The relationship between guanidino acetic acid and metabolisable energy level of diets on performance of broiler chickens

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Abstract

This work aimed to investigate the effects on performance and carcass characteristics of broiler chickens of 2 levels of guanidino acetic acid (GAA) in a commercial form (CreAMINO®) and 4 levels of metabolisable energy. The eight dietary regimens were tested for starter (0-10 d), grower (11-22 d) and finisher (23-35 d) period as well as cumulatively, while ME level is one of the major factors, which play an important role in regulating feed intake and feed efficiency in broilers (Lopez and Leeson, 2008).

In Saudi Arabia, there is a ban of animal by-product use in the poultry industry, consequently lacking creatine as this semi-essential nutrient is only present in raw materials of animal origin (Ringel et al., 2008). Creatine is naturally synthesised mainly in the liver and kidney of avian from guanidino acetic acid (GAA). Guanidino acetic acid is synthesised in the liver and kidney from arginine and glycine then acted upon by the enzyme transamidinase and subsequently methylated by S-adenosyl-methione to creatine (Borsook and Dubnoff, 1940; Wyss and Kaddurah-Daouk, 2000). The need for creatine is age-dependent, higher amounts are needed by growing animals for muscle growth vs adults (Brosnan et al., 2009). Furthermore, it has an important role as an energy carrier in the cells (Walker, 1979). Guanidino acetic acid is considered the only immediate precursor for creatine in the body of animals; moreover, it is more stable and less expensive than creatine and it could be a good additive for poultry (Wietlake et al., 2009). However, there is a lack of information about a possible interaction between GAA and ME in broilers.

Therefore, in the present study, the objective was to evaluate the efficacy of supplementing GAA (supplemented as CreAMINO®) to a typical corn soybean meal diets with graded levels of ME on performance and carcass characteristics of broiler chickens form 0 to 35 days of age.

Materials and methods

Animals, husbandry and treatments

A total of 200, one-day-old Ross 308 male broiler chicks were obtained from a commercial hatchery (Al-Wadi Poultry Farm Co., Riyadh, Saudi Arabia) and grouped by weight
in such a way as to reduce variation in mean body weight. Then chicks were allotted into 40 experimental cages with four chicks per cage (50 cm length, 60 cm width and 36 cm depth) in a four-deck cage system and received the experimental diets in electrically heated battery brooders with raised wire floors. Environmental temperature in the first week of life was kept at 35°C and then decreased to 22°C until the end of the experiment. The chicks were vaccinated against Marek’s disease, Newcastle disease and infectious bronchitis. The growth experiment was carried out from 0 to 35 days of age and samples were obtained at the end of the trial. Feed and water were provided ad libitum and birds were maintained a 24-h light schedule. The study was conducted under a protocol approved by King Saud University and complied with the current laws of Saudi Arabia. Typical starter (0-10 d), grower (11-22 d) and finisher (23-35 d) diets based on corn-soybean meal were formulated which met or exceeded the recommendations in commercial practice in Saudi Arabia except for metabolisable energy for each period and provided in mash form. The control diets contained 3000, 3075 and 3150 kcal/kg for starter, grower and finisher, respectively (Tables 1-3). The increments of ME were achieved by lowering the amount of oil added to the diets. Chicks received one of 8 treatments for each growing period (starter, grower and finisher) as follows: i) 75 kcal/kg less ME than the control; ii) 50 kcal/kg less ME than the control; iii) 25 kcal/kg less ME than the control; iv) commercial ME level (control); v) 75 kcal/kg less ME than the control+0.06% CreAMINO® (CreAM); vi) 50 kcal/kg less ME than the control+0.06% CreAM; vii) 25 kcal/kg less ME than the control+0.06% CreAM and viii) commercial ME level+0.06% CreAM. All the chemical analyses for the experimental diets were performed by Evonik Industries AG Feed Additives, Hanau, Germany. The metabolisable energy content of the diets was based on calculation.

