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Amino acids digestibility, metabolizable energy availability, carcass and digestive tract status in response to wheat screening and multi enzyme in Japanese quail

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In the first experiment nutrient retention was recorded by 120 Japanese quails (25-d old) to determine AME, AMEn, coefficient of apparent protein digestibility (based on excreta nitrogen) and amino acid digestibility (based on ileum content). Corn-soybean meal was used as a control diet and, in the other two treatments, wheat screening (W.S) (at a level of 40%) with and without enzyme as the test ingredient were supplemented to the basal diet. Chromic oxide was included in all diets (0.3%) as an indigestible marker. The second experiment was carried out with 480 Japanese quails to compare effects of wheat screening on carcass and digestive tract status. Quail chicks were randomly assigned to 8 treatments. Each treatment included 4 replicates and 15 quails chicken in each. Complete random designs (CRD) in factorial arrangement were modulated in 0, 5, 10 and 15% of W.S included 0 and 0.5 kg multi enzyme per ton. The first experiment results shown that dry mater digestibility and apparent metabolizable energy corrected for nitrogen (AMEn) not affected by multi enzyme addition ($P>0.05$). Improved apparent metabolizable energy, protein and amino acid digestibility were found by multi enzyme compared to control (enzyme absent) ($P<0.05$). The second experiment results shown that small intestine length was decreased significantly by 0% level of W.S in comparison to other levels ($P<0.05$). Duodenum and ileum length were significantly decreased by 0% W.S level compared to 10 and 15 % ($P<0.05$). Jejunum length was significantly decreased by 0 % of W.S in comparison to other levels ($P<0.05$). Caecum length was significantly decreased by 0% and 10 % of wheat screening compared to 15 % in this respect ($P<0.05$). Viscosity was reduced significantly in 0% W.S without enzyme rather than the other treatments. The results this study have shown that W.S Apparent metabolizable energy, protein and amino acid digestibility were improved by multi enzyme in Japanese quails.

Key words: Metabolizable Energy, Amino Acid Digestibility, Ileum, Excreta, Japanese quail.
AMEn: Apparent Metabolizable Energy corrected for nitrogen

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Introduction

Energy is one of the most expensive parts of a poultry ration, accurate knowledge of the available energy content of feedstuffs is necessary to formulate the most economical least-cost rations and to achieve profitable production (Sibbald, 1982). The digestibility and availability of nutrients in feed ingredients may vary depending to physiological status of the bird. A number of studies have indicated that the energy metabolisability and protein digestibility of feed ingredients were influenced by many variables including species, age, genotype, sex of bird and environmental conditions. The chemical composition and energy availability of wheat can vary (Kim *et al*., 2003). Therefore, there is a large variation in the chemical composition of wheat by-products such as W.S because of different sources of wheat (e.g., soft vs. hard) Kim *et al*., 2003) and differences in processing techniques. Feed ingredients of plant origin contain a number of components that cannot be digested by monogastric species because the lack or insufficiency of endogenous enzyme secretions. In addition to being unavailable to the animal, these components also lower the utilization of other dietary nutrients, leading to depressed performance. In recent years, with the development of enzyme products targeting specific substrates, the use of feed enzymes to ameliorate the effects of these antinutritive factors has received more attention (Ravindran *et al*., 1999). Research have indicated that Exogenous enzyme improve bird performance (Saleh *et al*., 2003; Yu and Chung, 2004; Cowieson and Adeola, 2005), AME (Meng *et al*., 2005), and ileal amino acid digestibility for some amino acids (Zanella *et al*., 1999). In contrast, other workers have shown no effect of supplementation of some enzyme preparations on AME (Scheideler *et al*., 2005) ileal digestible energy and nitrogen (Cowieson and Adeola, 2005) and protein; starch (Meng *et al*., 2005). The mechanism by which dietary AME content increases with enzyme supplementation is not clear. Zanella *et al*. (1999) and Meng *et al*. (2005) have found no improvement in starch (the primary energy source) digestion after enzyme supplementation with a variety of enzymes. The digestibility of amino acids (DAA) is considered a suitable measure of feed protein quality in order to formulate economically feasible poultry diets that improve the overall efficiency of protein use and reduce emission of nitrogenous gases from the farms. Most of the published data on digestible amino acids in feed ingredients have been obtained from excreta assays with roosters (Sibbald, 1986; Green *et al*., 1987; Parsons, 1991; NRC, 1994; Rhone-Poulenc, 1993). The majority of published values currently available on digestible amino acids for poultry are based on excreta analysis because of its simplicity and because the assay can be carried out on large numbers without sacrificing the birds (Huang *et al.*, 2006). It is being

increasingly recognized that the determination of amino acid digestibility in poultry should be based on the analysis of ileal digesta, because of the modifying and variable effects of caecal microflora (Ravindran *et al*., 1999), but data sources on ileal digestibility values are limited. All dietary components are important in poultry feed formulation, but critical attention should be paid to the dietary protein. Nitrogen intake may be influenced by metabolizable energy and consequently excreta nitrogen. For these reasons and the vital role energy as a more important regulator of feed intake, determination of metabolizable energy and protein digestibility is important in by products. Therefore, the present study was designed with the objective of amino acids digestibility and metabolizable energy availability of wheat screening in response to multi enzyme in Japanese quail.

