Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2016) 40: 583-589
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doi:10.3906/vet-1504-65

Gross responses and apparent ileal digestibility of amino acids and minerals in broiler chicken fed vegetable-based starter diets supplemented with microbial enzymes

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Received: 18.04.2015 • **Accepted/Published Online:** 03.03.2016 • **Final Version:** 02.11.2016

Abstract: This study was conducted to investigate the effects of diet and exogenous enzymes on growth responses and micronutrient digestibility of broilers. Day-old broilers (n = 256) were distributed into a 2 × 2 factorial design and were fed on two basal diets: soybean (T_1) or canola (T_2) meals as such, or supplemented with enzymes up to 21 days. Feed intake (FI) and live weight (LW) to 21 days on the T_2 diet was higher ($P < 0.001$) than that on T_1. Feed conversion ratio (FCR) was better ($P < 0.01$) on T_1 than on T_2 at 21 days. Enzymes generally improved ($P < 0.001$) FI, FCR, and LW. The digestibility of histidine was higher ($P < 0.05$) on T_2, but lysine digestibility was higher ($P < 0.01$) on T_1 at 21 days. Histidine, threonine, lysine, valine, isoleucine, and phenylalanine digestibility was improved ($P < 0.05$) in the supplemented diets. Mineral digestibility was unaffected ($P > 0.05$) by diet, but enzymes increased ($P < 0.05$) digestibility of P, K, Mn, and Cu at 21 days. The digestibility of Cu, Zn, and Mg was higher ($P < 0.05$) on T_2, whereas Ca digestibility was greater ($P < 0.05$) on the T_1 diet. Broilers' growth responded positively to enzyme diets, probably due to improvement in nutrient digestibility.

Key words: Broiler, digestibility, enzyme, growth, micronutrient, amino acid, mineral, vegetable diet

1. Introduction

Protein is the most essential and most costly component of feed. This challenge has driven the development of numerous techniques including processed animal protein (PAP), protein concentrates, synthetic amino acids, premixes, and microbial enzymes, which might help the poultry industry utilize feed proteins for broiler chickens more efficiently. PAP such as fish meal, meat and bone meal, and other animal by products is rich in available nutrients, which can more easily meet the protein and amino acid requirements of poultry than plant protein sources. Exclusion of PAP from diet formulation will not only decrease the nutritional value of the ration but also may create problems in balancing diets for nonruminant animals (1). However, some concerns about using PAP in poultry diets, including rising cost, zoonotic transmission, and poor shelf life, are driving feed formulators to explore alternative feed proteins for the poultry industry across the globe (2,3). Plant protein sources like soybean and canola meals are some of the alternatives being explored to address these concerns.

In light of the above, vegetable feedstuffs such as soybean meal (SBM) and canola meal (CM) are increasingly being used in feed formulation by the global poultry industry consistently, because these meals provide birds with good sources of plant nutrients that are cheaper and safer than PAP. However, the use of large amounts of vegetable feedstuffs in poultry diets is constrained by their high content of nonstarch polysaccharides (17.9% in CM vs. 14.5% in SBM) (4). Furthermore, broiler diets based solely on plant ingredients may increase the availability of dietary crude fiber level, which may suppress nutrient digestibility, affecting the birds' performance (5). Nonruminant animals have a different digestive mechanism compared to ruminant animals such as cattle and goats, and these animals cannot digest highly fibrous diets, because there is no microbial action in the stomach. Furthermore, plant feedstuffs such as CM contain high levels of phytate, which forms a complex bond with other nutrients (protein, minerals) and makes these nutrients unavailable in formulated diets (6,7). It was reported that nonruminant animals such as pigs and poultry have a scarcity of certain types and amounts of adequate enzymes (phytase) to digest plant phytate (8), which can adversely affect energy supply and the digestibility and availability of other nutrients (e.g., fat, amino acids, minerals) and, in turn, the performance of the birds (9). The uses of microbial enzymes along with other supplements in

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practical diets of broiler chickens is assumed to be very effective in reducing the negative impact of vegetable diets to an extent. Inclusion of enzymes in plant diets enables the birds to degrade the antinutritive feed components along with promoting the breakdown of starch, cell walls, and storage proteins (10). However, many previous researchers have reported that the efficacy of such enzymes seems to be unpredictable in some cases and depends partially on the substrate or the nature of the diet composition including many other factors (11–13). The current study was undertaken to assess the relative nutritional merits of the two leading vegetable protein sources, SBM and CM, when fed with or without carbohydrase (Avizyme) and phytase enzyme supplements.

