*Indian Journal of Animal Sciences* **86** (6): 696–701, June 2016/Article

# Effect of low-protein diet, gender and age on the apparent ileal amino acid digestibility in broiler chickens raised under hot-humid tropical condition

ELMUTAZ ATTA AWAD[1], IDRUS ZULKIFLI[2], ABDOREZA SOLEIMANI FARJAM[3], LOH TECK CHWEN[4], MOHAMMAD ABUL HOSSAIN[5] and AHMED ALJUOBORI[6]

*Universiti Putra Malaysia, (UPM) Serdang, Selangor 43400 Malaysia*

Received: 27 August 2015; Accepted: 1 October 2015

ABSTRACT

The study was undertaken to investigate the effects of feeding low-protein diet, gender, and age on the apparent ileal amino acid (AA) digestibility of broilers raised under hot-humid environmental condition. Broiler chicks (320) of either sex were fed on diets varying in crude protein (CP) levels (standard vs. low), and were assessed for apparent ileal digestibilities (AID) of CP and AA at 3 and 6 week of ages, respectively. Birds were fed on 2 diets, i.e starter (CP 22.2%; 16.2%) and finisher (19.5% and 13.5%) *ad lib.* from 1–42 days. Results showed that birds fed on low-CP diet significantly increased the apparent ileal digestibility of CP and all AA except for Lys, regardless of sex and age. Sex had no influence on the AID for CP and all AA except for Val and Cys, as measured in this study. At 42 d, a significant increase was found in the AID for CP, Ser, including other 7 essential amino acids (EAA), irrespective of sex and diet. In conclusion, feeding broilers with low-CP diets caused a higher AID for the CP and most of AA under tropical condition. Gender had no effect on the CP and AA digestibility, whereas birds' age influenced the AID highly at 42 days that of 21days.

**Key words**: Age, Amino acids, Broiler, Digestibility, Gender, Low protein diet

Poultry need precise and balanced amount of essential amino acids (EAA) and nitrogen for the synthesis of non-essential amino acids (NEAA), rather than crude protein *per se* (NRC 1994). Considering this as an important point, poultry diets are usually formulated to meet the digestible amino acid (AA) requirements instead of crude protein (CP) and total amino acids requirements (Baker 1997, Ravindran *et al.* 2005). As it is reported that amino acids are the building blocks of protein, and are the products of protein hydrolysis (Hossain *et al.* 2015), the amino acids play a significant role in structural and protective tissues in the animal body and are also important in enzyme and tissue functions (NRC 1994). For this reason, deficiency of particular amino acid in the diets adversely affects the body growth and development of the birds. Satisfactory broiler performance can be attained as long as diets are properly balanced with the limiting essential amino acids (Rokade *et al.* 2014). Therefore, it is necessary to ensure required amount of digestible amino acids in the formulation of poultry diets, for their optimum growth and performance. In addition, for optimizing the efficient diet formulation, and to secure maximum return from the poultry, it appears to be more important to assess the availability and digestibility of dietary amino acids including other nutrients of the feed ingredients (Hossain *et al.* 2015).

However, feeding broilers on a balanced EAA, low-CP diet (16.2%) under tropical environment, is highly associated with poorer growth performance (Awad *et al.* 2014a). A significant decrease in protein and amino acids digestibilities of heat-stressed broiler chickens was observed by previous investigators (Zuprizal *et al.*1993, Soleimani *et al.* 2010). In terms of growth performance, females responded differently to feeding low-CP diet than males, and there was a significant dietary protein level effect on the apparent ileal digestibility (AID) of CP under thermo-neutral zone (Hernández *et al.* 2012). Thus, this study was carried out to evaluate the interactive effects of feeding low-CP diet, gender, and age on CP and AA apparent ileal digestibilities in broilers under the hot and humid tropical conditions.