Material and methods

Experimental 1

Nutrient Retention: Nitrogen-corrected apparent metabolizable energy was determined according to the method of Newkirk et al. (2003) using 120 quail raised to 25 d of age. Ten birds were randomly allocated per cage and the cages (experimental units) were randomly assigned to one of three dietary treatments. The basal diet contained corn and soybean meal as the major ingredients. Corn-soybean meal was used as a control diet and, in the other two treatments, wheat screening (W.S) (at a level of 40%) with and without enzyme as the test ingredient were supplemented to the basal diet. The diets were fed to the birds for 7d. Chromic oxide was included in all diets as an indigestible marker. The basal and test diets are presented in Table 1. Feed and water were offered *ad libitum* at all times. Excreta were collected daily for the last 3 d and samples from each replicate were pooled on the final day and frozen at -20°C for further analysis (Scott *et al*., 1998). Before analysis, the frozen samples were removed from the freezer, dried and ground. Dry matter and crude protein of diets and excreta were determined by methods according to the Association of Official Analytical Chemists (AOAC, 1990). Upon final collection, all of the birds were euthanased and the contents of the distal ileum (Meckel's diverticulum to a point 3 cm proximal to the ileo-caecal junction) were collected and freeze-dried. Amino acid concentrations in diets and ileal digesta were determined by high performance liquid chromatography (HPLC) by the method of Ravindran and Bryden (1999). Apparent amino acid digestibility of diet was determined by the method described by Tendoeschate *et al*. (1993).

Chromic oxide was determined spectrophotometrically by the method of Fenton and Fenton (1979). Gross energy contents of diets and excreta were

determined using an adiabatic bomb calorimeter. The diet AMEn was multiplied by a factor of 1.0474 to compensate for the test and basal diets having 4.88% of premix (oyster shell, dicalcium phosphate, salt, chromic oxide, vitamin and mineral premix) per 95.12 of macro ingredients (Newkirk *et al*., 1997; Saki *et al*., 2008). Therefore, the AMEn of wheat screening was calculated as: AMEn of W. S= ((test diet AMEn-basal diet AMEn×0.6)/4) ×10) Tendoeschate *et al*. (1993).

Table 1. Formulation of rations and diet composition (%)

Ingredient (%)	**Wheat screening-enzyme**	**Wheat screening + enzyme**	**Basal diet**
Corn grain	27.37	27.32	69.55
Soybean meal	19.68	19.68	17.10
Wheat screening	40	40	0
Wheat bran	8	8	8
oil	0.1	0.1	0.5
Lysine	0.65	0.65	0.65
Met+Cys	0.2	0.2	0.2
Di calcium phosphate	1.4	1.4	1.4
Oyster shell	1.45	1.45	1.45
Salt	0.35	0.35	0.35
Vitamin permix[1]	0.25	0.25	0.25
mineral permix[2]	0.25	0.25	0.25
Enzyme[3]	0	0.05	0
Chromic oxide	0.3	0.3	0.3
Total (%) calculated	100	100	100
ME (Kcal/kg)	2628.11	2628.11	2839.6
CP (%)	18.06	18.06	14.83

1-Vitamin premix; $B_{1,}$ 3.3g; $B_{2,}$ 0.72g; K3, 1.6g; Vitamin E; 14.4g; Vitamin D, 7g; Vitamin A, 7.7 g; Pantothenic acid , 12g; Pyridoxine, 6.2, mg, B_{12} 14.4g; Coline chloride, 440 mg. 2-mineral premix(Concentration per kg); Mn 72 g; Cu 10 g; Zn 100 g; Fe 100 g; I 2 g; Co 0.2 g. 3-multi enzyme contain:phytase, lipase, β-glucanase, xylanase, α-amylase, protease, pantosanase, hemiselulase, selulase and pectinase.

