant protein [6, 7] and its rich source of vitamins and minerals.

This study therefore investigated the chemical, amino acids and antinutritional constituents in *Amaranthus cruentus* leaf meal (ACLM) as a prelude to incorporation into broiler finisher diets. Performance characteristics, nitrogen utilization, haematological indices and serum/liver metabolites of experimental birds were thereafter investigated as a measure of nutritional acceptability of ACLM in broiler finisher feed.

MATERIALS AND METHODS

Collection and preparation of *Amaranthus cruentus* leaf meal

Amaranthus cruentus plants were harvested fresh from maturing stems at about 20-30days 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 potential of the ACLM. Thereafter, the

ACLM was used to formulate diets along with other ingredients purchased locally such as maize, groundnut cake, soybean meal, fish meal, wheat offals, bone meal, oyster shell, NaCl, DL-methionine, lysine and vitamin/mineral premix.

Proximate, gross energy, amino acids and mineral content determination
Proximate composition of ACLM was determined [8] 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 [8]. The other mineral elements were determined after wet digestion with a mixture of nitric, sulphuric and hydrochloric acid using Atomic Absorption Spectrophotometer (AAS model SP9). Gross energy of the ACLM sample and the 6 experimental diets were determined against thermocouple grade benzoic acid using a Gallenkamp ballistic bomb calorimeter (Model CBB-330-0104L).

Determination of phytin and oxalate
The extraction and precipitation of phytin in the sun-dried ACLM were done by the described method [9] while iron in the precipitate was also determined as described [10]. Phytin was determined by using a 4:6 Fe/P ratio to calculate phytin phosphorus and multiplying the phytin phosphorus by 3.55 [11]. Oxalate content was determined by the titrimetric method [12] as modified [13]. Where extracts were intensely coloured, they were decolourised with activated charcoal [14].

Site preparation
The poultry house was thoroughly disinfected, fumigated with 1 part of Potassium permanganate pellets to 3 parts of formalin. Thereafter, the house was rested for 2 weeks before transferring the 28 day old birds into the experimental deep litter pens.

Experimental rations formulation
The feed ingredients used in ration formulation were purchased locally from a reputable commercial feed miller (Adedom Investment Holdings) located at Km 3, Ondo Road, Akure. The ACLM was sourced as earlier discussed. The results of the proximate compositions earlier determined were used as guides in the ration formulation of the six experimental diets. All diets were formulated to contain identical crude protein content (iso-nitrogenous) and gross energy (isocaloric). Diet 1 was the control diet and was formulated without the inclusion of ACLM. Diets 2, 3, 4, and 5 were formulated such that ACLM was incorporated at 5%, 10%, 15%, and 20%, respectively at the expense of other protein sources in the diets.

Other notable protein sources were fish meal at 2% inclusion levels in all diets, soybean meal at 8% inclusion levels in all diets and groundnut cake at 20%, 18%, 15%, 12%, and 10% in diets 1, 2, 3, 4, and 5, respectively. All diets were also supplemented with feed grade methionine and lysine.

Management of experimental birds and experimental design
A total of 160 day-old broiler chicks of the *anak* heavy strain were purchased from Zartech hatchery, a division of Zartech Farms, Ibadan, Oyo-State (150 birds were eventually used for the experiment at the finisher phase). All chicks were electrically brooded at the Gabof Research Farms, Aule Government Residential Area, Akure. They were fed a 24% crude protein broiler finisher commercial ration *ad libitum* for the first 3 weeks before

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commencement of experiment. The chicks were also sexed on the second day of brooding as described [15]. Clean drinkable water was also provided *ad libitum* with appropriate antibiotics and antistress particularly after arrival. The following medications were administered:

i. Intraocular vaccination against Newcastle disease at day one.
ii. Neoceryl (antibiotics) for a period of 4 days from 3 days of age.
iii. Coccidiostat for the treatment/control of coccidiosis and chronic respiratory diseases.
iv. Gumboro vaccine at 2 weeks of age
v. Lasota vaccine (New castle booster) administered in a day at about 3 weeks of age.

The experiment was conducted as a completely randomized design with a total of 15 experimental units (replicates). After the 3 weeks starter phase the birds (5 males and 5 females) were randomly distributed into 15 experimental units. The birds were assigned at the rate of 30 birds/diet in 3 replications of 10 chicks/replicate such that the mean group weights were similar at the beginning of the experiment. The birds were fed the experimental diet *ad libitum* for 15 days during which records on daily feed consumption and 3-day periodic weight changes were recorded. Four birds were randomly selected (2 males and 2 females) such that at least a bird comes from each replicate/treatment 5 days before the termination of the experiment to determine the nitrogen retention of birds on each diet. These birds were transferred into metabolic cages where the excreta could be collected for analyses.

