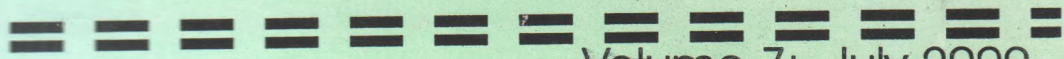




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Principal Contact

Dr. G.R.K. Sharma
Editor-in-Chief
Dept. of Vet & A. H. Extension
College of Vet. Science
A.N.G.R. Agricultural University
Tirupati - 517502
Andhra Pradesh
India
Email: sharmagrk@yahoo.com

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CORRESPONDENCE

Dr. A.E. Adekoya
Department of Agric. Ext. and Rural Development
University of Ibadan
Nigeria
Email: vichenfel@yahoo.com

PERFORMANCE OF BROILER CHICKENS FED DIETS SUPPLEMENTED WITH FEED GRADE ENZYME

O. A. Abu^{2*}, J. U. Igwebuike¹ & Judamat Z. Abu²¹Department of Animal Science, University of Maiduguri, Maiduguri, Nigeria²Department of Animal Science, University of Ibadan, Ibadan, NigeriaE-mail: oa.abu@mail.ui.edu.ng

ABSTRACT

The effect of dietary supplementation of a commercial multi-enzyme complex (Avizyme 1500TM) on the performance and nutrient digestibility by broiler finishers was investigated. A total of 120 d-old broiler chickens was assigned to four treatments each having two replicates of 15 birds in a 2 x 2 factorial block design. Avizyme was incorporated into the finisher diets at inclusion levels of 0 (high and low fibre diets), 1.0 and 2.0g/Kg diets in diets 1-4 respectively. The study covered the finisher phase (4-8 week period). Results obtained indicated that incorporation of Avizyme up to 2.0g/Kg diet into broiler finisher diet produced no significant ($P > 0.05$) effects on average daily feed intake and feed conversion efficiency of the birds but depressed daily weight gain ($P < 0.05$). The average final body weight followed the same trend as the daily weight gain, as the birds fed control diet and diet containing 1g/kg Avizyme did best ($P < 0.05$). The digestibility of nutrients did not differ significantly ($P > 0.05$) among treatments. Birds on the control diet used nutrients better than the rest treatments. The haematological parameters such as Packed Cell Volume (PCV), Haemoglobin (Hb), White Blood Cell (WBC), Mean Corpuscular Haemoglobin (MCH), and Mean Corpuscular Haemoglobin Concentration (MCHC) except for Red Blood Corpuscles (RBC) did not differ ($P > 0.05$) among treatments. Dressing percentage, cut-up parts and weight of organs expressed as a percentage of body weight did not differ significantly ($P > 0.05$). The results of this study showed that the inclusion of 1g/kg of the enzyme mixture into broiler finisher diet did not show any adverse effect on the performance and blood parameters of the broiler chickens. The 1g/kg level of inclusion of enzyme in the diet appears to be most beneficial to the farmer in view of its relatively better results.

Key words: multi-enzyme mixture supplementation, broiler, performance, growth, blood profile, and carcass characteristics

INTRODUCTION

Cereals provide the bulk of the energy in poultry diets. However the presence of high non-starch polysaccharides in these cereals has been implicated in the formation of viscous digesta leading to reduced nutrient utilization efficiency in birds (Bedford, 1995; Graham, 1996). Fungal enzymes have been reported useful in enhancing the nutritive value of livestock feed ingredients (Onifade and Babatunde, 1996, Abu *et al.*, 1998, Onifade *et al.*, 1999, Onilude and Oso, 1999, Abu *et al.*, 2000, Afolabi *et al.*, 2007). Various attempts have also been made to improve the nutritional value of high polysaccharide containing feeds by the addition of exogenous feed enzymes (Bedford, 1995; Fuente *et al.*, 1995). Xylanases act as fiber degrading enzymes thereby allowing the bird's own enzymes greater access to the nutrients (Graham, 1996). Feed grade mannanase enzymes have also been reported to improve performance of birds fed

palm kernel based diets (Abu *et al.*, 2009a, Abu *et al.*, 2009b) Proteases present in commercial enzymes have been found to degrade soybean proteins and the trypsin inhibitors, lectins and antigenic proteins present in soybean (Graham, 1996). In addition no nutritional or physiologically harmful effects have been reported on animals fed feeds supplemented with commercial enzymes (Hotten, 1991). The inclusion of multi-enzyme preparations in livestock feeds maximizes the release of protein from the aleurone layer of cereals (Mulder *et al.*, 1991). Most promising enzymes are those that are capable of degrading structural carbohydrates, such as cellulose and lignin, modification of proteins and utilization of phosphorus from complex compounds (Joskel *et al.*, 1996). The optimal and judicious use commercial enzymes in feeds may therefore be a major factor for consideration in attaining high profitability in the poultry industry. This experiment was conducted to evaluate the

effects of Avizyme 1500[®], a commercial enzyme, on the growth parameter indices and cost of production of finisher broilers.

