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The Availability or Retention of Micronutrient Contents in the Ilealdigesta of Broiler Chickens Raised on Plant Protein Diets with and Without Supplemental Enzymes

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ABSTRACT

The study was conducted to investigate the effects of protein sources or diets, amino acid and mineral retentions in the digesta of broiler chickens fed diets based on vegetable protein diets with and without supplementation of microbial enzymes. A total of 160 male broiler chicks (Ross 308) was assigned randomly into four dietary treatments, each replicated five times with eight chicks per replicate, in a (2 × 2) factorial design. Two basal diets were formulated with soybean ($SBM75^{-}$) and canola ($CM75^{-}$) meals at a ratio of 3:1 along with basal grains, and fed to the birds *ad libitum* as such or supplemented with enzymes from 1-35 days. Parameters measured are amino acids (Histidine, Arginine, Glycine, Threonine, Lysine, Methionine, Valine, Iso-leucine, Leucine, and Phenylalanine) and mineral contents (Mn, Cu, Zn, Ca, Mg, Na, K and P) from the digesta samples of broilers collected on 21d and 35 days, respectively. The concentrations of all amino acids measured here were increased ($P<0.05$; $P<0.01$) in the digesta of birds fed on $SBM75^{-}$ diet compared to those birds fed on $CM75^{-}$ diet on day 21. The retentions of almost all amino acids were similar ($P>0.05$) between two test diets, except for arginine which was increased ($P<0.05$) in $SBM75^{-}$ diet compared to $CM75^{-}$ diet when fed the birds on 35d. The retentions of Cu, Zn, and Ca found in ilelaldigest a were increased ($P<0.05$; $P<0.01$) in chicks fed on $SBM75^{-}$ diets, but not the contents of Mg, Na and P, which were higher ($P<0.01$) in $CM75^{-}$diets on day 21. Only the retentions of Cu, Zn, Ca, P, and Mg were increased ($P<0.05$; $P<0.01$) in chicks fed on $SBM75^{-}$ diets compared to those fed on $CM75^{-}$ diets on day 35d. Enzyme had influenced ($P<0.05$) the Na and Cu contents at 21d and 35days, respectively. It can be concluded that dietary protein sources influenced the micro-nutrients (amino acid and mineral) retentions in the digesta of broiler chickens with a little effect of enzymes on the mineral contents only.

Keywords: Broiler chickens, Plant proteins, Enzymes, Amino acids, Minerals, Ilealdigesta.

INTRODUCTION

Vegetable feedstuffs, i.e soybean and canola meal are a rich sources of plant protein, essential and non-essential amino acids, minerals and antioxidants. The protein quality of these feedstuffs depends greatly on the availability and digestibility of essential and non-essential amino acids along with other inherent nutrients. Dietary requirements for protein are actually requirements for amino acids. As we know that amino acids are the building blocks of protein and are the products of protein hydrolysis. These amino acids play an important role in structural and protective tissues in the body and are also important in enzyme and tissue functions (NRC, 1994). So it is important to know the digestibility and availability of amino acids in the feedstuffs in order to appraise the quality of protein. An important feature of protein quality for the feed industry is knowledge of the availability of amino acids in feedstuffs. Although the term digestibility and availability of nutrients denote different things, but these are very often used interchangeably (Ravindran and Bryden, 1999; Moughan, 2003). Availability of amino acid and mineral is defined as that proportion of amino acid or mineral which is suitable for digestion, absorption and assimilation by the birds. Therefore, measuring the accurate values or availability and digestibility of dietary amino acids and other nutrients of the feedstuffs is of prime significance, for optimizing the efficient diet formulation, and to secure maximum output from the poultry birds.