Estimation of nitrogen retention, apparent nitrogen digestibility and 'operative' protein efficiency ratio

Total excreta 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 [8]. Nitrogen retained was calculated as the algebraic difference between nitrogen intake and faecal nitrogen (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. The protein efficiency ratio was calculated as the ratio of weight gain to total protein consumed.

Statistical analysis

Data were analysed using the ANOVA (SPSS 11.0 for Windows) (SPSS Inc., Chicago IL, USA). Significance was accepted at 5% probability level ($P<0.05$).

RESULTS

Proximate composition, gross energy and amino acids content of ACLM are presented in Table 1 while the mineral composition is presented in Table 2. The *Amaranthus cruentus* leaf meal (ACLM) was relatively high in crude protein at 23.0%$\pm$0.55; fat at 5.4%$\pm$0.01 and sugar + starch (NFE) at 43.5%$\pm$0.52. The ACLM was remarkably rich in mineral elements such as Ca, K, Na, Mg, Fe and Zn compared to levels reported for most plant protein sources. The phytic acid and oxalate levels (Table 2) were relatively higher than most other plant protein sources at 680 mg/100g and 620 mg/100g, respectively. The phytin-P was also high at 160 mg/100g. The performance data are presented in Table 4.

The average weight gain (WG) value of experimental broiler finisher birds on the control diet (without ACLM) was the highest at 52.6+13.01 g/bird/day but similar (P>0.05) to the WG value obtained for birds on diet 3 (10% ACLM inclusion). However, WG values for birds on diets 2 and 3 were also similar (P>0.05). The WG values obtained for birds on diets 4 and 5 were similar (P>0.05) and lowest at 34.6+20.20 g/bird/day and 34.1+19.1 g/bird/day, respectively. The average feed consumption (FC) was highest for birds on diet 5 at 123.5+1.37 g/bird/day but similar to the FC value obtained for birds on diet 4 at 121.6+2.01 g/bird/day. The FC values obtained for birds on diets 3 and 4 were similar at 115.2+2.32 g/bird/day and 118.5+3.10 g/bird/day, respectively. The control diet had the lowest FC value of 112.0+3.92 g/bird/day but this was also similar to FC value obtained for diet 2. The feed efficiency (FE) value obtained for birds on the control diet (2.13+0.05) was significantly lower and better (P<0.05) than the other FE values. The FE values obtained for birds on diets 2 and 3 were similar at 2.44+1.10 and 2.44+2.14, respectively.

The FE values obtained for birds on diets 4 and 5 were also similar at 3.51+1.64 and 3.62+1.27, respectively. The protein efficiency ratio (PER) value obtained for the birds on the control diet was the highest (3.14+1.39) but similar to the PER value obtained for birds on diet 3 at 2.90+2.16. However, the PER values obtained for birds on diets 2 (2.33+2.09) and 3 were also similar. The PER values obtained for birds on diets 4 and 5 were similar and the lowest at 2.16+1.32 and 2.06+2.25, respectively. The nitrogen utilization indices presented in Table 5 revealed that the nitrogen intake (NI) values of birds on diets 2, 3 and 4 were similar at 3.69+1.06, 3.86+1.44 and 3.89+1.22, respectively.

The NI values of birds on diets 4 and 5 (3.95+1.26) were also similar. Birds on diet 1 also had similar NI value with birds on diet 2. The nitrogen retention (NR) and apparent nitrogen digestibility (AND) values followed similar pattern. The NR values of birds on diets 1 and 2 were similar at 2.52 +1.34 and 2.40+1.26, respectively. The AND values were also similar at 70.39%+8.75 and 65.04%+9.10, respectively. However, birds on diets 2, 3 and 4 also had similar for NR and NR values at 2.40+1.26, 2.32+1.27 and 2.32+2.03, respectively. Birds on diet 5 (20% ACLM inclusion) had the significantly lowest (P<0.05) NR value of 2.21+1.29. Similarly, the AND values obtained for birds on diets 2, 3 and 4 were not significantly different (P>0.05) at 65.04%+9.10, 64.77%+10.09 and 59.64%+8.29, respectively. Birds on diet 5 had the lowest AND value of 55.95%+9.55 but this was also similar to the value obtained for birds on diet 4.