### Table 1. Composition of experimental starter diets fed to broilers from 1 to 10 days.

| Ingredient, %       | Control group | CreAMINO® group |
|---------------------|---------------|------------------|
|                     | Low ME        | Normal ME        | Low ME     | Normal ME        |
|                     | 75 kcal       | 50 kcal          | 25 kcal    | 75 kcal          | 50 kcal           | 25 kcal          |
| ME group            | 2925          | 2950             | 2975       | 3000             | 2925              | 2950             | 2950           | 3000         |
| Ingredients, %      |               |                  |            |                 |                   |                 |                |
| Corn                | 65.13         | 64.57            | 64.02      | 63.46            | 65.04             | 64.49            | 63.93          | 63.38        |
| Soybean meal        | 30.86         | 30.96            | 31.05      | 31.15            | 30.87             | 30.97            | 31.07          | 31.17        |
| Dicalcium phosphate | 1.96          | 1.96             | 1.96       | 1.96             | 1.96              | 1.96             | 1.96           | 1.97         |
| Calcium carbonate   | 0.73          | 0.73             | 0.73       | 0.73             | 0.73              | 0.73             | 0.73           | 0.73         |
| D-L methionine 99%  | 0.25          | 0.25             | 0.25       | 0.25             | 0.25              | 0.25             | 0.25           | 0.26         |
| Salt                | 0.25          | 0.25             | 0.25       | 0.25             | 0.25              | 0.25             | 0.25           | 0.25         |
| Soy oil             | 0.20          | 0.12             | 1.12       | 1.57             | 0.21              | 0.67             | 1.13           | 1.58         |
| L-lysine            | 0.18          | 0.18             | 0.18       | 0.18             | 0.18              | 0.18             | 0.18           | 0.18         |
| Vitamin-mineral premix® | 0.20      | 0.20             | 0.20       | 0.20             | 0.20              | 0.20             | 0.20           | 0.20         |
| Sodium bicarbonate | 0.12          | 0.12             | 0.12       | 0.12             | 0.12              | 0.12             | 0.12           | 0.12         |
| L-threonine         | 0.07          | 0.07             | 0.07       | 0.07             | 0.07              | 0.07             | 0.07           | 0.07         |
| Choline chloride 60 | 0.05          | 0.05             | 0.05       | 0.05             | 0.05              | 0.05             | 0.05           | 0.05         |
| CreAMINO®           | 0.00          | 0.00             | 0.00       | 0.00             | 0.06              | 0.06             | 0.06           | 0.06         |
| Determined chemical composition, % | | | | | | | | |
| Crude protein       | 20.73         | 21.07            | 20.45      | 20.44            | 19.51             | 20.25            | 20.52          | 22.40        |
| Methionine          | 0.55          | 0.49             | 0.54       | 0.54             | 0.53              | 0.47             | 0.56           | 0.65         |
| Cysteine            | 0.36          | 0.36             | 0.35       | 0.35             | 0.34              | 0.33             | 0.35           | 0.37         |
| Lysine              | 0.91          | 0.84             | 0.88       | 0.88             | 0.87              | 0.90             | 0.91           | 1.10         |
| Methionine+cysteine | 0.91          | 0.84             | 0.88       | 0.88             | 0.67              | 0.80             | 0.91           | 1.01         |
| Arginine            | 1.57          | 1.57             | 1.54       | 1.53             | 1.27              | 1.23             | 1.34           | 1.46         |
| Threonine           | 0.85          | 0.87             | 0.82       | 0.83             | 0.78              | 0.78             | 0.85           | 0.93         |
| Leucine             | 1.79          | 1.82             | 1.76       | 1.74             | 1.68              | 1.68             | 1.74           | 1.83         |
| Isoleucine          | 0.85          | 0.86             | 0.84       | 0.84             | 0.79              | 0.77             | 0.81           | 0.90         |
| Valine              | 0.99          | 0.99             | 0.97       | 0.97             | 0.92              | 0.90             | 0.94           | 1.02         |
| Histidine           | 0.55          | 0.55             | 0.53       | 0.53             | 0.51              | 0.50             | 0.53           | 0.57         |
| Phenyalanine        | 1.03          | 1.04             | 1.01       | 1.00             | 0.96              | 0.94             | 1.00           | 1.08         |
| Glycine             | 0.85          | 0.85             | 0.82       | 0.82             | 0.79              | 0.77             | 0.83           | 0.90         |

ME, metabolisable energy; °Vitamin-mineral premix contains the following per kg: vitamin A, 10,000,000 U; vitamin D3, 5,000,000 U; vitamin E, 50,000 mg; vitamin K3, 3000 mg; vitamin B6, 2000 mg; vitamin B12, 2000 mg; vitamin B1, 2000 mg; vitamin B2, 6000 mg; vitamin B3, 60,000 mg; vitamin B5, 15,000 mg; vitamin B9, 3000 mg; vitamin B12, 10,000 meq; biotin, 200,000 meq; antioxidant, 25,000 mg; copper, 2000 mg; iodine, 1250 mg; iron, 40,000 mg; manganese, 120,000 mg; selenium, 40 mg; zinc, 120,000 mg.
Statistical analysis

Data were analyzed by using the general linear model procedure of SAS (2002) for randomised complete block design with 4 × 2 factorial arrangements of treatments, in which each experimental diet was fed to 5 replicate pens. The data were tested for the main effects of energy level (4 levels), CreAM (with or without) and for interaction effect for energy CreAM. The experimental unit was the pen mean. Orthogonal contrasts (linear, quadratic and cubic) were used for further investigating the significant energy level in cases where the term was significant. The overall level for statistical significance was set at P<0.05. All values were expressed as statistical means±standard error of the mean (SEM).

