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Amino Acids and Protein Digestibility and Metabolizable Energy Availability of Barley Ration in Response to Grind® Enzyme in Broiler Chickens

A. A. Saki*, S. Mirzayi[1], Sh. Ghazi[1], M. M. Moini[1] and R. Naseri Harsini

Department of Animal Science, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran

ABSTRACT : Increasing accuracy of broiler diet formulation based on amino acid digestibility in comparison to application of total amino acids could lead to more feed efficiency and productivity. This experiment was conducted for determination of sampling site (excreta and ileum) and recognition of the effects of a commercial enzyme (Grind®; Danisco, Finland) on metabolizable energy, protein and amino acid digestibility of barley. This study was modulated by a marker in 21-day old Arbor Acres chickens. Corn-soybean meal was used as a control diet and, in the other two treatments, barley (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 (0.5%) as an indigestible marker. Apparent metabolizable energy (AME), corrected by nitrogen (AMEn) and apparent digestibility of aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine, valine and methionine were significantly ($p<0.05$) higher in feces than ileum. Protein digestibility of diet and barley was significantly ($p<0.05$) higher in the ileum than in feces. Apparent digestibility of tryptophan, proline, methionine, phenylalanine and lysine was increased significantly ($p<0.05$) by enzyme supplementation. In contrast, no response was observed in AME, AMEn, and protein digestibility of the diet and barley by enzyme supplementation. The results of this study have shown that AME and amino acid digestibility were increased in feces, in contrast an adverse effect was observed for protein digestibility of the diet and barley. (**Key Words :** Metabolizable Energy, Protein Digestibility, Amino Acid Digestibility, Ileum, Excreta)

INTRODUCTION

Cereals are the major part of a poultry diet and primary sources of feed energy. Balancing the energy to protein ratio is a fundamental principle of feed formulation (Classen and Stevens, 1995). Birds adjust their feed intake to obtain a constant energy intake (Lesson et al., 1996). However, the energy a bird obtains from a cereal is variable and depends on its availability to the bird and the presence or concentration of anti-nutritive compounds such as soluble non- starch polysaccharide (NSP), especially β-glucan in barley. Effect of age of bird on feedstuffs which contain anti-nutrients, such as β-glucan in barley, is important. Decreasing growth rate by barley β-glucan in young chickens could be reduced by fungal or bacterial enzyme in the diet (Campbell and Bedford, 1992). Determination of nutritive values (available energy and protein) of the diet is critical in the poultry industry. Estimation of chemical energy in feedstuff is relatively easy; however, this estimation in birds is not precise and measurement of metabolizable energy is common (Scott et al., 1998). To improve dietary efficiency, it is necessary to elucidate the exact amino acid requirements of poultry (Ishibashi and Yonemochi, 2002). The quality of a feed protein depends not only on nitrogen content, but also on constituent amino acids and their digestibility (Ravindran and Bryden, 1999). Diet formulation based on digestible amino acids will allow the use of alternative protein sources with low digestibility coefficients, because such formulation will improve the precision of least-cost diets and reduce nitrogen excretion from poultry operations (Perttilä et al., 2002; Lemme et al., 2004). Although the advantages of the digestible amino acid system are recognized, diet formulation based on the total amino acid content is still widely used in many parts of the world. Because corn and soybean meal, most commonly used in poultry rations, have a high amino acid digestibility, the benefits of switching to the digestible amino acid

* Corresponding Author: Ali Asghar Saki. Tel: +98-918-3139775, Fax: +98-811-8273126, E-mail: dralisaki@yahoo.com

[1] Department of Animal Science, Faculty of Agriculture, Razi University, Kermanshah-Iran.

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system would be relatively small. In the future, however, economic reasons will compel the poultry industry to increase the use of an array of cheaper, alternative protein supplements with low digestibility coefficients in feed formulation. In broiler diet formulation, attention should be given to economical and maximum poultry performance (Schutte and Pack, 1995). Therefore, the main objective of the present study was to evaluate the influence of a commercial enzyme (Grind®; Danisco, Finland) and site of sampling (excreta and ileum) on estimation of metabolizable energy, protein and, particularly, amino acid digestibility of barley by marker in broiler chickens.