Plant ingredients either in raw form or if not properly processed, may contain numerous anti-nutritive factors (e.g. non-starch polysaccharides (NSP), tannins, trypsin inhibitor and phytic acid), which are resistant to animal enzymes and tend to suppress the nutritive value and digestibility of the feedstuffs (Hossain *et al.,* 2013). Feeds containing high NSP are comparatively cheaper in cost, and these products discourage the poultry integrators from using these feedstuffs in diet formulation due to the poor utilization of nutrients and low feed efficiency (Lee *et al.,* 2010). The presence of detrimental factors in the feedstuffs naturally limit the use of vegetable protein stuffs in the practical diets of poultry, as these factors are known to reduce the digestibility, absorption and assimilation of various nutrients (Gatel, 1994; Smits and Annison, 1996). These constraints inhibit the birds to utilize feed more efficiently, and deprive them from getting full nutritional benefits, which result in poor performance of the chicken (Hossain *et al.,* 2011). Some of these factors can be digested by exogenous enzymes, when supplemented in practical diet of poultry. But enzyme's response depends partly on the nature of the diet including many other factors, for example, feed composition, enzyme level, substrate, type, source, inclusion level of grains, extrinsic or intrinsic nature of enzymes, anti-nutritive factors, crude fibre, bird or strain, age of the birds, and so on (Almirall *et al*., 1995; Hew *et al.,* 1999; Ravindrand *et al.,* 2001; Selle *et al.,* 2003). Feed formulated entirely with the all-vegetable ingredients may increase dietary fibre level considerably, and young birds might suffer from feed indigestibility, resulting in poor performance. Young birds might be lacked of certain types and amounts of adequate enzymes which are necessary to utilize a high carbohydrate and vegetable protein diet at an early stage, thus affecting nutrient digestibility (Classen and Bedford, 1999). The beneficial response of including numerous enzymes to vegetable diets, has been demonstrated by many researchers (Zanella *et al*, 1999; Café *et al*., 2002; Bhuiyan *et al.,* 2010; Hossain *et al.,* 2014a).

Therefore, the present study has been undertaken using nutritionally adequate diets based on exclusively of plant ingredients (mainly maize, wheat, soybean and canola) with or without dietary supplementation of microbial enzymes cocktail (Avizyme 1502 and Phyzyme-XP), to assess the micro-nutrient (amino acids and minerals) availability or retentions of vegetable protein diets fed the broiler chickens.

MATERIALS AND METHODS

A 2×2 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 of micro-nutrient (essential amino acids and minerals) contents availability in the ilealdigesta of broiler chickens. A total of 160 male broiler chicks (Ross 308) were assigned randomly into four dietary treatments, each replicated five times, eight chicks per replicate in a (2 × 2) factorial design. The chicks were weighed on receipt, and were distributed randomly into 20 cages. The birds were housed in environmentally controlled house to rear those from 1 to 35 days, and birds had a free access to feed and water *ad libitum* on starter and finisher diets (pelleted) in a cage rearing system. 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 rest of the trial period.

Table1: Ingredient and nutrient composition of the starter diets (0-21 days)

	Diets			
	SBM75$^+$	**SBM75$^-$**	**CM75$^+$**	**CM75$^-$**
Ingredient composition (g/kg)				
Maize	405.9	405.9	377.5	377.5
Wheat	210.0	210.0	187.5	187.5
Vegetable oil	0.00	0.00	20.0	20.0
Soybean meal	246.9	246.9	93.8	93.8
Canola meal	82.3	82.3	281.25	281.25
Limestone	20.1	20.1	13.5	13.5
Dicalcium phosphate	18.4	18.4	10.9	10.9
DL-Methionine	2.0	2.6	1.4	2.0
Lysine	1.7	1.7	1.2	1.2
Sodium chloride	3.5	3.5	4.0	4.0
Vitamin-mineral premix[1]	2.5	2.5	2.25	2.25
Choline chloride	0.6	0.6	0.6	0.6
Avizyme-1502	0.5	0.0	0.5	0.0
Phyzyme-XP	0.1	0.0	0.1	0.0
Zinc Bacitracin	0.5	0.5	0.5	0.5
Nutrient composition (g/kg)				
ME(MJ/kg)	12.38	12.38	12.38	12.38
Crude protein	210.0	210.0	211.1	211.1
Crude fibre	30.5	30.5	37.0	37.0
Ether extract	24.0	24.0	28.4	28.4
Calcium	11.5	11.5	11.0	11.0
Available P	5.2	5.2	5.3	5.3
Sodium	1.8	1.8	1.8	1.8
Chlorine	2.5	2.5	2.7	2.7
Lysine	13.0	13.0	12.1	12.0
Methionine	5.5	5.5	5.2	5.2
Threonine	8.2	8.2	8.4	8.4
Arginine	14.2	14.2	13.0	13.0

[1]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, 80mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg and Mo, 1 mg.