2. Materials and methods

2.1. Enzyme composition

Two commercial microbial enzymes (Avizyme 1502 and Phyzyme XP) were used in this study to carry out this experiment. The exogenous enzyme Avizyme 1502 (containing amylase 800 U/g, xylanase 1200 U/g, protease 8000 U/g) was supplemented at the rate of 0.5 g/kg, while Phyzyme XP (1000 FTU) was included at 0.1 g/kg in diets, as per the specification of the manufacturing company (Danisco Animal Nutrition, UK).

2.2. Animal husbandry and bird management

Day-old Ross male broiler chicks (n = 256; 46.34 ± 0.27 g) were procured from a commercial hatchery and used for conducting this experiment from hatching to 21 days. The chicks were weighed initially and were immediately distributed randomly into four dietary treatments (details are given below) in a 2 × 2 factorial arrangement, each treatment replicated 8 times with 8 birds per replicate. The chicks were reared in brooder cages (42 × 75 × 25 cm), in a climate-controlled house up to the end of the trial period (21 days). The chicks were brooded at a temperature of 33 °C for the first couple of days; after that, the temperature was then decreased gradually to 24 °C until 19 days and maintained at this level until the end of trial period. Eighteen hours of lighting and 6 h of darkness per day were provided during the entire trial period except for the first week; during this period, chicks were exposed to continuous lighting (23 h light : 1 h darkness).

2.3. Diets

Two basal diets (T_1 and T_2) were formulated with corn, wheat, and vegetable oil as the main energy sources and SBM and CM as the protein sources, along with other nutrients, and later cold-pelleted, as shown in Table 1. All diets were formulated entirely with ingredients of plant origin to meet or exceed NRC recommendations. The diets were fed to the birds as such (T_1 and T_2), or as supplemented (T_{1+} and T_{2+}) diets incorporating carbohydrase (Avizyme 1502) and phytase (Phyzyme XP) enzymes as per the

Table 1. Ingredient and nutrient composition of the basal (starter) diet (0–21 days).

	Diets	
	T_1	T_2
Ingredient composition (%)		
Corn	40.66	36.36
Wheat	21.10	18.17
Vegetable oil	0.00	2.17
Soybean meal	24.69	9.64
Canola meal	8.23	29.00
Limestone	2.30	1.48
Dicalcium phosphate	1.70	2.10
DL-Methionine	0.20	0.17
Lysine	0.17	0.12
Sodium chloride	0.35	0.42
Vitamin–mineral premix 1	0.23	0.23
Choline chloride	0.06	0.06
Sodium bicarbonate	0.03	0.03
Avizyme 1502	0.00	0.00
Phyzyme XP	0.00	0.00
Zinc Bacitracin	0.05	0.05
Marker	0.50	0.50
Nutrient composition (%)		
ME (kcal/kg)	2954.52	2955.19
Crude protein	21.10	21.11
Crude fiber	3.10	3.62
Ether extract	2.40	2.81
Calcium	1.23	1.22
Available P	0.62	0.62
Sodium	0.20	0.20
Chlorine	0.25	0.27
Lysine	1.30	1.31
Methionine + cysteine	0.83	0.83

Provided per kg of diet (mg): vitamin A (as all-trans retinol), 3.6 mg; cholecalciferol, 0.09 mg; vitamin E (as d-α-tocopherol), 44.7 mg; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; vitamin B_{12}, 0.2 mg; biotin, 0.1 mg; niacin, 50 mg; D-calcium pantothenate, 12 mg; folic acid, 2 mg; Mn, 80 mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg; and Mo, 1 mg. T_1-is a SBM-predominant diet along with CM, whereas T_2 is a CM-predominant diet in addition to SBM at a 75:25 ratio, which is also followed for the former diet.

recommendation of the supplier companies (shown above). All the diets were isoenergetic and isonitrogenous. Titanium dioxide (TiO_2) was incorporated into each diet at a rate of 5g/kg as an indigestible marker to enable assessment of amino acid and mineral digestibility. Birds had free access to the starter diet and water ad libitum throughout the trial period (21 days).

2.4. Data and sample collection

Gross responses in terms of body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were recorded weekly. Three birds from each pen were randomly selected on day 21 and weighed and killed by cervical dislocation to collect digesta samples from the ileum for the assessment of amino acid and mineral digestibility. The digesta samples were pooled by pen, frozen immediately, and preserved until further chemical analyses were conducted.