MATERIAL AND METHODS

In this experiment, 90-day-old unsexed Arbor Acres broiler chicks were transported from a commercial hatchery to the poultry research farm at the Bu-Ali Sina University on January 20 through March 3 2007 (d 1). The animal care committee in Bu-Ali Sina University approved this experiment. Chicks were placed on wood shavings litter in environmentally controlled chambers. The temperature and lighting regime were arranged based on Arbor Acres commercial broiler chicken recommendations. Chicks were fed a commercial starter diet for a 16-d pre-experimental period and, after four h of feed deprivation, were randomly distributed into experimental groups (three treatments, six replicates and five birds in each) in such a way that all groups had a similar average weight. All diets were given in mash form with birds having free access to water and feed throughout the experiment. The basal diet used during the experimental period was based on corn and soybean meal as major ingredients (Table 1). Barley (as test ingredient) was included in the basal diet at level of 40% to form test diets. The crude protein and metabolizable energy contents of basal and experimental diets were 18.26%, 3,025.97 kcal/kg, and 14.62%, 2,859.23 kcal/kg, respectively. The basal diet was calculated to satisfy the chick's requirements according to Arbor Acres recommended requirements. Chromic oxide was included in all diets (0.5%) as an indigestible marker. The test diet divided into two portions and enzyme was supplemented to one of these at 0.5 kg/ton. The crude enzyme preparation used in this study was Grind enzyme (a commercial multienzyme complex produced from a selected strain of *Aspergillus niger* that hydrolyzes a broad range of carbohydrates). The supplier (Danisco, Finland)

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

Ingredients (%)	Barley-enzyme	Barley+enzyme	Basal diet
Corn	63.58	36.60	36.60
Soybean meal	29.22	16.15	16.15
Barley	-	40	40
Soybean oil	2.39	2.39	2.39
Oyster shell	1.50	1.50	1.50
Dicalcium phosphate[1]	1.91	1.91	1.91
Sodium chloride	0.33	0.33	0.33
Vitamin mix[2]	0.25	0.25	0.25
Mineral mix[3]	0.25	0.25	0.25
DL-met (98.5%)	0.07	0.07	0.07
enzyme	-	0.05	-
Chrome oxide	0.5	0.5	0.5
Calculated analysis			
ME (Kcal/kg)	3,025.97	2,859.23	2,859.23
CP (%)	18.26	14.62	14.62
Ca[4] (%)	1.08	1.12	1.12
NPP[5] (%)	0.47	0.49	0.49
Na (%)	0.15	0.22	0.22
Met (%)	0.37	0.31	0.31
Lys (%)	1.03	0.73	0.73
Met+cys (%)	0.70	0.59	0.59

[1] Contains 18.7% P and 22% Ca.

[2] Supplied per kg of vitamin mixture: Vitamin A, 7.2 g; Vitamin D, 7.0 g; Vitamin E, 14.4 g; Vitamin K_3, 1.6 g; Vitamin B_1, 0.72 g; Riboflavin, 3.3 g; Pantothenic acid, 12 g; niacin, 12,160 mg; Vitamin B_6, 6.2 mg; Biotin, 0.2 g; Vitamin B_{12}, 0.6 g; choline chloride, 440 mg.

[3] Supplied per kg of mineral mixture: manganese (oxide), 64 g; iron ($FeSO_4$), 100 g; zinc (oxide), 44 g; copper ($CuSO_4$), 16 g; iodine (calcium iodate), 64 g; selenium (1%), 8 g; cobalt, 0.2 g.

[4] Calculated from tabular values (NRC, 1994). [5] NPP, nonphytate P.

reported that the crude enzyme contained endo-1, 3 (4) β-glucanase (6,000 U/g), and endo-1,4-β-xylanase (12,000 U/g). The activity of each enzyme was determined by the supplier according to the Nelson-Somogyi method for the determination of reducing sugar content (Somogyi, 1960). The enzyme preparation was added directly to other ingredients according to the supplier's recommendations.

Apparent metabolizable energy, corrected by nitrogen, and its availability

At 16 days of age, birds were transferred to metabolism cages. After a 4-d (Kadim and Moughan, 1997) adaptation period to experimental diets (without marker), the birds were deprived of feed for 6 h, then allowed *ad libitum* feed and water consumption for 3 d. Total excreta was collected during the balance period (3 d) and frozen at -20°C. Then, chickens were fed experimental diets for a further day and were euthanized and the contents of the ileum (from the Meckel's diverticulum to 4 cm above the ileo-caecal junction) were collected (26 d of age) 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). 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 (Sibbald and Slinger, 1963; Newkirk et al., 1997; Saki et al., 2008). Therefore, the AMEn of barley was calculated as: AMEn of barley = ((test diet AMEn-basal diet AMEn×0.6)/4)×10).