Diet and wheat screening protein or amino acid digestibility

Protein digestibility or D.A.A of diets and wheat screening was determined using the procedures described by Tendoeschate *et al*. (1993) as follow:

$$DC_{diet}= 1-[(\frac{M\ diet}{Mi,e}) \times (\frac{Ci,e}{C\ diet})]$$

Where DC_{diet}, digestibility coefficient of protein or A.A in diet; M_{diet}, marker concentration in diet; $M_{i,e}$, marker concentration in ileal digesta (i) or excreta (e); C_{diet}, concentration of protein or A.A in diet; $C_{i,e}$, concentration of protein or A.A in ileal digesta (i) or excreta (e).

$$DC_{fs}=(DC_{test} \times C_{test} - DC_{basal} \times C_{basal} \times 0.6)/(C_{test} - C_{basal} \times 0.6)$$

Where DC_{fs}, digestibility coefficient of protein or A.A in the wheat screening; DC_{test}, digestibility coefficient of protein or A.A in the test diet; C_{test}, the concentration of protein or A.A in the test diet; DC_{basal}, digestibility coefficient of protein or A.A in the basal diet; C_{basal}, concentration of protein or A.A in basal diet.

Experimental 2

A total of 480 quail chicks unsexed in 10 day-old, were randomly assigned to 8 treatments in similar mean weight. Each treatment included 4 replicates and 15 quails chicken in each. Complete random designs (CRD) in factorial arrangement were modulated in 0, 5, 10 and 15% of W.S included 0 and 0.5 kg per ton multi enzyme. Rations were based on yellow corn, soybean meal, and wheat screening (Table 2). Diets were isoenergitic in all period and formulated by NRC (1994).

***Carcass and intestinal measurements*:** At 42 days of age, 2 birds per replicate were randomly selected and slaughtered. Immediately after dressing, the GIT was removed. The digestive tracts from the gizzard to the bile duct and from the bile duct to the Meckel's diverticulum were dissected and designated duodenum and jejunum, respectively. The tract between the Meckel's diverticulum and the ileocaecal junction was designated ileum. The caeca was also dissected. The lengths of all mentioned segments were recorded. In addition Breast meat, back, thigh, heart, liver, gizzard, pancreas, removed and weighed.

Table 2. Percentage composition, calculated and analyzed contents of main nutrient

Ingredient	T1	T2	T3	T4	T5	T6	T7	T8
Corn grain	55.75	52.80	49.85	46.90	55.70	52.75	49.80	46.85
Soybean meal	30.00	30.00	30.00	30.00	30.00	30.00	30.00	30.00
Wheat screening	0	5	10	15	0	5	10	15
Corn gluten	4.60	3.92	3.24	2.54	4.60	3.92	3.24	2.54
Fish meal	3	3	3	3	3	3	3	3
Oyster shell	2.98	2.57	2.15	1.74	2.98	2.57	2.15	1.74
Di calcium phosphate	2.78	1.86	0.94	0.02	2.78	1.86	0.94	0.02
Salt	0.32	0.31	0.3	0.3	0.32	0.31	0.3	0.3
Lysine	0.07	0.04	0.02	0	0.07	0.04	0.02	0
mineral permix[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin permix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Multi enzyme[3]	0	0	0	0	0.05	0.05	0.05	0.05
Total (%)	100	100	100	100	100	100	100	100
calculated								
ME(Kcal/kg)	2800	2800	2800	2800	2800	2800	2800	2800
Protein (%)	23.2	23.2	23.2	23.2	23.2	23.2	23.2	23.2
Crude Fiber (%)	3.5	4.02	4.54	5.05	3.5	4.02	4.54	5.05
Lysine (%)	1.26	1.26	1.26	1.27	1.26	1.26	1.26	1.27
Met+Cys (%)	0.79	0.79	0.79	0.79	0.79	0.79	0.79	0.79
Sodium (%)	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Calcium (%)	1.97	1.62	1.26	0.9	1.97	1.62	1.26	0.9
Available Phosphorus (%)	0.74	0.61	0.47	0.34	0.74	0.61	0.47	0.34
Electrolyte balance	208.9	216.74	224.28	231.87	208.9	216.74	224.28	231.87
Analysed								
Dry mater (%)	90.91	90.91	91.22	91.15	90.56	90.84	91.32	91.29
Crude Protein (%)	23.22	23.25	23.25	23.19	23.22	23.25	23.25	23.19
Crude Fiber (%)	3.98	4.40	4.90	5.15	3.98	4.40	4.90	5.15
Ether Extract (%)	4.25	4.25	4.10	4.18	4.28	4.25	4.15	4.19
Ash (%)	6.32	6.65	5.91	5.50	5.98	6.10	5.75	6.31

T1=0% W.S without enzyme. T2= 5% W. without enzyme. T3= 10%W.S without enzyme. T4= 15%W.S without enzyme. T5= 0%W.S with enzyme. T6=5% W.S with enzyme. T7= 10% W.S with enzyme. T8= 15% W.S with enzyme. 1- mineral premix(Concentration per kg); Mn 72 g; Cu 10 g; Zn 100 g; Fe 100 g; I 2 g; Co 0.2 g. 2-Vitamin premix; $B_{1,}$ 3.3g; $B_{2,}$ 0.72g; K3, 1.6g; Vitamin E; 14.4g; Vitamin D, 7g; Vitamin A, 7.7 g; Pantothenic acid , 12g; Pyridoxine, 6.2, mg, B_{12} 14.4g; Coline chloride, 440 mg. 3-multi enzyme contain:phytase, lipase, β-glucanase, xylanase, α-amylase, protease, pantosanase, hemiselulase, selulase and pectinas.