Table 2: Ingredient and nutrient composition of finisher diets

	Diets			
	$SBM75^-$	**$SBM75^+$**	**$CM75^-$**	**$CM75^+$**
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	8.3	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

Dietary Treatments

Four experimental diets coded as $SBM75^+$, $SBM75^-$, $CM75^+$ and $CM75^-$, 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 ($SBM75^-$) and canola meal ($CM75^-$) at a ratio of 75:25 to one another along with basal grains as shown in Tables 1 & 2. These were fed as such ($SBM75^-$ or $CM75^-$) or supplemented with microbial enzymes (Avizyme 1502 and Phyzyme XP, Danisco Animal Nutrition, UK) to create diets $SBM75^+$ and $CM75^+$. 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 isocaloric and isonitrogenous, 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 Collection and Analyses

Three birds on day 21, and two birds on 35 days from each pen were randomly selected, weighed and killed by cervical dislocation to collect digesta samples from the ileum of the birds to assess of micro-nutrients availability (amino acids and minerals). Collected ilealdigesta samples of birds were pooled by pen, frozen immediately after collection and subsequently freeze-dried. Dried ilealdigesta samples were ground to pass through a 0.5 mm sieve and stored in airtight containers at -20˚C for chemical analyses. The amino acid content of diets and ilealdigesta samples were analysed at the Australian Proteome Analysis Facility Ltd, Macquarie University, Sydney, NSW, Australia, using the pre-column derivatisation method (AccQTaq, Waters, Milford, MA, USA). Mineral concentrations (Ca, P, Mg, K, Zn, Cu, Na and Mn) of diets and digesta samples were measured as per the method described by Anderson and Henderson (1986) using inductively coupled plasma atomic emission spectrometry (ICPAES).

Statistical Analyses and Animal Ethics

Statistical analyses were performed using Minitab software (Minitab version 16, Minitab, 2000). All the data were subjected to GLM analyses of variance for factorial design and tested for significance between the dietary treatment means by Fisher's least significance difference at $P \leq 0.05$. The experiment was conducted at the Animal House of the University of New England (UNE), and approved by the Animal Ethics Committee of the UNE (Approval No: AEC11/067).

RESULTS

Retentions of Amino Acids in the Digesta of Broiler Chickens

The results of amino acids (histidine, arginine, glycine, threonine, lysine, methionine, valine, iso-leucine, leucine and phenylalanine) concentration in the ilealdigesta of broiler chickens measured at 21d, and 35 days are shown in Tables 3 & 4, respectively. Results from the present study indicate that neither enzymes nor interaction (Diet × enzyme) had influenced ($P>0.05$) the retentions of amino acids in ilealdigesta of broiler chickens; but the availability of amino acids differed significantly ($P<0.05$; $P<0.01$) between two test diets due to dietary protein sources (Tables 3 & 4). The retentions of all amino acids measured here were increased significantly ($P<0.05$; $P<0.01$) in the birds fed on $SBM75^-$ diet compared to those birds fed on $CM75^-$ diet on day 21 (Table 3). The retentions of almost all amino acids were similar ($P>0.05$) between two test diets, except for arginine, which was increased ($P<0.05$) in $SBM75^-$ diet compared to $CM75^-$ diet fed the birds on 35d (Table 4).

