Amino acids fortification of low-protein diet for broilers under tropical climate. 2. Nonessential amino acids and increasing essential amino acids

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Introduction

The availability of commercial amino acids (AA) in synthetic form has allowed to reduce crude protein (CP) in poultry diets. Low protein diets fortified with synthetic AA have been the subject of extensive research. Supplementing low-CP diets with synthetic AA has been shown to save cost (Dozier et al., 2008) and decrease nitrogen excretion (Bregendahl et al., 2002). However, excessive low-CP diets are still not recommendable even with AA fortification (Awad et al., 2014). Several studies investigated the potential of nonessential amino acids (NEAA) to enhance the performance of broilers fed low-CP, essential amino acids (EAA) supplemented diets with some discrepancies in their findings. Some studies showed similar growth performance when the low-CP diets were supplemented with NEAA either in individual (Dean et al., 2006; McGill et al., 2012a; Laudadio et al., 2012) or in combined form (Aletor et al., 2000; Fatufa and Rodehutsdorfi, 2005; Corzo et al., 2005; Dean et al., 2006). However, others failed to achieve performance equal to that of positive control group (Kidd et al., 1996; Hussein et al., 2001; Waldroup et al., 2005; Berres et al., 2010). In addition, many studies have been conducted to evaluate the effect of supplementing low-CP diets with EAA in levels higher than the requirements (Deschepper and DeGroote, 1995; Si et al., 2004; Waldroup et al., 2005). Although, Deschepper and DeGroote (1995) showed that equaling the EAA levels of low-CP diets to that of standard improved performance, Waldroup et al. (2005) and Si et al. (2004) found otherwise.

Most of previous research (Deschepper and DeGroote, 1995; Waldroup et al. 2005; Dean et al., 2006) dealing with NEAA or further EAA fortification to low-CP diets in poultry was carried out under temperate condition with little or no available data on such fortification effect under the hot and humid tropical condition. It is well-documented that high temperatures may alter CP and AA requirements in poultry (Ojano-Dirain and Waldroup, 2002; Balnave, 2004). Brake et al. (1998) showed that under heat stress condition, broilers required higher Arg:Lys ratio for optimum growth performance. Larbier et al. (1993) reported a significant decrease in protein and AA digestibility of rapeseed and soybean meals in broilers raised under heat stress condition. Thus, the objective of this study was to evaluate the effect of further EAA and NEAA supplementation to a low-CP (16.2% CP and already supplemented with all EAA) on growth performance, serum metabolites, organ weights, breast yield and abdominal fat weight in male broiler chicks during the starter period under the hot and humid tropical conditions.

Materials and methods

Birds and management

The study was conducted in accordance with the guidelines of the Research Policy on Animal Ethics of the Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Three hundred day-old male broiler chicks (Cobb 500) were obtained from a commercial hatchery and housed in floor pens. Each pen measured 1.5x1.5 m and equipped with one tube feeder, one bell drinker, and wood shavings as litter material in a conventional open sided house. The profile of in-house temperature and relative humidity was recorded daily (Table 1; Figure 1). Chicks were raised under a continuous photoperiod programme. On arrival, chicks (300) were weighed and allotted randomly to five dietary groups (60 birds per group). Each group had five replicates with 12 chicks per pen. Mash feed and water were provided ad libitum.

