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EFFECT OF DIETARY UTILIZATION OF WHEAT DISTILLERS GRAINS WITH SOLUBLES SUPPLEMENTED WITH ENZYMES ON CARCASS AND ORGANS CHARACTERISTICS OF ROSS BROILER CHICKENS

N. B. Rano

Department of Animal Science, Bayero University Kano, Nigeria

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ABSTRACT

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*Corresponding

Author:

nbrano.asc@buk.edu.ng

A study was conducted to evaluate the effect of feeding diets containing different levels of wheat distillers dried grains with solubles (wDDGS) and enzyme inclusion on carcass and internal organs characteristics of Ross broiler chickens. One hundred and eighty day-old Ross broiler chicks were fed diets in a completely randomized design in a 2 x 3 factorial arrangement comprising 2 wDDGS levels of 0 and 15% and 3 enzyme levels of no enzyme (NE), + enzyme A (EA) and + enzyme B (EB) designated as Diets A, B, C, D, E and F respectively. The experiment lasted for 6 weeks. The chicks were individually weighed, wing tagged and assigned to 6 groups (dietary treatments) of 30 chicks per treatment. Each group was further divided into 5 sub-groups consisting 6 birds per replicate. The experimental diets were iso-caloric with varying metabolisable energy. The diets and clean drinking water were provided *ad libitum* to these birds. The enzyme was included at 0.5 kg /tonne of complete diet. At 45 days of age, 2 birds per pen were killed, skinned and eviscerated and their relative weights measured and recorded. The data were statistically compared for the effects of wDDGS, Enzyme and wDDGS x Enzyme interaction at $P < 0.05$. The results of the present experiment indicated that inclusion of 15% wDDGS supported increased liver weight of broilers managed on deep litter, weights of thigh and wing of caged birds as well as that of empty gizzard of both caged and deep litter managed birds. The introduction of enzyme A to wDDGS-based diets can positively affect the cold carcass weight of caged broilers.

INTRODUCTION

The inclusion of wheat distiller's dried grains with soluble (wDDGS) characterised as fibrous agricultural by-product in poultry diets is limited due to the reduced starch and high fibre content. However, the continued increase in ethanol demand and its production, especially in the US, Europe and other countries, has led to production of enormous amounts of DDGS flooding into the animal feed industry for use. Meanwhile, the

major constraint associated with the use of DDGS for poultry is the high variability of the nutrient content and availability, as well as the poor digestibility of some amino acids such as lysine, tryptophan and arginine (Choct, 2006), in addition to the presence of high fibre, possible mycotoxins and some anti-nutrients e.g. phytate.

However, using enzymes to improve the nutrient availability of wDDGS, in particular the protein quality and the release of metabolisable energy (ME) could result in an improved product for poultry.

Meanwhile, inclusion of exogenous enzymes in wheat-based broiler diets is an increasingly common practice. It has been reported that enzyme supplementation in wheat-based diets for broilers improved performance (Annison, 1991). The aim of this paper was to determine the effect of utilization of isonitrogenous diets containing either wDDGS supplemented with enzymes on the carcass composition of broiler chickens and to evaluate the interaction between wDDGS levels and enzymes in diets fed to broiler chickens.

MATERIALS AND METHODS

Experimental Site

The study was conducted at Cockle Park farm, Newcastle University, under the ethical guidelines for non-regulated procedure of Newcastle University, Newcastle Upon Tyne, UK. Newcastle upon Tyne commonly known as Newcastle, is a city in Tyne and Wear, North East England, 103 miles (166 km) south of Edinburgh and 277 miles (446 km) north of London on the northern bank of the River Tyne, 8.5 mi (13.7 km) from the North Sea. The ground beneath the city is formed from Carboniferous strata of the Middle Pennine Coal Measures Group—a suite of sandstones, mudstones and coal seams which generally dip moderately eastwards. To the west of the city are the Upper Pennine Coal Measures and further west again the sandstones and mudstones of the Stainmore Formation, the local equivalent of the Millstone Grit (Bedrock Geology UK North).

