AJFAND ONLINE
Volume 7 No. 6 2007
December 2007
ISSN 1684-5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

AMARANTHUS CRUENTUS LEAF MEAL AS A PROTEIN SUPPLEMENT IN BROILER FINISHER DIETS
PART 2. HAEMATOLOGICAL RESPONSES, CARCASS CHARACTERISTICS AND RELATIVE ORGAN WEIGHTS

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ABSTRACT

Amaranthus cruentus leaf meal (ACLM) was prepared by sun-drying freshly harvested *Amaranthus cruentus* leaves and milling. ACLM was thereafter included into finisher broiler diets as a protein source 0% (control), 5%, 10%, 15% and 20%. All diets were isocaloric and isonitrogenous and were fed to 150 birds at the finisher phase of production for 15 days. Performance and nitrogen utilization of experimental birds were determined in the earlier part of this study. The present study examined the haematological parameters of the experimental birds which include packed cell volume (PCV), red blood cell (RBC), white blood cell (WBC), haemoglobin concentration (Hbc), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume (MCV) and erythrocyte sedimentation rate (ESR). Mean values for each parameter were similar across diets ($P > 0.05$) except for RBC and ESR. The total serum protein, albumin, globulin and albumin/globulin ratio were determined for the serum and liver. All serum and liver metabolites examined had similar mean values ($P > 0.05$). Some carcass traits examined include the dressed weight as a percentage of the liveweight, eviscerated weight also as a percentage of the liveweight, thigh, drumstick, back, backfat, shank, wing, head and neck. Except for dressed weight and backfat, all other carcass traits measured were similar ($P > 0.05$) among treatments. Some organs weights were taken relative to the body weight. Such organs include liver, kidney, heart, spleen, pancreas, bursa, gizzard and lungs. Only heart and gizzard had significant ($P < 0.05$) differences in their mean values. The relative weight, length and breadth of chest and thigh muscles were also measured and were found to be statistically similar ($P > 0.05$). The introduction of ACLM into broiler finisher diets did not predispose the birds to any known acute general infections, malformation of any kind or diseased condition.

Key words: *Amaranthus cruentus*, Haematology, Liver Biochemistry

INTRODUCTION

Haematological profiles both in human and animal sciences are important indices of the physiological state of the individual [1]. The ability to interpret the state of blood profile in normal and in diseased condition is among many primary objectives of haematological studies. Researchers have proved that definite changes occur in the profile of blood cells throughout life [2 - 7].

The haematological features have attracted many workers to look at these features in order to make clinical predictions of the health status of a particular animal. The blood picture changes with the advancement of the animal age and also varies with certain conditions as stress, bacteria/viral infections and intoxication [1, 4, 5]. The blood of the domestic fowl contains erythrocytes, thrombocytes, non-granular leukocytes and granular leukocytes, suspended in plasma [8, 9].

Blood with its myriad of constituents provides a valuable medium both for clinical investigations and nutritional evaluation of the organism [10]. The ingestion of numerous dietary components has measurable effects on blood constituents [11]. Nutrient levels in the blood and body fluids might not be valid indications of nutrients function at cellular level. They are considered to be proximate measures of long-term nutritional status [12]. Consequently, blood sampling for the assay of biochemical constituents and haematological traits are frequently employed in nutritional studies. Changes in the constituent components of blood when compared to normal values could be used to interpret the metabolic state of the animal as well as the quality of feed [13].

The green vegetable has long been recognised as the cheapest and most abundant potential source of proteins because of its ability to synthesize amino acids from a wide range of virtually unlimited and readily available primary materials such as water, CO_2, atmospheric N_2 (as in legumes). There is no doubt that vegetable leaf protein sources are cheaper sources of protein in animal nutrition particularly poultry because of their agronomic superiority over many plant protein sources. For instance, harvesting of amaranthus vegetable species is done 20-30 days after transplanting and then every 2-3weeks for a period of one to two months. They are also rich sources of proteins, vitamins and minerals [14, 15].

This study investigated the beneficial inclusion levels of *Amaranthus cruentus* leaf meal as supplementary source of protein in broiler finisher birds focusing on the carcass characteristics and haematological implications on the broiler finisher birds.

MATERIALS AND METHODS

A detailed methodology on housing and routine management of experimental birds is documented and can be found in the earlier part (part 1) of this work.

