ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF POULTRY SCIENCE

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 13 (10): 555-561, 2014
ISSN 1682-8356

Impact of Microbial Enzymes on Growth Performance, Micronutrient Digestibility, Tissue Protein Contents and Endogenous Enzyme Activities of Broiler Chickens Fed on Vegetable Protein Diets

M.A. Hossain, A.F. Islam and P.A. Iji
School of Environmental and Rural Science, University of New England, NSW-2351, Australia

Abstract: A study was conducted to investigate the effects of dietary protein sources and microbial enzyme supplementation on amino acid and mineral digestibility, tissue protein contents and endogenous enzyme activities of broiler chickens. Ross 308 day-old male broiler chicks (n = 160) were assigned randomly to four dietary treatments, each replicated five times, eight chicks per replicate, in a 2 x 2 factorial design. Two basal diets were formulated with soybean (SBM) and canola (CM) meals at a ratio of 75:25, respectively, along with basal grains and fed to the birds as such or supplemented with enzymes from 1 to 35 days. Enzymes had a positive effect on the gross responses (feed intake, body weight and feed conversion ratio) of the broiler chickens at 35 days. However, feed intake was significantly different ($p<0.001$) between protein sources but this did not affect ($p>0.05$) body weight and feed conversion ratio (FCR) between 1 and 35 days. Tissue protein contents and endogenous enzyme activities at 21 days (except for maltase) were unaffected by dietary sources and microbial enzyme supplementation. The activity of maltase was higher ($p<0.05$) on CM diets than on SBM diets, but this effect was absent when diets were supplemented with enzymes. The digestibility of threonine and lysine, measured at 35 days, was significantly ($p<0.01$) higher on SBM diets than on CM diets. Similarly the digestibility of valine, isoleucine and leucine was also higher ($p<0.05$) on SBM diet than in CM diet. Addition of enzymes also significantly ($p<0.05$) increased the digestibility of histidine, threonine, lysine and isoleucine, but not the digestibility of other amino acids measured at this period (35d). Enzymes had no effect ($p>0.05$) on mineral digestibility of broilers over the test period. The digestibility of Cu, Zn and Mg was increased ($p<0.05$) on CM diet, whereas Ca digestibility was higher on SBM diet at 35 days. It can be concluded that the improved growth of the birds might be a result of increased digestibility of amino acids and increase in feed consumption.

Key words: Enzyme, amino acid, mineral, digestibility, tissue protein, enzyme activities, vegetable-based diets, broiler chickens

INTRODUCTION

Protein is an essential nutrient required by broiler chickens for optimum growth and development. Proteins of animal origin, such as fish meal, meat meal or milk products, as a class, are superior to proteins of plant origin. No two protein sources are completely alike. The type of proteins used in feed formulation for broiler chickens not only affects the quality of the formulated diets but also influences the performance of the birds and the quality of meat. Diets containing animal proteins can meet the protein and amino acid requirements of the birds with a minimum intake. If the dietary proteins are deficient in one or more essential amino acids, they will not be able to provide proper protein nutrition even with excessive intake (Singh and Panda, 1992). However, globally the poultry industry is seeking alternative feed proteins due to rising cost, hazard of zoonoses in animal protein meals and rising feed demand by the poultry industry (CEC, 2000; FAO, 2004). Therefore, it might be beneficial to use vegetable ingredients instead of animal protein meals to forestall the cross-contamination as well as to reduce feed cost of poultry production. In this regard, proteins in diets containing only vegetable ingredients have the potential for providing safe and quality diet formulation for poultry. Soybean is the premier vegetable source of protein, while canola seed is also becoming popular and these are currently the largest protein meals produced worldwide (Hossain *et al*., 2012). These meals contain higher proportions of protein (37-48%) and in a typical diet for broiler chickens these meals can be used to provide up to 60 % of the crude protein (Newkirk and Classen, 2002). However, vegetable feedstuffs may also be potential resources and are rich sources of other micro-nutrients such as essential amino acids, vitamins, minerals and antioxidants (Omenka and Anyasor, 2010). In addition, these feed proteins are inexpensive and reduce production costs. Besides, in most poultry feeds, vegetable proteins are the major protein source and they account for about 33.5% of the

Corresponding Author: P.A. Iji, School of Environmental and Rural Science, University of New England, NSW-2351, Australia

total feed cost in commercial poultry production (Sarwar *et al.*, 2002). Therefore, nutritionists are exploring low cost and good quality feed ingredients for cost-effective diet formulation for poultry (Naseem *et al.*, 2006).