Retentions of Mineral Contents in the Digesta Samples of Broiler Chickens

The results of mineral (Mn, Cu, Zn, Ca, P, Mg, Na, K) concentrations in the ilealdigesta of broiler chickens measured at 21d, and 35 days are shown in Tables 5 & 6, respectively. Results demonstrated that except for Na, no other mineral contents was influenced ($P>0.05$) by enzymatic diets on day 21. The retention of Na was increased ($P<0.05$) in $CM75^+$ diet when fed enzyme supplemented diet at 21d (Table 4). Dietary protein sources also influenced ($P<0.05$; $P<0.01$) the mineral contents between treatments at this period. Except for Mn and K, the other mineral (Cu, Zn, Ca, P, Mg, and Na) contents differed significantly ($P<0.05$; $P<0.01$) between two diets at 21d. The retentions of Cu, Zn, and Ca found in ilelaldigesta were increased ($P<0.05$; $P<0.01$) in chicks fed on SBM75- diets, but not the contents of Mg, Na and P, which were higher ($P<0.01$) in $CM75^-$ diets on day 21 (Table 5). Enzymes and diet interaction (Diet × enzyme) had no significant ($P>0.05$) effects on the retentions of mineral contents in ilealdigesta of broiler chickens at 21d. Only Cu content was significantly ($P<0.05$) increased in SBM75+ diet compared to $CM75^+$ diet when the birds fed the enzyme supplemented diets at 35d. The Ca concentration tended to be significant ($P<0.053$) between two enzyme diets (Table 6). Except for Mn, Na and K, the remaining mineral (Cu, Zn, Ca, P, Mg) contents differed significantly ($P<0.05$; $P<0.01$) between two test diets at 35d. The retentions of Cu, Zn, Ca, P, and Mg were increased ($P<0.05$; $P<0.01$) in chicks fed on SBM75- diets compared to those fed on $CM75^-$ diets on day 35d (Table 6). Only Ca content was influenced ($P<0.05$) by interaction (Diet × enzyme) in ilealdigesta of broiler chickens during finisher period (35d).

Table 3: Retentions of amino acid contents in the ilealdigesta samples taken at 21 days on the plant-based diets with or without supplemental enzymes

Treatments	Enzymes	Essential amino acid contents (mg/g) -21d									
		His	Arg	Gly	Thr	Lys	Met	Val	Ile	leu	Phe
SBM75	SBM75^{-}	3.62^{a}	6.36^{a}	7.74^{a}	7.70^{a}	6.56^{a}	1.18^{a}	8.16^{a}	6.44^{a}	12.18^{a}	6.78^{a}
	SBM75^{+}	3.64^{ab}	6.54^{ab}	7.74^{ab}	7.92^{ab}	6.52^{ab}	1.24^{ab}	8.26^{ab}	6.54^{ab}	12.52^{ab}	6.94^{ab}
CM75	CM75^{-}	3.06^{b}	5.16^{b}	7.04^{b}	7.26^{b}	6.00^{b}	0.98^{b}	7.4^{b}	5.56^{b}	10.14^{b}	5.46^{b}
	CM75^{+}	2.96^{ab}	5.24^{ab}	6.90^{ab}	7.00^{ab}	5.76^{ab}	1.02^{ab}	7.14^{ab}	5.36^{ab}	9.72^{ab}	5.28^{ab}
SEM		0.0704	0.1507	0.136	0.1151	0.1353	0.0343	0.150	0.1279	0.265	0.151
Level of significance											
Diets (A)		0.01	0.01	0.012	0.01	0.027	0.01	0.01	0.01	0.01	0.01
Enzymes (B)		0.78	0.672	0.80	0.932	0.612	0.476	0.794	0.847	0.941	0.974
A × B		0.675	0.0870	0.80	0.313	0.716	0.886	0.558	0.566	0.485	0.582

[Data represent means of three chicken of five replicate groups at age of 21 days, ^{a,b}Means bearing uncommon superscripts within column are significantly different shown in the table; **SBM75**-it contains 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; SEM= Pooled standard error of mean.]

Table 4: Retentions of amino acid contents in the ilealdigesta samples taken at 35 days on the plant-based diets with or without supplemental enzymes