Blood collection for analysis

At the end of the feeding trial, a male bird per replicate was randomly selected, weighed and sacrificed by severing the jugular vein and blood allowed to flow freely into labeled bottles one of which contained a drop of EDTA while the other without EDTA was processed for serum. The serum was kept deep frozen prior to analysis. Packed cell volume (PCV%) was estimated by spinning about 75:l of each blood sample in heparinized capillary tubes in an haematocrit micro centrifuge for 5 minutes while the total red blood cell (RBC) count was determined using normal saline as the diluting fluid. The haemoglobin concentration (Hbc) was estimated using cyanomethaemoglobin method [16] while the mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH) and the mean corpuscular volume (MCV) were calculated. Total serum protein was determined colorimetrically using the SIGMA assay kits. The albumin and the globulin were determined using the described method [16].

Carcass, muscle and organ measurements
After slaughtering, the carcasses were scalded at 75^{o}C in a water bath for about 30 sec before defeathering. The dressed chicks were later eviscerated. The measurement of the carcass traits (dressed weight %, eviscerated weight %, thigh, drumstick, shank, chest, back, neck, wing, bellyfat and head) were taken before dissecting out the organs. The organs measured were the liver, kidneys, lungs, pancreas, heart, spleen, bursa of fabricus and gizzard. The following muscles: inner chest muscle (*Supra coracoideus*) outer chest muscle (*Pectoralis thoracicus*) and thigh (*Gastrocnemius*) were carefully dissected out from their points of origin and insertion. Measurements of the fresh weight, length and breadth of these muscles were taken. All the carcass traits, except the dressed and eviscerated weights, were expressed as percentages of the live weight while the organs and muscles were expressed in gkg^{-1} body weight. The length and breadth of the muscles were expressed in $cmkg^{-1}$ body weight.

Statistical analysis
Data were analysed using the ANOVA (SPSS 11.0 for Windows) (SPSS Inc., Chicago IL, USA) based on the principles of the coefficient of variation analysis and analysis of variance [17, 18]. Significant differences found between the means were compared using the Duncan Multiple Range Test [19] and accepted at 5% probability level ($P<0.05$).

RESULTS

The proximate composition, gross energy and amino acids content and the composition of the experimental diets are already shown in part 1 of this work. Results of haematological parameters are shown in Table 1. Except for RBC and erythrocyte sedimentation rate (ESR) for which significant variations in treatment mean values ($P < 0.05$) were observed, no significant differences were seen for all other parameters examined. Red blood cell counts were 3.6 ± 0.2 and 3.6 ± 0.3 in diets 1 and 4, respectively, as the highest RBC counts and 3.1 ± 1.0 and 3.1 ± 0.3 in diets 3 and 5, respectively, as the lowest counts. The ESR ranged from 4.3 ± 0.6 in diet 1 to 5.5 ± 0.5 in diet 5.

The significant variation in the ESR for birds across the dietary treatments appeared to increase across dietary treatments from the control diet (without ACLM) to diet 5 (20% ACLM dietary inclusion). The serum biochemistry is shown in Table 2. All parameters examined (total serum protein, serum albumin, serum globulin, albumin/globulin ratio for serum, total liver protein, liver albumin, liver globulin and albumin/globulin ratio for liver) were not significantly different. Carcass traits were similar ($P > 0.05$) among birds across dietary treatments except for dressed weight and backfat (Table 3). Backfat values varied upwards for birds across the dietary treatments from 2.5±0.7 gkg^{-1} body weight in diet 1 to 3.5±1.1 gkg^{-1} body weight in diet 5 (20% ACLM dietary inclusion).

The relative organ weights are shown in Table 4. Only the heart and gizzard relative weights differed significantly ($P < 0.05$) among dietary treatments. The weight of heart increased across the dietary treatments from diet 1 with 7.2±0.3 gkg^{-1} body weight to diet 5 with 8.9±1.5 gkg^{-1} body weight. This trend was similar for the gizzard which also increased across the dietary treatments from diet 1 of 36.8±3.8 $gkg^{-}1$ body weight to diet 5 with 38.9± 0.9 gkg^{-1} body weight.

All values obtained for the relative weights of chest and thigh muscles and also for the length and breadth of chest muscles were similar (Tables 5 and 6).

DISCUSSION

The similarity in the PCV values of all birds sampled among dietary treatments indicated that PCV was not affected by diets. The PCV values obtained for all dietary treatments also conformed with values reported [20]. The significant variation in the RBC values across dietary treatments is in agreement with previous reports [21]. It was concluded by most researchers that a degree of variation for RBC is considered normal [20, 21, 22]. The increment in ACLM inclusion rates in the diets from diet 2 to diet 5 seemed to enhance erythrocyte sedimentation rate. This is supported by previous work that RBC and other parameters such as Hbc and ESR of a bird vary among species, breed, sex and the nutrition supplied to the bird [22].