Experimental enzymes, wDDGS and Diets

Description and composition of enzymes used in the experiment

The enzyme A was a mixture of xylanase, amylase and protease and it was designed to increase the efficiency of wheat-based broiler, turkey and duck feeds. It is specifically developed for use in mixed-grain poultry diets, containing vegetable and other protein sources and some high fibre feeds. The enzymes were derived from *Bacillus subtilis* and *Trichoderma reesei* fermentation processes and mixed with wheat flour and calcium propionate as carriers and binders. This enzyme is recommended for its addition to prepared vitamin and mineral premixes that were then mixed at appropriate levels with different broiler feeds. Each gram of this enzyme was expected to have enzymic activities of 9200 U endo-1, 4-beta-xylanase, 16000 U of subtilisin as protease and 1600 U of alpha amylase (Bedford, 2000).

The enzyme B was declared to be derived from *Trichoderma reesei* fermentation and it also contained variable amounts of sodium sulphate, poly-vinyl alcohol, starch, hydrated magnesium silicate and sucrose. Each g of this enzyme was declared to contain activities of 12200 U endo-1, 4-beta-xylanase, and 1520 U of endo-1, 3 (4) -beta-glucanase (Bedford, 2000).

Experimental diets

Three isonitrogenous diets were formulated by substituting wDDGS for coarsely ground wheat and soybean meal, allowing variation in metabolisable energy and fibre content. The diets were identified by combinations of numbers and letters depending upon the wDDGS levels and enzyme types.

Table 1. Composition of the experimental diets for one-phase feeding system (42 days)

Ingredients (g/kg)	Diets(%wDDGS Inclusion)							
	0		15		0		15	
	NE0	NE15	EA0	EA15	EB0	EB15		
	A	B	C	D	E	F		
wDDGS	0	150	0	150	0	150		
Wheat	647	575	646.5	574.5	646.5	574.5		
Soybean meal	296	218	296	218	296	218		
Soybean oil	10	10	10	10	10	10		
Limestone	20	20	20	20	20	20		
MCP	10	10	10	10	10	10		
Salt	2.5	2.5	2.5	2.5	2.5	2.5		
Premix*	12.5	12.5	12.5	12.5	12.5	12.5		
Methionine	1	1	1	1	1	1		
Lysine	1	1	1	1	1	1		
Enzyme	0	0	0.5	0.5	0.5	0.5		
Total	1000	1000	1000	1000	1000	1000		

Table 1. Ingredient and estimated nutrient composition of broiler diets (kg/ tonne) (continued)

	DIETS (%wDDGS Inclusion)					
	0		15		0	
	NEo	NE15	EAo	EA15	EBo	EB15
	A	B	C	D	E	F
Estimated Analysis (g/kg) (unless otherwise stated)						
Crude Protein	226	223	226	223	226	223
ME (MJ/kg)	14	13	14	13	14	13
Starch	380.1	339.9	380.1	339.9	380.1	339.9
Crude Fibre	34	76	34	76	34	76
Ether Extract	76	67	76	67	76	67
Calcium	11	10	11	10	11	10
Phosphorus	5	6	5	6	5	6
Amino Acids						
Arginine	14.7	13.6	14.7	13.6	14.7	13.6
Histidine	6.1	5.9	6.1	5.9	6.1	5.9
Isoleusine	9.7	9.4	9.7	9.4	9.7	9.4
Leusine	17.3	17.2	17.3	17.2	17.3	17.2
Lysine	11.6	10.0	11.6	10.0	11.6	10.0
Methionine	4.5	4.3	4.5	4.3	4.5	4.3
Phenylalanine	11.7	11.6	11.7	11.6	11.7	11.6
Threonine	8.3	8.1	8.3	8.1	8.3	8.1
Tryptophan	3.1	2.9	3.1	2.9	3.1	2.9
Valine	10.8	10.8	10.8	10.8	10.8	10.8
Total AA	97.7	93.7	97.7	93.7	97.7	93.7

NE = No Enzyme, EA= Enzyme A, EB= Enzyme B, ME = Metabolisable Energy, MCP= Monocalcium Phosphate, AA = Amino Acids *Premix of 12.5kg contained Vitamin A 12,500 IU, Vitamin D3 3,000iu, Vitamin E-150g, Vitamin K 3g, Vitamin B1 2g, Vitamin B2 8g, Vitamin B6 5g, Vitamin B12 30mg, Nicotinamide 50g, D-cal Pan 10g, Folic Acid 2g, Biotin 150mg, Vitamin C 4g, Choline 300g, Copper (Copper Sulphate pentahydrate) 10g, Manganese (Manganese Sulphate monohydrate) 80g, Zinc (Zinc Oxide) 60g, Iron (Ferrous Sulphate monohydrate) 30g, Iodine (Calcium Iodate) 2g, Selenium (Sodium Selenite 0.25mg, Selenium rich yeast 0.08mg), Molybdenum (Sodium molybdate) 0.20mg, Calcium (30%), Sodium chloride (8%)