The major factor limiting the use of vegetable proteins in practical diets is the presence of naturally occurring anti-nutritional factors, including non-starch polysaccharides (NSP), tannins and trypsin inhibitors, which have adverse effects on nutrient digestibility and absorption (Gatel, 1994; Smits and Annison, 1996). Some of these constraints can be overcome by supplementation with microbial enzymes. It has been reported that addition of feed enzymes to diets improves amino acid digestibility and metabolizable energy value of the diets (Bryden *et al.*, 2009). However, the response to feed enzymes is dependent on multiple factors, for example, diet composition, and nature and level of enzyme addition (Hew *et al.*, 1999; Ravindran *et al.*, 2000, 2001; Selle *et al.*, 2003), age and others factors.

The current study was undertaken to investigate the growth performance, nutrient digestibility and endogenous enzyme activities of broiler chickens fed on all-vegetable diets containing microbial enzymes.

MATERIALS AND METHODS

All care, handling and management of birds in this experiment were approved by the Animal Ethics Committee of the University of New England (Approval No: AEC11/067) and compiled with the Australian Code of Practice for the Care and Use of Live Animals for Teaching and Scientific Research Purposes.

Experimental design and conduct: A 2 x 2 factorial experiment, involving two main vegetable protein sources (soybean or canola) and two enzyme levels (with or without) was conducted to examine the effect of diets and the addition of microbial enzymes to the diets on the gross responses and digestibility of amino acids and minerals by broiler chickens. A total of 160 day-old male Ross 308 broiler chicks were obtained from a local commercial hatchery and tested from hatch to 35 days in a cage rearing system in a climate-controlled house. The chicks were weighed on receipt and were distributed randomly into four dietary treatments (described below), each treatment replicated five times with eight birds per replicate. For the first two days the birds were provided with a temperature of 33°C. The temperature was then gradually reduced by 1 or 2°C every 1 or 2 days until the chicks were 19 days old at which point the temperature was maintained at 24°C for the rest of the trial. Birds were managed in brooder cages for the first three weeks and then transferred to larger metabolic cages for the rest of the trial period.

Dietary treatments: Four experimental diets were formulated with maize, wheat and vegetable oil as the main energy sources, along with soybean and canola meals as the main protein sources and cold-pelleted. The diets were formulated exclusively with ingredients of plant origin with or without addition of enzymes. Two basal diets were formulated with soybean meal (SBM) and canola meal (CM) at a ratio of 75:25 to one another along with basal grains as shown in Table 1 and 2. These were fed as such (SBM or CM) or supplemented with microbial enzymes (Avizyme 1502 and Phyzyme XP, Danisco Animal Nutrition, UK) to create diets SBM+ and CM+. Avizyme 1502 (containing amylase 800, xylanase 1200, 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. The diets were iso-caloric and iso-nitrogenous in nature and further supplemented with Zinc Bacitracin (0.5 g/kg) and a marker, titanium dioxide (5 g/kg). The birds were fed starter diets *ad libitum* for the first three weeks and finisher diets for the last two weeks of the experiment.

Data and sample collection: On day 35, feed intake (FI), body weight (BW) and feed conversion ratio (FCR) were calculated replicate-wise. Three birds from each pen on day 21 were randomly selected, weighed and killed by cervical dislocation. Pancreatic and jejunal tissue samples were collected to assess the tissue protein contents (pancreas and jejunum) and internal enzyme (chymotrypsin amidase, alkaline phosphatase, sucrase and maltase) activities. Besides, two birds on day 35, were randomly selected, weighed and slaughtered in a similar way to collect digesta samples from the ileum for the assessment of amino acid and mineral digestibility. Collected digesta samples were pooled by pen, frozen immediately after collection and subsequently freeze-dried. The dried samples were ground to pass through a 0.5 mm sieve and stored in airtight containers at -20°C for chemical analyses.