## MATERIALS AND METHODS

The study was conducted following the guidelines of the Research Policy on Animal Ethics of the Universiti Putra Malaysia. Male (160) and female (160) day-old broiler chicks were obtained from a commercial hatchery. Upon arrival, the chicks were weighed and assigned to 20 floor pens (1.5 m × 1.5 m) in a conventional open-sided house. Each gender group was randomly and equally distributed into 2 dietary protein treatments (normal and low); each replicated 5 times with 16 chicks/ replicate pen. Each pen

Present address: [1](motazata83@gmail.com), Department of Poultry Production, University of Khartoum, 13314, Khartoum North, Khartoum, Sudan. [2](zulidrus@upm.edu.my), [3](abdoreza @upm.edu.my), [4](tcloh@upm.edu.my), [5](rony745@gmail.com), [6](ahmedawj@yahoo.com), Institute of Tropical Agriculture.

was equipped with one tube feeder, one automated bell drinker, and bedded with wood shaving as litter material. During brooding period, whenever needed pens were heated with electric pin bulbs. House maximum and minimum temperatures and relative humidity were recorded daily (Fig 1). Birds had a free access to feed and water for the entire trial period. Each pen was equipped with a 60-watt scroll bulb to maintain a continuous light during the night. Before diet formulation, representative corn and soybean samples were taken for CP and AA determination. Two experimental normal and low protein diets were formulated for starter (1–21 d) and finisher (22–42 d) feeding periods. The standard (control) protein diets consisted of 22.2% and 19.5% CP during starter and finisher periods, respectively. While, the low-CP diets consisted of 16.2% starter diet and 13.5% CP finisher diet. The starter and finisher low-CP diets were fortified with a crystalline amino sources to meet the ideal AA profile as suggested by Baker (1997), and to meet or exceed the specifications of NRC (1994) amino acids as well. The ingredient and nutritional compositions of the experimental diets are presented in Table 1. The digestibility study consisted of eight treatments using a 2 (protein diets) × 2 (genders) × 2 (ages) factorial arrangement in a completely randomized design.

Apparent ileal digestibility of CP and AA were determined at the end of each feeding period. The method used in the current trial was modified from our previous study (Aljuobori *et al.* 2014). Briefly, at both 19 and 40 d of ages, 0.5% titanium dioxide ($TiO_2$) as an indigestible marker was incorporated into the starter and grower diets, respectively. Birds had access to these test diets at all times over three days (19–21d during the starter and 40–42 d during finisher periods). At the end of 21 d, one bird from each pen was slaughtered humanely and ileal (starting from Meckel's diverticulum and ending at a point approximately 40 mm proximal to the ileo-cecal junction) contents were collected by gentle flushing with distilled water. The digesta samples were then freeze-dried, finely ground using a mortar and pestle, and kept at –20°C until further analysis. Following the same procedure above, digesta samples were collected from two slaughtered birds from each pen (ileal digesta samples from birds in the same pen were pooled together) when the finisher period was completed. Determination of $TiO_2$ concentrations in feed and digesta samples were done following the procedures of Short *et al.* (1996). The AID coefficients of CP and AA were calculated