Treatments	Enzyme	Essential amino acid contents (mg/g) -35d									
		His	Arg	Gly	Thr	Lys	Met	Val	Ile	leu	Phe
SBM75	SBM75^{-}	2.98	5.54^{a}	6.32	6.34	5.34	0.80	6.74	5.42	9.92	5.72
	SBM75+	2.62	4.84^{ab}	5.64	5.76	4.76	0.72	5.94	4.66	8.50	4.86
CM75	CM75^{-}	2.62	4.42^{b}	5.90	6.10	4.82	0.74	6.20	4.62	8.46	4.52
	CM75+	2.68	4.60^{ab}	6.06	6.14	5.10	0.84	6.36	4.76	8.62	5.36
SEM		0.058	0.121	0.109	0.104	0.105	0.022	0.126	0.106	0.213	0.210
Level of significance											
Diet (A)		0.213	0.013	1.00	0.741	0.674	0.501	0.814	0.118	0.134	0.418
Enzymes (B)		0.213	0.30	0.252	0.214	0.486	0.821	0.221	0.163	0.157	0.981
A × B		0.088	0.089	0.073	0.156	0.058	0.056	0.074	0.053	0.081	0.061

[Data represent means of two chickens of five replicate groups at age of 35 days, ^{a,b}Means bearing uncommon superscripts within column are significantly different as shown in the table]

Table 5: Retentions of mineral contents in the ilealdigesta samples taken at 21 days on the plant-based diets with or without supplemental enzymes

Treatments	Enzyme	Minerals contents (mg/g) -21d							
		Mn	Cu	Zn	Ca	Mg	Na	K	P
SBM75	SBM75-	0.384	0.047[a]	0.42[a]	37.2[a]	6.52[b]	6.44[c]	4.02	10.56[b]
	SBM75+	0.438	0.048[ab]	0.41[ab]	40.40[ab]	6.06[ab]	7.68[b]	4.06	11.22[ab]
CM75	CM75-	0.370	0.038[b]	0.37[b]	27.80[b]	7.56[a]	8.00[b]	3.86	14.42[a]
	CM75+	0.382	0.038[ab]	0.36[ab]	29.32[ab]	7.14[ab]	8.34[a]	3.36	12.94[ab]
SEM		0.058	0.0094	0.0011	0.0086	0.971	0.1812	0.1651	0.1013
Level of significance									
Diet (A)		0.213	0.082	0.01	0.013	0.01	0.01	0.01	0.054
Enzymes (B)		0.213	0.099	0.93	0.819	0.242	0.242	0.029	0.273
A × B		0.088	0.282	0.861	0.909	0.641	0.957	0.192	0.201

[Data represent means of three chicken of five replicate groups at age of 21days, [a,b]Means bearing uncommon superscripts within column are significantly different shown in the table].

Table 6: Retentions of mineral contents in the ilealdigesta samples taken at 35 days on the plant-based diets with or without supplemental enzymes

Treatments	Enzyme	Minerals contents (mg/g) -21d							
		Mn	Cu	Zn	Ca	Mg	Na	K	P
SBM75	SBM75-	0.394	0.050[a]	0.490[a]	44.8[a]	6.42[a]	7.74	5.58	19.08[a]
	SBM75+	0.376	0.052[a]	0.496[ab]	44.40[a]	6.60[ab]	8.76	5.70	19.18[ab]
CM75	CM75-	0.394	0.044[c]	0.460[b]	38.10[b]	5.68[b]	9.12	5.86	18.72[b]
	CM75+	0.416	0.048[b]	0.464[ab]	41.60[a]	5.98[ab]	9.10	5.42	18.60[ab]
SEM		0.058	0.0056	0.0007	0.0068	0.373	0.072	0.228	0.255
Level of significance									
Diet (A)		0.213	0.095	0.01	0.028	0.01	0.01	0.074	1.0
Enzymes (B)		0.213	0.846	0.04	0.829	0.054	0.116	0.279	0.758
A × B		0.088	0.095	0.549	0.943	0.019	0.683	0.0578	0.591

[Data represent means of three chicken of five replicate groups at age of 35days, [a,b]Means bearing uncommon superscripts within column are significantly different shown at the above table].

DISCUSSION

The protein requirement for poultry is actually a requirement for amino acids (NRC, 1994). The main function of dietary protein is to provide amino acids, and protein quality is measured by both the nitrogen content and amino acid constituents of the protein (NRC, 1994; Ravindran and Bryden, 1999). The term digestibility refers to the process of digestion and absorption and reflects enzymatic hydrolysis and microbial fermentation of ingested protein, peptides, and absorption of amino acids from the gastrointestinal lumen (Fuller, 2003).Thus, the quality of dietary proteins relies not only its nitrogen content but also on other constituents such as amino acids, their availability, mode of digestibility, and physiological utilization of specific amino acid after ingestion by the birds (Bryden *et al*., 2000; Bryden and Li, 2003).