Experimental diets

Prior to diet formulation, representative samples from the corn and soybean were analysed for CP using Kjeldahl method according to the procedure of AOAC (1990) and AA
content using high performance liquid chromatography (HPLC) as described previously (Ahmed et al., 2014) (Table 2). Briefly, 0.1 to 0.2 g of sample was hydrolysed by 5 mL of 6N HCl at 110°C for 24 h. Afterwards, a 4 mL of internal standard (L-9 amino-N-butyric acid; AABA) was added to the hydrolysate (analysis of all AA except Met, Cys, and Trp). After paper and syringe filtering, 10 µL of the sample was mixed with 70 µL of borate buffer and 20 µL of ACQ reagent (Waters Corporation, Milford, MA, USA). Then, an AA column (AccQ Tag 3.9 150 mm; Waters) was used for peaks separation. The latter was detected by a fluorescent detector (2475; Waters) using HPLC. Cystine and methionine were analysed as cystic acid and methionine sulphone, respectively by oxidation with performic acid for 16 h at 4°C and neutralisation with hydrobromic acid before hydrolysis. Tryptophan contents were determined following alkaline hydrolysis of sample with 4.3N LiOH•H2O for 16 h at 120°C and neutralisation with 6N HCl. Five isocoric [3000 metabolisable energy (ME)/kg] experimental diets were used as follows: i) 22.2% CP (positive control; PC); ii) 16.2% CP + all EAA to meet both National Research Council recommendations (1994) and the ideal AA ratios suggested by Baker (1997) (negative control; NC); iii) NC + further EAA to equal the levels in the PC diet; iv) NC + NEAA (Glycine, Proline, Glutamic, Alanine, and Asparatic) to equal the levels in the PC; and v) NC + EAA and NEAA to equal the AA levels in the PC diet. The compositions of the experimental diets are presented in Tables 3 and 4.

Data collection and sampling

Body weights (pen basis) were recorded on day 1 and 21, and weight gain (WG) was calculated accordingly. Feed intake (FI) from day 1 to 21 was measured, and feed conversion ratios (feed/gain) (FCR) were calculated. Protein efficiency ratio (PER) was calculated as the body weight/protein intake. Mortality was expressed as a percentage of the body weight (BW). Abdominal fat pad was defined as the tissue surrounding the gizzard and intestines, extending within the ischium, and surrounding the cloaca, bursa of Fabricius and adjacent to the abdominal muscles (Fancher and Jensen, 1989). Blood samples (3 mL) were collected during neck cut and placed in tubes and kept in ice. The blood samples were centrifuged at 4000×g at 4°C for 20 min. The haemolysis-free serum samples were collected and stored at -20°C. Serum concentrations of albumin (Alb), triglyceride (TG), total protein (TP), and uric acid (UA) were determined using an automated chemistry analyser (Hitachi 902 Automatic Analyzer; Hitachi, Tokyo, Japan) with commercial test kits (Roche Diagnostics, Basel, Switzerland).

Statistical analysis

Data were subjected to ANOVA using the GLM procedure of SAS software (SAS, 2005). Comparison among means was done by Duncan’s multiple-range test. Mortality data were analysed by chi-square test. Significance was considered at P≤0.05.

Results

The analysed values of the dietary AA are in close agreement with the calculated values (Table 4). The results of growth performance and mortality rate are presented in Table 5. Feeding the chicks with NC diet resulted in lower BW (P≤0.0003), DWG (P≤0.0003), and poorer FCR (P≤0.0003), and the highest PER (P≤0.0001) compared to PC and NC+NEAA groups. Supplementing the NC diet with further EAA to provide the same levels as in the PC diet failed to improve the BW, DWG, and FCR of the birds, although FI was not significantly different from the PC group. However, NEAA supplementation to the NC diet resulted in a similar BW, DWG, FCR, and better PER in the birds comparable to those fed PC diet. The BW, DWG, FI, FCR, and PER of the birds fed NC diet supplemented with both EAA and NEAA were not significantly different from birds fed PC diet. Mortality rate was not affected by diet.

Dietary treatment had no significant effect on serum Alb and TP (Table 6). However, there was a significant increase in serum UA when the NC diet was supplemented with EAA, NEAA, or the combination of EAA+NEAA. Serum TG of group fed NC diet was significant-

| Table 1. Mean environmental temperature and relative humidity inside the house during the experimental period. |
|-----------------------------------------------|
| Temperature, °C | 8:00 am | 2:00 pm | 8:00 pm |
| Humidity, % | 27 | 36 | 31 |
| Humidity, % | 82 | 57 | 69 |