Viscosity measurement

Total intestinal digesta were removed from two birds on days 42 and pooled in collection tubes. The digesta samplings were gently removed with hand in collection tubes. Samples were freezed at –20°*C* until analyzing. Intestinal digesta viscosity measured by the method of Shoemaker *et al*. (1981).

Statistical analysis

In finally total data was statistically analyzed. One-way analysis of variance was performed using the general linear model procedure of the SAS software (SAS Institute, 2004). Duncan's multiple range test was used to compare treatments ($P<0.05$). Complete random designs (CRD) in factorial arrangement were considered in Experiment 2.

$$Y_{ijk}=\mu+F_i+L_j+F_iL_j+e_{ijk}+\varepsilon_{ijkl}$$

Where Y_{ijk}, observed trait; μ, overall mean; F_i, effect of wheat screening, L_j, effect of enzyme; $Fi \times Lj$, interaction of L_j and F_i; and e_{ijk}, random error; ε_{ijkl}, sampeling error.

A complete random design (CRD) was applied for Experiment 1 with 3 experimental diets, 4 replicates and 10 chickens in each.

$$Y_{ijk} = \mu + T_i + e_{ij}$$

Where Y_{ijk}, observed trait; μ, overall mean; $T_{i,}$ effect of treatment; e_{ijk}, random error.

Results

Nutrient Retention: The chemical compositions of wheat screening have shown in Table 3 expressed an as-fed basis. The nutrient retention of wheat screening is presented in Table 4. This result indicated that Dry Mater (DM) digestibility and apparent metabolizable energy corrected by nitrogen (AMEn) were not affected by multi enzyme addition ($P>0.05$). Apparent metabolizable energy, protein and amino acid digestibility improved with multi enzyme ($P<0.05$). Amino acid digestibility (Ile, Val, Ala, Gly and Phe) were improved by multi enzyme compared with amino acid digestibility without multi enzyme ($P<0.05$) (Table 6).

Table 3. Chemical composition of wheat screening on as-fed basis (%)

Wheat Screening	DM	CP	CF	EE	ASH	GE*
	90.95	14	10.56	2.74	7.47	4003.90

*Gross energy (Kcal/kg)

Table 4. Wheat screening dry mater, protein digestibility and Metabolizable energy in excreta in-25 day-old quails (%)

Treatment	DM Digestibility (%)	Protein digestibility (%)	AME (kcal/kg)	AMEn (kcal/kg)
Wheat screening - enzyme	0.08±0.67	0.03±0.70 [b]	177.00±2816.86 [b]	214.66±2731.43
Wheat screening +enzyme	0.05±0.79	0.05±0.79 [a]	121.37±2979.25 [a]	120.97±2857.53
SEM	0.04	0.01	34.86	52.66
P	0.16	0.02	0.04	0.19

Means with common superscripts in same column are not significantly different ($P < 0.05$). Means ± Standard Deviation

Carcass and intestinal measurements

There were no significant differences between the percentage of gastronomical tract section weight ($P>0.05$). Duodenum and ileum length significantly increased by 10 and 15% wheat screening level compared to control (0% W.S) ($P<0.05$). Jejunum length significantly increased by 5, 10 and 15% of wheat screening in comparison to control (0% W.S) ($P<0.05$). Caecum length was significantly increased by 15% compared to 0 and 10 % W.S in this respect ($P<0.05$).

Viscosity measurement

Viscosity have shown significant increased by 10 and 15% wheat screening without enzyme rather than the 5, 10 and 15% wheat screening with 0.05% enzyme ($P<0.05$).

Discussion

Nutrient Retention: Chemical composition of wheat screening indicated that little variation in dry matter (DM), crude protein (CP), ether extract (EE) and gross energy (GE) content, but the similar variation were reported by Slominski *et al*. (2004), Ahmadi and Karimov (2010), Mazhari *et al*. (2011). A big variation in the content of CF and Ash indicated that the fiber fraction in the W.S is more susceptible to vary with the source of grains than other proximate components. This variation may be due to growing conditions, wheat cultivars, the amount of weeds and postharvest status storage (Gutierrez *et al*., 2008).