MATERIAL AND METHODS

Site of Experiment

This study was conducted at the University of Maiduguri Teaching and Research Farm, Nigeria. The location is latitude 11° 15' N and longitude 30° 5' on an altitude of 345m above sea level. It is in the semi-arid zone of Nigeria, characterized by hot and dry climates and short duration of erratic annual rainfall of between 3-4 months. It has a long dry season and rainfall varies from 150-160mm with ambient temperature in December-January ranging from 15°C to 19°C and high by March to June, ranging from 33°C to 43°C and low relative humidity ranging from 5% to 43.5% (Alaku, 1983).

Animals and diets

One hundred and twenty Hybro day-old broiler chicks were fed on a commercial starter diet for four weeks. The chicks were then randomly allotted to four groups of 30 chicks per treatment with each having two replicates of 15 birds per replicate. The birds were placed in identical pens (1.55 x 1.30x 1.13m) in an open-sided poultry house. The chicks were fed *ad libitum* a standard commercial diet for four weeks. Four diets were formulated each representing a treatment. Diet 1 (treatment 1) was the positive control. Diet 2, 50% of maize was replaced by maize offal (negative control diet). Diets 3 and 4 had 50% of maize replaced by maize offal and Avizyme 1500[™] (Finnfeeds International Ltd., Marlborough, Wiltshire, UK) added at 1 and 2g/Kg, respectively. Avizyme 1500[™] contained the following activities, xylanase (I.U.B No. EC 3.2.1.18) 300 µg/g; (I.U.B No. EC 3.4.24.20) protease 4000u/g and amylase (I.U.B No. EC 3.2.1.1) 400u/g.

Parameters Assessed

Feed intake and body weight changes were measured daily and weekly respectively for four weeks. Feed conversion ratio was calculated based on the values obtained from the feed intake and body weight gain. The costs of the feed ingredients used were calculated based on the prevailing market prices. At the end of the feeding trial, three birds close to the respective treatment means were randomly selected from each replicate and blood samples were collected from the jugular veins. The birds were then starved of feed for 12 hr to allow for post-absorptive conditioning after which they were slaughtered by rapid

severance of the trachea and esophagus along the large blood vessels of the neck as described by Gill, (1991). The slaughtered birds were hung upside down to allow bleeding-out. The birds were scalded for 10 minutes, defeathered and carcass cut up and the internal organs weighed fresh. The slaughtered weights, dressed weight, weight of internal organs were recorded immediately.

Chemical and Statistical Analyses

The diets were chemically analyzed for proximate composition by methods of AOAC (1994). All the data collected, except so indicated, were subjected to analysis of variance (ANOVA) and means were separated using Duncan's Multiple Range Test Duncan, (1955).

RESULTS AND DISCUSSION

Table 1 showed the composition of the experimental diets. The major fiber sources were maize offal (except for diet 1 without maize offal) and wheat offal while the major plant protein source was full fat soybean. Fishmeal and blood meal supplied protein of animal origin. The replacement of 50% maize in the diet with maize offal did not seem to alter appreciably the crude protein and the energy levels of the diets. These nutrient levels therefore met the requirements of broiler chickens (ARC, 1975). However, the crude fiber level in the diets rose from 3.72% to 6.5% when compared with the control diet. The quantity of protein in each diet did not seem to vary. However the protein quality is expected to differ as maize has a superior protein quality when compared to maize offal.