Chemical analyses: Digestive enzyme activities and protein concentrations were assessed on jejunal tissues after the samples were processed according to the method described by Shirazi-Beechey *et al.* (1991) and modified for poultry (Iji *et al.*, 2001). The pancreatic tissue was processed in a similar way to the jejunal tissues, except that Milli-Q water (Millipore, Australia) was used in lieu of buffer and the entire organ was homogenized (Nitsan *et al.*, 1974; Iji *et al.*, 2001). The specific activities of jejunal and pancreatic enzymes were assessed by incubation with fixed substrate concentrations as standardized for poultry (Iji *et al.*, 2001). Both jejunal and pancreatic tissue protein concentrations were measured according to the procedure described by Bradford (1976) using Comassie dye-binding. All the raw data for protein concentration were processed through the Lowry software (McPherson, 1985) before statistical analysis.

Table 1: Ingredient and nutrient composition of the starter diets (0-21 days)

	Diets			
	SBM	SBM+	CM	CM+
Ingredient composition (g/kg)				
Maize	406.6	406.6	363.6	363.6
Wheat	211.0	211.0	181.7	181.7
Vegetable oil	0.0	0.0	21.7	21.7
Soybean meal	246.9	246.9	96.4	96.4
Canola meal	82.3	82.3	290.0	290.0
Limestone	20.3	20.0	14.8	14.5
Dicalcium phosphate	17.0	17.0	21.0	21.0
DL-Methionine	2.0	2.0	1.7	1.7
Lysine	1.7	1.7	1.2	1.2
Sodium chloride	3.5	3.5	4.2	4.2
Vitamin-mineral premix[2]	2.3	2.3	2.3	2.3
Choline chloride	0.6	0.6	0.6	0.6
Sodium bicarbonate	0.3	0.0	0.3	0.0
Avizyme 1502	0.0	0.5	0.0	0.5
Phyzyme X P	0.0	0.1	0.0	0.1
Zinc bacitracin	0.5	0.5	0.5	0.5
Marker	5.0	5.0	5.0	5.0
Nutrient composition (g/kg)				
ME (MJ/kg)	12.37	12.37	12.38	12.38
Crude protein	211.0	211.0	211.1	211.1
Crude fibre	31.0	31.0	36.2	36.2
Ether extract	24.0	24.0	28.1	28.1
Calcium	12.3	12.3	12.2	12.2
Available P	6.2	6.2	6.2	6.2
Sodium	2.0	2.0	2.0	2.0
Chlorine	2.5	2.5	2.7	2.7
Lysine	13.0	13.0	13.1	13.1
Methionine+cysteine	8.3	8.3	8.3	8.3
Threonine	8.3	8.3	8.4	8.4
Arginine	14.1	14.1	14.2	14.2

1: SBM contained predominantly soybean meal along with canola meal at 3:1 ratio, whereas CM contains predominantly canola meal in addition with soybean meal at 3:1 ratio].

[2]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

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 pre-column derivatization method (AccQTaq, Waters, Milford, MA, USA). The mineral concentrations of diets and digesta samples were measured as per the method described by Anderson and Henderson (1986) using inductively coupled plasma atomic emission spectrometry (ICP-AES). The titanium dioxide (TiO_2) contents of the diets and digesta samples were measured according to the method of Short *et al*. (1996). The apparent ileal digestibility coefficient of nutrients (amino acids and minerals) was calculated using the following equation:

$$\text{Digestibility coefficient} = 1 - \frac{\text{digesta nutrient (g/kg) / digesta Tio}_2\text{ (g/kg)}}{\text{diet nutrient (g/kg) / diet Tio}_2\text{ (g/kg)}}$$

Statistical analyses: Statistical analyses were performed using Minitab software (Minitab version 16, Minitab, 2000). The data were subjected to GLM analyses of variance for factorial design. Differences between the dietary treatment means were tested for significance by Fisher's least significant difference at $p \leq 0.05$.