Data collection

Carcass measurements

At the end of the experiment, eight birds per treatment (i.e. 2 birds per replicate) were randomly selected for carcass analysis. The birds were killed by cervical dislocation and dressed manually by removing the skin along with feathers to obtain their cold carcasses and weighed. The carcasses were eviscerated and dressed by removing the neck and the shanks and then the cut up parts weighed. The dressing percentage was calculated using the formula below:

$$\frac{\text{Dressing percent (g)}}{\text{Weight of live chicken (g)}} \times 100 = \text{Dressing percent (\%)}$$

Parts such as thighs, drumsticks, wings, breast muscle and bone) were determined using knife. These parts were then weighed and expressed in percentage of their respective cold carcass weights. The thighs and drumsticks were removed from the carcass by cutting above the thigh, towards the acetabulum and behind the pubic bone (the pelvic/thigh incision). Then the drumsticks were separated from the thighs by cutting perpendicular to the joint between the drumstick and thigh bones. The wings were removed by shoulder incision through the joint (articulation) surface of the scapula. The breasts were separated by rib incision i.e. a cut perpendicular to the ventral joints of ribs. The back-pelvis separations were performed by cutting perpendicularly to the ventral column at the final vertebral level. The internal organs such as liver, heart, gizzard, intestines, crop and proventriculus were also carefully separated and weighed, however, gizzard and crop were emptied prior to weighing. The components were expressed as percentages of their respective cold carcass weights. The cold carcass weight expressed excludes the weights of internal organs. The digestibility trial took place after the feeding trial and lasted for a week (i.e. 3 days

adaptation period and 4 days total collection of excreta). The birds here attained 52 days of age. Similarly, 2 birds per replicate were selected based on their uniform live weights across the pens, sacrificed, processed, weighed and dissected to study the carcass characteristics and organ weights in the same way as described above to compare the performance of these birds on deep litter versus metabolic cages.

Data Analysis

The collected data were subjected to the analysis of variance (ANOVA) using a general linear model (GLM) procedure in a Minitab 16 statistical package. Where the effect of a factors were significantly affected by treatment, means of were significantly ($P < 0.05$) compared using the Tukey's test.

RESULTS AND DISCUSSION

The effects of different levels of wDDGS and enzyme inclusion on carcass characteristics and digestive organs are shown in Table 2. There was no significant ($P > 0.05$) effect on cold carcass weight either for different levels of wDDGS or for enzyme inclusion. Much research has investigated the composition of the carcass in chickens. Jourdain (1980) found that eight-week old broiler chickens have carcass yields of 64%, with regard to those raised in the tropics. Salami *et al.* (2004) found that the most ideal carcass yield for table birds should be between 65-70%. Previous research by Oluyemi and Roberts (1998) dissected broiler chicken meat and found yield levels of 17.4%, 12.95%, 11.67%, 8.21% and 4.38% for the breast, thighs, drumsticks, wings and neck, respectively.

Inclusion of 15% wDDGS significantly ($P < 0.05$) reduced the weight of breast with bone and increased the proportional weight of liver and empty gizzard. Meanwhile, enzyme B addition, significantly ($P < 0.05$) increased the dressing percent and liver weight compared with no enzyme. The reduction in breast meat yield could be attributed to the varying energy content as the level of wDDGS was increased as reported by Waldroup *et al.* (1981) who found a decline in performance of broilers fed 15% or more of wDDGS levels.

The main effects of wDDGS and Enzyme on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets for a further week during the digestibility trial are presented in Table 4. The digestibility trial took place after the feeding trial and lasted for a week (i.e. 3 days adaptation period and 4 days total collection of excreta). The birds here attained 52 days of age. Inclusion of 15% wDDGS significantly ($P < 0.05$) decreased the weights of breast with bone and heart. However, it significantly increased the weights of thighs and wings. There was also a significant ($P < 0.05$) increase with the 15% wDDGS in the proportional weight of empty gizzard, total gut as well as empty crop. This agrees with the findings of Sundu *et al.* (2008) who reported in a similar study that a high fibre diet has been found to affect the mass and size of digestive organs, thereby affecting the quality of the carcass.