Feed intake, Body weight, Feed conversion

Table 2 showed the performance of the chickens. The daily feed intake was not affected ($P > 0.05$) in all the diets. Supplementation with or without enzyme therefore had no effect on feed intake. This finding was contrary the findings that addition of amylase and protease enzymes to diets produces a significant decrease in the feed intake (Collier and Hardy, 1986; Inborr and Ogle, 1988; Ogbonna *et al.*, 1996). In spite of the high fiber level of diet 2 a reduced feed intake was not observed as report indicate that increased fiber level in the diet reduces feed intake as a result of dilution of energy of such diet. The feed intake varied between 108.1 and 112.5g/bird/day. Birds fed the positive control diet had the best feed conversion ratio, which was significant to other diets. The FCR values obtained in this study were however better than

those reported by Faniyi (1997) when mango seed kernel based diets were fed to broilers but the values were comparable to those reported by Fanimó *et al.*, 1998. The inclusion of the enzyme at 2g/Kg depressed daily weight gain when compared to values obtained in the other treatments. Inclusion of the enzyme at 1g/kg diet improved significantly ($P < 0.05$) the final body weight of birds on high fiber diets but not at 2g/kg inclusion rate but similar to the control ($P > 0.05$). Inborr and Bedford (1994) reported a linear improvement in live weight gain and feed conversion efficiency in birds with increased levels of enzymes (Avizyme SX) addition contrary to the results obtained in this study. High fiber significantly ($P < 0.05$) depressed body weight gain of birds but addition of enzyme at 1g/kg diet did not ($P > 0.05$) when compared with normal fiber diet. However supplementation of high fiber diet with enzyme at 2g/kg diet further reduced final body weight even when compared with high fiber unsupplemented diet (treatment 2). The body weight gains recorded in treatments 1, 2, and 3 were similar to those reported by Esonu *et al.*, 1999 but differ from those reported by Nworgu and Egbunike (1999) in broilers fed soybean based diets.

Nutrient Digestibility

The apparent digestibility of nutrients is presented in Table 3. DM digestibility was 90.0, 97.0, 97.0 and 96.0% for treatments 1-4 respectively. There was no significant ($P > 0.05$) difference in these values. The observation is however contrary to that of Maquardt *et al.* (1996) who observed otherwise. Crude protein digestibility did not differ ($P > 0.05$) among treatments. Treatment effects did not affect dry matter, crude fiber, and crude protein ($P > 0.05$) retentions. This is contrary to reports that the addition of exogenous feed enzymes improves the nutrient extraction efficiency of the chicken (Bedford, 1995; Fuente *et al.*, 1995). It may be safe to conclude that the addition of the exogenous enzyme in this experiment did not elicit the desired response possibly because of the nature of and type of cereal (maize) and cereal by-product (maize offal) used in this experiment. However ether extract, ash and nitrogen free extract were significantly affected ($P < 0.05$).

Mortality

Mortality was between 3.33 and 6.67% and was not attributed to effects of dietary treatments. The litter material in treatment 1 and 2 were changed three times during the

feeding trial while those of treatments 3 and 4 were changed twice. Birds on treatments 3 and 4 gave drier drooping unlike those on treatments 1 and 2. Earlier studies (Classen and Bedford, 1991; Bedford, 1995; Balnave, 1996; Classen, 1996) indicated that birds fed commercial enzymes were able to extract more efficiently nutrients from feed hence produced firmer and drier droppings than those unsupplemented diets. Birds on enzyme supplemented diets gave firmer and drier droppings hence cleaner litter materials but they did not show efficient nutrient extraction as reflected in their performance when compared against those birds fed diets unsupplemented with enzyme. Birds fed enzyme-supplemented diets also had cleaner and unsoiled plumage. This is reflected in the vent pasting values obtained (Table 2). Such birds may command higher monetary value when sold. This is therefore an economic advantage for the broiler producer. The inclusion of β -glucanase (pentosanase) in rye-based diets had earlier been linked with improved litter quality (Pettersson and Åman, 1998; Bedford *et al.*, 1991) and reduced wet excreta output (Bedford, 1995).

Blood Parameters

Data for the blood parameters are shown in Table 4. They include the packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). The mean RBC counts for the treatments groups were 4.21, 4.71, 4.27 and $3.62 \times 10^{12}/\mu\text{l}$ for treatments 1-4 respectively. However a significant ($P < 0.05$) difference among the values for RBC was observed. The RBC values observed in this study for treatments 1, 2 and 3 were above the normal range of $2-4 \times 10^{12}/\text{mm}^3$ except for treatment 4 that was within the normal range. The WBC values for the treatments were 18.58, 21.73, 20.21 and $19.55 \times 10^3/\text{mm}^3$ for the treatments. The values obtained were consistent with the normal range ($9-31 \times 10^3/\text{mm}^3$) for healthy birds of similar age. The Hb values for the treatments were 10.10, 11.10, 10.20 and 9.23 g/dl for treatments 1-4 respectively. There were however no significant ($P > 0.05$) differences between the control and other treatments. The Hb values for all the treatments fell within the normal range (7-13 g/100ml) for chickens.