| Table 2. Crude protein and amino acids content of corn and soybean (as-fed basis). |
|-----------------------------------------------|
| Corn | Soybean |
| CP*, % | 8.5 | 46 |
| EAA, % | | |
| Lys | 0.25 | 2.59 |
| Arg | 0.42 | 3.50 |
| Met | 0.18 | 0.66 |
| Thr | 0.32 | 1.91 |
| Val | 0.45 | 2.39 |
| Ile | 0.31 | 2.12 |
| Phe | 0.45 | 2.55 |
| Trp | 0.05 | 0.61 |
| His | 0.26 | 1.35 |
| Leu | 1.12 | 3.68 |
| NEAA, % | | |
| Cys | 0.19 | 0.69 |
| Tyr | 0.18 | 1.29 |
| Glu | 0.36 | 2.14 |
| Ser | 0.46 | 2.25 |
| Glu | 1.66 | 6.84 |
| Pro | 0.80 | 2.52 |
| Ala | 0.66 | 1.99 |
| Asp | 0.60 | 5.18 |

*CP: crude protein; EAA, essential amino acids; NEAA, nonessential amino acids; Lys, lysine; Arg, arginine; Met, methionine; Thr, threonine; Val, valine; Ile, isoleucine; Phe, phenylalanine; Trp, tryptophan; His, histidine; Leu, leucine; Cys, cysteine; Tyr, tyrosine; Glu, glutamic; Ser, serine; Gln, glutamine; Pro, proline; Ala, alanine; Asp, aspartic. °Each value represents the mean of three samples; †each value represents the mean of two samples. |

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ly higher compared to PC and NC+EAA+NEAA groups. Diet had no significant (P>0.05) on relative weights of heart, liver, abdominal fat, and breast meat yield (Table 7). Diet had no significant (P>0.05) effect on the aforementioned parameters.

**Discussion**

Feeding chicks with low-CP diet (NC) suppressed the growth performance of broilers under the hot and humid tropical conditions. This is in agreement with our earlier findings (Awad et al., 2014) and it could be attributed to insufficient nitrogen sources for NEAA synthesis (Han et al., 1992; Aletor et al., 2000; Corzo et al., 2005; Dean et al., 2006). In a standard broiler diet, the NEAA were synthesized from the extra balance of EAA (Waldroup, 2007). In the present study, further addition of EAA to NC did not improve growth performance of broilers. This finding supported the notion that the depressed growth performance in the NC group was probably not due to the reduced EAA concentrations. Work by Deschepper and DeGroote (1995), and Waldroup et al. (2005) under thermoneutral conditions suggested that the reduction in growth performance of broilers was not overcome by equaling the EAA levels of diets with 19 and 16% CP to that of 23 and 22% CP, respectively. The present findings suggested that the BW, DWG, FI and FCR of birds fed NC+NEAA were similar to those of PC. Previous work under temperate condition suggested that boilers provided low-CP diets with a combination of NEAA supplementation had similar growth performance to control groups (Deschepper and DeGroote, 1995; Aletor et al., 2000; Fatufe and Rodehutscord, 2005; Corzo et al., 2005; Dean et al., 2006). Others reported that supplementation with only single NEAA such as Glu (Han et al., 1992; McGill et al., 2012a) or Gly (Dean et al., 2006) also resulted in similar growth performance as control birds. In contrast, Waldroup et al. (2005) reported that isocaloric and low-CP diets with NEAA fortification failed to improve growth performance of broilers. There is no clear explanation for the discrepancies. However, the inconsistencies could be associated with factors such as CP level in the control diet, and concentration of supplemented AA (McGill et al., 2012b), energy level (Sklan and Plavnik, 2002), gender (Hernández et al., 2012), and age of chickens (Deschepper and DeGroote, 1995). Interestingly, in our study birds fed NC+EAA+NEAA diet had significantly poorer FCR comparable with those provided