2.5. Chemical analyses

The amino acid contents of diets and ileal digesta samples were analyzed at the Australian Proteome Analysis Facility Ltd., Macquarie University, Sydney, NSW, Australia, using the precolumn derivatisation method (AccQTaq, Waters, Milford, MA, USA). The mineral concentrations of the diets and digesta samples were measured as per the method described by Anderson and Henderson (14) using inductively coupled plasma atomic emission spectrometry (ICP-AES). The TiO_2 contents of the diets and digesta samples were measured according to the method of Short et al. (15). The apparent ileal digestibility coefficient of nutrients (amino acids and minerals) was calculated using the following equation:

$$\text{Digestibility coefficient} = 1 - \frac{\text{digesta nutrient (g/kg) / digesta } TiO_2 \text{ (g/kg)}}{\text{diet nutrient (g/kg) / diet } TiO_2 \text{ (g/kg)}}$$

2.6. Statistical analyses and animal ethics

Statistical analyses were performed using Minitab software. The data were subjected to GLM analyses of variance for a factorial design and tested for significance between the dietary treatment means by Fisher's least significance difference at $P \leq 0.05$. All the management, care, and handling of these experimental birds were approved by the Animal Ethics Committee of the University of New England, Australia.

3. Results

3.1. Gross performances of the broiler chickens fed starter-based vegetable diets

The results of gross response of broilers in Table 2 show that protein source had no effect ($P > 0.05$) on feed intake (FI) of chickens up to 7 days, but FI of birds on the canola meal (T_2) diet to 14 and 21 days was significantly ($P < 0.001$) higher than that on the soybean meal (T_1) diets. Body weight (BW) on the T_2 diet was also greater than on

Table 2. Feed intake (FI), live weight (LW), and feed conversion ratio (FCR) of broiler chickens between hatch and 7, 14, and 21 days.

Diet	Enzyme	FI (g/bird)			LW (g/bird)			FCR		
		D7	D14	D 21	D7	D14	D21	D7	D14	D21
T_1	-	110.6[b]	433.3[c]	1017.7[c]	119.8[c]	333.8[b]	678.2[c]	1.49	1.51[b]	1.62[b]
	+	150.2[a]	512.0[b]	1155.7[b]	152.4[a]	432.4[a]	811.8[b]	1.41	1.32[c]	1.51[c]
T_2	-	132.3[b]	519.8[b]	1181.4[b]	134.7[b]	350.9[b]	714.4[b]	1.52	1.70[a]	1.77[a]
	+	148.6[a]	598.7[a]	1266.6[a]	156.4[a]	444.7[a]	838.6[a]	1.35	1.50[b]	1.60[b]
SEM		3.44	12.52	18.10	8.78	9.48	13.14	0.04	0.03	0.018
Significance										
Diet (A)		0.156	0.001	0.001	0.003	0.068	0.011	0.828	0.001	0.002
Enzymes (B)		0.001	0.001	0.001	0.001	0.001	0.001	0.124	0.001	0.001
A × B		0.101	0.999	0.145	0.073	0.763	0.688	0.607	0.839	0.410

Data represents means of 8 replicate groups consisting of 8 birds per replicate during 1–21 days; [a, b, c, d]: Means bearing different superscripts within a column are significantly different at the levels shown in the above table; the T_1 diet contains predominantly SBM in addition to CM at a 3:1 ratio, whereas the T_2 diet is predominantly CM along with SBM at the same ratio followed in the former diet; SEM = pooled standard error of means.

the T_1 diet when fed for 7 ($P < 0.01$) and 21 ($P < 0.05$) days, respectively. Protein sources had no significant effect ($P > 0.05$) on FCR at 7 days of age, but FCR was significantly ($P < 0.01$) better in the chicks on the T_1 diet when fed for 21 days only. However, FI and BW were improved significantly ($P < 0.001$) in chickens as a result of enzyme supplementations of diets to 7, 14, and 21 days. Except for days 1–7, FCR was also improved ($P < 0.001$) by supplemental enzymes when fed for 14 and 21 days, respectively. There were no significant effects ($P > 0.05$) of diet and enzyme interaction on the gross responses of the broiler chickens.