Addition of EA significantly ($P < 0.05$) increased the cold carcass weight compared with NE but weight was similar to EB. EA also increased drumstick and heart weights, which were better than EB, and supported highest wing weight but decreased the weight of empty crop. Similarly, addition of EB significantly ($P < 0.01$) increased the dressing percentage and breast with bone weight compared with NE and EA. EB also significantly decreased the weight of thighs compared with NE and EA. Both EA and EB significantly reduced the weight of total gut relative to NE.

The wDDGS levels x enzyme interaction (Table 3) had no significant effect on cold carcass, thighs as well as breast with bone weights and total gut. The dressing percent was significantly better at 15% wDDGS when supplemented with enzyme B than enzyme A. There were significant interactions ($P < 0.05$) for the proportional weights of liver, heart, and empty gizzard. Inclusion of 15% wDDGS with EA reduced wings weight of broiler chickens compared to 15% wDDGS with EB, though similar to 0% wDDGS with no enzyme. Similarly, 15% wDDGS supplemented with enzyme A had the best heart weight. It also presented better empty gizzard compared to 15% wDDGS with EB, though similar to 0% wDDGS with no enzyme. Meanwhile, 0%

wDDGS with NE presented higher empty crop and proventriculus weights compared to 0% wDDGS with enzyme A.

As body weight gain was higher at 0% wDDGS level, the cold carcass weight was also higher in birds fed that level. Among the meat, the breast meat is considered by humans to be the best meat which was also higher at 0 and 15% wDDGS levels. As enzyme reduced body weight gain of broilers at 15% wDDGS, it also reduced cold carcass weight at the same level of wDDGS. Like feed intake, enzyme slightly increased cold carcass weight of birds for 15% wDDGS level, though the weights of these groups of birds were still lower than 0 % wDDGS level. As the ultimate outcome for a broiler is its carcass weight, low carcass weight at 15% wDDGS level will reduce the profit margin of farmers and even the enzyme supplementation will not be able to recover the profit.

Manipulating the diets and nutrients of broiler chickens has been found to affect the composition of the carcass, muscles and organs. Additionally, it affects the quality features in meat chickens at the finisher stage (Juzl *et al.*, 2012). The live weight gain of birds in this present study was lower for 15% wDDGS than 0% wDDGS was reflected in the cold carcass weight (g) in both caged and deep litter birds. This is a confirmation of the findings of Ranoet *al.* (2012) who reported the lower carcass yield and organ weights recorded could be attributed to poor digestibility and poor utilization of feed by birds on higher wheat-based wDDGS levels. However, the supplementation of enzyme A used in this research increased the cold carcass weight of caged birds which is the primary concern in broiler production. Some internal organs such as the empty crop, total gut, empty gizzard and liver may have had a higher weight due to the 15% wDDGS's fibre content but this has reduced economic value.

Almost all of the data obtained for the components of the carcass and internal organs on caged and deep litter birds were numerically comparable except for the final live weight and cold carcass weight, where the former increased while the latter decreased.

At the end of the trial in the cages the final live weight had considerably risen and the reason that the cold carcass weight was lower than that of the deep litter birds is not clearly understood. Increased thigh and wing weights were observed with 15% wDDGS inclusion however, a decreased breast weight was also exhibited which is an undesirable result. Wang *et al.* (2007) found that the inclusion of high percentages of DDGS also reduced the breast meat yield which they attributed to the overall reduced performance possibly due in part to a deficiency of some amino acids that were not typically accounted for in formulation and in part to reduced feed

quality associated with higher inclusion of DDGS.

Conclusion

It appeared that inclusion of 15% wDDGS in the diets of broilers supported increased liver weight of broilers managed on deep litter, weights of thigh and wing of caged birds as well as that of empty gizzard of both caged and deep litter managed birds. Meanwhile, enzyme supplementation enhanced the cold carcass weight of caged broilers.