| Table 3. Composition of the experimental diets (as-fed basis). |
|------------------|-----|-----|-----|-----|-----|
| Ingredient, %    | PC  | NC  | NC+EAA | NC+NEAA | NC+EAA+NEAA |
| Corn             | 52.80 | 63.48 | 63.48 | 63.48 | 63.48 |
| Soybean meal     | 37.81 | 17.48 | 17.48 | 17.48 | 17.48 |
| Palm oil         | 5.40 | 6.72 | 6.72 | 6.72 | 6.72 |
| Dicalcium phosphate | 1.62 | 1.86 | 1.86 | 1.86 | 1.86 |
| Limestone        | 1.23 | 1.24 | 1.24 | 1.24 | 1.24 |
| Sodium Chloride  | 0.50 | 0.50 | 0.50 | 0.50 | 0.50 |
| Sand             | 0.00 | 5.57 | 3.76 | 1.81 | 0.00 |
| Vitamin premix°  | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Mineral premix°  | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 |
| L-lys•HCl        | 0.12 | 0.58 | 0.62 | 0.58 | 0.62 |
| L-arg            | 0.00 | 0.46 | 0.67 | 0.46 | 0.67 |
| DL-met           | 0.20 | 0.42 | 0.44 | 0.42 | 0.44 |
| L-thr            | 0.02 | 0.30 | 0.37 | 0.30 | 0.37 |
| L-val            | 0.00 | 0.27 | 0.44 | 0.27 | 0.44 |
| L-ile            | 0.00 | 0.25 | 0.40 | 0.25 | 0.40 |
| L-phe            | 0.00 | 0.33 | 0.64 | 0.33 | 0.64 |
| L-his            | 0.00 | 0.07 | 0.25 | 0.07 | 0.25 |
| L-gly            | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| L-trp            | 0.00 | 0.07 | 0.12 | 0.07 | 0.12 |
| L-leu            | 0.00 | 0.02 | 0.06 | 0.02 | 0.06 |
| L-pro            | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| L-ala            | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| L-glu acid       | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| L-aspar acid     | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ChCl             | 0.00 | 0.08 | 0.08 | 0.08 | 0.08 |

PC, positive control; NC, negative control; EAA, essential amino acids; NEAA, nonessential amino acids; Lys, lysine; Arg, arginine; Met, methionine; Thr, threonine; Val, valine; Ile, isoleucine; Phe, phenylalanine; His, histidine; Gly, glycine; Trp, tryptophan; Leu, leucine; Pro, proline; Ala, alanine; Glu, glutamic; Asp, aspartate. Lys, Met, Thr, and Trp were feed grade, while the rest of supplemented amino acids were pharmaceutical grade.°Supplied per kilogram of diet: vitamin A, 10,000 U; vitamin D3, 1000 U; vitamin E, 30 U; vitamin K3, 2.5 mg; vitamin B6, 1 mg; vitamin B12, 0.01 mg; niacin, 30 mg; d-biotin, 0.045 mg; vitamin C, 50 mg; d-pantothenate, 8 mg; folic acid, 0.5 mg.°Supplied per kilogram of diet: Mn, 70 mg; Fe, 35 mg; Zn, 70 mg; Cu, 8 mg; I, 1 mg; Se, 0.25 mg; Co, 0.2 mg.

![Figure 1](image-url)
Table 4. Nutrients composition of the experimental diets.