There is a negative correlation between fiber content and protein digestibility (Mandal *et al*., 2005). The lower protein digestibility of wheat screening in this study in comparison to the other results probably is due to higher fiber content (10.56%). This finding implies fiber impedes protein utilization (Symbaya *et al*., 1996). This mechanism in not clear, but the indigestible protein fraction in the wheat screening may be bound to, or encapsulated by fibrous components. Variable fiber fraction probably is the predominant factor influencing the ME of W.S (Villamide and San Juan, 1998; Wang *et al*., 2009). Kim *et al*. (2005) reported a wide range of variations in the fiber fraction of W.S because of the wheat variety. The differences in AMEn values of W.S have been attributed to the fiber and NSP contents as well as the age and type of birds and also the basal diet used for estimation of AME values. Logically, greater arabinoxylan content in a feed or feedstuff will increase the quantity of entrapped nutrients and thus provide a greater positive effect with enzyme supplementation (Nortey *et al*., 2008; Table 4). In this study the amino acid contents of wheat screening are nearly similar with NRC (1994), Van Wijk *et al*. (1998) and Kadim *et al*. (2002) (Table 5). Uptake of amino acids and other nitrogenous compounds is not thought to take place after the terminal ileum (Webb, 1990). Therefore amino acid disappearance may have arisen by some other routes, for instance by microbial fermentation. Microbial metabolism of amino acids in the poultry hindgut comprises the degradation and synthesis these materials (Ravindran *et al*., 1999; Kadim *et al*., 2002). Therefore the disappearance of amino acids in poultry hindgut is determined by balance of catabolism and anabolism of amino acids. When the net result is a good catabolism, the output of amino acids in excreta will be decreased, resulting in the overestimation of amino acid digestibility. But, when the net result is anabolism of amino acids, under estimation of digestibility may be occurred. In net catabolism, ammonia may be absorbed but not utilized by the birds and completely excreted in the urine as uric acid (Salter, 1974). According to Twombly and Meyer (1961) reports, endogenous nitrogen secretions between birds are varied which is due to feed with different diets. Also, Yen *et al*. (2001) have stated that wheat and barley contain soluble non-starch polysaccharides and high fiber that increased endogenous secretion in broiler chickens and affected this cereals digestibility. Present study results were agreement with Huang *et al*. (2007) and were lower than results reported by Saki *et al*. (2009; Table 6. But variations exist due to differences in methodology followed, site of measurement (ileal or excreta) and correction for endogenous losses (true or apparent), age and sex of birds (Batal and Parsons, 2002; Huang *et al*., 2006). It can be speculated that part of the observed differences in apparent ileal digestibility among the class of birds was caused

by differences in endogenous losses (Ravindran *et al*., 2004). Another reason may be ordained to the relationship between amino acid intake and digestibility, because Payne *et al*. (1971) and Sibbald (1979) documented that the greater intake of an amino acid, could lead to the higher digestibility.

Table 5. Amino acid contents in wheat screening fed to quail (mg/g as-fed)

Ingredients	Glu	Asp	Ser	Ile	Val	Ala	Lys	Phe	Met	Thr	Leu	Gly	Arg	Tyr	His
wheat screening	23.20	5.37	3.65	4.75	5.28	3.14	4.20	4.61	4.52	3.14	6.75	3.05	4.85	3.60	4.69

Table 6. Amino acids digestibility in 25 -day-old quail (%)

Treatment /amino acids	W.S - enzyme	W.S + enzyme	SEM	P
Lys	0.04±0.62	0.02±0.65	0.01	0.33
Glu	0.07±0.64 [a]	0.05±0.50 [b]	0.01	0.007
Asp	0.05±0.61	0.09±0.64	0.02	0.51
Ser	0.11±0.59	0.07±0.65	0.04	0.64
Ile	0.05±0.60 [b]	0.06±0.70 [a]	0.01	0.01
Val	0.06±0.53[b]	0.07±0.61[a]	0.01	0.01
Ala	0.04±0.62 [b]	0.03±0.67 [a]	0.008	0.04
Met	0.07±0.57	0.11±0.59	0.06	0.85
Thr	0.08±0.50	0.09±0.59	0.03	0.16
Leu	0.02±0.45[b]	0.06±0.70[a]	0.01	0.001
Gly	0.03±0.49[b]	0.06±0.61[a]	0.02	0.04
Arg	0.09±0.48	0.08±0.64	0.05	0.13
Tyr	0.05±0.50	0.10±0.61	0.04	0.17
His	0.14±0.66	0.05±0.69	0.03	0.56
Phe	0.02±0.57[b]	0.08±0.67[a]	0.02	0.04

Means with common superscripts in same raw are not significantly different ($P < 0.05$). Means ± Standard Deviation