Feeds containing estrogenic substances easily predisposed birds to increase their ESR value and decrease their circulating erythrocytes per unit of blood [23]. Generally, the haematological values obtained for the experimental birds were analogous with that of the normal ranges mentioned in earlier works stated as follows: PCV = 30.6%; RBC = 2.5 – 3.2 millions/mm3; Hbc = 6.5 – 9.0g/100ml; WBC = 20-30 thousands/mm3; MCV = 115-125c.u; MCH = 25-27pg and MCHC = 21-23% [19, 20].

Of all the carcass traits examined, only dressed weight and backfat showed significant variations among the treatment means obtained for the sampled experimental birds. The heart and gizzard also varied significantly in their relative weights among the organs measured. It is suspected that ACLM may have estrogenic factors possibly inducing hyperlipemia in birds [23, 24]. This hyperlipemia is approximately proportional to the degree of sedimentation, keeping other factors equal [25].

Fattening is evidenced by the increased backfat deposition and the increased relative weight of the heart across diets from the reference diet 1 to diet 5 that had 20% of ACLM dietary inclusion. The deposition of fat around the heart ostensibly occasioned the increased weights of the hearts of birds across the diets. This assertion is supported by previous workers [23, 25, 26]. The upward variation in the weights of the gizzard across diets may be linked to the increased bulkiness of the diets as a result of the increased ACLM inclusion. Gizzards are known to naturally increase in size to accommodate the introduction of fibre (bulkiness) in diets [27].

The similarity in the relative weights of the chest and thigh muscles of the experimental birds indicated a uniform growth pattern and muscle development in all birds on the ACLM based diets compared with the control diets. The uniformity in growth rate and muscle development compared favourably with previous reports on avian growth pattern and muscle development [28, 29].

CONCLUSION

The introduction of ACLM at measured quantities into broiler finisher diets had nutritional benefits without any deleterious effects on the response criteria measured. There is no predisposition to acute general infection, malignant tumor, malformation or diseased condition even at the highest inclusion level of 20% in broiler finisher diets.

The carcass traits, organ weights and muscle development also supported the potentiality of ACLM as a veritable source of protein in broiler finisher diets.

Table 1: Haematological indices of broiler finisher birds fed ACLM-based diets

Parameters	Diets				
	% Inclusion levels of ACLM				
	0	5	10	15	20
PCV (%)	25.1[a]±1.7	24.7[a]±1.4	24.5[a]±2.3	25.0[a]±1.1	24.7[a]±2.3
RBC count x10^6/mm^3)	3.6[a]±0.2	3.3[ab]±0.1	3.1[b]±1.0	3.6[a]±0.3	3.1[b]±0.3
WBC count (g/100ml)	1.8[a]±2.1	1.8[a]±2.5	1.8[a]±2.0	1.8[a]±3.2	1.8[a]±1.9
Hbc (g/100ml)	2.2[a]±0.1	2.1[a]±0.3	2.1[a]±0.3	2.1[a]±0.4	2.2 [a]±0.2
MCHC (%)	30.4[a]±0.9	30.4[a]±0.4	30.3[a] ±0.7	30.3[a]±0.2	30.1[a]±0.4
MCH (pg)	18.2[a]±0.3	18.3[a]±0.4	18.1[a]±0.7	18.3[a]±0.8	18.1[a]±0.5
MCV (µm^3)	129.2[a]±12.0	128.9[a] ±10.0	129.1[a]±11.0	127.9[a]±13.0	128.3[a]±12.0
ESR (mm)	4.3[a]±0.6	4.5[a]±0.5	4.8[b]±0.4	5.1[b]±0.3	5.5[c]±0.5

ACLM= *Amaranthus cruentus* 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.

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

Values are mean ± SD.

Table 2: Serum and Liver Metabolites of broiler finisher birds fed ACLM-based diets