Availability of Amino Acids in the Digesta of Broiler Chickens

The results of amino acids retentions in the ilealdigesta samples of broiler chickens were not influenced by addition of exogenous microbial enzymes or interaction of enzymes and diets in the plant protein diets in this study. It implies that enzymes exerted similar impact on the retention of amino acids in the ilealdigesta of broiler chickens fed vegetable protein diets. Furthermore, it may be due to the similar pattern of absorption and utilization of both enzymes diets used in this trial by the birds. The pattern of amino acid retentions was different between the two vegetable protein diets fed the birds. The retentions of all amino acids measured on the early period (21d) were improved in the birds fed on soybean meal diet compared to those birds fed on canola meal diets. The increased amino acid retentions of soybean meal diets in the digesta of the birds may possibly be due to the higher protein quality and quantity of soybean meal compared to both quality and quantity of canola meal diets. Furthermore, soybean meal is generally higher in protein content, lower in fibre content and other anti-nutritive factors than the canola meals. These characteristics of the two vegetable protein diets might influence the availability of amino acid retentions in the digesta of the birds (Hossain *et al*., 2013, 2014a, b). The reason might be due to the availability of protein sources of soybean meal, as soybean contains higher amount of protein percentage than the canola meal. The reduced amount of amino acids in the digesta of birds fed on canola meal diet might be due to higher fibre content of canola meal. Le Goff and Noblet (2001) reported that most of the variation in the nutrients is related to the presence of dietary fibre. Apart from these, the higher content of anti-nutritive factors, particularly, phytic acid in canola diet might influence the retention and availability of amino acids including other nutrients (Selle *et al*., 2003). Besides, the differences in response between the two main VP sources may be attributed by differences in nutrient profile, characteristic of proteins, and level of anti-nutritive factors (Singh and Panda, 1992; Hossain *et al.*, 2014a). Besides these, the chemical and physical protein characteristics, nature of protein and amino acids, dietary fibre contents, processing method might also affect the digestibility and availability of feed proteins (Mahmoudnia *et al*., 2011; Hossain *et al*., 2011; 2013).

Only significantly higher amount of arginine content was found in the soybean meal diet than in canola meal diet; although the remaining other essential amino acids measured in this study was identical between two test diets during finisher stage (35d). The higher proportion of this amino acids in the ilealdigesta of broiler chickens might be a result of indigestion or lower suitability of this amino acid to be digested, absorbed and utilized by the intestinal tissues of the broiler chickens fed the soybean meal diets. Conversely, lower concentration of amino acid in the ilealdigesta of broiler chickens fed canola meal diet indicates that, birds absorbed and utilized higher proportion of amino acids by the intestinal tissue to give rise to improved body growth for the broiler chickens. The reduced amino acid content in the digesta of canola meal (CM) diet may have been due to presence of high level of phytate content in CM (Selle *et al*., 2003), which can bind multivalent minerals and amino acids, thus reducing their availability (Van der Klis *et al.*, 1997; Camden *et al.*, 2001; Rutherfurd *et al.*, 2002). The prevalence of proteases or trypsin inhibitors in the oil-seed meals might decrease the efficacy of the enzymes (trypsin and chymotrypsin) in mono-gastric animals, which in turn suppress the digestibility and availability of protein and amino acids (Opapeju *et al*. 2006; Coca-Sinova *et al*. 2008). Besides, the use of different diet formulations, birds per se, feeding regimes, nature of feedstuffs, fibre, anti-nutrient components etc., can be considered as responsible factors for these differences (Pirgozliev *et al*., 2011) in the availability or retention of nutrient contents found in the ilealdigesta of the broiler chickens.

