Bangladesh Journal of Animal Science
Journal homepage: http://www.banglajol.info/index.php/BJAS

Effect of high protein supplementation on growth and nutrient digestibility of broiler

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Abstract

This study was undertaken to compare the growth performance and nutrient digestibility (energy, protein and amino acid) of broilers fed diets containing only vegetable protein (soybean) with birds that received Pro-EL as protein supplement in their diets. Day-old male broiler chicks (n=60; Cobb 500) were randomly distributed into 2 dietary treatment groups, namely control (T_1) and supplemented (T_2) diets, with 5 birds per replication in a CRD. Birds had a free access to iso-caloric and iso-nitrogenous starter diets in cages up to 21d. The results of FI, LW, LWG and FCR of broilers up to 21 days were poorer (P<0.01) on T_2 diet than the broilers fed on T_1 diet. The ileal nutrient digestibility of GE and CP was identical (P>0.05), but the majority of the AAs digestibility values were influenced (P<0.05; P<0.01) by treatments except for Gly, Thr, Cys, Val, Ile, Leu, and Phe. The digestibility of Asp, His, Arg, Ala, Pro, Ser, Glu, Tyr and Lys was impaired (P<0.05; P<0.01) in the T_2 diet compared to T_1 diet except for Met, which was improved (P<01) in T_2 diet. It can be concluded that the negative response of broilers fed on protein supplemented diet might arise from the reduced nutrient digestibility of the diet.

Key words: growth, pro-el, nutrient digestibility, broiler chicken

 Bang. J. Anim. Sci. 2017. 46 (1): 44-50

Introduction

Protein is an expensive element and indelibly requires to formulate diet, and to meet the essential nutrient requirement of the broiler chickens. It is well-recognized that greater portion of costs associated with poultry production involves meeting the requirements of protein or amino acids (AAs) of the birds (May *et al*., 1998; Corzo*et al*., 2004). It is reported that protein requirements incur 45% of the total cost of poultry production (Ahmad *et al.,* 2006). Deficiency of protein and amino acids in poultry diet can adversely affect their optimum growth and development (Awad *et al.,* 2016). Moreover, proteins play a crucial role in structural and protective tissues in the body and are also important in enzymes and tissue functions (NRC, 1994). However, it can be assumed that broiler diets supplemented with the high protein sourced ingredient might be potential to ensure the better productivity of the broiler chickens. Moreover, the supplementation of a novel protein ingredient into broiler diet might reduce the lacks and gaps of protein requirement as well as the feed cost of rearing broiler chicken under hot humid tropical condition. For this reason, poultry scientists and feed formulators are exploring cheap sources of feed ingredients and feed supplements to manufacture the quality poultry diet. In this regard, protein supplement such as Pro-EL can be incorporated into poultry diet as a rich source of protein ingredient to enhance the quality and quantity of diet. This ingredient is available in the commercial market as a synthetic form or protein supplement and cheaper in cost, which contains more than 80 % protein with a good profile of both essential and non-essential amino acids, as observed by our lab analytical process.

The introduction of this novel protein supplement (Pro-EL) into poultry diet might be beneficial to be used as a rich source of protein to meet the actual protein requirement and enhanced production. However, the growth responses of broiler chickens are the key performance indicator, which can be attained or assessed by feeding birds with a different sort of feedstuffs or diets, available in the nature or commercially.

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Basically, the nutritional value of diets fed to chicken can be evaluated by their productivity and nutrient digestibility. Besides, the knowledge of the digestibility of feed ingredients is the basic requirement to formulate the balanced diet of chicken. Furthermore, determining feed nutrient digestibility is also considered as an important parameter in evaluating the nutrient adequacy or availability and efficiency of the feedstuffs (Noreen, 2006). However, the information on nutrient digestibility of all feedstuffs available in the nature is limited. Without accurate digestible data of the available feedstuffs, diet formulators, researchers and nutritionists might run a risk of over-fortification, which can raise the cost of feeding the birds, or under-fortification might results in reduced growth rate or poor performance of the birds. However, there is a lack of information regarding the nutritional value and digestibility of many unconventional feed resources such as novel synthetic ingredients, crab meal and there is a lack of inconsistency in the methods used to determine digestibility (Applegate *et al*., 2008; Garcia *et al.,* 2007). Considering this view, the present study was undertaken to investigate the gross responses and the apparent ileal digestibility of gross energy (GE), crude protein (CP) and amino acids (AAs) of protein supplemented diet (Pro-EL) diets fed the broiler chickens.