Table 2: Ingredient and nutrient composition of finisher diets

	Diets			
	SBM	SBM+	CM	CM+
Ingredient composition (g/kg)				
Maize	414.1	414.1	382.2	382.2
Wheat	211.0	211.0	190.0	190.0
Vegetable oil	0.0	0.0	18.3	18.3
Soybean meal	227.0	227.0	89.0	89.0
Canola meal	75.6	75.6	267.0	267.0
Limestone	25.0	24.6	21.0	21.0
Dicalcium phosphate	24.0	24.0	20.4	20.4
DL-Methionine	2.2	2.2	1.8	1.5
L-Lysine	2.3	2.3	1.6	1.4
Sodium chloride	5.0	5.0	5.0	5.0
Sodium bicarbonate	5.1	5.1	0.1	0.0
Vitamin-mineral premix[1]	2.5	2.5	2.5	2.5
Choline chloride	0.6	0.6	0.6	0.6
Avizyme 1502	0.0	0.5	0.0	0.5
Phyzyme XP	0.0	0.1	0.0	0.1
Zinc bacitracin	0.5	0.5	0.5	0.5
Marker (TiO_2)	5.0	5.0	5.0	5.0
Nutrient composition (g/kg)				
ME(MJ/kg)	12.41	12.41	12.42	12.42
Crude protein	191.4	191.4	191.2	191.2
Crude fibre	29.4	29.4	36.0	36.0
Ether extract	24.0	24.0	28.0	28.0
Calcium	14.3	14.3	14.2	14.2
Av. P	6.3	6.3	6.2	6.2
Sodium	2.3	2.3	2.2	2.2
Chlorine	3.3	3.3	3.3	3.3
Lysine	12.4	12.4	12.3	12.3
Methionine+cysteine	8.2	8.2	8.1	8.1
Threonine	8.0	8.0	8.1	8.1
Arginine	13.1	13.3	13.0	13.0

[1]Composition as in Table 1

RESULTS

Growth response: The results of gross response of broilers showed that feed intake (FI) of birds on the CM diet to 35 days was significantly ($p<0.001$) higher than that of SBM diets (Table 3). Diets had no significant effect ($p>0.05$) on feed conversion ratio (FCR) of broilers at 35 days of ages, but enzyme-supplemented diets promoted ($p<0.01$) feed efficiency compared to the birds fed on non-supplemented diets. However, FI and live weight (LW) were improved significantly ($p<0.001$) in chickens as a result of enzyme supplementation of diets at 35 days. Although diets had no significant ($p>0.05$) effect on FCR, birds fed on SBM diet showed somewhat better feed efficiency than the birds fed on CM diets. There were no significant effects ($p>0.05$) of diet x enzyme interaction on the gross response of the chickens.

Amino acid digestibility: The digestibility of threonine and lysine was significantly ($p<0.01$) higher on SBM diet than on CM diet measured at 35 d (Table 4). Similarly

Table 3: Feed intake (FI), live weight (LW) and feed conversion ratio (FCR) of broiler chickens at 35 days

Diet	Enzyme	FI (g/b)	LW (g/b)	FCR
SBM	SBM	3577.6^{d}	1930.5^{b}	1.90^{a}
	SBM+	3681.8^{b}	2088.0^{a}	1.80^{b}
CM	CM	3633.1^{c}	1942.2^{b}	1.92^{a}
	CM+	3796.4^{a}	2111.1^{a}	1.84^{b}
Pooled SEM		7.83	12.96	0.014
significance	Diet (A)	0.001	0.506	0.287
	Enzyme (B)	0.001	0.001	0.004
	A × B	0.069	0.827	0.805

Data represent means of eight replicate groups consisting five birds during 35 days.

^{a,b,c,d}Means bearing uncommon superscripts within a column are significantly different at the levels shown in the above Table.

SEM = Pooled standard error of means.

the digestibility of valine, isoleucine and leucine was also higher ($p<0.05$) on SBM diet than on CM diet. The digestibility of histidine and arginine in SBM diet tended to be better than on CM diet. Apart from this, addition of enzymes also significantly ($p<0.05$) increased the digestibility of histidine, threonine, lysine and isoleucine, but not the digestibility of other amino acids measured at this period (35 d). Furthermore, the digestibility of leucine in birds on enzyme-supplemented diets also increased marginally ($p = 0.09$) compared to the birds fed the non-supplemented diets. There was no significant ($p>0.05$) effect of diet x enzyme interaction on the digestibility of amino acids at 35 days.