| Ingredient | PC | NC | NC+EAA | NC+NEAA | NC+EAA+NEAA |
|------------|----|----|--------|---------|-------------|
| ME, kcal/kg | 3000 | 3000 | 3000 | 3000 | 3000 |
| CP, % | 22.2 (22.1) | 16.2 (16.7) | 18.0 (18.3) | 19.9 (20.4) | 21.8 (21.9) |
| ME:CP ratio | 135 | 185 | 167 | 151 | 138 |
| Ca, % | 0.94 | 0.94 | 0.94 | 0.94 | 0.94 |
| Available P, % | 0.45 | 0.45 | 0.45 | 0.45 | 0.45 |
| Total AA#, % | | | | | |
| Lys | 1.23 (1.22) | 1.19 (1.11) | 1.23 (1.03) | 1.19 (1.08) | 1.23 (1.09) |
| Arg | 1.55 (1.45) | 1.34 (1.33) | 1.55 (1.42) | 1.34 (1.34) | 1.55 (1.54) |
| Met | 0.54 (0.53) | 0.65 (0.65) | 0.67 (0.55) | 0.65 (0.54) | 0.67 (0.66) |
| Met+cys | 0.90 (0.91) | 0.88 (0.88) | 0.91 (0.89) | 0.89 (0.90) | 0.91 (0.89) |
| Thr | 0.91 (0.90) | 0.84 (0.88) | 0.91 (0.85) | 0.84 (0.77) | 0.91 (0.87) |
| Val | 1.14 (1.08) | 0.97 (0.98) | 1.14 (1.06) | 0.97 (1.00) | 1.14 (1.14) |
| Ile | 0.97 (0.93) | 0.82 (0.83) | 0.97 (0.92) | 0.82 (0.84) | 0.97 (0.97) |
| Leu | 1.98 (1.75) | 1.37 (1.27) | 1.98 (1.78) | 1.37 (1.58) | 1.98 (1.52) |
| Phe+tyr | 1.65 (1.56) | 1.34 (1.36) | 1.65 (1.55) | 1.34 (1.41) | 1.65 (1.62) |
| Trp | 0.26 | 0.21 | 0.26 | 0.21 | 0.26 |
| His | 0.65 (0.68) | 0.47 (0.53) | 0.65 (0.58) | 0.47 (0.50) | 0.65 (0.58) |
| Gly+ser | 2.09 (1.99) | 1.29 (1.31) | 1.29 (1.41) | 2.10 (1.93) | 2.09 (2.03) |
| Glu | 3.64 (3.58) | 2.25 (2.34) | 2.25 (2.67) | 3.46 (3.39) | 3.46 (3.45) |
| Ala | 1.10 (0.97) | 0.77 (0.71) | 0.77 (0.79) | 1.10 (1.02) | 1.10 (1.04) |
| Asp | 2.28 (2.01) | 1.29 (1.23) | 1.29 (1.39) | 2.28 (1.86) | 2.28 (1.99) |
| Pro | 1.38 (1.23) | 0.95 (0.91) | 0.95 (0.87) | 1.38 (1.42) | 1.38 (1.43) |

True digestible AA#, %

| Item | PC | NC | NC+EAA | NC+NEAA | NC+EAA+NEAA |
|------|----|----|--------|---------|-------------|
| Lys | 1.12 | 1.12 | 1.16 | 1.12 | 1.16 |
| Arg | 1.41 | 1.26 | 1.47 | 1.26 | 1.47 |
| Met | 0.52 | 0.63 | 0.65 | 0.63 | 0.65 |
| Met+cys | 0.83 | 0.83 | 0.83 | 0.83 | 0.83 |
| Thr | 0.79 | 0.76 | 0.83 | 0.76 | 0.83 |
| Val | 1.03 | 0.90 | 1.07 | 0.90 | 1.07 |
| Ile | 0.89 | 0.77 | 0.92 | 0.77 | 0.92 |
| Leu | 1.83 | 1.27 | 1.88 | 1.27 | 1.88 |
| Phe+tyr | 1.49 | 1.23 | 1.54 | 1.23 | 1.54 |
| Trp | 0.23 | 0.19 | 0.24 | 0.19 | 0.24 |
| His | 0.59 | 0.43 | 0.63 | 0.43 | 0.63 |
| Gly+ser | 1.75 | 1.06 | 1.06 | 1.06 | 1.06 |
| Glu | 3.11 | 2.03 | 2.03 | 3.24 | 3.24 |
| Ala | 0.96 | 0.68 | 0.68 | 1.02 | 1.02 |
| Asp | 1.94 | 1.09 | 1.09 | 1.09 | 1.09 |

PC, positive control; NC, negative control; EAA, essential amino acids; NEAA, nonessential amino acids; ME, metabolisable energy; CP, crude protein; Ca, calcium; P, phosphorus; AA, amino acids; Lys, lysine; Arg, arginine; Met, methionine; Gly, glycine; Val, valine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; Tyr, tyrosine; Trp, tryptophan; His, histidine; Glu, glutamic; Ala, alanine; Asp, asparatic; Pro, proline. Tryptophan, Gly, Ser, Glu, Ala, and Asp were calculated using apparent ileal digestibility coefficients from Huang et al. (2006). Tryptophan was calculated using standardised ileal digestibility coefficients from Hoofer et al. (2016).