Results and discussion

Feed consumption and efficiency

Data related to chick’s performance such as FI, BWG and FCR for starter, grower, finisher and cumulative periods are presented in Tables 4-7. Table 4 shows the performance results for the starter period (0 to 10 days of age). No significant differences in BWG, FI and FCR were found related to the ME level of the diet, which may be explained by the small quantity of feed consumed during the first 10 days. However, a significant difference in FCR was observed due to CreAM supplementation (P<0.05), birds, which had received CreAM converted feed to body weight more efficiently as compared to the other group (1.45 vs 1.51). However, Stahl et al. (2003) demonstrated that creatine monohydrate did not significantly affect FCR of broilers during weeks one and two. When examining the chemical analyses of the diets used in this period, it was noted that diet which contained 50 kcal lower ME had lower methionine and lysine content due to unknown reason.

Table 4 shows the performance results for the grower period (11 to 22 days of age). A significant two-way interaction (CreAM energy) was observed for FI (P<0.05), chicks consumed less feed at all levels of energy when diets were supplemented with CreAM except at the level of 3050 kcal/kg. Chicks in this group consumed more feed when the diet was sup-

Table 2. Composition of experimental grower diets fed to broilers from 11 to 22 days.

|                     | Control group | CreAMINO® group |
|---------------------|---------------|-----------------|
|                     | Low ME        | Normal ME       | Low ME        | Normal ME       |
|                     | 75 kcal       | 50 kcal         | 25 kcal       | 75 kcal         | 50 kcal         | 25 kcal         |
| ME group            | 3000          | 3025            | 3050          | 3075            | 3000            | 3025            | 3050            | 3075            |
| Ingredients, %      |               |                 |               |                 |                 |                 |                 |                 |
| Corn                | 68.70         | 68.15           | 67.59         | 67.04           | 68.62           | 68.06           | 67.51           | 66.95           |
| Soybean meal        | 26.88         | 26.98           | 27.08         | 27.18           | 26.90           | 26.99           | 27.09           | 27.19           |
| Dicalcium phosphate | 1.95          | 1.95            | 1.95          | 1.95            | 1.95            | 1.95            | 1.95            | 1.95            |
| Calcium carbonate   | 0.76          | 1.22            | 1.68          | 2.13            | 0.77            | 1.23            | 1.69            | 2.14            |
| D-L methionine 99%  | 0.64          | 0.64            | 0.64          | 0.64            | 0.64            | 0.64            | 0.64            | 0.64            |
| Salt                | 0.25          | 0.25            | 0.25          | 0.26            | 0.25            | 0.25            | 0.25            | 0.26            |
| Soy oil             | 0.25          | 0.25            | 0.25          | 0.25            | 0.25            | 0.25            | 0.25            | 0.25            |
| L-lysine            | 0.18          | 0.17            | 0.17          | 0.17            | 0.18            | 0.17            | 0.17            | 0.17            |
| Vitamin-mineral premix°| 0.15        | 0.15            | 0.15          | 0.15            | 0.15            | 0.15            | 0.15            | 0.15            |
| Sodium bicarbonate  | 0.11          | 0.11            | 0.11          | 0.11            | 0.11            | 0.11            | 0.11            | 0.11            |
| L-threonine         | 0.08          | 0.08            | 0.08          | 0.08            | 0.08            | 0.08            | 0.08            | 0.08            |
| Choline chloride 60 | 0.04          | 0.04            | 0.04          | 0.04            | 0.04            | 0.04            | 0.04            | 0.04            |
| CreAMINO®           | 0.00          | 0.00            | 0.00          | 0.00            | 0.06            | 0.06            | 0.06            | 0.06            |
| Determined chemical composition, % |             |                 |               |                 |                 |                 |                 |                 |
| Dry matter          | 88.00         | 88.00           | 88.00         | 88.00           | 88.00           | 88.00           | 88.00           | 88.00           |
| Crude protein       | 18.96         | 19.36           | 19.72         | 19.03           | 18.66           | 19.65           | 17.94           | 19.64           |
| Methionine          | 0.49          | 0.50            | 0.52          | 0.59            | 0.53            | 0.53            | 0.42            | 0.54            |
| Cysteine            | 0.33          | 0.34            | 0.33          | 0.32            | 0.32            | 0.32            | 0.30            | 0.33            |
| Lysine              | 1.09          | 1.15            | 1.10          | 1.12            | 1.10            | 1.11            | 0.92            | 1.15            |
| Methionine+cysteine | 0.82          | 0.84            | 0.86          | 0.90            | 0.85            | 0.86            | 0.72            | 0.87            |
| Arginine            | 1.21          | 1.29            | 1.24          | 1.19            | 1.18            | 1.23            | 1.05            | 1.25            |
| Threonine           | 0.85          | 0.87            | 0.82          | 0.83            | 0.78            | 0.78            | 0.55            | 0.53            |
| Leucine             | 1.64          | 1.68            | 1.69          | 1.63            | 1.64            | 1.69            | 1.52            | 1.63            |
| Isoleucine          | 0.76          | 0.79            | 0.79          | 0.74            | 0.76            | 0.78            | 0.66            | 0.77            |
| Valine              | 0.88          | 0.92            | 0.91          | 0.86            | 0.89            | 0.91            | 0.78            | 0.89            |
| Histidine           | 0.50          | 0.52            | 0.50          | 0.49            | 0.49            | 0.50            | 0.44            | 0.50            |
| Phenylalanine       | 0.93          | 0.96            | 0.96          | 0.91            | 0.91            | 0.95            | 0.83            | 0.93            |
| Glycine             | 0.76          | 0.79            | 0.77          | 0.75            | 0.75            | 0.76            | 0.66            | 0.77            |