Mineral digestibility: The digestibility of K, Na, P and Mn at 35d of age was identical between the two test diets (Table 5). The digestibility of Cu, Zn and Mg was significantly ($p<0.05$) higher in birds on the CM diet than in those on the SBM diet. Calcium digestibility was, however, higher ($p<0.05$) on the SBM diet than on CM diet. Apart from this, the K digestibility of broiler chickens on SBM diet was also marginally ($p = 0.09$) higher than that of birds fed the CM diet. There was no significant ($p>0.05$) effect of enzymes on mineral digestibility at 35 d. Diet x enzyme interaction on the digestibility of minerals at 35 days was also similar between treatments.

Tissue protein growth and digestive enzyme activities: There was no significant effect ($p>0.05$) of diet or enzyme supplementation on pancreatic tissue protein content and chymotrypsin amidase activities (Table 6). However, tissue protein content in the pancreas tended to be higher ($p = 0.054$) in birds on the enzyme-supplemented diets than on control diets. Jejunal mucosal protein content was also not different between treatments. Sucrase, maltase and alkaline phosphatase activities in the jejunum were not influenced ($p>0.05$) by addition of enzymes to diets, but the activity of maltase was significantly ($p<0.05$) higher in birds on the CM diet than on the SBM diet. Apart from this, the activity of sucrase was marginally increased in birds fed on diets with enzyme supplementation, but this effect was not significant ($p>0.05$). The activities of alkaline phosphatase in chicks on enzyme-supplemented diets declined, although the difference between the two test diets was insignificant ($p = 0.53$). There was no significant ($p>0.05$) effect of diet x enzyme interaction on the tissue protein contents and enzyme activities.

DISCUSSION

The results of this experiment demonstrate a significant improvement in FI, LW and FCR of broilers on vegetable protein (canola and soybean meal) diets with enzyme supplementation throughout the trial period. This may be due to an improvement in nutrient digestibility, enabling the birds to grow to their full potential. These results agree with the reports of previous researchers (Zanella *et al*., 1999; Bhuiyan *et al*., 2010; Pirgozliev *et al*., 2011) who found similar growth response by supplementation of exogenous enzymes in plant-based broiler diets.

However, although broilers from both diet groups (SBM and CM) with or without enzyme supplementation showed a similar trend in gaining body weight up to 35 days, birds from the CM diet group attained greater body weight than those from the SBM diet group during this period. This improved growth of broiler chickens fed on CM diet might be due to a higher consumption of the particular diets. The FCR was significantly better in the SBM diet groups with or without enzyme supplementation. The amino acid digestibility of the SBM diets was significantly better than that of the CM diets. In general, supplementation of exogenous enzymes to diets can also improve the feed efficiency of broiler chickens (Peng *et al*., 2003).

The pattern of amino acid digestibility was different between the two vegetable diets, particularly at the finisher period. Digestibility of lysine, threonine, valine, iso-leucine and leucine was increased in birds fed on the SBM diet when compared to CM. Histidine and arginine digestibility was also slightly improved on the SBM diet at the finisher period as well. The improved amino acid digestibility of SBM diets may possibly be due to the higher protein quality of soybean meal compared to the quality of canola meal. Furthermore, SBM is generally lower in fibre content and other anti-nutritive factors than CM. Le Goff and Noblet (2001) reported that most of the variation in digestibility of nutrients is related to the presence of dietary fibre. However, enzyme addition to the two VP diets improved the digestibility of key amino acids at 35 days of age. The results are in agreement with the reports of previous researchers (Ravindran *et al*., 2000; Cowieson and Ravindran, 2008). Exogenous enzymes in VP diets might enable the broilers to break down the anti-nutritive factors, especially cell wall polysaccharides, to expose nutrients that can be digested by endogenous enzymes. Moreover, microbial enzymes may decrease the digesta viscosity and facilitate improved contact between

Table 4: Ileal digestibility coefficients of amino acids in birds on diets with or without supplemental enzymes