Carcass and intestinal measurements

Significant differences between the percentages of gastronomical tract sections weight were not observed by wheat screening and enzyme levels in Table 7. Sarica *et al.* (2009) did not observe any differences with addition enzyme in wheat based diets on carcass of quail. It is reported that more changes in the all intestine length and small intestine length are associated with fiber level of diet, digestibility and chemical compounds (Sarica *et al*., 2009). Matthias and Abdel Rahmaan (2003) have reported that high-fiber diet (40% and 45% oats) in quail diets increased gizzard weight, intestinal length and

decreased liver weight. With regard to lower wheat screening levels, (less fiber) in the present study Compared with the high amount of fiber in Matthias and Abdel Rahmaan (2003), this value has no effect on organ weights. It is expected that in treatments 8 and 4 (15% wheat screening with and without enzyme) due to higher viscosity, increases in length of small intestine could be observed. This result was agreement with other researches (Viveros *et al*., 1994; Smits *et al*., 1997; Simon, 1998; Iji *et al*., 2001). The effect of viscosity on increase small intestinal length is due to increase the microbial activity, that it is observed in this study. Fenna and Boag (1974) have found no differences in gut size between quail fed a low-fiber diet and those fed the same diet containing 300g cellulose/kg.

Table 7. Effect of different levels of wheat screening and multi enzyme on carcass characteristics of Japanese quail (42 day old)

Effects	levels	Carcass Weight (% of live weight)	Breast (% of Carcass)	Legs (% of Carcass)	Back (% of Carcass)	Neck (% of Carcass)	Wings (% of Carcass)
W.S (%)	0	3.65±62.20	0.99±37.92	0.93±24.75	0.79±21.84	0.38±8.56	1.37±6.93
	5	3.50±60.86	1.28±36.91	1.63±25.28	1.20±22.39	0.27±8.70	1.61±6.69
	10	52.51±60.59	1.12±37.10	2.10±25.37	1.41±22.36	0.32±8.46	2.08±6.68
	15	1.86±60.56	1.72±37.01	2.01±23.87	1.48±22.56	0.37±8.53	1.57±8.00
SEM		0.95	0.27	0.61	0.25	0.05	0.56
Multi Enzyme (%)	0	3.62±61.09	1.21±37.57	1.49±24.97	1.27±220.4	0.33±8.48	1.89±6.92
	0.05	2.23±61.01	1.40±36.89	2.06±24.67	1.19±22.54	0.34±8.63	1.56±7.24
SEM		0.67	0.38	0.43	0.36	0.08	0.40
Treatment 1-8	0% W.S[1]	4.14±64.05	1.22±37.84	0.71±24.74	0.61±21.36	0.35±8.54	0.91±7.50
	5% W.S	3.44±59.72	1.11±37.01	1.85±25.82	1.20±22.39	0.30±8.58	1.90±6.16
	10%W.S	3.25±60.18	1.08±37.77	1.85±24.99	1.59±22.45	0.26±8.29	2.90±6.48
	15%W.S	2.21±60.43	1.44±37.67	1.06±24.32	1.39±21.96	0.38±8.51	1.17±7.52
	0%w.s+0.05M E.[2]	1.91±60.36	0.78±38.00	1.16±24.77	0.66±22.33	0.42±8.51	1.57±6.37
	5%w.s+0.05M.E	3.40±61.99	1.51±36.80	1.27±24.75	1.28±22.39	0.18±8.83	1.17±7.21
	10%w.s+0.05M.E	1.62±61.00	0.71±36.43	2.38±25.76	1.30±22.27	0.28±8.64	0.88±6.88
	15%w.s+0.05M.E	1.56±60.70	1.81±36.34	2.66±23.42	1.39±23.17	0.39±8.55	1.83±8.49
SEM		1.35	0.54	0.87	0.51	0.11	0.54
P-value	W.S	0.58	0.24	0.31	0.54	0.23	0.32
	Enzyme	0.93	0.08	0.64	0.18	0.07	0.58
	treatment	0.43	0.21	0.61	0.46	0.17	0.51
	W.S× Enzyme	0.18	0.97	0.69	0.44	0.34	0.51

Treatment1=0% W.S without enzyme. Treatment2= 5% W. without enzyme. Treatment3= 10%W.S without enzyme. Treatment4= 15%W.S without enzyme. Treatment5= 0%W.S with enzyme. Treatment6=5% W.S with enzyme. Treatment7= 10% W.S with enzyme. Treatment8= 15% W.S with enzyme. Means with common superscripts in same column are not significantly different ($P < 0.05$). Means ± Standard Deviation. 2- M.E= Multi Enzyme.