The values for MCV, MCH and MCHC are shown in Table 4. There was no significant ($P > 0.05$) difference for MCV, MCH and MCHC for

all the treatments. These are indices of RBC and are used to measure the size and haemoglobin content of erythrocytes, which make them vital in the diagnosis various anaemic conditions. The MCV values in this study were lower than the 127fl normal value for birds and this indicates that birds were somehow anaemic. However, this was not so severe as to depress the performance of the chickens. The high MCV values in treatment 4 are an observation consistent with the RBC count. In general, the blood parameters fell within the normal ranges as given by Anon (1980). These observations point to the nutritional adequacy of the experimental diets.

Carcass Evaluation

The carcass characteristics of the chickens are presented in Table 5. The birds fed high enzyme diet (2g/kg diet) had the least live weight and the least carcass weight compared to those fed on the control diet. The dressing percentages for the treatments were 65.66, 68.37, 67.94 and 69.93% for treatments 1-4 respectively. The control had a significantly ($P < 0.05$) lower dressing percent than the other groups. The initial weight of the bird and weight of the birds after blood drained in all the treatments were also significant ($P < 0.05$). However the dressing percentage obtained in all the treatments were lower than those reported by Biswas and Wakita (2001) when Japanese green tea powder was supplemented in the diets of broilers. The cut-up carcasses, which include the wings, back, neck and ribs did not differ significantly ($P > 0.05$). The values for thighs reported were higher than those reported by Fanimu *et al.* (1998) when broilers were fed shrimp waste meal-based diets supplemented with amino acids. The neck values obtained in this study were however lower than reported by the same authors. The drumsticks and breast were significantly affected by dietary treatment. Table 6 shows the visceral organs of the birds. The heart, gall bladder, kidneys, gizzard and crop were not affected by dietary treatments ($P > 0.05$). There seems to be an interactive effect of high fiber and enzyme inclusion on spleen weight. The high fiber and inclusion of enzyme lowered the spleen weight ($P < 0.05$) but did not affect other visceral organs but for the ceca weight which was also affected by dietary treatment.

Economy of Production

The economic performance data is shown in Table 2. The cost/kg diet varied between ₦22.26 and ₦25.16/kg. The feed cost decreased with increased substitution of maize

for maize offal. However the addition of enzyme in the diet 3 and 4 at the rate of 1g/kg and 2g/kg diet respectively increased the cost/kg of these diets from ₦22.26 to ₦22.76 for diets 3 and 4 respectively. Despite the enzyme inclusion, diet 3 and 4 were cheaper than the control diet that had no maize offal inclusion. The highest and lowest cost per/kg diets were therefore obtained in diets 1 and 2 respectively. However the total feed cost showed a different trend. The highest in diet 1 (₦79.25) followed by diet 3 (₦73.97), then diet 4 (₦70.47) while diet 2 cost the least (₦69.22). The feed cost/kg gain showed that diet 2 (high fiber diet without enzyme supplementation) had the least (₦59.68) while diet supplemented with enzyme at inclusion rate of 2g/kg diet (diet 4) had the highest value of ₦66.48. However when these values were expressed in feed cost/kg eviscerated weight the diet 2 had the highest value of ₦19.49 while diet 1 had the least (₦16.27).

CONCLUSIONS

- The overall performance of the broilers indicated that supplementation of the highest fiber diet with Avizyme at 1g/kg diet inclusion rate gave the best results.
- Feed cost and feed quality are two factors, among other major factors militating against the expansion of the poultry industry. The feed are very expensive and usually of poor quality. Most often, the poor quality is caused by high level of fiber and/or low crude protein inclusion. Being monogastrics, poultry have poor ability to digest fiber efficiently; as a result most of the feed is not efficiently utilized.
- Birds fed Avizyme had cleaner plumage with less vent pasting. This may be of economic value as birds with cleaner and unsoiled plumage may command higher market value.
- Inclusion of Avizyme in the diet reduced litter moisture and hence improved litter quality.