Availability of Minerals Contents in the Digesta of Broiler Chickens

Enzymes had minor influence on the mineral concentrations of broiler chickens as observed from our current study. Only Na and Cu contents were significantly increased in the ilealdigesta of broiler chickens when they fed enzyme-supplemented diets during starter and finisher phases. The concentration of Ca was also slightly improved when the birds fed enzymatic diet at this finisher stage (35d). The improved mineral concentrations (Na, Cu, Ca) in the digesta content may be a result of enzymatic action on the vegetable diets. The increased utilization of minerals and energy and their nutrient digestibility were observed by several researchers (Zyla *et al*., 2001; Selle *et al.,* 2000) when broiler fed wheat-based diets supplemented with microbial enzymes (phytase). The efficient utilization of nutrients might happen at that time when it becomes available in the intestinal lumen of the chickens. As it is reported that enzymes break down the anti-nutritive factors, cell wall polysaccharides into smaller ligand, storages nutrients and thereby increase their nutritive value (Wyatt, 1992; Troce *et al.*, 2007; Cowieson and Ravindran, 2008). Certain trace minerals (Cu, Zn) along with enzymes might play active catalytic role in processes like digestion, absorption and assimilation (Hossain *et al.,* 2014b). However, the interactive action of diets and enzymes exerted similar impact on the mineral contents of ilealdigesta of broiler chickens fed plant protein diets.

The retentions of mineral contents were different between the two vegetable protein (VP) diets fed the birds. Significantly higher retentions of Cu, Zn, Ca, Mg and P were found in the ilealdigesta of broiler chickens fed soybean meal diet than those of canola meal diet. The increased mineral retentions of soybean meal diets in the digesta of the birds may possibly be due to the higher mineral quality and quantity of soybean meal compared to the quality and quantity of canola meal diets. Furthermore, the higher proportion or availability indicates that these mineral contents are undigested, or in less suitable form to be digested, absorbed and assimilated by the intestinal tissue of the birds as compared to the birds of other diet group. Moreover, excess dietary nutrients may be utilized by the birds inefficiently, because the nutrients are assumed to be surplus to the bird's requirement, and it would be voided by the excreta of the birds (Kamran *et al.*, 2004). Conversely, reduced content of mineral matters in the digesta of broilers fed canola meal diets might be due to increased absorption by intestinal tissue of the birds. Variation of mineral contents in the ilealdigesta of the birds fed plant protein diets may have been due to many factors, for example, feed composition, fibre level, deleterious or toxic factors, bird per se, ingredient diversity, protein quality, physical and chemical characteristics of protein, nature of feedstuffs, processing methods, and so on (Almirall *et al*., 1995; Ravindran *et al*., 2001; Mahmoudnia *et al*., 2011; Pirgozliev *et al*., 2011). The difference in mineral contents between the two plant protein diets may be caused by differences in phytate content (not measured), which has a strong effect on availability of nutrients, particularly minerals (Thompson and Yoon, 1984; Sebastian *et al.,* 1997). It is reported that phytic acid is a common anti-nutritive or toxic factor, and is predominantly found in all plant feedstuffs. VP, particularly canola meal, contains relatively high level of phytate average 76.4% of total P (Selle *et al*., 2003). The presence of phytate in the feed form protein and mineral-phytatebond, which results in unavailability of protein and minerals in the diets (Khare, 2000; Leeson and Summers, 2001; Al-Kaiesy *et al*., 2003). In addition, this phytate content affects the digestibility of starch through forming tertiary complexes with starch materials or binary complexes with the mineral calcium (Cowieson, 2005). Many researchers reported that, the mineral, phosphorus (P) present in phytate is mostly not available in the plant feedstuffs, and phytate can bind multivalent minerals and amino acids, thus reducing their availability (Van der Klis *et al.*, 1997; Camden *et al.*, 2001; Rutherfurd *et al.*, 2002). Although our diet is supplemented with exogenous enzymes, but the anti-nutritive properties of phytate cannot be fully eliminated by phytase enzyme supplementation, because degradation of phytate is incomplete (Selle and Ravindran, 2007).

CONCLUSIONS

An overview of the results obtained in this study revealed that, neither enzymes nor interaction of diet and enzymes had influenced the retention of amino acids in the digesta of broiler chickens. From the results it would appear that enzymes had a minor effect on the mineral contents only, but different VP sources influenced the availability of both amino acid and mineral contents in the ilealdigesta of the broiler chickens.

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