DISCUSSION

The protein level and amino acid composition of ACLM make it ranks along side other conventional protein sources especially of plant origins [16]. The consideration of the protein (23.0%+0.55 CP) and the amino acid profile of ACLM particularly its methionine and lysine levels [6], suggests its probable incorporation into practical feed manufacturing. Methionine and lysine are the limiting amino acids in most plant proteins. It has been reported that about 75% of the total nitrogen in most vegetables is protein-nitrogen although this proportion varied with vegetable species [16]. The ash (mineral) content was remarkably high revealing ACLM as a rich source of Ca, Mg and Fe and to a lesser extent K, Na and Zn [7]. The notable antinutritional factors (ANFs) found in ACLM are phytins and oxalates [6, 7]. However, the processing effect of sun-drying the freshly harvested amaranthus leaves might have significantly reduced the toxic effects of these ANFs on the experimental broiler starter chicks as no mortality was recorded and haematological studies compared favourably with records obtained for chicks raised under normal conventional management and nutritional practices [17-20].

Even though the average weight gain, average feed consumption, feed efficiency and protein efficiency ratio values of birds on the control diet were consistently higher and better than other values obtained for birds on other diets, there were similarity in some cases with values obtained for WG, FC and PER of birds on diets 2 and 3. It is noteworthy that birds on diets 1 and 3 had similarity in their WG and PER values. This may be connected with the rich amino acid profile and certain growth factors reported in some amaranth plants [16, 6].
The decline in performance characteristics experienced by birds on diets 4 and 5 with 15% and 20% inclusions, respectively, may be attributed to the earlier observations that the major drawbacks to the use of vegetable materials as major sources of nutrients by monogastrics (including man) are their high fibre and bulkiness which call for large quantities to be consumed to provide adequate levels of nutrients [7]. Another possible drawback is the anti-nutritional factors noticeably phytates and oxalates. Phytic acid can bind with proteins to form phytate-protein complexes [21].

These complexes can adversely affect the digestibility of protein [22] by inhibiting a number of digestive enzymes in the gastro-intestinal tract such as pepsin [23], trypsin [24] and chymotrypsin [25] thereby reducing the digestibility of proteins and amino acids. Appreciable quantities of oxalates in amaranth plant leaves are present in soluble forms and this can induce kidney stones when ingested in excess [26].

The nitrogen utilization indices (nitrogen intake, nitrogen retention and apparent nitrogen digestibility) of the experimental broiler finisher birds on diets 1,2,3 and 4 compared favourably with data obtained from previous works on broiler birds [27, 28]. Even though, the nitrogen utilization indices of birds on the control diet were clearly superior to the values obtained for birds on other diets, nitrogen utilization indices obtained for birds on diets 2, 3 and even 4 compared favourably with the control diet and in some cases similar to the values obtained for diet 1. The nitrogen balance data actually compared favourably with data obtained for known conventional nitrogen sources in livestock feeds [29, 30].

CONCLUSION

In conclusion, results of the study showed that the proximate, gross energy, amino acids content and mineral composition all revealed that ACLM is a potentially rich source of nutrients in monogastric feed formulation. The processing effect of sun-drying might have drastically reduced the antinutritional factors (ANFs) to innocuous levels that enhanced higher tolerant levels in the broiler finisher birds. Inclusion level of 10% of ACLM in broiler finisher birds was found to be most suitable in facilitating better performance characteristics. The proximate composition, amino acid content and mineral content distinguished ACLM as a potential nutritive feed resource. The performance characteristics and nitrogen utilization indices were also in favour of an inclusion level of 10% in broiler finisher diets without any adverse nutritive condition.

Table 1: Proximate composition (g/100g), gross energy (kcal/g) and amino acid content (%) 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 (kcal/g)	3.25±0.01
Amino acids	
Alanine	1.24
Aspartic acid	1.78
Arginine	2.11
Glycine	0.63
Glutamic acid	0.12
Histidine	0.61
Isoleucine	1.02
Lysine	2.01
Methionine	3.52
Cystine	0.81
Meth. + Cys.	4.33
Leucine	1.85
Serine	0.81
Threonine	0.52
Phenylalanine	1.51
Valine	1.04
Tyrosine	0.94
Tryptophan	0.64

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Table 2: Mineral composition, Phytic acid, phytin-P and oxalic acid content of *Amaranthus cruentus* leaf meal (ACLM) (means, n = 2)

ACLM	Ca	P	K	Na	Mg	Fe	Mn	Cu	Zn
			g/100g				(ppm)		
	2.4	1.8	5.8	7.2	3.1	1175	198	36	890

ACLM	Phytic acid mg/100g	Phytin-P (mg/100g)	Phytin-P (as % of total P)	Oxalate (mg/100g)
	680	160	12.2	620

Table 3: Composition of experimental diets (g/100g)

