**Effect of different amino acids density diets on lysine, methionine and protein efficiency in Arian broiler**

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**Abstract**

The advantages provided by amino acid (AA) densities to broiler performance have been well documented, but little research has been reported on comparing the effect of different densities, i.e. high, medium, standard and low amino acid levels (HAA, MAA, SAA, and LAA), on protein and energy efficiency in broiler. This study evaluated the effects of the four different amino acid densities in a completely randomized experimental design on 800 male (10 replicates per treatment) broilers. All diets were isocaloric and isonitrogenous. In broilers receiving HAA, there had been a significant increase in body weight at Day 42. Feeding broilers with HAA diets significantly increased protein and energy intake in the grower period and during the overall study period (0-42 days of age) (P<0.05). There was a significant difference in efficiency of lysine and methionine during all time periods (P<0.05) and HAA levels were significantly higher than SAA. Protein efficiency ratio (PER) and energy efficiency ratio (EER) were affected by an increase in AA density. AA levels had a significant effect on production efficiency factor (PEF). The results of this study suggest that additional lysine and methionine at 120% and other AA at 110% of National Research Council recommendations in starter and grower diets significantly improved body weight and PEF.

**Materials and methods**

The study was carried out on 1-6 week old Arian male broilers. At Day 1, 800 Arian male chickens (four treatments with ten replicates) were placed in 40 floor pens (20 chicks per pen and 0.12m² floor space per chick) with straw bedding. Water and feed were supplied ad libitum. A continuous lighting regimen was used with 24 h of light each day for the first three days and then standard 23 h light:1 h darkness until the end of the trial. The basic chemical composition of the feed materials was determined according to the AOAC (2006). Before formulation of the experimental diets, samples of the ingredients contributing protein (corn, soybean meal) were analyzed for total amino acid concentration. The total amino acid values of the ingredients were assayed by high-pressure liquid chromatography analysis. The following treatments were applied: Diet 1 with high amino acid (HAA), Diet 2 with medium amino acid (MAA), Diet 3 with standard amino acid (SAA), and Diet 4 with low amino acid (LAA) levels. The average analyzed differences between HAA, MAA and LAA vs SAA for amino acid density in the starter and grower periods were +10, +5 and -5%, respectively. These differences for lysine and methionine were +20, +10 and -10%, respectively. Broiler starter diets were formulated according to NRC (1994) recommendations to contain 22.5% CP and 3,034 kcal of ME/kg in starter diets, and 18.97% CP and 3,170 kcal of ME/kg in grower diets. The formulated diets were isoenergetic and isonitrogenic (Table 1).

Body weights (BW) and feed consumption were obtained at 21 and 42 days of age. There was no mortality during any of the treatments. The following parameters were calculated to the end of the study period:

- production efficiency factor \(P_{EF} = (\text{final bird weight, kg} \times \text{livability %})/(\text{age days \times feed conversion ratio} \times 100)\) (Lemme et al., 2006);
- protein efficiency ratio (PER): weight gain divided by protein intake (Kamran et al., 2008);
- energy efficiency ratio (EER): weight gain x 100/total ME intake (Kamran et al., 2008);
- lysine efficiency (Lys E): lysine intake (mg)/weight gain (g);
- methionine efficiency (Met E): methionine intake (mg)/weight gain (g).

Data were analyzed by a completely randomized experimental design using ANOVA (SAS, 2004) and means were compared with the Duncan multiple range tests when significance was reached. Output data were expressed as means with SEM.

**Results and discussion**

Results of body weight (BW) are shown in Table 2. Increasing AA density improves body weight at 42 days of age. Maximum BW occurred with the HAA diet from 0 to 42 days of age (2293g). There was a significant improvement in BW (at 42 days of age) in broilers fed the HAA diet, being 202 g higher than those of birds fed the SAA diet. This result is similar to those of Kidd et al. (2004) and Corzo et al. (2004). Final live performance results (0-42 days old, Table 2) demonstrate that the best BW was achieved when birds were fed HAA starter and grower diets in comparison with birds fed LAA, SAA and MAA diets. These results indicate that the amino acid requirement of Arian male broilers for maximum body weight gain was higher than those reported by the NRC (1994) and close to those of reported for other strains (Han and Baker, 1991). Nasr and Kheiri (2011a,b) demonstrated that increasing lysine and AA density (120%) (NRC, 1994) in starter and grower periods improved body weight gain.