Diet and barley protein digestibility

Protein digestibility of diets and barley was determined using the procedures described by Ten Doeschate et al. (1993) as follow:

$$DC_{diet} = 1 - \left[\left(\frac{M_{diet}}{M_{i,e}} \right) \times \left(\frac{C_{i,e}}{C_{diet}} \right) \right]$$

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

$$DC_{barley} = \left[\frac{DC_{test} \times C_{test} - DC_{basal} \times C_{basal} \times 0.6}{C_{test} - C_{basal} \times 0.6} \right]$$

Where DC_{barley}, digestibility coefficient of protein in the barley; DC_{basal}, digestibility coefficient of protein in the basal diet; DC_{test}, digestibility coefficient of protein in the test diet; C_{basal}, digestibility coefficient of protein in basal diet; C_{test}, the concentration of protein in the test diet.

Amino acid digestibility

Amino acid concentrations in diets, excreta, 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 Ten Doeschate et al. (1993) as follows:

$$AD_{AA} = 1 - \left[\left(\frac{M_{diet}}{M_{i,e}} \right) \times \left(\frac{A_{i,e}}{A_{diet}} \right) \right]$$

Where AD_{AA}, apparent digestibility of individual amino acid; M_{diet}, marker concentration in diet; $M_{i,e}$, marker concentration in ileal digesta (i) or excreta (e); A_{diet}, concentration of each amino acid in diet; $A_{i,e}$, concentration of each amino acid in ileal digesta (i) or excreta (e).

Statistical analysis

The experiment was designed and statistically analyzed as a 2×2 factorial arrangement of two sites of sampling and two levels of enzyme based on a completely randomized design. Body weight at 16d was used as a covariate. The following statistical model (SAS, 2004) was used to assess the main effect of sampling site (S); the main effect of enzyme (D); and the corresponding interaction S×D:

$$Y_{ijk} = \mu + S_i + D_j + S_i \times D_j + e_{ijk}$$

Where Y_{ijk}, observed trait; μ, overall mean; S_i, effect of sampling site; D_j, effect of enzyme; $S_i \times D_j$, interaction of D_j and S_i; and e_{ijk}, random error.

Duncan's multiple-range test was used to determine significant difference among treatment means.

RESULTS

Apparent metabolizable energy, corrected by nitrogen, and its availability

Results on AME, AMEn and AME availability are summarized in Table 2. No significant effects of enzyme inclusion were found on AME, AMEn and AME availability ($p>0.05$). The AME, AMEn and AME availabilities based on ileal measurement were significantly lower than the corresponding measurement based on excreta samples ($p<0.05$). The interaction of site of sampling by enzyme was not significant for these parameters ($p>0.05$).

Table 2. Comparison of AME, AMEn and availability of AME

Source of variation		AME[1] (kcal/kg)	AMEn[2] (kcal/kg)	AME availability (%)
Sample source (S)	ileum	2,009.3[b]	1,856.8[b]	0.48[b]
	excreta	2,740.5[a]	2,634.4[a]	0.65[a]
	p	<0.0001	<0.0001	<0.0001
Enzyme level (D)	0	2,369.7[a]	2,236.9[a]	0.56[a]
(kg/ton)	0.5	2,380.2[a]	2,254.3[a]	0.56[a]
	p	0.6244	0.4020	0.4815
S×D	p	0.0744	0.0918	0.0635
Combination effects				
Sample source×enzyme level	0.5×ileum	2,084.21±326[b]	1,933.1±0.31[b]	0.49±0.07[b]
	0×ileum	1,934.43±216[b]	1,780.4±0.21[b]	0.46±0.05[b]
	0.5×excreta	2,804.91±167[a]	2,693.4±0.14[a]	0.67±0.04[a]
	0×excreta	2,676.12±125[a]	2,575.4±0.12[a]	0.63±0.03[a]
	p	0.0031	0.0015	0.0031
	MSE	4,943.27	4,480.65	0.0028

Means with common superscripts in same column are not significantly different (p<0.05).
[1] Apparent metabolizable energy. [2] Apparent metabolizable energy corrected by nitrogen.