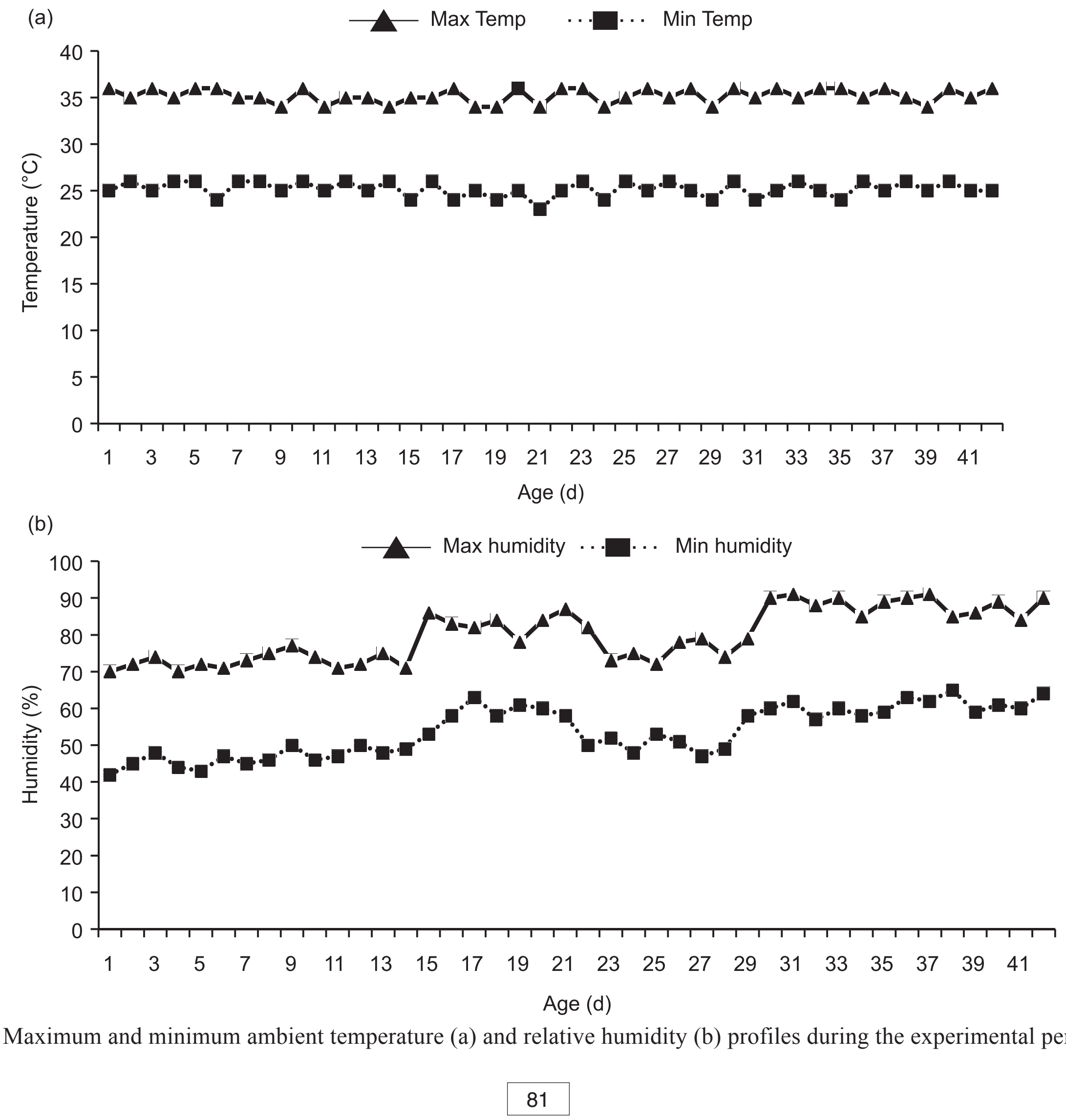


Fig. 1. Maximum and minimum ambient temperature (a) and relative humidity (b) profiles during the experimental period.

using the following formula (Sebastian *et al.* 1997):

$$\text{AID (\%)} = 100 - [(\text{Nutrient}_{digesta} / \text{Nutrient}_{diet}) \times (\text{Marker}_{diet} / \text{Marker}_{digesta}) \times 100]$$

Crude protein of feed ingredients, diets, and digesta samples was calculated after determining the nitrogen content in the samples using Kjeldahl method according to the procedure of AOAC (1990), CP calculated as N × 6.25. Amino acid concentrations were determined using high performance liquid chromatography (HPLC) as described previously (Awad *et al.* 2014b). Feed samples and digesta samples were dried in a drying oven at 105°C for 24 h to determine the dry matter (DM) following the Association of Analytical Chemists (1990) procedure.

Data were subjected to GLM using SAS software (SAS Institute 2005). Comparison between means was done by Duncan's multiple-range test. Significance is considered at $P<0.05$.

## RESULTS AND DISCUSSION

The digestibility data (Table 2) demonstrated that diet, age, diet and gender interaction have influenced the CP digestibility of broilers except for sex. The CP digestibility was found to be significantly ($P<0.001$) increased in the birds fed low-protein diet compared to those fed on the normal diet. The CP digestibility rate was higher ($P<0.05$) at d 42 than that on d 21. Diet influenced all the AA measured in this study herein except for lysine. Birds fed on low-CP diet significantly ($P<0.001$) increased apparent ileal digestibilities (AID) for all AA except for Lys. Both diet and age affected AID of most AA (Met, Thr, al, IIe, Arg, Phe, Leu, Ser, Pro, Cys) measured in this study. Only valine was influenced by diet, sex and age, with no interactive effects amongst other AA. The AID of Met, Thr, Val, Leu, Ile, Phe, and Ser were significantly ($P<0.05$) higher at d 42 than their respective coefficients at d 21. There was no significant interactions ($P>0.05$) for Val, Glu, Gly, Asp, Phe, Arg, Ala, Cyst and Tyr, except for other AA as measured herein, which were influenced significantly by their associated interactive actions (diet × age, diet × sex, diet × age × sex etc.).