Materials and Methods

Animal Husbandry and experimental design

A total of sixty (Cobb 500) day-old male broiler chicks were procured from a local commercial hatchery to conduct this experiment up to 21 days. The chicks were weighed on receipt, and then equally assigned to two dietary treatment groups (T_1 and T_2), each diet replicated 6 times with five birds per replication in a completely randomized block design (CRD). All the chicks were allotted into 12 battery cages within an open-sided housing condition. The chicks were fed a broiler starter diet from day1 to 21d (Table 1). The following two diets were used: (i) standard corn-soybean based broiler diet (control, T_1) and (ii) broiler diet containing15% Pro-EL (T_2 or test diet). The ingredient and nutrient compositions of T_1 and T_2 were shown in Table 1. Both diets were iso-caloric and iso-nitrogenous in nature and contained titanium dioxide (TiO_2) as marker to enable assessment of nutrient digestibility (GE, CP, AAs). Birds had a free access to feed (mash) and water entire the trial period.

Table 1. Ingredients and nutrient composition of experimental diets

Ingredient composition (%)	Treatment	
	T_1	T_2
Corn	53.37	61.50
Protein supplement (Pro-EL)	**0.00**	**15.00**
Soybean meal	37.20	12.52
Palm oil	4.50	2.30
DCP	2.00	2.00
Limestone	1.40	1.40
Common salt	0.10	0.10
Sodium sulphate	0.40	0.40
Choline Cl-70%	0.05	0.15
Sand	0.00	4.00
Vitamin- premix[1]	0.05	0.05
Mineral- premix[1]	0.10	0.10
L-Lysine	0.48	0.31
DL-Methionine	0.25	0.00
L-Threonine	0.10	0.17
Nutrient composition (%) (calculated value)		
ME (MJ/kg)	12.71	12.71
CP	22.00(23.65)	22.00(23.80)
CF	2.30	2.70
Ca	0.95	0.94
Available P	0.46	0.45
Lysine	1.45 (1.9)	1.44 (1.4)
Methionine	0.54 (0.6)	0.53(0.67)
Threonine	0.94	0.94

[T_1 diet refers to control or basal diet whereas T_2 is test diet having 15% protein supplement, *i.e* Pro-EL; Figures inside the bracket only indicate analyzed value whereas figures outside the bracket indicate calculated values; [1]Provided per kg of diet (mg): vitamin A (as all-trans retinol), 3.6 mg; cholecalciferol, 0.09 mg; vitamin E (as dα-tocopherol), 44.7 mg; vitamin K3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; vitamin B12, 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.]

Data collection

Live weight, feed intake and feed conversion ration were calculated weekly, to assess the gross responses of the broiler chicks. Three chicks from each replicate cage on day 21, were selected randomly, weighed and killed by humanely to collect ileal digesta contents from the ileum of the birds. Collected digesta were pooled by pen in a plastic container and later dried by freeze –dryer machine. The dried samples were ground to pass through a 0.5 mm sieve, and stored in airtight containers at -20^{o}C for chemical analyses.

Composition of Pro-EL ingredient (analyzed value)

It's a synthetic protein supplement which contains ME, 3200 Kcal/kg, CP, 84.12%, NPN, 3.42%, lysine 4.6%, methionine 3.0 %, cysteine 0.013%, leucine 5.3%, histidine, 1.27%, threonine 2.73 %, valine 3.3%, glycine 2.26%, arginine 4.3%.

Chemical analyses

The starter diets and digesta samples were analyzed for GE, CP and AA digestibility. Samples were dried at 105 ^{o}C in a drying oven for 24h for dry matter (DM) determination. The titanium dioxide (TiO_2) concentrations in the diets and digest samples were measured after ashing the samples and treating the ash with boiling 7.4 M sulphuric acid according to the method of Short *et al*. (1996). Gross energy content of diets and ileal digesta were determined using an IKA bomb calorimeter (IKA-WERKE, C7000, Staufen, Germany). Nitrogen content of the samples was determined by Kjeldahl method using standard laboratory procedures (AOAC, 2000). Crude protein (CP) equivalent was calculated as N (%) × 6.25.