Diet	Enzyme	His	Arg	Thr	Lys	Met	Val	Ile	Leu	Phe
SBM	SBM	0.81^{b}	0.85	0.73^{b}	0.85^{b}	0.92	0.77^{a}	0.78^{b}	0.80^{a}	0.80
	SBM+	0.84^{a}	0.87	0.76^{a}	0.87^{a}	0.93	0.79^{a}	0.81^{a}	0.82^{a}	0.83
CM	CM	0.80^{b}	0.84	0.69^{c}	0.83^{c}	0.92	0.74^{b}	0.76^{c}	0.78^{b}	0.77
	CM+	0.82^{b}	0.85	0.72^{b}	0.84^{b}	0.93	0.77^{a}	0.78^{b}	0.79^{a}	0.79
Pooled	SEM	0.004	0.003	0.005	0.003	0.002	0.005	0.005	0.004	0.012
Significance	Diet (A)	0.07	0.06	0.01	0.01	0.36	0.02	0.05	0.04	0.12
	Enzyme (B)	0.01	0.12	0.01	0.03	0.23	0.27	0.05	0.09	0.92
	A × B	0.62	0.31	0.79	0.30	0.47	0.62	0.38	0.52	0.20

Data represent means of two chickens from five replicate groups at 35 days of age.

[a,b,c]Means bearing uncommon superscripts within a column are significantly different at the levels shown in the above Table.

Table 5: Ileal minerals digestibility coefficient of broilers fed on vegetable protein diets with or without supplemental enzymes

Diet	Enzyme	Mn	Cu	Zn	Ca	Mg	K	P	Na
SBM	SBM	0.46	0.44^{b}	0.53^{b}	0.65^{a}	0.54^{b}	0.88	0.63	0.39
	SBM+	0.49	0.47^{a}	0.55^{a}	0.66^{a}	0.56^{a}	0.89	0.63	0.42
CM	CM	0.44	0.49^{a}	0.57^{a}	0.62^{b}	0.57^{a}	0.86	0.61	0.42
	CM+	0.46	0.49^{a}	0.57^{a}	0.65^{a}	0.57^{a}	0.86	0.63	0.47
Pooled SEM		0.006	0.009	0.006	0.004	0.006	0.006	0.004	0.018
Significance	Diet (A)	0.11	0.04	0.04	0.01	0.02	0.08	0.14	0.25
	Enzyme (B)	0.53	0.53	0.90	0.26	0.92	0.76	0.11	0.74
	A × B	0.10	0.75	0.94	0.47	0.74	0.52	0.19	0.33

Data represent means of two chickens from five replicate groups at 35 days of age.

[a,b]Means bearing uncommon superscripts within a column are significantly different at the levels shown in the above Table.

Table 6: Tissue protein content and enzyme activities of broiler chickens at day 21 with or without microbial enzyme supplementation

		Pancreas		Jejunum			
Diet	Enzyme	Protein (mg/g tissue)	CA[2]	Protein (mg/g tissue)	AP[3] (ηmol/mg protein)	Maltase	Sucrase (ηmol/mg protein)
SBM	SBM	55.1	3.8	64.5	5.8	0.48^{b}	0.15
	SBM+	58.8	4.9	66.7	5.6	0.52^{a}	0.15
CM	CM	57.3	4.8	57.2	7.1	0.58^{a}	0.14
	CM+	61.8	5.6	62.5	6.0	0.64^{a}	0.18
Pooled SEM		1.03	0.35	2.01	0.48	0.022	0.006
Level of significance	Diet (A)	0.213	0.258	0.193	0.352	0.023	0.295
	Enzyme (B)	0.054	0.193	0.389	0.539	0.224	0.09
	A × B	0.838	0.839	0.722	0.663	0.799	0.200

Data represent means of five replicates at 21 days of age.

CA[2], Chymotrypsin amidase (çmol/mg protein).

AP[3], Alkaline phosphatase (μmol/min/mg protein).

[a,b]Means bearing uncommon superscripts within a column are significantly different at the levels shown.

endogenous enzymes and nutrients, thereby improving digestibility (Lazaro *et al.*, 2004).

Enzymes had no effect on the mineral digestibility of broiler chickens at the stage tested. However, significant differences in mineral digestibility were observed between the dietary sources. The difference in mineral digestibilities of the two test diets might be caused by differences in phytate contents or other anti-nutrient factors found in plant feed ingredients. The presence of such factors in many plant ingredients can affect nutrient utilization and this has been highlighted by many researchers (Thompson and Yoon, 1984; Wappnir, 1989; Sebastian *et al.*, 1997). However, the use of different diet formulations, the bird strain, feedstuffs, crude fibre level, anti-nutrient components etc., may also be responsible for these differences in the digestion of mineral nutrients (Pirgozliev *et al.*, 2011).