Table 8. The weight of different segments of the gastro-intestinal tract (% of live weight) of quail fed on w.s and enzyme in 42 days old

Effects	Levels	Total intestinal	Proventriculus	Gizzard	Liver	Heart	Pancreas	Spleen
W.S (%)	0	1.87±10.73	0.07±0.37	0.05±3.07	0.23±1.93	0.18±0.90	0.10±0.23	0.02±0.112
	5	1.41±10.98	0.08±0.43	0.31±3.21	0.25±2.21	0.11±0.88	0.08±0.31	0.97±0.113
	10	1.47±11.36	0.05±0.39	0.26±3.34	0.46±2.18	0.15±0.93	0.83±0.27	0.99±0.103
	15	0.69±11.18	0.07±0.38	0.23±3.33	0.16±2.14	0.09±0.87	0.03±0.32	0.02±0.114
SEM		0.48	0.21	0.11	0.10	0.05	0.02	0.005
Multi Enzyme (%)	0	1.33±10.80	0.07±0.38	0.33±3.16	0.34±2.04	0.13±0.90	0.07±0.30	0.02±0.10[b]
	0.05	1.46±11.33	0.07±0.40	0.37±3.32	0.27±2.19	0.15±0.89	0.09±0.27	0.02±0.11[a]
SEM		0.34	0.01	0.08	0.07	0.03	0.02	0.003
Treatment 1-8	0% W.S[1]	2.01±10.18	0.05±0.34	0.35±2.82	0.16±1.75	0.24±0.90	0.12±0.25	0.02±0.10[ab]
	5% W.S	1.27±10.31	0.07±0.41	0.16±3.07	0.27±2.14	0.07±0.90	0.05±0.32	0.01±0.09[b]
	10%W.S	0.07±11.12	0.06±0.42	0.18±3.49	0.50±2.16	0.10±0.91	0.03±0.30	0.03±0.10[ab]
	15%W.S	0.42±11.58	0.07±0.36	0.15±3.27	0.19±2.12	0.05±0.89	0.01±0.30	0.02±0.10[ab]
	0%w.s+0.05ME.[2]	1.66±11.28	0.07±0.39	0.54±3.32	0.14±2.11	0.13±0.91	0.08±0.21	0.03±0.12[ab]
	5%w.s+0.05M.E	1.27±11.66	0.09±0.46	0.37±3.36	0.22±2.27	0.15±0.87	0.09±0.29	0.01±0.12[a]
	10%w.s+0.05M.E	1.99±11.60	0.03±0.36	0.26±3.19	0.46±2.21	0.19±0.95	0.10±0.25	0.01±0.09[a]
	15%w.s+0.05M.E	0.69±10.77	0.07±0.41	0.29±3.39	0.15±2.16	0.12±0.84	0.05±0.35	0.03±0.12[ab]
SEM		0.68	0.03	0.16	0.15	0.07	0.04	0.007
P.value	W.S	0.58	0.31	0.34	0.28	0.86	0.18	0.45
	Enzyme	0.28	0.32	0.18	0.20	0.91	0.23	0.02
	treatment	0.65	0.31	0.15	0.44	0.99	0.36	0.06
	W.S× Enzyme	0.41	0.29	0.11	0.72	0.94	0.73	0.1

Treatment1=0% W.S without enzyme. Treatment2= 5% W. without enzyme. Treatment3= 10%W.S without enzyme. Treatment4= 15%W.S without enzyme. Treatment5= 0%W.S with enzyme. Treatment6=5% W.S with enzyme. Treatment7= 10% W.S with enzyme. Treatment8= 15% W.S with enzyme. Means with common superscripts in same column are not significantly different ($P < 0.05$). Means ± Standard Deviation. 2- M.E= Multi Enzyme.

Table 9. The lengths of different segments of the gastro-intestinal tract (cm) of quail fed on wheat screening and enzyme in 42 days old

Effects	Levels	Small intestine	Duodenum	Jejunum	Ileum	Caeca
W.S (%)	0	5.36±50.37^{b}	0.76±10.43^{b}	2.69±22.38^{b}	1.90±16.97^{b}	0.70±6.89^{c}
	5	3.66±56.46^{a}	0.71±11.33^{ab}	2.65±24.09^{a}	2.38±18.21^{ab}	1.02±7.94^{ab}
	10	3.05±57.15^{a}	0.86±12.60^{a}	4.06±27.00^{a}	1.88±18.89^{a}	1.08±7.69^{bc}
	15	4.17±58.24^{a}	0.07±11.35^{a}	1.73±27.12^{a}	1.75±19.50^{a}	0.66±8.66^{a}
SEM		1.54	0.28	1.10	0.57	0.31
Multi Enzyme (%)	0	5.55±53.73	1.20±11.30	3.66±25.51	2.02±17.81	1.12±7.88
	0.05	4.11±54.87	1.09±11.55	3.30±24.78	2.18±18.97	1.04±7.71
SEM		1.09	0.19	0.78	0.40	0.22
Treatment 1-8	0% W.S[1]	7.23±50.63	0.89±10.32^{d}	1.88±23.51^{ab}	1.94±16.89^{b}	0.50±6.67^{c}
	5% W.S	4.48±55.97	0.45±11.8^{abc}	2.87±24.32^{ab}	1.67±17.37^{ab}	0.14±7.87^{abc}
	10%W.S	1.71±57.12	1.03±12.32^{a}	5.80±27.01^{a}	1.96±17.81^{ab}	0.77±8.18^{ab}
	15%W.S	4.93±58.21	1.20±10.78^{bcd}	1.42±27.20^{a}	2.08±19.71^{ab}	0.81±8.80^{a}
	0%w.s+0.05ME.[2]	3.02±50.10	0.65±10.53^{cd}	3.01±21.25^{b}	1.99±17.05^{b}	0.83±7.11^{bc}
	5%w.s+0.05M.E	2.85±56.95	0.62±10.86^{bcd}	2.08±23.86^{ab}	2.79±19.05^{ab}	0.97±8.01^{abc}
	10%w.s+0.05M.E	4.13±57.17	0.59±12.87^{a}	1.29±26.98^{a}	1.04±19.97^{a}	1.16±7.20^{bc}
	15%w.s+0.05M.E	1.87±58.27	0.53±11.92^{ab}	2.10±27.04^{a}	1.41±19.83^{a}	0.49±8.53^{ab}
SEM		2.81	0.398	1.56	0.81	0.447
P Value	W.S	0.017	0.002	0.013	0.029	0.005
	Enzyme	0.46	0.4	0.51	0.055	0.59
	treatment	0.092	0.008	0.092	0.04	0.03
	W.S× Enzyme	0.72	0.09	0.88	0.60	0.43