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Table 1. Composition of Experimental Diets (%)

Ingredients (%)	T1	T2	T3	T4
	No Enzyme (low fibre)	No Enzyme (high fibre)	+ 1g/kg Enzyme (high fibre)	+ 2g/kg Enzyme (high fibre)
Maize	56.0	28.0	28.0	28.0
Maize offal	0.0	28.0	28.0	28.0
Wheat offal	10.0	10.0	10.0	10.0
Full fat soybean	25.0	25.0	25.0	25.0
Fish meal	3.0	3.0	3.0	3.0
Blood meal	2.0	2.0	2.0	2.0
Bone meal	2.0	2.0	2.0	2.0
Oyster shell	1.0	1.0	1.0	1.0
DL Methionine	0.2	0.2	0.2	0.2
Premix*	0.5	0.5	0.5	0.5
Salt	0.3	0.3	0.3	0.3
Total	100	100	100	100
<i>Cal. proximate analysis (%)</i>				
Crude protein	21.35	21.63	21.63	21.63
Crude fiber	3.72	6.5	6.5	6.5
ME MJ/g	12.67	12.12	12.12	12.12
Ca	1.70	1.7	1.7	1.7
Available P	0.6	0.6	0.6	0.6

* Each 1Kg premix contains Vit A i.u., 5,000,000 Vit D₃ i.u., 1,000,000; Vit E, 16,000 mg; Vit K₃, 800 mg; Vit B₁, 1,200 mg; Vit B₂, 2,200 mg; Niacin, 22,000 mg; Calcium pantothenate 4,600 mg; Vit B₆, 2,000 mg; Vit B₁₂ 1,000 mg; Folic Acid, 400 mg; Biotin, 32 mg; Choline Chloride, 500,000 mg; Mn, 120,000 mg; Fe, 100,000 mg; Zn, 80,000mg; Cu, 8,500 mg; I₂, 600 mg; Co, 120 mg; Se, 48 mg; Anti-Oxidant, 48mg.

Table 2. Effects of Avizyme 1500® On the Growth and Economics of Production of Finisher Broilers Fed High Fiber Diets

	Diets				Means	SEM
	1	2	3	4		
Ave. initial body wt (g)	700.0	755.0	730.0	720.0	726.3	
Ave. initial feed intake (g/bird)	112.5	111.1	116.1	108.1	111.9	2.57 (NS)
Total wt. Gain (g/bird)	1963.72 ^a	1917.56 ^b	1946.43 ^a	1780.0 ^c	1901.93	*
Total weight gain (g/bird)	1263.92	1162.56	1215.2	1096.76	1178.8	56.11*
Ave. daily wt. Gain (g/bird/day)	45.14 ^a	41.52 ^b	43.40 ^a	39.17 ^c	42.31	2.04*
Feed conversion ratio	2.44 ^c	2.86 ^a	2.69 ^b	2.90 ^a	2.72	0.14*
Feed cost/kg (N)	25.16	22.26	22.76	23.26	23.36	-
Total feed cost (N)	79.25	69.22	73.97	70.47	73.23	-
Cost/kg eviscerated wt. (N)	16.27	19.49	17.60	17.53	17.42	-
Feed cost/kg gain (N)	62.89	59.68	60.63	66.48	62.42	-
Mortality	6.67	6.67	3.33	6.67	5.84	-
Vent pasting (% birds affected)	16.67	33.3	3.33	0.00	53.3	

NS= Not significant (P > 0.05) 1 US = (N) 130 at March 2006

Table 3. Mean apparent nutrient digestibility by broiler fed diets supplemented with Avizyme

Nutrient (%)	Diets				Mean	SEM
	1	2	3	4		
Level of Avizyme (g/kg diet)	0	0	1.0	2.0	-	-
Dry matter	96.0	97.0	97.0	96.0	96.50	0.73 (NS)
Crude protein	71.70	65.70	58.70	68.0	65.85	4.19 (NS)
Crude fiber	39.0	45.70	35.70	40.0	40.10	10.7 (NS)
Ether extract	80.70 ^{ab}	86.70 ^a	72.70 ^b	84.0 ^a	81.03	3.82*
Ash	57.0 ^a	44.0 ^b	45.60 ^b	46.30 ^b	48.23	2.38*
Nitrogen free extract	61.30 ^a	54.30 ^b	64.30 ^a	64.60 ^a	61.13	1.50*

Table 4. Effect of Avizyme supplementation on hematological indices of Broilers fed high fiber diets (5-6 weeks of age)