Diet and barley protein digestibility

Results on protein digestibility of diet and barley are summarized in Table 3 and 4, respectively. No reaction was observed on protein digestibility of diets by enzyme inclusion (p>0.05). The ileal protein digestibility of diets was significantly higher than the excreta protein digestibility (p<0.05, Table 3). Barley protein digestibility at the ileum was significantly higher rather than in excreta (p<0.05). No reaction was found by inclusion of enzyme on barley protein digestibility (p>0.05). The sampling site×enzyme interaction for protein digestibility of diet and barley was also not significant (p = 0.2117, and p = 0.0561, respectively).

Amino acid digestibility

Results of apparent digestibility of amino acids are summarized in Table 5. Apparent digestibilities of diet aspartic acid, glutamic acid, serine, glysine, alanine, valine, tyrosine and methionine were significantly (p<0.05) higher in excreta in comparison to ileal samples. Significant increases in digestibility of tryptophan, proline, methionine, phenylalanine and lysine were achieved by enzyme inclusion (p<0.05). Interaction between sampling site and enzyme was not significant for apparent digestibility of aspartic acid, glutamic acid, histidine, glycine, arginine, phenylalanine, isoleucine, alanine, tryptophan, and serine (p>0.05).

Table 3. A comparison of *in vivo* diet protein digestibility (%)

Source of variation		Diet protein digestibility
Sample source (S)	ileum	60.24[a]
	excreta	41.80[b]
	p	0.0004
Enzyme level (D)	0	52.20[a]
(kg/ton)	0.5	50.20[a]
	p	0.4889
S×D	p	0.2117
Combination effects		
Sample source ×enzyme level	0.5×ileum	59.96±0.06[a]
	0×ileum	60.52±0.03[a]
	0.5×excreta	43.88±0.07[b]
	0×excreta	39.71±0.02[b]
	p	0.0024
	MSE	0.0028

Means with common superscripts in same column are not significantly different (p<0.05).

Table 4. A comparison of *in vivo* barley protein digestibility (%)

Source of variation		Barley's protein digestibility
Sample source (S)	ileum	52.77[a]
	excreta	41.79[b]
	p	0.0001
Enzyme level (D)	0	44.26[a]
(kg/ton)	0.5	50.30[a]
	p	0.4889
S×D	p	0.0561
Combination effects		
Sampling site ×enzyme level	0.5×ileum	60.90±1.85[a]
	0×ileum	44.64±8.48[b]
	0.5×excreta	39.71±2.23[b]
	0×excreta	43.88±7.61[b]
	p	0.2544
	MSE	0.0112

Means with common superscripts in same column are not significantly different (p<0.05).

Table 5. i) Amino acid digestibility of diets (%)

Source of variation		Aspartic acid	Glutamic acid	Histidine	Glycine	Arginine
Sample source (S)	ileum	65.06[b]	73.24[b]	76.02[a]	68.66[b]	67.59[a]
	excreta	71.17[a]	83.63[a]	73.64[a]	76.27[a]	71.41[a]
	p	0.0001	<0.0001	0.0061	0.0001	0.0005
Enzyme level (D)	0	66.14[a]	76.72[a]	73.09[a]	72.19[a]	69.83[a]
(kg/ton)	0.5	70.09[a]	80.15[a]	76.57[a]	72.74[a]	69.17[a]
	p	0.0884	0.3681	0.2436	0.9923	0.6724
S×D	p	0.0741	0.6423	0.4330	0.3679	0.9264
Combination effects						
Sample source ×enzyme level	0.5×ileum	67.44±1.07[ab]	73.66±1.61[c]	78.08±1.29[a]	69.75±1.76[b]	66.80±0.27[a]
	0×ileum	62.69±3.06[b]	72.83±0.26[c]	73.97±0.02[ab]	67.57±1.29[b]	68.39±2.84[a]
	0.5×excreta	72.75±0.55 [a]	86.65±0.77[a]	75.07±1.45[ab]	75.74±0.79[a]	71.55±0.63[a]
	0×excreta	69.60±3.39[a]	80.62±3.39[b]	72.22±3.39[b]	76.81±3.39[a]	71.28±3.29[a]
	p	0.2168	0.8760	0.1472	0.4759	0.7605
	MSE	16.38	23.01	3.82	40.7	8.49

Means with common superscripts in same column are not significantly different (p<0.05).