Ingredients	Diets 1	2	3	4	5
	% inclusion levels of ACLM				
	0	5	10	15	20
Maize (11.0% CP)	40.00	40.00	40.00	40.00	40.00
Groundnut cake (45.0% CP)	20.00	18.00	15.00	12.00	10.00
Soybean meal (45.0% CP)	8.00	8.00	8.00	8.00	8.00
Fish meal (68.0% CP)	2.00	2.00	2.00	2.00	2.00
Wheat offals (15.0%)	25.7	22.7	20.7	18.7	15.7
ACLM* (23.0% CP)	-	5.00	10.00	15.00	20.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Oyster shell	0.50	0.50	0.50	0.50	0.50
Nacl	0.50	0.50	0.50	0.50	0.50
DL-methionine	0.15	0.15	0.15	0.15	0.15
DL-Lysine	0.15	0.15	0.15	0.15	0.15
Premix**	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00
Calculated composition:					
Crude protein (%)	20.00	20.00	20.00	20.00	20.00
Crude fibre (%)	4.52	4.57	4.71	4.70	4.73
Ether extract (%)	7.21	6.41	6.40	6.71	6.51
GE*** (kcal/100g)	462.4	462.3	462.1	461.5	461.7
Analysed composition:					
Crude protein (%)	19.46	19.51	19.53	19.49	20.01
Crude fibre (%)	5.02	6.21	6.37	6.41	6.38
Ether extract (%)	7.61	7.65	7.80	7.81	7.83
GE (kcal/100g)	454.2	452.6	453.1	451.8	452.0

*ACLM, *Amarathus cruentus* leaf meal

* *contained vitamins A (4,000,000iu); D(800,000 iu); E (14,000 iu); K (760mg); B12 (7.6mg); Riboflavin (2,800mg); Pyridoxine (1,520mg); Thiamine (880mg); D Pantothenic acid (4,400mg); Nicotinic acid (18,000mg); Folic acid (560mg); Biotin (45.2mg); and Trace elements as Cu (3,200mg); Mn (25,600mg); Zn (16,000mg); Fe (12,800mg) Se (64mg); I_2 (320mg) and other items as Co (160mg); Choline (190,000mg); Methionine (20,000mg); BHT (2,000mg) and Spiramycin (2,000mg) per kg.

GE*** (kcal/100g) calculated based on 5.7kcal/g protein; 9.5kcal/g lipid; 4.0kcal/g carbohydrate. (Ng and Wee, 1989)

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Table 4: Performance of Broiler Fed ACLM-based diets from age 28-42 days

Parameters	Diets				
	% Inclusion levels of ACLM				
	0	5	10	15	20
Initial weight (g/bird)	786.5±12.92	802.1±15.62	792.7±18.10	772.5±15.10	767.3±11.20
Final Weight (g/bird)	1259.5±13.10	1228.2±14.12	1230.4±17.10	1083.7±13.21	1074.3±10.80
Average Weight Gain (g/bird/day)	52.6^{a}±13.01	47.3^{b}±14.87	48.6^{ab}±17.10	34.6^{c}±20.20	34.1^{c}±19.1
Average Feed Consumption (g/bird/day)	112.0^{a}±3.92	115.2^{ab}±2.32	118.5^{b}±3.10	121.6^{bc}±2.01	123.5^{c}±1.37
Feed Efficiency	2.13^{a}±0.05	2.44^{b}±1.10	2.44^{b}±2.14	3.51^{c}±1.65	3.62^{c}±1.27
'Operative' Protein Efficiency (OPER)	3.14^{a}±1.39	2.33^{b}±2.09	2.90^{ab}±2.16	2.16^{c}±1.32	2.06^{c}±2.25

Means are for 30 birds/diet.
Means with different superscripts within row are significantly different ($P<0.05$).
Values are mean ± SD.

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Table 5: Nitrogen utilization of broiler fed *Amaranthus cruentus* Leaf Meal based diets

Parameters	DIETS				
	% Inclusion levels of ACLM				
	0	5	10	15	20
Nitrogen Intake gN/bird/day	$3.58^{a}\pm1.20$	$3.69^{ab}\pm1.06$	$3.86^{b}\pm1.44$	$3.89^{bc}\pm1.22$	$3.95^{c}\pm1.26$
Nitrogen Retention gN/bird/day	$2.52^{a}\pm1.34$	$2.40^{b}\pm1.26$	$2.32^{b}\pm1.27$	$2.32^{b}\pm2.03$	$2.21^{c}\pm1.29$
Apparent Nitrogen Digestibility (%)	$70.39^{a}\pm8.75$	$65.04^{ab}\pm9.10$	$64.77^{b}\pm10.09$	$59.64^{bc}\pm8.29$	$55.95^{c}\pm9.55$

Means with different superscripts within a row are significantly different ($P < 0.05$).

Birds used for nitrogen studies are subsets of the original 30 birds/diet.

Values are mean $\pm$ SD.

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