ME, metabolisable energy °Vitamin-mineral premix contains the following per kg: vitamin A, 10,000,000 IU; vitamin D₃, 5,000,000 IU; vitamin E, 50,000 IU; vitamin K₁, 3000 mg; vitamin B₁, 2000 mg; vitamin B₂, 6000 mg; vitamin B₃, 60,000 mg; vitamin B₆, 15,000 mg; vitamin B₁₂, 3000 mg; vitamin B₁₂, 2000 mg; vitamin B₁₂, 16,000 mcg; biotin, 200,000 mcg; antioxidant, 25,000 mg; copper, 2000 mg; iodine, 1250 mg; iron, 40,000 mg; manganese, 120,000 mg; selenium, 40 mg; zinc, 120,000 mg.
plemented with CreAM (877 vs 809 g feed, respectively). No significant differences in BWG were observed during this period due to energy level, CreAM supplementation or their interaction (P>0.05). As a result of the dissimilarities of FI during this period, significant differences in FCR were observed due to main effects (energy level and CreAM supplementation) (P<0.05); birds converted feed more efficiently as the level of dietary energy increased in a linear trend (P<0.01). On the other hand, birds converted feed more efficiently when diets were supplemented with CreAM (1.438 vs 1.488). Table 6 shows the performance results for the finisher period (23 to 35 days of age). For this period, FI was not affected by the energy level or CreAM supplementation (P>0.05).

However, there was a trend due to CreAM on BWG (P=0.06), such that birds received CreAM gained 39 g more body weight as compared to the group, which did not receive CreAM. As a result, birds which had received the diets supplemented with CreAM had a better FCR (P<0.005) as compared to the other group (1.71 vs 1.80, respectively). This is in line with observations of Stahl et al. (2003) who found improvement of FCR from weeks three to four after creatine monohydrate supplementation, they linked that improvement to muscle cell hydration and increase in weight gain as GAA plays a significant role in muscle tissues in animal tissue.

When examining the cumulative period of the trial (0 to 35 days of age) (Table 7), two-way interactions were significant for FI and BWG (P<0.05 and P<0.05, respectively). Birds, which had received the control level of energy, 75 and 50 kcal/kg lower ME than the control consumed less feed when these diets were supplemented with CreAM as compared to the unsupplemented diets. On the contrary, CreAM supplementation to the diet which had 25 kcal/kg less of ME increased feed intake by 144 g. On the other hand, birds which had received diets lower in 75 or 50 kcal/kg ME gained less weight when these diets were supplemented with CreAM as compared to the other groups. Birds, which received (25 kcal/kg) lower ME or the control energy levels had gained more weight when these diets were supplemented with CreAM. However, the magnitude of BWG