3.2. Amino acid digestibility of broiler chickens

There was no significant effect ($P > 0.05$) of protein source on amino acid digestibility as measured at 21 days of age except for histidine and lysine (Table 3). The digestibility of histidine was highest ($P < 0.05$) in birds on diet T_2, whereas the digestibility of lysine was highest ($P < 0.01$) on the T_1 diet. Enzyme supplementation increased the digestibility of all indispensable amino acids except for arginine, methionine, and leucine. Methionine digestibility tended to be significantly different ($P = 0.07$) between the two test diets. Similarly, the digestibility of leucine was also improved marginally ($P = 0.09$) in enzyme-supplemented diets compared to those fed the control diets. The digestibility of histidine, valine, isoleucine, and phenylalanine was similar in birds on the two enzyme-supplemented diets, but the digestibility of threonine ($P < 0.001$), lysine ($P < 0.01$), and the remaining amino acids was improved ($P < 0.05$) due to enzyme supplementation. There was no effect ($P > 0.05$) of diet × enzyme interaction on the digestibility of amino acids at 21 days.

3.3. Mineral digestibility of broiler chickens fed test diets

The ileal digestibility of minerals in chicks fed the two vegetable protein diets to 21 days is shown in Table 4. There was no significant effect ($P > 0.05$) of diets on mineral digestibility. However, the digestibility of Zn tended to be different ($P = 0.06$) between the two test diets. The digestibility of Cu was significantly ($P < 0.01$) increased by enzyme supplementation of both diets, while enzyme supplementation improved ($P < 0.05$) the digestibility of P, Mn, and K (Table 4). Additionally, the digestibility of Mg on supplemented diets tended to be higher ($P = 0.08$) than the value in birds on diets with no enzymes.

4. Discussion

In this study, the results showed that diets (vegetable protein sources) and enzymes, as individual factors, had greater effects on the gross responses of broiler chickens than interactions between them did. Birds of the CM-predominant (T_2) diet groups consumed a significantly higher amount of feed than those fed the SBM-predominant (T_1) diets, regardless of enzyme supplementation. The results agree with the reports of previous studies (16,17,13). The reason for the greater feed consumption of broiler chickens on enzyme-supplemented diets may be a result of increased fiber digestion, as the fiber tends to create a gut fill. Once such fiber is digested, chicks are able to increase feed intake to meet their nutrient requirements (18). The higher feed intake on CM (T_2) diets could also be caused by faster growth of the birds and the consequent higher nutritional requirements. Moreover, enzyme supplementation of diets may enhance the availability of certain nutrients, including trace minerals (e.g., Mn, Cu,

Table 3. Ileal digestibility of amino acids in birds on diets with or without supplemental enzymes.

Diet	Enzyme	His	Arg	Thr	Lys	Met	Val	Ile	Leu	Phe
T_1	-	0.76^{c}	0.83	0.66^{b}	0.82^{b}	0.89	0.72^{c}	0.74^{b}	0.75	0.76^{b}
	+	0.79^{b}	0.84	0.71^{a}	0.85^{a}	0.90	0.74^{b}	0.75^{b}	0.77	0.77^{b}
T_2	-	0.78^{b}	0.83	0.67^{b}	0.80^{c}	0.90	0.73^{c}	0.74^{b}	0.76	0.76^{b}
	+	0.81^{a}	0.85	0.70^{a}	0.83^{b}	0.91	0.76^{a}	0.77^{a}	0.78	0.80^{a}
Pooled SEM		0.004	0.004	0.004	0.003	0.003	0.005	0.005	0.006	0.006
Significance										
Diet (A)	0.03	0.27	0.99	0.00	0.07	0.15	0.44	0.23	0.94	
Enzyme (B)	0.01	0.20	0.00	0.00	0.26	0.01	0.02	0.09	0.01	
A × B	0.94	0.98	0.70	0.96	0.61	0.79	0.91	0.82	0.90	

Data represent means of 3 chickens from 5 replicate groups at 21 days of age; [a, b, c]: Means bearing different superscripts within a column are significantly different at the levels shown in the above table.

Table 4. Ileal digestibility of minerals of broilers fed on vegetable protein diets with or without supplemental enzymes.

Diet	Enzyme	Mn	Cu	Zn	Ca	Mg	K	P
T_1	-	0.42[b]	0.45[b]	0.46	0.52	0.47	0.91[b]	0.63[b]
	+	0.45[b]	0.47[b]	0.52	0.58	0.52	0.90[b]	0.67[a]
T_2	-	0.43[b]	0.44[b]	0.44	0.52	0.48	0.90[b]	0.60[c]
	+	0.52[a]	0.52[a]	0.45	0.53	0.54	0.92[a]	0.67[a]
Pooled SEM		0.010	0.008	0.011	0.013	0.014	0.002	0.010
Significance								
Diet (A)		0.10	0.95	0.06	0.42	0.67	0.17	0.63
Enzyme (B)		0.02	0.00	0.16	0.20	0.08	0.01	0.05
A × B		0.23	0.50	0.28	0.34	0.98	0.16	0.71

Data represent means of 3 chickens from 5 replicate groups at 21 days of age; [a, b]: Means bearing different superscripts within a column are significantly different at the levels shown in the above table.