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Table 2. Means for the main effects of wDDGS and enzyme on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets (45 days of age)

Parameters (%)*	DIETS								
	0	wDDGS (%) 15	SEM	P value	NE	Enzyme Inclusion		SEM	P value
					EA	EB			
FLW(g)	3150	2956	70.8	0.064	3075	3141	2944	86.8	0.283
Cold Carcass (g)	2848	2751	69.2	0.335	2825	2816	2759	84.7	0.836
Dressing percent	70	72	0.9	0.144	69 ^b	71 ^{ab}	74 ^a	1.10	0.010
Major Carcass Components									
Breast + Bone	33	31	0.57	0.026	32	32	32	0.7	0.945
Thighs	21	22	0.5	0.154	21	22	22	0.6	0.890
Drumsticks	10	10	0.2	0.925	10	10	11	0.2	0.889
Neck & Back	15	16	0.3	0.076	16	16	16	0.3	0.330
Wings	7	7	0.2	0.325	7	7	7	0.2	0.867
Edible Organ Components									
Liver	2.8	3.2	0.05	0.023	2.8 ^b	3.2 ^{ab}	3.1 ^a	0.06	0.025
Heart	0.74	0.63	0.073	0.287	0.74	0.66	0.64	0.089	0.699
Empty Gizzard	1.24	1.51	0.041	0.001	1.37	1.40	1.36	0.050	0.871
Non-Edible Components									
Total Gut	6.7	6.8	0.26	0.856	7.4	6.4	6.4	0.32	0.086
Empty Crop	0.54	0.59	0.072	0.640	0.51	0.47	0.72	0.088	0.134
Proventriculus	0.44	0.50	0.027	0.101	0.50	0.45	0.50	0.033	0.605

*a, b Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means, *unless otherwise stated, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B, FLW= Final Live Weight*

Table 3. Means for the interaction effect of wDDGS x enzyme on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets (45 days of age)

Parameters (%)*	Enzyme Inclusion						SEM	P value
	No Enzyme			+ Enzyme				
	0 NE	15 NE	wDDGS (%) 0 EA	15 EA	0 EB	15 EB		
Final Live Weight (g)	3181	2969	3159	3122	3111	2777	122.7	0.486
Cold Carcass (g)	2885	2764	2803	2828	2855	2662	119.8	0.654
Dressing percent	68	72	71	70	70	77	1.6	0.123
Major Carcass Components								
Breast + Bone	34	32	34	30	32	31	1.0	0.164
Thighs	20	22	21	22	22	22	0.9	0.533
Drumsticks	11	10	10	10	10	11	0.3	0.862
Neck & Back	15	16	15	17	16	16	0.4	0.137
Wings	7	7	7	7	7	7	0.3	0.630
Edible Organ Components								
Liver	2.5 ^b	3.1 ^a	3.1 ^a	3.2 ^a	2.9 ^{ab}	3.3 ^a	0.09	0.004
Heart	0.89	0.59	0.68	0.65	0.65	0.63	0.126	0.452
Empty Gizzard	1.22	1.52	1.30	1.49	1.21	1.51	0.070	0.674
Non-Edible Components								
Total Gut	7.4	7.3	6.4	6.5	6.4	6.5	0.451	0.968
Empty Crop	0.6 ^a	0.5 ^{ab}	0.5 ^b	0.4 ^{ab}	0.4 ^{ab}	1.0 ^a	0.124	0.018
Proventriculus	0.43	0.51	0.44	0.45	0.44	0.55	0.02	0.517

a, b, c, d Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means,

**unless otherwise stated, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B*

Table 4. Means for the main effects of wDDGS and enzyme on carcass characteristics and internal organs (% carcass weight) of broilers fed the experimental diets during the one week extended period in cages (52 days of age)