### Table 3. Composition of experimental finisher diets fed to broilers from 23 to 35 days.

| Ingredients, % | Control group | CreAMINO® group |
|----------------|---------------|-----------------|
|                | Low ME        | Normal ME       | Low ME       | Normal ME       |
|                | 75 kcal       | 50 kcal         | 25 kcal      | 75 kcal         | 50 kcal         | 25 kcal       |
| ME group       | 3075          | 3100            | 3125         | 3150            | 3075            | 3100          | 3125         | 3150 |
| Ingredients, % |               |                 |              |                 |                 |               |             |     |
| Corn           | 72.42         | 71.86           | 71.31        | 70.75           | 72.33           | 71.78         | 71.22        | 70.66 |
| Soybean meal   | 22.77         | 22.87           | 22.97        | 23.06           | 22.78           | 22.88         | 22.98        | 23.08 |
| Dicalcium phosphate | 1.86     | 1.86            | 1.86         | 1.86            | 1.86            | 1.86          | 1.86         | 1.86  |
| Calcium carbonate | 0.60      | 0.60            | 0.60         | 0.60            | 0.60            | 0.60          | 0.60         | 0.60  |
| L-methionine 99% | 0.25      | 0.25            | 0.25         | 0.25            | 0.25            | 0.25          | 0.25         | 0.25  |
| Salt           | 0.25          | 0.25            | 0.25         | 0.26            | 0.25            | 0.25          | 0.25         | 0.26  |
| Soy oil        | 1.29          | 1.75            | 2.21         | 2.66            | 1.30            | 1.76          | 2.22         | 2.67  |
| L-lysine       | 0.18          | 0.17            | 0.17         | 0.17            | 0.15            | 0.17          | 0.17         | 0.17  |
| Vitamin-mineral premix | 0.15   | 0.15            | 0.15         | 0.15            | 0.15            | 0.15          | 0.15         | 0.15  |
| Sodium bicarbonate | 0.11   | 0.11            | 0.11         | 0.11            | 0.11            | 0.11          | 0.11         | 0.11  |
| L-threonine    | 0.09          | 0.09            | 0.09         | 0.09            | 0.09            | 0.09          | 0.09         | 0.09  |
| Choline chloride 60% | 0.04 | 0.04            | 0.04         | 0.04            | 0.04            | 0.04          | 0.04         | 0.04  |
| CreAMINO®      | 0.00          | 0.00            | 0.00         | 0.00            | 0.06            | 0.06          | 0.06         | 0.06  |
| Determined chemical composition, % |               |                 |              |                 |                 |               |             |     |
| Dry matter     | 88.00         | 88.00           | 88.00        | 88.00           | 88.00           | 88.00         | 88.00        | 88.00 |
| Crude protein  | 16.51         | 16.96           | 16.54        | 16.14           | 16.96           | 19.72         | 16.64        | 17.89 |
| Methionine     | 0.39          | 0.47            | 0.43         | 0.43            | 0.43            | 0.67          | 0.42         | 0.52  |
| Cysteine       | 0.31          | 0.30            | 0.30         | 0.29            | 0.30            | 0.33          | 0.30         | 0.31  |
| Lysine         | 0.89          | 0.94            | 0.90         | 0.88            | 0.92            | 1.20          | 0.91         | 1.05  |
| Methionine+cysteine | 0.70    | 0.77            | 0.72         | 0.72            | 0.73            | 1.00          | 0.71         | 0.83  |
| Arginine       | 1.02          | 1.05            | 1.00         | 0.97            | 1.02            | 1.25          | 1.01         | 1.11  |
| Threonine      | 0.65          | 0.69            | 0.66         | 0.66            | 0.66            | 0.83          | 0.66         | 0.74  |
| Leucine        | 1.47          | 1.46            | 1.48         | 1.44            | 1.48            | 1.59          | 1.47         | 1.53  |
| Isoleucine     | 0.62          | 0.64            | 0.64         | 0.61            | 0.65            | 0.75          | 0.64         | 0.70  |
| Valine         | 0.75          | 0.77            | 0.75         | 0.73            | 0.77            | 0.89          | 0.78         | 0.83  |
| Histidine      | 0.44          | 0.44            | 0.43         | 0.42            | 0.44            | 0.50          | 0.43         | 0.46  |
| Phenylalanine  | 0.79          | 0.80            | 0.79         | 0.77            | 0.80            | 0.91          | 0.78         | 0.84  |
| Glycine        | 0.65          | 0.65            | 0.63         | 0.62            | 0.65            | 0.77          | 0.64         | 0.70  |

ME, metabolisable energy. "Vitamin-mineral premix contains the following per kg: vitamin A, 10,000,000 I.U.; vitamin D3, 5,000,000 I.U.; vitamin E, 50,000 mg; vitamin K3, 3000 mg; vitamin B1, 2000 mg; vitamin B2, 6000 mg; vitamin B6, 60,000 mg; vitamin B12, 15,000 mg; vitamin B6, 3000 mg; vitamin B6, 2000 mg; vitamin B12, 16,000 meq; biotin, 200,000 meq; antioxidant, 25,000 mg; copper, 2000 mg; iodine, 1250 mg; iron, 40,000 mg; manganese, 120,000 mg; selenium, 40 mg; zinc, 120,000 mg."
Table 4. Feed intake, body weight gain and feed conversion ratio of broiler chickens given experimental diets at 10 d (0-10 d).