Zn), which are known to promote greater feed intake of broiler chickens (19), enabling them to reach their full growth potential. The FCR was significantly better in chickens on the SBM (T_1) diets irrespective of enzyme supplementation. This may be due to better protein quality of T_1 than T_2. The improvement achieved with enzyme supplementation is supported by the findings of several other researchers (20, 21,13).

The amino acid digestibility of the two test diets differed significantly for the broiler chickens' vegetable-based starter diets, as observed in this present study. Amino acid (e.g., lysine, threonine, valine, isoleucine, and leucine) digestibility was promoted in the birds fed the SBM (T_1) diet as compared to the CM (T_2) diet. The higher protein quality and quantity of soybean meal than that of canola meal may be responsible for the improved amino acid digestibility (13). Apart from this, as is evident from the formulation profile, the reduced fiber content of the soybean meal (T_1) diet may also be liable for creating the differences in digestibility of the two plant-based diets (22). However, microbial enzyme supplementation with the two test diets improved the digestibility of the main amino acids for broiler chickens fed plant-based starter diets. The results agree with the reports of many previous researchers (11,23). Similar responses were also observed in another study when broiler chickens were fed vegetable-based finisher diets supplemented with exogenous enzymes (13).

The results from the present study indicate that the amino acid digestibility was improved in the broilers when they were fed vegetable-based starter diets supplemented with microbial enzymes. It is obvious that the improvement of nutrient (amino acid) digestibility is an outcome of enzyme supplementation in the vegetable protein diets. The reason for this increased nutrient digestibility might have been the addition of exogenous enzymes to the test diets, as enzymes enable the birds to degrade the antinutrient feed components, along with promoting the breakdown of starch, cell wall matrix, and storage proteins (10). Studies in ruminant models have also suggested that the use of exogenous enzymes can disrupt cell wall-associated proteins, which facilitates microbial colonization of the substrate (24). Exogenous enzymes decrease the detrimental effects of nonstarch polysaccharides and enhance the digestion of nutrients in poultry diets (25). The enzyme increased the degree of protein hydrolysis and thereby increased the proportion of soluble low-molecular-size proteins, hence making the protein more available for uptake by the chickens (26).

However, the impact of enzymes on digestibility was more pronounced than that of protein sources in this study. Digestibility of the majority of essential amino acids (histidine, threonine, lysine, valine, isoleucine, and phenylalanine) was increased by supplemental enzymes during the middle growing period (21 days). This implies that enzymes in the sort of diets tested exerted more action on amino acid digestibility during the early stage of growth. The pronounced efficacy of this enzyme at this stage may possibly be due to digestive function relative to age of the growing birds, because young chicks might have insufficiency of some intrinsic enzymes at an early age due to lack of proper functioning of the secretory glands and organs of the birds during this period. This is supported by Classen and Bedford (27), who reported that young birds might have limited amounts of certain type of enzymes, which might influence the enzyme activities and digestibility of feed nutrients (e.g., amino acids).

Mineral digestibility was not influenced by the interaction of diet and enzyme, but there was a significant separate effect of protein source and enzymes. The variation in mineral digestibility between the two test diets might be caused by differences in phytate contents or other antinutritive factors found in the plant feedstuffs. That the presence of such factors in many plant ingredients can affect nutrient utilization has been highlighted by earlier researchers (28). Moreover, many previous researchers have reported that the presence of this phytate in feed causes a complex bond that results in reduced availability of nutrients, particularly minerals (7,29). However, the improvement in the digestibility of some minerals due to enzyme supplementation during the starter period of growth as observed in our study partly agrees with the results of Selle et al. (12), which showed a positive effect of dietary enzymes (phytase) on the utilization of minerals and energy and nutrient digestibility in wheat-based diets. The use of different diet formulations, the bird strain, feedstuffs, crude fiber level, antinutrient components, etc. may also be responsible for these differences in the digestion of mineral nutrients (30).

The results generally demonstrated similar performance in terms of gross response as the differences between the two test diets were not significant. However, both protein sources possess different advantages and would be better combined, as is done in practical diets. The improved growth response of chicks on the enzyme-supplemented diets is a result of improved nutrient digestibility, suggesting that vegetable-based starter diets can support optimum growth with this intervention.

Acknowledgment
The project was jointly funded by the University of New England, Australia, and Danisco Animal Nutrition, UK.

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