Parameters (%)*	Diets				Enzyme Inclusion				
	0	15	SEM	P value	NE	EA	EB	SEM	P value
Final Live weight (g)	3954	4079	0.1	0.001	3867 ^c	4225 ^a	3958 ^b	0.2	0.001
Cold Carcass (g)	2738	2638	38.0	0.075	2582 ^b	2820 ^a	2661 ^{ab}	46.5	0.005
Dressing percent	61	59	1.0	0.401	58 ^b	58 ^b	66 ^a	1.3	0.001
Major Carcass Components									
Breast + Bone	35.3 ^a	32.3 ^b	0.47	0.001	32.6 ^b	33.2 ^b	35.7 ^a	0.48	0.003
Thighs	13.6 ^b	14.7 ^a	0.29	0.002	14.4 ^a	14.8 ^a	13.2 ^b	0.28	0.001
Drumsticks	11.9	11.8	0.11	0.712	11.8 ^{ab}	12.2 ^a	11.6 ^b	0.12	0.016
Neck & Back	17	18	0.33	0.251	17	18	17	0.5	0.331
Wings	7.0 ^b	7.3 ^a	0.07	0.021	7.5 ^a	6.8 ^c	7.1 ^b	0.09	0.001
Edible Organ Components									
Liver	2.7	2.5	0.07	0.060	2.7	2.5	2.6	0.08	0.544
Heart	0.7 ^a	0.6 ^b	0.003	0.001	0.7 ^a	0.7 ^a	0.68 ^b	0.004	0.001
Empty Gizzard	1.2 ^b	1.4 ^a	0.02	0.001	1.4 ^a	1.3 ^b	1.2 ^b	0.02	0.001
Non-Edible Components									
Total Gut	10	11	0.2	0.025	11 ^a	10 ^b	10 ^b	0.3	0.002
Empty Crop	0.4 ^b	0.5 ^a	0.03	0.005	0.5 ^a	0.3 ^b	0.5 ^{ab}	0.04	0.015
Proventriculus	0.3	0.4	0.01	0.056	0.4	0.3	0.4	0.002	0.196

a, b, c, Means bearing different letters within rows are significantly different (P<0.05), SEM=Standard Error of means

**unless otherwise stated, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B*

Table 5. Means for the effects of wDDGS x enzyme on carcass characteristics and digestive organs (% carcass weight) of broilers fed the experimental diets during the one week extended period in cages (52 days of age)

Parameters (%)*	Enzyme Inclusion		wDDGS (%)				SEM	P value
	No Enzyme	+ Enzyme	No Enzyme		+ Enzyme			
	o NE	15 NE	o EA	15 EA	o EB	15 EB		
Parameters (%)*								
Final Live weight (g)	3676 ^f	4057 ^c	4255 ^a	4193 ^b	3931 ^e	3985 ^d	0.2	0.001
Cold Carcass weight (g)	2558	2607	2894	2746	2762	2560	65.7	0.155
Dressing percent	61 ^{abc}	54 ^c	59 ^{bc}	56 ^{bc}	63 ^{ab}	68 ^a	1.8	0.007
Major Carcass Components								
Breast + Bone	34	32	34	32	38	34	0.8	0.228
Thighs	14	15	14	16	13	13	0.4	0.328
Drumsticks	11.3 ^b	12.2 ^a	12.1 ^a	12.2 ^a	12.2 ^a	11.0 ^b	0.17	0.001
Neck & Back	17	17	18	18	16	18	0.7	0.076
Wings	7.3 ^{abc}	7.8 ^a	7.0 ^{bcd}	6.6 ^d	6.8 ^{cd}	7.4 ^{ab}	0.12	0.001
Edible Organ Components								
Liver	3.0 ^a	2.3 ^b	2.6 ^{ab}	2.4 ^b	2.5 ^{ab}	2.7 ^{ab}	0.12	0.007
Heart	0.7 ^c	0.7 ^d	0.6 ^e	0.8 ^a	0.8 ^b	0.6 ^f	0.01	0.001
Empty Gizzard	1.4 ^a	1.4 ^a	1.1 ^b	1.4 ^a	1.2 ^b	1.2 ^b	0.03	0.001
Non-Edible Components								
Total Gut	11	11	10	11	9	10	0.4	0.070
Empty Crop	0.5 ^a	0.5 ^a	0.2 ^b	0.5 ^a	0.4 ^{ab}	0.5 ^a	0.05	0.027
Proventriculus	0.4 ^a	0.3 ^{ab}	0.3 ^b	0.4 ^a	0.4 ^{ab}	0.4 ^a	0.02	0.002

a,b,c,d Means bearing different letters within rows are significantly different ($P < 0.05$), SEM=Standard Error of means,

*unless otherwise stated, NE= No Enzyme, EA= Enzyme A, EB= Enzyme B.