Parameters	Treatments (diets)				X	SEM
	1	2	3	4		
Haematocrit (%)	32.67	34.67	32.17	29.0	32.13	0.99 (NS)
Haemoglobin (Hb, %)	10.10	11.10	10.20	9.23	10.16	0.32 (NS)
WBC (x 10 ³ µl)	18.58	21.73	20.21	19.55	20.02	0.69 (NS)
RBC (x 10 ⁶ µl.)	4.21 ^{ab}	4.71 ^a	4.27 ^{ab}	3.62 ^b	4.20	0.17*
MCV (fl)	77.94	73.64	75.75	80.57	76.98	1.23 (NS)
MCH (pg)	24.10	23.56	24.05	25.72	24.36	0.41 (NS)
MCHC (%)	30.92	31.99	31.77	32.24	31.73	0.30 (NS)

SEM = Standard Error of the Mean, NS= Not significant (p > 0.05), *= Significant (p < 0.05).

Table 5. Effect of Avizyme supplementation on carcass characteristics of broiler finishers fed high fibre diets

Parameters	Treatments (Diets)				X	SEM
	1	2	3	4		
Final body wt (g)	1963.33 ^a	1973.33 ^a	1916.67 ^a	1766.67 ^b	1905.0	24.69*
Wt. of bird after blood drain (g)	1926.67 ^a	1926.67 ^a	1853.33 ^b	1740.0 ^b	1861.67	0.75*
Wt of defeathered bird (g)	1740.00 ^b	1906.67 ^a	1683.33 ^c	1660.0 ^c	1747.50	0.026.11*
Carcass weight (g)	1644.40 ^a	1671.77 ^a	1584.93 ^b	1491.30 ^c	1598.21	31.58*
+ Eviscerated weight (g)	1289.17 ^a	1349.2 ^a	1302.20 ^a	1235.56 ^b	1294.03	1.89*
Dressing percentage (%)	65.66	68.37	67.97	69.93	67.98	0.88 (NS)
Cut-up carcass as % of carcass wt						
Drumsticks	12.50 ^{ab}	11.79 ^b	8.23 ^c	13.08 ^a	11.40	1.09*
Thighs	14.49	14.11	13.81	13.44	13.96	0.22 (NS)
Wings	9.07	9.35	9.69	9.72	9.46	0.15 (NS)
Breast	20.95 ^a	15.5 ^b	22.06 ^a	20.50 ^a	19.76	1.45*
Back	9.37	9.28	9.38	9.95	9.50	0.15 (NS)
Neck	6.32	6.31	6.19	7.06	6.47	0.20 (NS)
Ribs	7.12	6.04	6.19	6.96	6.58	0.27 (NS)

X = Mean, SEM = Standard Error of Mean; NS = Not significant ($P > 0.05$) a, b, cMeans in the same row bearing different superscripts differ significantly ($p < 0.05$).

+ Calculated as $\frac{\text{Eviscerated carcass weight (g)} \times 100}{\text{Live weight (g)}}$

Table 6. Effect of Avizyme supplementation on visceral organ characteristics of broiler finishers fed high fibre diets

Parameters	Treatments (Diets)				X	SEM
	1	2	3	4		
Wt of organs as % of carcass						
Heart	0.62	0.58	0.63	0.55	0.60	0.02 (NS)
Lungs	0.72	0.75	0.65	0.60	0.69	0.03 (NS)
Liver	2.84	2.53	2.44	2.50	2.58	0.02 (NS)
Ceca weight	0.91 ^a	0.77 ^b	0.66 ^c	0.89 ^a	0.81	0.06*
Ceca length (cm)	20.57	19.83	19.83	19.83	19.89	0.10 (NS)
Gall bladder	0.15	0.06	0.09	0.09	0.10	0.02 (NS)
Kidney	0.76	0.74	0.83	0.72	0.76	0.02 (NS)
Full gizzard	3.47	4.14	4.18	4.20	3.99	0.18 (NS)
Empty gizzard	2.48	2.50	2.78	2.91	2.67	0.10 (NS)
Spleen	0.39 ^a	0.20 ^b	0.18 ^b	0.20 ^b	0.24	0.05*
Proventriculus	0.68	0.67	0.57	0.66	0.65	0.03 (NS)
Empty crop	0.48	0.50	0.38	0.49	0.46	0.03 (NS)
Abdominal fat	1.62 ^c	1.72 ^{bc}	2.52 ^a	1.91 ^b	1.95	0.20*

X = Mean, SEM = Standard Error of Mean; NS = Not significant ($P > 0.05$) a, b, cMeans in the same row bearing different superscripts differ significantly ($p < 0.05$).