Table 5. ii) Amino acid digestibility of diets (%)

Source of variation		Tyrosine	Methionine	Isoleucine	Phenylalanine	Lysine
Sample source (S)	ileum	67.78[b]	64.30[b]	73.08[a]	74.45[a]	74.37[a]
	excreta	72.47[a]	81.35[a]	75.32[a]	79.77[a]	74.72[a]
	p	0.0031	0.0001	0.06318	0.0566	0.5942
Enzyme level (D)	0	71.89[a]	62.37[b]	72.76[a]	71.61[b]	71.49[b]
(kg/ton)	0.5	68.36[a]	83.28[a]	75.64[a]	82.61[a]	77.59[a]
	p	0.3679	0.0023	0.2895	0.0001	0.0311
S×D	p	0.0286	<0.0001	0.8442	0.0539	0.0454
Combination effects						
Sample source ×enzyme level	0.5×ileum	69.50±0.70[b]	79.15±1.20[b]	75.13±1.23[a]	83.05±1.34[a]	77.06±1.32[a]
	0×ileum	66.06±0.66[b]	49.45±3.39[c]	71.03±3.39a	65.85±3.39[b]	69.68±3.39[b]
	0.5×excreta	67.22±1.09[b]	84.41±0.83[a]	76.15±1.20a	82.17±1.17[a]	76.12±1.23[a]
	0×excreta	77.73±3.39[a]	75.29±0.31[b]	74.50±2.45a	77.37±7.84[ab]	73.31±1.83[ab]
	p	0.0105	0.0001	0.2669	0.0503	0.0447
	MSE	3.41	3.44	5.12	19.04	4.53

Means with common superscripts in same column are not significantly different (p<0.05).

Table 5. iii) Amino acid digestibility of diets (%)

Source of variation		Serine	Tryptophan	Alanine	Proline	Valine
Sample source (S)	ileum	68.71[b]	70.42[a]	70.49[b]	79.64[a]	65.09[b]
	excreta	72.49[a]	63.69[b]	77.99[a]	82.89[a]	74.64[a]
	p	0.0052	0.0004	0.0001	0.0652	0.0001
Enzyme level (D)	0	70.11[a]	63.42[b]	72.37[a]	76.56[b]	69.41[a]
(kg/ton)	0.5	71.09[a]	70.69[a]	76.11[a]	85.97[a]	70.32[a]
	p	0.2391	0.0001	0.1517	0.0001	0.2834
S × D	p	0.5626	0.0638	0.08291	0.0138	0.0095
Combination effects						
Sample source ×enzyme level	0.5×ileum	69.45±0.77[a]	72.78±0.44[a]	71.75±3.18[b]	83.45±0.77[ab]	67.27±1.03[bc]
	0×ileum	67.97±0.85[a]	68.06±1.63[a]	69.23±1.08[b]	75.84±3.39[b]	62.92±4.13[c]
	0.5×excreta	72.73±0.52[a]	54.06±1.32[b]	80.47±0.74[a]	88.50±2.12[a]	73.37±0.88[ab]
	0×excreta	72.26±3.39[a]	73.33±3.39[a]	75.51±3.39[ab]	77.29±5.86[b]	75.91±1.28[a]
	p	0.1484	0.0019	0.0351	0.0696	0.0148
	MSE	3.28	4.03	5.84	12.76	5.15

Means with common superscripts in same column are not significantly different (p<0.05).

Methionine and glutamic acid digestibility were significantly ($p<0.05$) higher in excreta collected from chicks fed diet with enzyme than on other treatments. In contrast, tryptophan digestibility in this treatment was significantly lower compared to other treatments ($p<0.05$). Maximum lysine digestibility between treatments was observed from the ileum of chicks fed enzyme supplemented diet, and a similar trend was observed for phenylalanine.

Treatments effects on digestibility of isoleucine, arginine, and serine were not significant ($p>0.05$).