The AAs concentrations of diets and ileal digesta samples were analyzed following the procedure as described by Strydom and Cohen(1994), using the pre-column derivatization method (AccQ Tag, Waters, Milford, Ma, USA), high performance liquid chromatography (HPLC). Cysteine and methionine were analyzed as cysteic acid and methionine sulfone by oxidation with performic acid for 16 h at 4°C, and neutralization with hydrobromic acid before hydrolysis. The apparent ileal digestibility coefficient of nutrients (GE, CP and AAs) was calculated using the following equations:

a) Digestibility coefficient for diet

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

Statistical analyses

All collected data were statistically analyzed using Minitab software (Minitab Version 16, 2000). The data were analyzed using one-way ANOVA with diet as factor. The significance of differences between means was determined by Fisher's least significant difference*P≤.05.*

Results

The results of gross responses of broilers denote that FI, LW, LWG and FCR fed supplemented diet (T_2) were poorer (P<0.01) than the responses of broiler fed control diet (T_1) as shown below in Table 2. The apparent ileal digestibility values of CP and GE were not influenced (P>0.05) by dietary treatment (Table 3). The ileal digestibility values of most of the AAs were influenced significantly (P<0.05; P<0.01) by dietary treatment measured in this study (Table 3). The ileal digestibility values of Gly, Thr, Cys, Val, Ile, Leu and Phe were not influenced (P>0.05) by dietary treatment. The ileal digestibility values of Asp, His, Arg, Ala, Pro, Ser, Glu, Tyr and Lys were decreased (P<0.05; P<0.01) in the supplemented diet (T_2) compared to basal diet (T_1) except for Met. Only the digestibility of Met was improved (P<0.01) in the birds fed supplemented diet (T_2).

Table-2. Feed intake (FI), live weight (LW), live weight gain (LWG) and feed conversion ratio (FCR) of broiler chickens fed protein supplemented diet from d1-21 days

Parameters	Age (days)	Treatment		Pooled SEM	P-values
		T_1	T_2		
FI (g/b)	1-14	547.33[a]	394.00[b]	8.500	0.01
	1-21	1199.17[a]	830.17[b]	15.242	0.01
LW(g/b)	1-14	416.33[a]	246.83[b]	5.112	0.01
	1-21	889.33[a]	475.00[b]	18.142	0.01
LWG (g/b))	1-14	371.67 [a]	202.00 [b]	5.113	0.01
	1-21	844.67[a]	430.17[b]	18.161	0.01
FCR	1-14	1.42[b]	1.95[a]	0.018	0.01
	1-21	1.48[b]	1.96[a]	0.045	0.01

[Each value refers to mean value of six replicates consisting of five birds per replicate cage from d1 to 21; [ab]Means bearing uncommon superscripts within a row are significantly different at $^{**}P<0.01$; SEM, Pooled standard error of mean; T_1, basal diet while T_2, supplemented diet].

DISCUSSION

Growth performance in broiler chickens has been regarded as the primary criterion for determining the feed nutrient requirements because the broiler chick is an ideal research tool with a limited nutrient store, high nutrient demand and rapid growth rate (Ammerman, 1995). In this study, overall growth responses of the broilers were influenced adversely by feeding supplemented (T_2) diet. The reduced performance of broilers on supplemented diet could be due to poor feed intake of the birds as observed in our study. The poor feed intake of broilers on T_2 diet could also be caused by poor weight gain of the birds and the consequent lower nutritional requirement (Hossain *et al.,* 2016). Though palatability test of the diet was not done, but it can be assumed that the less palatability and poor nutrient (AAs) digestibility of the diet could be reasons which might influence the feed intake and growth performance of the birds (Jackson *et al.,* 1982; Mahmoudnia *et al*., 2011). It is reported that the AAs imbalances in diets decreased the biological value and feed intake of the diets (Jackson *et al*., 1982).

Besides, other factors such as organoleptic traits (e.g colour, smell, odour, flavor, taste and texture) of diet might also affect the feed ingestion and feed regulation of broiler chickens (Cruze *et al*., 2005). Further, dietary composition and sources of protein use in the practical diet could also influence the feed intake and feed preference of broiler chicken (Hossain, 2013). However, it is obvious from the current study that broilers fed protein supplemented diet (T_2) grew poorly, most probably as a result of reduced feed consumption, inferior FCR and poor nutrient (AAs) digestibility of the birds. The impaired FCR of birds on T_2 diet indicates that the broilers are less efficient to convert this feed to meat more rapidly than the birds fed on control diet (Hossain *et al.,* 2011). Apart from these, the impaired amino acids (AAs) digestibility of the supplemented diet might be another reason for the poor growth responses of the birds as is evidence from our present study.