In this study, maltase activity was higher in birds on CM diets than those on SBM diets. This might stimulate the terminal digestion of starch and enable the birds to grow faster. Although there was a slight increase in pancreatic tissue protein content and activity of sucrase in enzyme-treated diets, the activity of alkaline phosphates declined somewhat in birds on the supplemental diets in this study. The activities of enzymes might exert a more stimulating effect on chyme passing through the digestive tract (Duke, 1986). Some of the results are in contradiction to those of Jiang *et al.* (2008) who reported that the activities of pancreatic lipase and proteases, including trypsin, when assessed directly in the tissue, were not influenced by exogenous enzymes. The researchers, however, observed an increase in the activity of trypsin in the intestinal lumen of the treated broiler chickens.

Conclusion: Enzyme supplementation significantly improved growth performance through an improvement in the digestibility of certain amino acids but not mineral digestibility, tissue protein contents, or internal enzyme activities. In this study, the differences in activities of endogenous enzymes measured at the early stage are not so pronounced. The differences in response between the two main vegetable protein sources may be attributable to differences in nutrient profiles, nature or characteristics of the proteins and levels of anti-nutritive factors.

ACKNOWLEDGEMENTS

The project was funded by the UNE, Australia and partly by Danisco Animal Nutrition, UK.

REFERENCES

Anderson, D.L. and L.J. Henderson, 1986. Sealed chamber digestion for plant nutrient analysis. Agron. J., 78: 937-938.

Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Bioch., 72: 248-254.

Bhuiyan, M.M., A.F. Islam and P.A. Iji, 2010. Response of broiler chickens to diets containing artificially dried high-moisture maize supplemented with microbial enzymes. S. Afr. J. Anim. Sci., 40: 348-362.

Bryden, W.L., X. li, G. Ravindran, L.I. Hew and V. Ravindran, 2009. Ileal digestible amino acid values in feedstuffs for poultry. RIRDC Publication No. 09/071, p.5.

Cowieson, A.J. and V. Ravindran, 2008. Effect of exogenous enzymes in maize-based diets varying in nutrient density for young broilers: growth performance and digestibility of energy, minerals and amino acids. Br. Poult. Sci., 49: 37-44.

CEC, 2000. Council Regulation 2000/766 concerning certain protection measures with regard to transmissible spongiform encephalopathies and the feeding of animal protein. Off. J. Eur. Comm., 43: 32-33.

Duke, G.E., 1986. Alimentary canal: secretion and digestion, special digestive functions and absorption. In P.D. Sturkie (Ed.), Avian Physiology (pp: 289-302): Springer-Verlag, New York.

FAO, 2004. Protein sources for the animal feed industry. ftp://ftp. fao.org /docrep fao/007/y 5019 e /y5019e00.

Gatel, F., 1994. Protein quality of legume seeds for non-ruminant animals: A literature review. Anim. Feed Sci. Technol., 45: 317-348.

Hew, L.I., V. Ravindran, G. Ravindran, P.H. Pittolo and W.L. Bryden, 1999. The apparent metabolizable energy and amino acid digestibility of wheat, triticale and wheat middlings for broiler chickens as affected by exogenous xylanase supplementation. J. Sci. Food and Agric., 79: 1727-1732.

Hossain, M.A., A.F. Islam and P.A. Iji, 2012. Energy Utilization and Performance of Broiler Chickens Raised on Diets with Vegetable Proteins or Conventional Feeds. Asian J. Poult. Sci., 6: 117-128.

Iji, P.A., A. Saki and D.R. Tivey, 2001. Body and intestinal growth of broiler chicks on a commercial starter diet. 2. Development and characteristics of intestinal enzymes. Br. Poult. Sci., 42: 514-522.

Jiang, Z., Y. Zhou, F. Lu, Z. Han and T. Wang, 2008. Effects of different levels of supplementary-alpha-amylase on digestive enzyme activities and pancreatic amylase mRNA expression of young broilers. Asian-Australas. J. Anim. Sci., 21: 97-102.

Le Goff, G. and J. Noblet, 2001. Comparative digestibility of dietary energy and nutrients in growing pigs and adult sows. J. Anim. Sci., 79: 2418-2427.

Lazaro, R.M., A. Latorre, P. Medel, M. Garcia and G.G. Mateos, 2004. Feeding regimen and enzyme supplementation to rye-based diets for broiler. Poult. Sci., 83: 152-160.