Treatment1=0% W.S without enzyme. Treatment2= 5% W. without enzyme. Treatment3= 10%W.S without enzyme. Treatment4= 15%W.S without enzyme. Treatment5= 0%W.S with enzyme. Treatment6=5% W.S with enzyme. Treatment7= 10% W.S with enzyme. Treatment8= 15% W.S with enzyme. Means with common superscripts in same column are not significantly different ($P < 0.05$). Means ± Standard Deviation. 2- M.E= Multi Enzyme.

Viscosity measurement

Increasing viscosity in the intestine with the cereals grain has been proven by most researchers and our results was consistent with those (Marpuardt, 2000; Preston *et al*., 2000; Mathlouthi *et al*., 2001; Wang *et al*., 2005). But enzyme supplementation in this study had no effect on viscosity reduction. It may be due to adaptation of birds to viscose diet in 42 days of age (Almirall *et al*., 1995). It is associated with many factors including development of gastrointestinal tract, adaptation of the gut microflora to enzymes secreted for NSP hydrolysis and development of animal enzymatic activity. However, recent evidence suggests that the positive responses from enzyme supplementation are not always associated with a decrease in digesta viscosity (Slominski *et al*., 2000; McCracken and Miller, 2002). Lavinia *et al*. (2011) concluded that supplementation with the wheat-specific enzyme of the compound feeds where wheat participates in a proportion of 60 % (LE3) or 30

% wheat and 30 % barley (LE5), determine viscosity decrease at duodenum and jejunum level as well. Olso Mathlouthi *et al*. (2001) have stated that xylanase in cereals is more effective than its byproducts.

Table 10. Digesta viscosity (mPa·s) of total intestinal content in quail fed to different levels of W.S

Effects	Levels	Viscosity
W.S (%)	0	0.03±1.12[c]
	5	0.02± 1. 23[b]
	10	0.01± 1.25[a]
	15	0.02± 1.26[a]
SEM		0.008
Multi Enzyme (%)	0	0.05±1.22
	0.05	0.06±1.21
SEM		0.006
Treatment 1-8	0% W.S[1]	0.04±1.13[c]
	5% W.S	0.02±1.24[ab]
	10%W.S	0.01±1.26[a]
	15%W.S	0.01±1.27[a]
	0%w.s+0.05M E.[2]	0.01±1.11[c]
	5%w.s+0.05M.E	0.03±1.22[b]
	10%w.s+0.05M.E	0.01±1.25[ab]
	15%w.s+0.05M.E	0.02±1.26[ab]
SEM		0.012
P Value	W.S	0.0001<
	Enzyme	0.0002
	treatment	0.0001<
	W.S× Enzyme	0.0001<

Treatment1=0% W.S without enzyme. Treatment2= 5% W. without enzyme. Treatment3= 10%W.S without enzyme. Treatment4= 15%W.S without enzyme. Treatment5= 0%W.S with enzyme. Treatment6=5% W.S with enzyme. Treatment7= 10% W.S with enzyme. Treatment8= 15% W.S with enzyme. Means with common superscripts in same Colum are not significantly different ($P < 0.05$). Means ± Standard Deviation. 2- M.E= Multi Enzyme.

Implications

According to results of the current study, multi enzyme addition can increase apparent digestibility of some amino acids, protein digestibility and AME. In contrast, no improvements were achieved in AMEn of wheat screening. Finally, the results of study have showed that segments of gastrointestinal tract vary in length and weight with dietary composition.

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