The findings of our present experiment clearly revealed that dietary protein levels, bird's age and their associated interactions significantly influenced the CP and almost all AA digestibilites of broiler chickens as measured in this study. The pronounced digestibility of CP and AA was observed in the birds fed low-protein diets compared to those fed with the normal protein diet. The increased digestibility of CP and AA in broilers could be attributed to better quality and quantity of low-protein diets compared to the normal protein diets. Furthermore, the protein and AA contents of low-protein diet assumed to be more efficient, or suitable form to be digested, absorbed and assimilated by the intestinal tissue of the birds as compared to the birds of other diet group (Hossain *et al.* 2015). Conversely, the reason of poor digestibility of CP and AA of birds fed normal protein diets could be explained by the fact that 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 through the excreta of the birds (Kamran *et al.* 2004). Apart from these, the variation of nutrient (CP and AA) digestibility in the ileal digesta of the birds fed low-protein diets may have been due to many factors, for example, feed composition, fiber level, anti-nutritional factors, bird *per se*, ingredient diversity, protein quality, physical and chemical characteristics of protein, nature of feedstuffs, processing methods and so forth (Almirall *et al*. 1995, Ravindran *et al*. 2001, Hossain *et al.* 2014).

However, our results further revealed that the apparent ileal digestibility of CP and AA was significantly increased on d 42 as compared to d 21. It implies that digestibility of birds was comparatively better during the older stage of growth than that of the younger stage. The reason behind this could be simply explained by the age of the birds relative to their secretory glands, organ growth and secretion of enzymes, which can also influence the nutrient digestibility of the broilers.The results suggest that adult birds appear to be more efficient to cope with the intestinal viscosity and digestive enzyme activities than the young birds (Almirall *et al.* 1995). The pronounced efficacy of the enzyme was observed at the elderly stage of birds fed finisher diets, which may be possibly due to digestive endogenous enzyme function relative to age of the growing birds (Luo *et al.* 2009). All the enzyme secretory glands and organs of young chicks do not grow and develop properly during early age, which might cause insufficient secretion of endogenous enzymes, that are necessary for digesting high carbohydrate and vegetable protein diets (Uni *et al*. 1999, Classen and Bedford 1999, Odetallah *et al.* 2005).

These results demonstrated that the digestible requirements of AA for broilers is less than levels provided in the commercial diets that are formulated to meet a particular protein level. It also confirms that formulating diets on a digestible basis to meet an ideal amino acids profile could be more beneficial (Baker 2009). Although Wallis and Balnave (1984) reported higher AA digestibility in males than females in broilers exposed to high environmental temperatures. In this study gender did not affect the AID of CP and most of AA. This discrepancy could be due to the differences in heat stress condition since Wallis and Balnave (1984) conducted their study at a constant high temperature. Nonetheless, Kim and Corzo (2012) also noted no differences between males and females of AID of AA. In this study, age had a significant effect on the AID. Many previous studies have investigated the effect of age on the AID in feedstuffs using broilers. Huang *et al.* (2005) determined the AID in corn and soybean among other feed ingredients and reported higher AID on d 42 for all AA in the corn and soybean as well as higher AID for CP in corn but not in soybean. However, Kim and Corzo (2012) noted no age effect on the AID of AA in soybean. In the present study, the AID of CP, Met, Thr, Val, Leu, Ile, Phe, and Ser were significantly ($P<0.05$) higher on d 42