Mcpherson, G.A., 1985. Lowry program: Elsevier-BIOSOFT, 68 Hills Rd, Cambridge, CB2, ILA, UK.

Minitab, 2000. Minitab Statistical Software User's Guide 2: Data Analysis and Quality Tools. Minitab Inc., State College, PA.

Newkirk, R.W. and H.L. Classen, 2002. The effects of toasting canola meal on body weight, feed conversion efficiency and mortality in broiler chickens. Poult. Sci., 81: 815-825.

Nitsan, Z., Y. Dror, I. Nir and I. Shapira, 1974. The effects of force-feeding on enzymes of the liver, kidney, pancreas and digestive tract of chicks. Br. J. Nutr., 32: 241-247.

Naseem, M.Z., S.H. Khan and M. Yousuf, 2006. Effects of different levels of canola meal on broiler production performance during two phases of growth. Pak. Vet. J., 26: 129-134.

Omenka, R.O. and G.N. Anyasor, 2010. Vegetable-based feed formulation on poultry meat quality. Afr. J. Food Agric. Nutr. Develop., 10: 2001-2011.

Peng, Y.L., Y.M. Guo and J.M. Yuam, 2003. Effects of microbial phytase replacing partial inorganic phosphorus supplementation and xylanase on the growth performance and nutrient digestibility in broiler fed wheat-based diets. Asian-Aust. J. Anim. Sci., 16: 239-247.

Pirgozliev, V., M.R. Bedford, T. Acamovic, P. Mares and M. Allymehr, 2011. The effects of supplementary bacterial phytase on dietary energy and total tract amino acid digestibility when fed to young chickens. Br. Poult. Sci., 52: 245-254.

Ravindran, V., S. Cabahug, G. Ravindran, P.H. Selle and W.L. Bryden, 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. II. Effects on nutrient digestibility and retention. Br. Poult. Sci., 41: 193-200.

Ravindran, V., P.H. Selle, G. Ravindran, P.C.H. Morel, A.K. Kies and W.L. Bryden, 2001. Microbial phytase improves performance, apparent metabolizable energy and ileal amino acid digestibility fed a lysine-deficient diets. Poult. Sci., 80: 338-344.

Singh, K.S. and B. Panda, 1992. Poultry Nutrition. Kalyani Publishers, New Delhi, India. pp: 57-61.

Short, F.J., P. Gorton, J. Wiseman and K.N. Boorman, 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol., 59: 215-221.

Selle, P.H., V. Ravindran and W.L. Bryden, 2000. The role of dietary enzymes in improving amino acids digestibility. Asian-Aust. J. Anim. Sci., 13: 75.

Smits, C.H.M. and G. Annison, 1996. Non-starch polysaccharides in broiler nutrition-towards a physiologically valid approach to their determination. World's Poult. Sci. J., 52: 203-221.

Sarwar, M., M.A. Khan and Iqbal, 2002. Status paper on feed resources for livestock in Pakistan. Int. J. Agric. Biol., 4: 186-192.

Shirazi-Beechey, S.P., M.W. Smith, Y. Wang and P.S. James, 1991. Post-natal development of lamb intestinal digestive enzymes is not regulated by diet. J. Physiol., 437: 691-698.

Sebastian, S., S.P. Touchburn, E.R. Chavez and P.C. Lague, 1997. Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. Poult. Sci., 76: 1760-1769.

Selle, P.H., V. Ravindran, G. Ravindran, R.A. Caldwell and W.L. Bryden, 2003. Phytate and phytase: consequences for protein utilization. Nutr. Res. Rev., 13: 255-278.

Thompson, L.U. and J.H. Yoon, 1984. Starch digestibility as affected by polyphenol and phytic acid. J. Food Sci., 49: 1228-1229.

Wappnir, R.W., 1989. Protein digestion and absorption of mineral elements. In: Mineral absorption in the mono-gastric GI tract, pp. 95-115 [R. D Frederick and Laszlo, J. A., editors], New York and London: Plenum press.

Zanella, I., N.K. Sakomura, F.G. Silversides, A. Fiqueirdo and M. Pack, 1999. Effect of enzyme supplementation of broiler diet based on corn and soybeans. Poult. Sci., 78: 561-568.