Parameters	Diets				
	% Inclusion levels of ACLM				
	0	5	10	15	20
Serum Metabolites: Total Serum Protein (g/100g)	9.8[a]±0.4	9.6[a]±1.4	9.7[a]±1.2	9.3[a]±1.3	9.4[a]±1.2
Albumin (g/100g)	0.6[a]±0.1	0.7[a]±0.2	0.6[a]±0.2	0.7[a]±0.3	0.7[a]±0.4
Globulin (g/100g)	9.1[a]±0.5	9.2[a]±0.4	9.0[a]±0.2	9.1[a]±0.2	9.3[a]±0.3
Albumin/Globulin Ratio	0.1[a]±0.2	0.1[a]±0.2	0.1[a]±0.1	0.1[a]±0.1	0.1[a]±0.1
Liver Metabolites: Total Liver Protein (g/100g)	10.1[a]±0.4	9.9[a]±0.1	10.0[a]±0.4	10.1[a]±0.2	9.9[a]±0.1
Albumin (g/100g)	2.6[a]±0.1	2.7[a]±0.2	2.6[a]±0.3	2.6[a]±0.1	2.5[a]±0.1
Globulin (g/100g)	7.4[a]±0.4	7.3[a]±0.3	7.5[a]±0.3	7.5[a]±0.2	7.4[a]±0.1
Albumin/Globulin Ratio	0.3[a]±0.1	0.3[a]±0.2	0.4[a]±0.1	0.3[a]±0.2	0.3[a]±0.1

Means with different superscripts in the same horizontal row are significantly different ($P<0.05$).

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AJFAND ONLINE
Volume 7 No. 6 2007
December 2007
ISSN 1684-5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

Table 3: Carcass traits of broiler finisher birds fed ACLM-based diets

Parameters	Diets				
	% Inclusion levels of ACLM				
	0	5	10	15	20
Live weight (g)	1250.1 ± 13.2	1225.2 ± 12.0	1230.4 ± 15.2	1074.3 ± 13.1	1075.0 ± 14.7
Dressed weight (%)	$89.7^{a}\pm0.9$	$90.7^{ab}\pm1.3$	$89.5^{ab}\pm1.0$	$90.0^{bc}\pm1.2$	$91.4^{c}\pm0.8$
Eviscerated weight (%)	$82.0^{a}\pm0.8$	$81.9^{a}\pm0.5$	$81.5^{a}\pm0.7$	$82.0^{a}\pm0.5$	$81.5^{a}\pm0.4$
Thigh (gkg^{-1} body weight)	$46.8^{a}\pm3.5$	$46.1^{a}\pm2.8$	$46.3^{a}\pm2.7$	$46.1^{a}\pm1.3$	$46.5^{a}\pm1.7$
Drumstick (gkg^{-1} body weight)	$102.1^{a}\pm2.7$	$101.5^{a}\pm1.8$	$101.3^{a}\pm2.1$	$102.0^{a}\pm1.8$	$101.8^{a}\pm1.8$
Back (gkg^{-1} body weight)	$81.2^{a}\pm0.5$	$81.3^{a}\pm0.9$	$80.5^{a}\pm0.3$	$80.8^{a}\pm0.3$	$60.6^{a}\pm0.1$
Backfat (gkg^{-1} body weight)	$2.5^{a}\pm0.7$	$2.9^{ab}\pm0.1$	$3.2^{bc}\pm0.3$	$3.2^{bc}\pm0.5$	$3.5^{c}\pm1.1$
Shank (gkg^{-1} body weight)	$29.9^{a}\pm3.7$	$30.1^{a}\pm2.1$	$30.3^{a}\pm1.8$	$30.8^{a}\pm1.8$	$31.0^{a}\pm1.9$
Wing (gkg^{-1} body weight)	$39.1^{a}\pm3.3$	$40.1^{a}\pm3.5$	$39.7^{a}\pm4.3$	$39.9^{a}\pm5.4$	$40.0^{a}\pm1.7$
Head (gkg^{-1} body weight)	$42.9^{a}\pm5.9$	$43.1^{a}\pm6.0$	$43.0^{a}\pm6.3$	$42.9^{a}\pm6.1$	$43.1^{a}\pm7.4$
Neck (gkg^{-1} body weight)	$63.4^{a}\pm2.6$	$63.0^{a}\pm3.4$	$63.1^{a}\pm3.5$	$62.9^{a}\pm7.0$	$61.9^{a}\pm0.4$

Means with different superscripts in the same horizontal row are significantly different ($P<0.05$); ACLM, *Amaranthus cruentus* leaf meal.

Birds used for serum and liver metabolites determinations are subsets of the original 30 birds/diet.

Values are mean ± SD.