Table 1. Ingredient and nutrient composition of starter and grower diets

| Item | Control | | Low protein | |
|---|---|---|---|---|
| | Starter | Grower | Starter | Grower |
| *Ingredient (%)* | | | | |
| Corn | 50.32 | 58.7 | 67 | 74.23 |
| Soybean meal | 39.02 | 31.68 | 16.31 | 7.52 |
| Palm oil | 6.67 | 6.15 | 4.54 | 4.5 |
| Dicalcium phosphate | 1.62 | 1.18 | 1.86 | 1.44 |
| Limestone | 1.22 | 1.31 | 1.24 | 1.33 |
| Sodium chloride | 0.5 | 0.42 | 0.5 | 0.42 |
| Sand | 0 | 0 | 4.93 | 6.24 |
| Vitamin premix[1] | 0.05 | 0.05 | 0.05 | 0.05 |
| Mineral premix[2] | 0.25 | 0.25 | 0.25 | 0.25 |
| L-Lysine.HCL | 0.15 | 0.11 | 0.79 | 0.81 |
| L-Arginine | 0 | 0 | 0.43 | 0.52 |
| DL-Methionine | 0.2 | 0.15 | 0.43 | 0.4 |
| L-Threonine | 0 | 0 | 0.33 | 0.38 |
| L-Valine | 0 | 0 | 0.28 | 0.34 |
| L-Isoleucine | 0 | 0 | 0.28 | 0.36 |
| L-Phenylalanine | 0 | 0 | 0.34 | 0.51 |
| L-Histidine | 0 | 0 | 0.08 | 0.1 |
| L-Tryptophan | 0 | 0 | 0.07 | 0.1 |
| L-Leucine | 0 | 0 | 0.15 | 0.15 |
| Glycine | 0 | 0 | 0.06 | 0.27 |
| Choline chloride | 0 | 0 | 0.08 | 0.08 |
| *Nutrient composition, (%) unless noted* | | | | |
| *Metabolizable energy, Kcal/kg* | 3130 | 3200 | 3130 | 3200 |
| Crude protein[3] | 22.2 (22.3) | 19.5 (19.4) | 16.2 (16.2) | 13.5 (13.2) |
| Calcium | 0.94 | 0.86 | 0.94 | 0.86 |
| Available phosphorus | 0.45 | 0.35 | 0.45 | 0.35 |
| Total Lys | 1.22 (1.22) | 1.03 (1.06) | 1.21 (1.18) | 1.03 (0.91) |
| Total Arg | 1.51 (1.39) | 1.31 (1.28) | 1.27 (1.23) | 1.10 (1.09) |
| Total Met+Cys | 0.90 (0.88) | 0.79 (0.77) | 0.90 (0.90) | 0.78 (0.75) |
| Total Thr | 0.86 (0.83) | 0.76 (0.76) | 0.83 (0.85) | 0.74 (0.73) |
| Total Val | 1.11 (0.96) | 0.98 (0.91) | 0.94 (0.93) | 0.83 (0.83) |
| Total Ile | 0.95 (0.82) | 0.83 (0.76) | 0.81 (0.79) | 0.73 (0.72) |
| Total Phe+Tyr | 1.73 (1.53) | 1.51 (1.46) | 1.34 (1.33) | 1.21 (1.23) |
| Total His | 0.61 (0.55) | 0.54 (0.53) | 0.45 (0.42) | 0.38 (0.37) |
| Total Trp | 0.26 | 0.22 | 0.2 | 0.18 |
| Total Leu | 1.82 (1.56) | 1.65 (1.57) | 1.35 (1.36) | 1.11 (1.12) |
| Total Gly+Ser | 2.02 (1.92) | 1.78 (1.74) | 1.25 (1.24) | 1.14 (1.22) |
| Digestible Lys | 1.11 | 0.93 | 1.14 | 0.97 |
| Digestible Arg | 1.39 | 1.2 | 1.19 | 1.05 |
| Digestible Met+Cys | 0.8 | 0.7 | 0.84 | 0.73 |
| Digestible Thr | 0.75 | 0.66 | 0.76 | 0.69 |
| Digestible Val | 1 | 0.88 | 0.87 | 0.78 |
| Digestible Ile | 0.88 | 0.76 | 0.76 | 0.69 |
| Digestible Phe+Tyr | 1.56 | 1.36 | 1.23 | 1.14 |
| Digestible His | 0.55 | 0.49 | 0.42 | 0.36 |
| Digestible Trp | 0.23 | 0.2 | 0.18 | 0.17 |
| Digestible | 1.68 | 1.52 | 1.26 | 1.04 |
| Digestible Gly+Ser | 1.7 | 1.48 | 1.04 | 0.98 |

[1]Supplied per kilogram of the diet: vitamin A, 8,000 IU; vitamin $D_3$, 1,000 IU; vitamin E, 30 IU; vitamin $K_3$, 2.5 mg; vitamin $B_1$, 2 mg; vitamin $B_2$, 5 mg; vitamin $B_6$, 2, mg; vitamin $B_{12}$, 0.01 mg; niacin, 30 mg; d-biotin, 0.045 mg; vitamin C, 50 mg; d-pantothenate, 8 mg; folic acid, 0.5 mg. [2]Supplied per kilogram of the diet: Mn, 70 mg; Fe, 35 mg; Zn, 70 mg; Cu, 8 mg; I, 1 mg, Se, 0.25 mg; Co, 0.2 mg. [3]Values in parenthesis represent analyzed crude protein and amino acids. The digestible amino acids were calculated using (National Research Council, 1994) true digestibility coefficients. Tyr, Gly, Ser, Glu, Ala, and Asp were calculated using apparent ileal digestibility coeficients from (Huang *et al.* 2006). Crystalline amino acids were considered 100% true digestible.

compared with their respective coefficients at d 21of age. The slower feed rate of passage in older birds, which increases the utilization of nutrients (Shires *et al.* 1987) might contribute to the resultant higher AID in older birds in this study. The increased in AA absorbability as a consequence to the increased intestinal mass (Wakita *et al.* 1970) also can explain such results.