| Diet | CreAmino, % | Energy, kcal/kg | FI, g | BWG, g | FCR, g.g |
|------|-------------|-----------------|-------|--------|---------|
| 1    | 0           | 2925            | 264   | 173    | 1.528   |
| 2    | 0           | 2950            | 258   | 160    | 1.623   |
| 3    | 0           | 2975            | 262   | 178    | 1.483   |
| 4    | 0           | 3000            | 267   | 190    | 1.404   |
| 5    | 0.6         | 2925            | 275   | 187    | 1.481   |
| 6    | 0.6         | 2950            | 241   | 167    | 1.451   |
| 7    | 0.6         | 2975            | 278   | 190    | 1.457   |
| 8    | 0.6         | 3000            | 246   | 174    | 1.422   |
| SEM± |             |                 | 11.1  | 10.2   | 0.046   |
| P (CreAmino×energy) | ns | ns | ns |
| Energy |             |                 |       |        |         |
| 2925 |             | 270             | 180   | 1.505  |
| 2950 |             | 249             | 163   | 1.537  |
| 2975 |             | 270             | 184   | 1.470  |
| 3000 |             | 256             | 182   | 1.413  |
| SEM± |             | 7.8             | 7.2   | 0.03   |
| P contrast | ns | ns | ns |
| Energy: linear | ns | ns | ns |
| Energy: quadratic | ns | ns | ns |
| Energy: cubic | ns | ns | ns |

Table 5. Feed intake, body weight gain and feed conversion ratio of broiler chickens given experimental diets at 22 d (11-22 d).

| Diet | CreAmino, % | Energy, kcal/kg | FI, g | BWG, g | FCR, g.g |
|------|-------------|-----------------|-------|--------|---------|
| 1    | 0           | 3000            | 896   | 578    | 1.549   |
| 2    | 0           | 3025            | 879   | 601    | 1.468   |
| 3    | 0           | 3050            | 809   | 558    | 1.455   |
| 4    | 0           | 3075            | 875   | 591    | 1.481   |
| 5    | 0.6         | 3000            | 852   | 564    | 1.508   |
| 6    | 0.6         | 3025            | 828   | 578    | 1.434   |
| 7    | 0.6         | 3050            | 877   | 603    | 1.453   |
| 8    | 0.6         | 3075            | 819   | 607    | 1.356   |
| SEM± |             |                 | 24.5  | 21.0   | 0.034   |
| P (CreAmino×energy) | * | ns | ns |
| Energy |             |                 |       |        |         |
| 2925 |             | 874             | 571   | 1.529  |
| 2950 |             | 854             | 590   | 1.451  |
| 2975 |             | 843             | 581   | 1.454  |
| 3000 |             | 847             | 599   | 1.419  |
| SEM± |             | 17.3            | 14.8  | 0.024  |
| P contrast | ns | ns | * |
| Energy: linear | ns | ns | ** |
| Energy: quadratic | ns | ns | ns |
| Energy: cubic | ns | ns | ns |

Fl, feed intake; BWG, body weight gain; FCR, feed conversion ratio. *Means in the same column with different superscripts differ significantly. *P<0.05; ns, not significant.
change was the greatest for birds which had received (25 kcal/kg) lower energy as compared to all other levels of energy (195 g difference between CreAM supplemented and unsupplemented group). Feed conversion ratio was influenced by the energy level and the CreAM (P<0.05 and P<0.001, respectively). A cubic trend for energy level on FCR was observed (P<0.05). Birds, which received the control energy level had the best FCR followed by those received 50, 25 and 75 kcal/kg less ME. This could explain the cubic trend. Supplementing the diet with CreAM improved FCR by more than 7 points as compared to the unsupplemented group (1.61 vs 1.68, respectively; P<0.0001). Ringel et al. (2008) showed similar weight gain in birds which had received vegetable diet that was supplemented with CreAM and those which received meat and bone meal diet for the period from 14 to 35 days. However, FCR was improved to a higher degree in birds which had received CreAM supplemented diet. Positive improvements in broiler’s performance due to CreAM supplementation were reported by Lemme et al. (2007a); however, a hierarchy of GAA requirements was reported in their experiment. Males requirement for maximum gain was lower than that for FCR (0.08 to 0.12%, respectively) for both starter and grower periods while, best performance for females was achieved at 0.12% dietary GAA for the starter (0 to 21 days) and 0.08% for the grower period (22 to 42 days of age). Energy is an expensive component of broiler’s diet due the relative high price of inedible fats. Dietary energy content influences the intake of all other nutrients; however, broilers display an exceptional ability to control their energy intake by regulating their feed intake as diet energy concentration changes (Lopez and Leeson, 2008). Saleh et al. (2004) reported that decreasing the amount of energy in the diet decreases growth rate and feed efficiency, however their experiment involved a wider range of ME (270 kcal/kg) as compared to this study (75 kcal/kg). However, in this trial ME content of the diet was changed in small increments (25 kcal/kg). The results are in line with those obtained by Noy and Sklan (2002). Feed intake was not influenced by the ME level of the diet, implicating that the energy requirement may not have been low enough to affect feed intake.