The results of efficiency of protein, energy, Lys and Met in three study periods (0-21, 22-42 and 0-42 days of age) are shown in Tables 2, 3 and 4. There was a significant difference in protein and energy intake in the grower and overall study periods. Protein and energy intake were the highest in broiler fed the HAA diet in the starter and overall study periods, and this difference was significant (P<0.05). Body weight, protein and energy intake were the highest for birds fed the HAA in starter and grower diets, and this difference was significant (P<0.05). However, there was no significant difference in PER and EER when compared to SAA and MAA diets (P>0.05). PER and EER were unaffected by an increase in dietary AA levels. The diets in this study were formulated according to the same protein (isonitrogenic) and energy (isenergetic) requirements but differed in Lys, Met, Cys, Arg, Ile, Thr, Trp and Val levels. Therefore, broiler fed HAA have a higher Lys, Met, Cys, Arg, Ile, Thr and Trp intake than those fed other diets.

There was a significant increase in Lys E (lysine intake(mg)/weight gain(g)) in broil-

### Table 1. Composition of experimental diets in starter (0-21 day) and grower (22-42 day) periods.

| Ingredient                  | Starter | Grower |
|-----------------------------|---------|--------|
|                             | High    | Medium | Standard | Low   | High    | Medium | Standard | Low   |
| Composition                 |         |        |          |       |         |        |          |       |
| Metabolizable energy, Mcal/kg| 3.034   | 3.034  | 3.034    | 3.034 | 3.17    | 3.17   | 3.17     | 3.17  |
| Protein, %                  | 22.50   | 22.50  | 22.50    | 22.50 | 18.97   | 18.97  | 18.97    | 18.97 |
| Ether extract, %             | 5.12    | 5.17   | 5.16     | 4.93  | 5.62    | 5.61   | 5.60     | 5.61  |
| Linoleic acid, %             | 2.58    | 2.74   | 2.65     | 2.54  | 3.21    | 3.10   | 3.20     | 3.22  |
| Crude fibre, %              | 4.62    | 4.45   | 4.45     | 4.52  | 4.34    | 4.39   | 4.39     | 4.39  |
| Calcium, %                  | 0.09    | 0.09   | 0.09     | 0.07  | 0.06    | 0.03   | 0.03     | 0.03  |
| Available phosphorus, %     | 0.49    | 0.44   | 0.44     | 0.44  | 0.44    | 0.43   | 0.40     | 0.43  |
| Potassium, %                | 0.03    | 0.02   | 0.01     | 0.01  | 0.07    | 0.08   | 0.07     | 0.07  |
| Sodium, %                   | 0.15    | 0.15   | 0.15     | 0.15  | 0.13    | 0.13   | 0.13     | 0.13  |
| Total amino acids analyzed  |         |        |          |       |         |        |          |       |
| ARG, %                      | 1.462   | 1.43   | 1.35     | 1.272 | 1.377   | 1.285  | 1.257    | 1.194 |
| ILE, %                      | 1.01    | 0.966  | 0.953    | 0.905 | 0.832   | 0.8    | 0.781    | 0.767 |
| LYS, %                      | 1.33    | 1.22   | 1.1      | 1.01  | 1.25    | 1.135  | 1.03     | 0.93  |
| MET, %                      | 0.612   | 0.561  | 0.508    | 0.458 | 0.461   | 0.421  | 0.382    | 0.344 |
| CYS, %                      | 0.387   | 0.377  | 0.371    | 0.36  | 0.354   | 0.339  | 0.328    | 0.315 |
| THR, %                      | 1.01    | 0.98   | 0.92     | 0.862 | 0.768   | 0.758  | 0.74     | 0.699 |
| TRP, %                      | 0.262   | 0.258  | 0.25     | 0.237 | 0.259   | 0.252  | 0.246    | 0.24  |
| VAL, %                      | 1.19    | 1.126  | 1.083    | 0.996 | 0.915   | 0.911  | 0.888    | 0.867 |