In conclusion, feeding broilers on low-CP diets may

Table 2. Effects of feeding low-CP, glycine + essential amino acids diet, gender, and age on the apparent ileal digestibility (%) of crude protein and amino acids in broiler chickens at 21 and 42 days

| | | Indispensable amino acids | | | | | | | | | Dispensable amino acids | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Factor | CP | Met | Lys | Thr | Val | Ile | Arg | Phe | Leu | His | Asp | Ser | Glu | Gly | Pro | Ala | Cys | Tyr |
| *Diet (protein level)* | | | | | | | | | | | | | | | | | | |
| Normal | 82.1$^{b}$ | 89.8$^{b}$ | 90.9 | 74.0$^{b}$ | 79.4$^{b}$ | 81.2$^{b}$ | 85.5$^{b}$ | 81.9$^{b}$ | 82.5$^{b}$ | 83.7$^{b}$ | 81.8$^{b}$ | 79.1$^{b}$ | 84.9$^{b}$ | 78.2$^{b}$ | 80.9$^{b}$ | 81.5$^{b}$ | 85.0$^{b}$ | 86.4$^{b}$ |
| Low | 86.4$^{a}$ | 90.5$^{a}$ | 91.0 | 86.8$^{a}$ | 86.1$^{a}$ | 89.8$^{a}$ | 91.4$^{a}$ | 91.8$^{a}$ | 87.3$^{a}$ | 88.4$^{a}$ | 83.0$^{a}$ | 84.3$^{a}$ | 88.1$^{a}$ | 83.7$^{a}$ | 84.6$^{a}$ | 88.9$^{a}$ | 85.6$^{a}$ | 89.5$^{a}$ |
| SEM | 0.32 | 0.21 | 0.37 | 0.78 | 2.08 | 0.76 | 1.17 | 1.22 | 0.83 | 0.92 | 0.76 | 0.58 | 0.75 | 3.20 | 0.72 | 1.43 | 0.71 | 0.92 |
| *Gender* | | | | | | | | | | | | | | | | | | |
| Male | 84.3 | 90.1 | 90.9 | 80.2 | 83.6$^{a}$ | 85.5 | 88.5 | 87.1 | 84.8 | 86.1 | 82.2 | 81.7 | 86.7 | 81.3 | 83.0 | 85.0 | 85.6$^{a}$ | 88.1 |
| Female | 84.2 | 90.2 | 91.0 | 80.6 | 81.9$^{b}$ | 85.6 | 88.4 | 86.6 | 84.8 | 86.1 | 82.5 | 81.8 | 86.4 | 80.6 | 82.5 | 85.3 | 85.0$^{b}$ | 87.7 |
| SEM | 0.32 | 0.21 | 0.37 | 0.78 | 2.08 | 0.76 | 1.17 | 1.22 | 0.83 | 0.92 | 0.76 | 0.58 | 0.75 | 3.20 | 0.72 | 1.43 | 0.71 | 0.92 |
| *Age (day)* | | | | | | | | | | | | | | | | | | |
| 21 | 84.0$^{b}$ | 90.0$^{b}$ | 91.0 | 80.1$^{b}$ | 80.9$^{b}$ | 85.1$^{b}$ | 88.1 | 86.4$^{b}$ | 84.1$^{b}$ | 85.9 | 82.2 | 81.4$^{b}$ | 86.4 | 81.1 | 82.5 | 85.2 | 85.1 | 87.8 |
| 42 | 84.4$^{a}$ | 90.3$^{a}$ | 90.9 | 80.7$^{a}$ | 84.6$^{a}$ | 86.0$^{a}$ | 88.8 | 87.3$^{a}$ | 85.6$^{a}$ | 86.3 | 82.6 | 82.0$^{a}$ | 86.6 | 80.8 | 83.0 | 85.1 | 85.6 | 88.1 |
| SEM | 0.32 | 0.21 | 0.37 | 0.78 | 2.08 | 0.76 | 1.17 | 1.22 | 0.83 | 0.92 | 0.76 | 0.58 | 0.75 | 3.20 | 0.72 | 1.43 | 0.71 | 0.92 |
| *P-value* | | | | | | | | | | | | | | | | | | |
| Diet | <.0001 | <.0001 | 0.8007 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0003 | <.0001 | <.0001 | <.0001 | <.0001 | <.0001 | 0.0256 | <.0001 |
| Gender | 0.5453 | 0.0527 | 0.3116 | 0.2111 | 0.0299 | 0.6011 | 0.8993 | 0.3167 | 0.6993 | 0.9308 | 0.2524 | 0.6044 | 0.3051 | 0.5130 | 0.0717 | 0.5674 | 0.0277 | 0.2558 |
| Age | 0.0116 | 0.0236 | 0.8066 | 0.0379 | <.0001 | 0.0026 | 0.1157 | 0.0457 | <.0001 | 0.2135 | 0.2307 | 0.0050 | 0.4462 | 0.8250 | 0.0503 | 0.7540 | 0.0556 | 0.4899 |
| Diet × Gender | 0.0312 | 0.0101 | 0.7834 | 0.0004 | 0.5362 | 0.4987 | 0.7467 | 0.5017 | 0.0905 | 0.0300 | 0.0590 | 0.4096 | 0.0063 | 0.9366 | 0.2511 | 0.0999 | 0.7996 | 0.2869 |
| Diet × Age | 0.3491 | 0.0286 | 0.7984 | 0.0112 | 0.2585 | 0.3954 | 0.3983 | 0.3163 | 0.0357 | 0.1351 | 0.7732 | 0.0131 | 0.0654 | 0.6348 | 0.8147 | 0.6156 | 0.5485 | 0.4412 |
| Gender × Age | 0.7671 | 0.2215 | 0.0024 | 0.7313 | 0.3531 | 0.0546 | 0.0625 | 0.5618 | 0.0670 | 0.2775 | 0.1543 | 0.4250 | 0.8917 | 0.1234 | 0.3696 | 0.5359 | 0.7086 | 0.3948 |
| Diet × Gender × Age | 0.3999 | 0.0023 | 0.0410 | 0.3846 | 0.9414 | 0.3294 | 0.4050 | 0.5049 | 0.7259 | 0.9882 | 0.4393 | 0.7691 | 0.7250 | 0.1703 | 0.8532 | 0.7815 | 0.1308 | 0.3425 |