AJFAND ONLINE
Volume 7 No. 6 2007
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ISSN 1684-5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

Table 4: Relative organs weights (gkg^{-1} body weight) of broiler finisher birds fed ACLM-based diets

	Diets				
	% Inclusion levels of ACLM				
Parameters	0	5	10	15	20
Liver	$19.2^{a}\pm1.6$	$20.0^{a}\pm 0.3$	$19.7^{a}\pm0.4$	$19.3^{a}\pm1.5$	$19.6^{a}\pm1.8$
Kidney	$6.8^{a}\pm0.1$	$6.5^{a}\pm0.7$	$6.6^{a}\pm0.4$	$6.5^{a}\pm1.2$	$6.7^{a}\pm1.5$
Heart	$7.2^{a}\pm0.3$	$7.5^{a}\pm1.8$	$8.0^{b}\pm0.4$	$8.4^{bc}\pm1.3$	$8.9^{c}\pm1.5$
Spleen	$1.1^{a}\pm0.2$	$1.2^{a}\pm0.3$	$1.1^{a}\pm0.3$	$1.2^{a}\pm0.1$	$1.4^{a}\pm0.5$
Pancreas	$2.9^{a}\pm0.3$	$2.9^{a}\pm0.5$	$3.1^{a}\pm0.3$	$3.0^{a}\pm0.7$	$2.9^{a}\pm0.5$
Bursa	$2.7^{a}\pm0.7$	$2.8^{a}\pm0.4$	$2.8^{a}\pm0.4$	$2.8^{a}\pm0.9$	$2.7^{a}\pm0.7$
Gizzard	$36.8^{a}\pm3.8$	$37.1^{ab}\pm0.5$	$37.4^{ab}\pm1.8$	$37.8^{ab}\pm1.9$	$38.9^{b}\pm0.9$
Lung	$6.7^{a}\pm1.0$	$6.7^{a}\pm1.8$	$6.7^{a}\pm2.1$	$6.6^{a}\pm2.5$	$6.7^{a}\pm1.2$

Means with different superscripts in the same horizontal row are significantly different ($P<0.05$);

ACLM, *Amaranthus cruentus* leaf meal.

Birds used for serum and liver metabolites determinations are subsets of the original 30 birds/diet.

AJFAND ONLINE Volume 7 No. 6 2007
December 2007
ISSN 1684-5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

Table 5: Relative weight (gkg^{-1} body weight) of chest and thigh muscles of broiler finisher birds fed ACLM-based diets

	Diets				
	% Inclusion levels of ACLM				
Muscle	0	5	10	15	20
Inner chest muscle (*Supra coracoideus*)	$8.6^a \pm 0.7$	$8.6^a \pm 0.2$	$8.5^a \pm 0.9$	$8.6^a \pm 0.6$	$8.6^a \pm 0.8$
Outer chest muscle (*Pectoralis thoracicus*)	$23.4^a \pm 2.8$	$23.7^a \pm 3.2$	$24.0^a \pm 1.9$	$23.8^a \pm 1.2$	$23.5^a \pm 1.8$
Thigh muscle (*Gastrocnemius*)	$31.2^a \pm 1.8$	$31.4^a \pm 1.9$	$31.5^a \pm 2.0$	$31.3^a \pm 0.9$	$31.8^a \pm 1.8$

Means with different superscripts in the same horizontal row are significantly different ($P<0.05$); ACLM, *Amaranthus cruentus* leaf meal

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AJFAND ONLINE
Volume 7 No. 6 2007
December 2007
ISSN 1684-5374
AFRICAN JOURNAL OF FOOD AGRICULTURE NUTRITION AND DEVELOPMENT

Table 6: Relative length and breadth ($cmkg^{-1}$ body weight) of chest muscle of broiler finisher birds fed ACLM-based diets

Muscle	Diets				
	% Inclusion levels of ACLM				
	0	5	10	15	20
Length of inner chest muscle (*Supra coracoideus*)	$19.1^{a} \pm 4.1$	$19.3^{a} \pm 5.2$	$19.4^{a} \pm 6.3$	$19.1^{a} \pm 0.5$	$19.2^{a} \pm 5.6$
Length of outer chest muscle (*Pectoralis thoracicus*)	$21.2^{a} \pm 5.7$	$22.0^{a} \pm 6.1$	$21.7^{a} \pm 7.2$	$21.8^{a} \pm 8.1$	$21.7^{a} \pm 6.2$
Breadth of inner chest muscle (*Supra coracoideus*)	$3.4^{a} \pm 0.2$	$3.5^{a} \pm 0.3$	$3.6^{a} \pm 0.4$	$3.6^{a} \pm 1.2$	$3.6^{a} \pm 0.8$
Breadth of outer chest muscle (*Pectoralis thoracicus*)	$6.9^{a} \pm 0.8$	$7.1^{a} \pm 0.5$	$7.1^{a} \pm 0.8$	$7.1^{a} \pm 0.6$	$7.0^{a} \pm 1.2$

Means with different superscripts in the same horizontal row are significantly different ($P<0.05$);

ACLM, *Amaranthus cruentus* leaf meal

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KARI-NARL Complex, Westlands, off Waiyaki Way
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Nairobi, KENYA
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