*Provides per kg of diet: vitamin A, 7000 UI; vitamin D₃, 1400 UI; vitamin E, 16.65 mg; vitamin K₁, 1.5 mg; vitamin B₁₂, 0.6 mg; vitamin B₆, 0.013 mg; biotin, 0.15 mg; choline, 1.54 g; pantothenic acid, 9.32 mg; niacin, 30.12 mg; folic acid, 1.42 mg; selenium, 0.85 mg; iodine, 0.35 mg; iron, 57.72 mg; copper, 12.30 mg; zinc, 141.48 mg; manganese, 175 mg; ARG, arginine; ILE, isoleucine; Lys, lysine; Met, methionine; CYS, cystine; THR, threonine; TRP, tryptophan; Val, valine.
ers fed the HAA diet in the starter, grower and overall study periods. The highest Lys E was obtained with the HAA diet (25.06 mg/g) compared to the SAA treatment (23.05 mg/g) from 0 to 42 days of age (P<0.001), and this difference was significant. There was a significant increase in Met E (methionine intake(mg)/weight gain(g)) in broilers fed the HAA diet in the starter, grower and overall study periods. Met E was the highest in the HAA diet (10.1 mg/g) compared to the SAA diet (9.01 mg/g) from 0 to 42 days of age (P<0.001), and this difference was significant. This study, similar to that of Nasr et al. (2011), showed that an increase in the lysine level (120%) (NRC, 1994) in broiler starter and grower diets significantly increased protein and energy intake, Lys E and PEF. Protein and energy intake, Lys E and PEF in the HAA diet were significantly higher than in the SAA diet (Tables 2 and 3). This study showed the HAA diet was better able to transform protein and energy intake into tissue synthesis and accretion. This is possibly related to a higher AA availability to synthesize muscle. Diets formulated with HAA levels promoted a better conversion of AA into body weight gain. Differences in dietary AA density responses among those reported in the literature (Corzo et al., 1991; Han and Baker, 1991) differs between different AA intake, lysine and methionine efficiency, by 0.21% and 1.09% compared to broilers fed a diet with standard amino acid levels from 0 to 42 days of age, respectively. Body weight at 42 days of age of broilers fed a diet with high amino acid levels were 202 g higher (P<0.05) than those of broilers fed the diet with standard amino acid levels. This study showed the high amino acid diet was better able to transform protein and energy intake into tissue synthesis and accretion while increasing amino acid density in the studied diets did not satisfy ideal protein requirements.

Table 2. Effects of amino acid density on body weight, protein intake and protein efficiency ratio.

| Amino acid density | Body weight, g | PER 0-21 d | PER 22-42 d | PER 0-42 d | Protein intake 0-21 d, g | Protein intake 22-42 d, g | Protein intake 0-42 d, g |
|--------------------|----------------|------------|-------------|------------|------------------------|------------------------|------------------------|
| Low                | 1935<sup>a</sup> | 2.19       | 2.54<sup>b</sup> | 2.44<sup>b</sup> | 228.9                  | 570.7<sup>a</sup>       | 799.6<sup>a</sup>       |
| Standard           | 2901<sup>ab</sup> | 2.37       | 2.65<sup>ab</sup> | 2.56<sup>ab</sup> | 235.4                  | 583.6<sup>a</sup>       | 799.0<sup>a</sup>       |
| Medium             | 2125<sup>ab</sup> | 2.36       | 2.77<sup>ab</sup> | 2.65<sup>ab</sup> | 243.3                  | 544.5<sup>a</sup>       | 787.8<sup>a</sup>       |
| High               | 2293<sup>ab</sup> | 2.20       | 2.71<sup>ab</sup> | 2.56<sup>ab</sup> | 259.3                  | 620.8<sup>a</sup>       | 880.1<sup>a</sup>       |
| P value            | 0.040           | 0.627      | 0.039        | 0.044       | 293.9                  | 0.025                  | 0.031                  |
| SEM                | 52.6            | 0.118      | 0.053        | 0.041       | 7.9                    | 17.6                   | 21.4                   |

PER, protein efficiency ratio; <sup>a-b</sup> means followed by different superscript letters are significantly different (P<0.05).