[a,b]Means within column-subgroups with no common superscripts are significantly different at P<0.05.

enhance the apparent ileal CP and AA digestibilities under the hot and humid tropical conditions. Moreover, such enhancement could be higher in older birds.

## ACKNOWLEDGMENT

This work was supported by the Malaysian Ministry of Education through the Long Term Research Grant Scheme.

## REFERENCES

Aljuobori A, Zulkifli I, Soleimani A F, Abdullah N, Liang J B and Awad E A. 2014. Effect of solid state fermentation on nutrient content and ileal amino acids digestibility of canola meal in broiler chickens. *Italian Journal of Animal Science* **13**: 410 –14.

Association of Analytical Chemists. 1990. *Official methods of Analysis*. 15th edition. Arlington, VA. AOAC Inc.

Awad E A, Fadlullah M, Zulkifli I, Soleimani A F and Loh T C. 2014a. Amino acids fortification of low-protein diet for broilers under tropical climate: ideal essential amino acids profile. *Italian Journal of Animal Science* **13**: 270–74.

Awad E A, Zulkifli I, Soleimani A F and Loh T C. 2014b. Amino acids fortification of low-protein diet for broilers under tropical climate. 2. Nonessential amino acids and increasing essential amino acids. *Italian Journal of Animal Science* **13**: 631–36.

Almirall M, Francesch M, Perez-Vendrell A M, Brufau J J and Esteve-Garcia E. 1995. The differences in intestinal viscosity produced by barley and 0–Glucanase alter digesta enzyme activities and heal nutrient digestibilities more in broiler chicks than in cocks. *Journal of Nutrition* **125**: 947–55.

Baker D H. 1997. Ideal amino acid profiles for swine and poultry and their applications in feed formulation, Biokyowa Technical Review. Cape Girardeau, MO: Biokyowa (Vol. 9, pp. 15–19).

Baker D H. 2009. Advances in protein–amino acid nutrition of poultry. *Amino acids* **37**: 29–41.

Classen H L and Bedford M R. 1999. The use of enzymes to improve the nutritive valueof poultry feeds. In: Recent Development in Poultry Nutrition 2. Eds Wiseman, J. And Garnsworthy, P.C. Pp: 285–308. Nottingham University Press, Notingham,UK.