DISCUSSION

AME and AMEn were significantly higher in feces (2,740.5 and 2,634.4kcal/kg) than ileum (2,009.3 and 1,856.8 kcal/kg). These observations may be related to the higher fiber and β-glucan content of barley. The undigested fiber provided a significant feed source for an adaptable gut microflora population. Also, a mature gut (e.g., capacity) and a mature endogenous enzyme system provide other positive conditions in the bird gut to access these fibrous substance (Graham and Aman, 1991; Schutte et al., 1992). The values of AME and AMEn measured in this study were lower than results of Scott et al. (1998). The difference may be related to composition of diet (proportion of test ingredient in diet, 20 versus 40% barley in Scott et al. (1998) and our investigations, respectively). Therefore, this variation may be related to other factors such as β-glucan, variety and seed quality (Slominski et al., 1999), and availability of protein and amino acid (Zhang et al., 1994). Anti-nutritional effects of β-glucan can be decreased by utilization of enzymes. Numerous studies made since the 1960s have confirmed that addition of enzyme preparations containing β-glucanase to diets containing a high proportion of barley increases the AME content (Chesson, 1992). Subsequent works by Broz and Frigg (1986) and Brufau et al. (1991) suggested that these observations can be generalized to the destruction of any gel-forming polysaccharide leached from barley cell wall which depressed AME in the diet. AME content has an adverse relationship with β-glucan level and viscosity in the ileum. Scott et al. (1998) reported that addition of enzymes in the diet increased AME, but in the current study no such outcome was obtained ($p>0.05$). Differences between AME content of excreta and ileum, were higher in the diet with enzyme than when enzyme was absent. These differences could be due to microbial fermentation in the distal intestine in this experiment. Similar results were achieved by Scott et al. (1998).

Enzyme inclusion had no significant effect on protein digestibility of the diet as well as barley ($p>0.05$). In recent years, protein digestibility by ileum sampling has been accepted in pigs, since superior estimation of digestibility was obtained rather than from excreta; however, in poultry less attention was given in this respect, because microbial processing effects in the cecum and colon of poultry on nutrient digestibility have less importance. Feed digestibility coefficient is affected by age, sex, genotype and experiment method, and it is necessary to evaluate accurately digestibility coefficients for these factors. Zenella et al. (1999) noted that protein digestibility was reduced by aging while amino acid digestibility increased, but no response was found to sex in this respect. Occasionaly, apparent digestibilities of amino acids in excreta were higher than at the ileum. This means that amino acids have disappeared during passage from the terminal ileum to the end of the digestive tract. 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 of these materials (Ravindran et al., 1999; Kadim et al., 2002). Therefore, the disappearance of amino acids in poultry hindgut is determined by a balance of catabolism and anabolism of amino acids. When the net result is 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 occur. In net catabolism, ammonia may be absorbed but not utilized by the birds and completely excreted in the urine as uric acid (Salter, 1974). In addition, fiber impedes protein utilization in the small intestine of poultry. The mechanism of fiber on digestibility of protein is not clear, but the indigestible protein fraction in the feed may be bound to, or encapsulated by fibrous components of the feed (Jensen et al., 1995). Therefore, with lower digestible ingredients such as barley, more undigested nitrogenous substance will reach the hindgut. Grind enzyme also increased apparent digestibility of excreta amino acids in several cases. Ileum digestibility had advantages rather than excreta digestibility in this regard. Nutrient digestibility in feedstuff may aid diet formulation, improve protein utilization and reduce nitrogen excretion. In past years, digestibility of amino acids was obtained by the Sibbald technique (1979) and precise feeding. This procedure is simple but had great problems. Therefore, ileum sampling could remove the undesirable effect of microbial fermentation in the large intestine. However, the influence of hindgut microflora on protein nutrition in chickens is not clearly established. In contrast to our results, Papadopoulos (1985) reported that the influence of the avian hindgut on protein nutrition is insignificant and that there is little advantage, over conventional excreta analysis,

in using other methods to determine amino acid digestibility in chickens. Commonly adult roosters were used for digestibility assay, and results were used for diet formulation in chickens; however, roosters are physiologically different from chicks. Thus, it is better to use chicks in digestibility assays. In barley, anti-nutritional factors are important agents in reduction of apparent protein digestibility. Inclusion of enzyme in broiler diets could increase apparent digestibility of amino acids and destroy anti-nutritional factors of feedstuffs. Increased endogenous enzymes and diminished fermentation in the gastrointestinal tract were achieved by β-glucanase and thereby increased nutrient absorption. These outcomes are in agreement with the results of Perttilä et al. (2001), Biadoo et al. (1998) and Ten Doeschate et al. (1993).

IMPLICATIONS

According to results of the current study, Grind enzyme addition can increase apparent digestibility of some amino acids. In contrast, no improvements were achieved in AME, AMEn, and protein digestibility of diets and barley by enzyme inclusion. Sampling site is important to estimate digestibility of protein and amino acids that is dependent on type of animal and diet. Apparent digestibility of aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine, valine and methionine were greater in feces than ileum. However in other amino acids, there were no differences between digestibility coefficients achieved by ileum or excreta samples. Values of AME and AMEn in feces are more valid than in the ileum; on the other hand, estimation of protein digestibility in the ileum shows better results than feces in poultry.

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