Table 5. Effect of amino acids supplementation to a low-protein diet from 1 to 21 days on growth performance and mortality rate of broilers.

| Item | PC | NC | NC+EAA | NC+NEAA | NC+EAA+NEAA | RMSE | P |
|------|----|----|--------|---------|-------------|------|---|
| BW, g | 933a | 823c | 853bc | 933a | 882a | 37.6 | 0.0003 |
| DWG, g | 42.3c | 37c | 38.5c | 42.2a | 39.9a | 1.79 | 0.0003 |
| FL, g | 1198b | 1132b | 1173a | 1175a | 1171a | 42.6 | 0.2301 |
| FCR | 1.35b | 1.46a | 1.45a | 1.33 | 1.40a | 0.04 | 0.0003 |
| PER | 3.34b | 4.23a | 3.83b | 3.77a | 3.28c | 0.12 | 0.0001 |

Mortality, %

| Item | PC | NC | NC+EAA | NC+NEAA | NC+EAA+NEAA | RMSE | P |
|------|----|----|--------|---------|-------------|------|---|
| Mortality, % | 3.3 | 8.3 | 6.7 | 6.7 | 3.3 | - | - |

PC, positive control; NC, negative control; EAA, essential amino acids; NEAA, nonessential amino acids; RMSE, root-mean-square error; BW, body weight; DWG, daily weight gain; FL, feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio. Data are mean for 5 replications of 12 birds per pen. *Means within rows followed by different superscript letters are significantly different (P<0.05).

Conclusions

Under the hot and humid environmental conditions, reduced growth performance of the broilers fed low-CP 16.2% CP diets with minimum EAA fortification can be restored by addition of NEAA. Moreover, increasing EAA more than requirements is unnecessary for optimum performance.
Table 6. Effect of amino acids supplementation to a low-protein diet on blood metabolites.

| Diet                          | PC     | NC   | NC+EAA | NC+NEAA | NC+EAA+NEAA | RMSE | P       |
|-------------------------------|--------|------|--------|---------|-------------|------|---------|
| Alb, g/L                      | 18.6   | 17.5 | 18.5   | 18.3    | 18.2        | 4.16 | 0.9825  |
| TG, mmol/L                    | 0.50<sup>a</sup> | 0.72<sup>b</sup> | 0.56<sup>a</sup> | 0.56<sup>a</sup> | 0.49<sup>b</sup> | 0.21 | 0.1343  |
| TP, g/L                       | 38.1   | 35.1 | 37.4   | 36.8    | 38.3        | 7.97 | 0.8999  |
| UA, umol/L                    | 579<sup>c</sup> | 238<sup>d</sup> | 463<sup>c</sup> | 470<sup>c</sup> | 603<sup>d</sup> | 204  | 0.0019  |

PC: positive control; NC: negative control; EAA: essential amino acids; NEAA: nonessential amino acids; RMSE: root-mean-square error. All birds per pen. Means within rows followed by different superscript letters are significantly different (P < 0.05).

Table 7. Effect of amino acids supplementation to a low-protein diet on relative weights of heart, liver, abdominal fat, and breast meat yield.

| Diet                           | PC     | NC   | NC+EAA | NC+NEAA | NC+EAA+NEAA | RMSE | P       |
|--------------------------------|--------|------|--------|---------|-------------|------|---------|
| Heart                          | 0.57   | 0.61 | 0.61   | 0.56    | 0.59        | 0.57 | 0.1799  |
| Liver                          | 2.11   | 2.46 | 2.45   | 2.23    | 2.19        | 0.34 | 0.1026  |
| Abdominal fat                  | 1.61   | 1.92 | 1.79   | 1.62    | 1.60        | 0.33 | 0.1326  |
| Breast                         | 20.5   | 20.3 | 20.3   | 20.6    | 20.4        | 1.73 | 0.9928  |

PC: positive control; NC: negative control; EAA: essential amino acids; NEAA: nonessential amino acids; RMSE: root-mean-square error. Data are means±SEM of 5 replications of 2 birds per pen.

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