Table 3. Effects of amino acid density on body weight, protein intake and protein efficiency ratio.

| Amino acid density | Body weight, g | PER 0-21 d | PER 22-42 d | PER 0-42 d | Protein intake 0-21 d, g | Protein intake 22-42 d, g | Protein intake 0-42 d, g |
|--------------------|----------------|------------|-------------|------------|------------------------|------------------------|------------------------|
| Low                | 1935<sup>a</sup> | 2.19       | 2.54<sup>b</sup> | 2.44<sup>b</sup> | 228.9                  | 570.7<sup>a</sup>       | 799.6<sup>a</sup>       |
| Standard           | 2901<sup>ab</sup> | 2.37       | 2.65<sup>ab</sup> | 2.56<sup>ab</sup> | 235.4                  | 583.6<sup>a</sup>       | 799.0<sup>a</sup>       |
| Medium             | 2125<sup>ab</sup> | 2.36       | 2.77<sup>ab</sup> | 2.65<sup>ab</sup> | 243.3                  | 544.5<sup>a</sup>       | 787.8<sup>a</sup>       |
| High               | 2293<sup>ab</sup> | 2.20       | 2.71<sup>ab</sup> | 2.56<sup>ab</sup> | 259.3                  | 620.8<sup>a</sup>       | 880.1<sup>a</sup>       |
| P value            | 0.040           | 0.627      | 0.039        | 0.044       | 293.9                  | 0.025                  | 0.031                  |
| SEM                | 52.6            | 0.118      | 0.053        | 0.041       | 7.9                    | 17.6                   | 21.4                   |

PER, protein efficiency ratio; <sup>a-b</sup> means followed by different superscript letters are significantly different (P<0.05).

Table 4. Effects of amino acid density on energy intake, energy efficiency ratio and production efficiency factor.

| Amino acid density | Energy intake 0-21 d, Mcal | Energy intake 22-42 d, Mcal | Energy intake 0-42 d, Mcal | EER 0-21 d | EER 22-42 d | EER 0-42 d | PEF |
|--------------------|---------------------------|-----------------------------|---------------------------|------------|------------|------------|-----|
| Low                | 3103                      | 9871<sup>a</sup>            | 12974<sup>ab</sup>       | 16.15      | 14.68<sup>b</sup> | 15.03<sup>b</sup> | 226c |
| Standard           | 3191                      | 9749<sup>a</sup>            | 12940<sup>ab</sup>       | 17.44      | 16.03<sup>a</sup> | 16.39<sup>a</sup> | 262<sup>ab</sup> |
| Medium             | 3288                      | 9418<sup>a</sup>            | 12716<sup>ab</sup>       | 17.78<sup>ab</sup> | 24.42<sup>a</sup> | 25.06<sup>a</sup> | 25.06<sup>a</sup> |
| High               | 3515                      | 10739<sup>a</sup>           | 14254<sup>ab</sup>       | 20.92<sup>b</sup> | 20.24<sup>b</sup> | 20.38<sup>b</sup> | 20.38<sup>b</sup> |
| P value            | 0.283                     | 0.025                       | 0.000                     | 0.020      | 0.000      | 0.000      | 0.000 |
| SEM                | 146.6                     | 313.8                       | 347.8                     | 0.59       | 0.24       | 0.28       | 3.9  |

EER, energy efficiency ratio; PEF, production efficiency factor; <sup>a</sup> means followed by different superscript letters are significantly different (P<0.05).

Conclusions

Feeding broilers a diet with high amino acid levels (Lys and Met +20% NRC, Arg, Ile, Cys, Thr, Trp, Val +10%) (NRC, 1994) increased protein and energy intake by 81.1 g, 1314 Kcal, lysine and methionine efficiency, by 2.01% and 1.09% compared to broilers fed a diet with standard amino acid levels from 0 to 42 days of age, respectively. Body weight at 42 days of age of broilers fed a diet with high amino acid levels were 202 g higher (P<0.05) than those of broilers fed the diet with standard amino acid levels. This study showed the high amino acid diet was better able to transform protein and energy intake into tissue synthesis and accretion while increasing amino acid density in the studied diets did not satisfy ideal protein requirements.
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