### Carcass characteristics

The mean percentage of carcass parts is documented in Tables 8 and 9. Energy level had a significant effect on leg and thigh percent (P<0.05), both leg and thigh responded to the level of energy in a cubic trend (P<0.05). CreAM, on the other hand, had no influence on all parameters measured except for liver percent. CreAM supplementation decreased liver percent from 3.07 to 2.84% (P<0.05). Previous studies that involved creatine supplementation to swine (O’Quinn et al., 2000) and broilers (Stahl et al., 2003) found that dietary treatment had little to no effect on dressing percentage or fat deposition.

### Table 6. Feed intake, body weight gain and feed conversion ratio of broiler chickens given experimental diets at 35 d (23-35 d).

| Diet | Energy, kcal/kg | FL, g | BWG, g | FCR, g | SEM± | P (CreAmino x energy) |
|------|----------------|-------|--------|--------|------|-----------------------|
| CreAmino, % |                   |       |        |        |      |                       |
| 1    | 0              | 3075  | 2206   | 1241   | 1.777|                       |
| 2    | 0              | 3100  | 2166   | 1218   | 1.779|                       |
| 3    | 0              | 3125  | 2172   | 1156   | 1.679|                       |
| 4    | 0              | 3150  | 2207   | 1208   | 1.744|                       |
| 5    | 0.6            | 3075  | 2151   | 1234   | 1.743|                       |
| 6    | 0.6            | 3100  | 2079   | 1231   | 1.690|                       |
| 7    | 0.6            | 3125  | 2233   | 1294   | 1.727|                       |
| 8    | 0.6            | 3150  | 2145   | 1280   | 1.681|                       |
| SEM± |               | 45.2  | 28.3   | 0.038  |      |                       |
| P (CreAmino x energy) |               | 0.057 |        |        |      |                       |
| Energy |               |       |        |        |      |                       |
| 2925 | 2178           | 1237  | 1.760  |       |      |                       |
| 2950 | 2123           | 1225  | 1.734  |       |      |                       |
| 2975 | 2203           | 1225  | 1.803  |       |      |                       |
| 3000 | 2176           | 1274  | 1.712  |       |      |                       |
| SEM± | 32.0           | 20.0  | 0.027  |       |      |                       |
| P    | ns             | ns    | ns     |       |      |                       |
| Energy: linear |               |       |        |        |      |                       |
| Energy: quadratic |            |       |        |        |      |                       |
| Energy: cubic |               |       |        |        |      |                       |
| CreAmino |               |       |        |        |      |                       |
| Without | 2188           | 1221  | 1.795* |       |      |                       |
| With   | 2152           | 1260  | 1.710* |       |      |                       |
| SEM± | 22.6           | 14.1  | 0.019  |       |      |                       |
| P    | ns             | 0.06  | **     |       |      |                       |

Fl, feed intake; BWG, body weight gain; FCR, feed conversion ratio. *Means in the same column with different superscripts differ significantly. **P<0.01, ns, not significant.
Table 7. Feed intake, body weight gain and feed conversion ratio of broiler chickens given experimental diets at 35 d (0-35 d).

| Diet | CreAmino, % | Energy, kcal/kg | FI, g | BWG, g | FCR, g:g |
|------|-------------|-----------------|------|--------|----------|
| 1    | 0           | 2925 S 3000 G 3075 F | 3367 | 1993   | 1.689    |
| 2    | 0           | 2950 S 3025 G 3100 F | 3303 | 1979   | 1.668    |
| 3    | 0           | 2975 S 3050 G 3125 F | 3245 | 1894   | 1.715    |
| 4    | 0           | 3000 S 3075 G 3150 F | 3349 | 2049   | 1.636    |
| 5    | 0.6         | 2925 S 3000 G 3075 F | 3280 | 1986   | 1.651    |
| 6    | 0.6         | 2950 S 3025 G 3100 F | 3150 | 1977   | 1.591    |
| 7    | 0.6         | 2975 S 3050 G 3125 F | 3389 | 2089   | 1.623    |
| 8    | 0.6         | 3000 S 3075 G 3150 F | 3211 | 2061   | 1.558    |