Hernández F, López M, Martínez S, Megías M D, Catalá P and Madrid J. 2012. Effect of low-protein diets and single sex on production performance, plasma metabolites, digestibility, and nitrogen excretion in 1–to 48–day-old broilers. *Poultry Science* **91**: 683–92.

Huang K H, Li X, Ravindran V and Bryden W L. 2006. Comparison of apparent ileal amino acid digestibility of feed ingredients measured with broilers, layers, and roosters. *Poultry Science* **85**: 625–34.

Huang K H, Ravindran V, Li X and Bryden W L. 2005. Influence of age on the apparent ileal amino acid digestibility of feed ingredients for broiler chickens. *British Poultry Science* **46**: 236–45.

Hossain M A, Islam F A and Paul I J. 2015. The availability or retention of micronutrient contents in the ileal digesta of broiler chickens raised on plant protein diets with and without supplemental enzymes. *Global Journal of Animal Scientific Research* **3**: 423–34.

Hossain M A, Islam F A and Paul I J. 2014. Impact of microbial enzymes on growthperformance, micro-nutrient digestibility, tissue protein contents and endogenous enzyme activities of broiler chickens fed on vegetable protein diets. *International Journal of Poultry Science* **13**: 555–61.

Kim E J and Corzo A. 2012. Interactive effects of age, sex, and strain on apparent ileal amino acid digestibility of soybean meal and an animal by-product blend in broilers. *Poultry Science* **91**: 908–17.

Kamran Z, Mirza M A, Haq A U and Mahmood S. 2004. Effects of decreasing dietary protein levels with optimal amino acid profile on the performance of broilers. *Pakistan Veterinary Journal* **24**: 165–68.

Luo D, Yanga F, Yang X, Yao J, Shi B and Zhou Z. 2009. Effects of Xylanase on Performance, blood parameters, intestinal morphology, microflora and digestive enzyme activities of broilers fed wheat-based diets. *Asian-Australasian Journal of Animal Sciences* **22**: 1288–95.

National Research Council. 1994. Nutrient requirements of poultry. 9th Revised Edition. National Academy Press, Washington, D. C, USA.

Odetallah N H, Wang J J, Garlich J D and Shih J C H. 2005. Versazyme supplementation broiler diets improves market growth performance. *Poultry Science* **84**: 858–64.

Ravindran V, Hew L, Ravindran G and Bryden W. 2005. Apparent ileal digestibility of amino acids in dietary ingredients for broiler chickens. *Animal Science* **81**: 85–97.

Ravindran V, Selle P H, Ravindran G, Morel P C H, Kies A K and Bryden W L. 2001. Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. *Poultry Science* **80**: 338–44.

Rokade J J, Thyagarajan D, Omprakash A V and Karunakaran R. 2014. Effect of low protein diet with balanced amino acids with protease on performance and litter quality of commercial broiler. *Indian Journal of Animal Sciences* **84**: 691–93.

Sebastian S, Touchburn S P, Chavez E R and Lague P C. 1997. Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. *Poultry Science* **76**: 1760–69.

Shires A, Thompson J R, Turner B V, Kennedy P M and Goh Y K. 1987. Rate of passage of corn-canola meal and corn-soybean meal diets through the gastrointestinal tract of broiler and white leghorn chickens. *Poultry Science* **66**: 289–98.

Short F J, Gorton P, Wiseman J and Boorman K N. 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. *Animal Feed Science and Technology* **59**: 215–21.

Soleimani A F, Kasim A, Alimon A R, Meimandipour A, Zulkifli I. 2010. Ileal endogenous amino acid flow of broiler chickens under high ambient temperature. *Journal of Animal Physiology and Animal Nutrition* **94**: 641–47.

Uni Z, Noy Y and Sklan D. 1999. Posthatch development of small intestinal function in the poult. *Poultry Science* **78**: 215–22.

Wakita M, Hoshino S and Morimoto K. 1970. Factors affecting the accumulation of amino acid by the chick intestine. *Poultry Science* **49**: 1046–50.

Wallis I R and Balnave D. 1984. The influence of environmental temperature, age and sex on the digestibility of amino acids in growing broiler chickens. *British Poultry Science* **25**: 401–07.

Zuprizal, Larbier M, Chagneau A M and Geraert P A. 1993. Influence of ambient temperature on true digestibility of protein and amino acids of rapeseed and soybean meals in broilers. *Poultry Science* **72**: 289–95.