Our results further revealed that test diets had no influence on the macro-nutrient (energy and protein) digestibility, but micro-nutrient, particularly AAs digestibility values were influenced significantly in this study. The digestibility values of Asp, His, Arg, Ala, Pro, Ser,

Glu, Tyr and Lys were found to be reduced in the supplemented diet (T_2) compared to basal diet (T_1) except for only Met. Although no synthetic Met was added into the diet, the Met digestibility was found to be increased in the supplemented diet. The higher content of Met in the supplemented diet might influence the improved digestibility of Met. Apart from the Met digestibility, the reason for the reduced digestibility of AA on supplemented diet (T_2) is not obvious, but it can be said that the quality and quantity of the protein of the test diet possibly become a factor which can cause a difference in digestibility between two diets (Hossain *et al.,* 2014). Further, the reduced AAs digestibility of birds on supplemented diet might be due to less absorption, utilization and assimilation by the intestinal tissue of the broiler chickens (Hossain *et al.,* 2015).

In fact, the protein requirement for poultry is actually a requirement for amino acids (NRC, 1994). It is reported that 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). As is observed from the formulation data herein this study, the crude fibre content of the supplemented diet appeared to be a bit higher than the basal diet which might influence the AAs digestibility of broiler chicken. Le Goff and Noblet (2001) reported that most of the variation in digestibility of nutrients is related to the presence of dietary fibre of the feed. However, it is difficult to say truly that what factors are actually responsible for the variation of digestibility between two diets as it is a novel work, and so no data are available regarding this experiment. However, it can be assumed that the differences in AAs digestibility between the two diets could be attributed by differences in nutrient profile, characteristic of proteins, level of anti-nutritive factors, *the physical and chemical composition of protein, diet characteristics,* nature of protein and amino acids, fibre contents, biological value, processing method, bird age, bird per se, strain etc. (Singh and Panda, 1992; Mahmoudnia *et al*., 2011; Pirgozliev *et al.,* 2011; Hossain*et al*., 2011, 2013, 2014).

Table 3. Apparent ileal gross energy (GE), crude protein (CP) and amino acids (AAs) digestibility of broiler chickens fed supplemented or test diet on 21 days

Treatment	Energy & protein		AAs							
	GE	**CP**	**Asp**	**Ser**	**Glu**	**Gly**	**His**	**Arg**	**Thr**	**Ala**
T_1	0.75	0.84	0.82^a	0.82^a	0.87^a	0.81	0.85^a	0.88^a	0.83	0.83^a
T_2	0.73	0.82	0.74^b	0.73^b	0.80^b	0.76	0.81^b	0.85^b	0.79	0.75^b
SEM	0.014	0.012	0.022	0.019	0.015	0.018	0.013	0.009	0.015	0.023
P-value	0.413	0.208	0.05	0.01	0.01	0.13	0.046	0.044	0.210	0.043

Treatment	**Pro**	**Cys**	**Tyr**	**Val**	**Met**	**Lys**	**Ile**	**Leu**	**Phe**
T_1	0.83^a	0.83	0.85^a	0.82	0.85^b	0.92^a	0.83	0.83	0.84
T_2	0.78^b	0.76	0.78^b	0.79	0.95^a	0.87^b	0.79	0.81	0.80
SEM	0.015	0.037	0.017	0.017	0.017	0.008	0.016	0.014	0.015
P-value	0.05	0.202	0.01	0.191	0.01	0.01	0.121	0.22	0.083

[Each value refers to mean value of six replicates consisting of three birds per replicate cage on 21d; ^{ab}Means bearing uncommon superscripts within a row are significantly different at **P*<0.05 and ***P*<0.01; GE, gross energy; CP, crude protein; Asp, aspartic acid;Ser, serine;Glu, glutamic acid;Gly, glysine; His, histidine; Arg, arginine; Ala, alanine; Pro, proline; Cys, cysteine; Tyr, tyrosine; Val, valine; Met, methionine; Lys, lysine; Ile, iso-leucine;Leu, leucine;Phe, phenylalanine]

Conclusion

Birds fed on protein supplemented diet responded negatively as a result of impaired feed consumption, body growth, feed conversion ratio and poor nutrient (AAs) digestibility of the broiler chickens. Although birds had a negative responses on the current level of protein supplement in the diet, further study may be undertaken to observe the different level of high protein supplement (Pro-EL) inclusion in poultry diet and its productivity including suitability.

Acknowledgement

The authors are greatly acknowledged for the funding and executing the project by the ITA, UPM, Serdang, Malaysia

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