SEM± 54.2 36.5 0.024

**P (CreAmino×energy)**

| Energy | SEM± | PP | P |
|--------|------|----|---|
| 1°     | 3323 | 1990 | 1.670 |
| 2°     | 3226 | 1978 | 1.630 |
| 3°     | 3317 | 1991 | 1.669 |
| 4°     | 3280 | 2055 | 1.597 |
| SEM±   | 38.3 | 25.8 | 0.017 |
| P      | ns   | ns  | **|
| Energy: linear | ns | ns | * |
| Energy: quadratic | ns | ns | ns |
| Energy: cubic   | *   | ns  | ns  |

CreAmino

| Without | SEM± | P |
|---------|------|---|
|         | 3316 | 1979 | 1.677^* |
| With    | 3257 | 2028 | 1.606^* |
| SEM±    | 27.1 | 18.2 | 0.012 |
| P       | ns   | 0.06 | *** |

FI, feed intake; BWG, body weight gain; FCR, feed conversion ratio; S, starter; G, grower; F, finisher. *Birds received 2855 kcal/kg for S period, 2950 kcal/kg for G period and 3075 kcal/kg for F period; °birds received 2850 kcal/kg for S period, 3025 kcal/kg for G period and 3100 kcal/kg for F period; ÷birds received 2975 kcal/kg for S period, 3050 kcal/kg for G period and 3125 kcal/kg for F period; *birds received 3000 kcal/kg for S period, 3075 kcal/kg for G period and 3150 kcal/kg for F period. °°°Means in the same column with different superscripts differ significantly. *P<0.05; **P<0.01; ***P<0.001; ns, not significant.
Table 8. Effect of treatments on parts yield as percentages of broiler dressed weight at 35 days.

| Diet | CreAmino, % | Energy, kcal/kg | Dressing, % | Breast¹, % | Leg¹, % | Drumstick², % | Thigh², % |
|------|-------------|-----------------|-------------|------------|---------|---------------|-----------|
| 1    | 0           | 3075            | 70.62       | 34.41      | 31.53   | 14.16         | 17.15     |
| 2    | 0           | 3100            | 70.59       | 34.16      | 31.39   | 14.07         | 17.29     |
| 3    | 0           | 3125            | 69.88       | 33.75      | 32.31   | 13.74         | 18.50     |
| 4    | 0           | 3150            | 72.06       | 34.46      | 31.47   | 14.52         | 16.90     |
| 5    | 0.6         | 3075            | 69.91       | 33.14      | 31.97   | 14.13         | 17.94     |
| 6    | 0.6         | 3100            | 69.70       | 35.59      | 31.15   | 14.00         | 17.08     |
| 7    | 0.6         | 3125            | 71.53       | 33.58      | 32.95   | 14.72         | 17.77     |
| 8    | 0.6         | 3150            | 71.31       | 34.74      | 31.00   | 14.46         | 16.68     |
| SEM± |             |                 | 0.67        | 0.83       | 0.56    | 0.34          | 0.43      |
| P (CreAmino×energy) | ns | ns | ns | ns | ns | ns | ns |

Energy

| Energy | SEM± | P | Energy: cubic | SEM± | P | Energy: linear | SEM± | P | Energy: quadratic | SEM± | P | Energy: cubic | SEM± | P |
|--------|------|---|---------------|------|---|----------------|------|---|-------------------|------|---|----------------|------|---|
| 2925   |      |   |               |      |   |                |      |   |                   |      |   |                |      |   |
| 2950   |      |   |               |      |   |                |      |   |                   |      |   |                |      |   |
| 2975   |      |   |               |      |   |                |      |   |                   |      |   |                |      |   |
| 3000   |      |   |               |      |   |                |      |   |                   |      |   |                |      |   |
| SEM±   | 0.47 |   | 0.59          | 0.39 |   | 0.24           | 0.31 |   |                   |      |   |                |      |   |
| P      | ns   | ns | ns            | *    |   | ns             | ns   |   |                   |      |   |                |      |   |

CreAmino

| Without | SEM± | P | Energy: cubic | SEM± | P | Energy: linear | SEM± | P | Energy: quadratic | SEM± | P | Energy: cubic | SEM± | P |
|---------|------|---|---------------|------|---|----------------|------|---|-------------------|------|---|----------------|------|---|
| 70.79   |      |   |               |      |   |                |      |   |                   |      |   |                |      |   |
| 70.61   |      |   |               |      |   |                |      |   |                   |      |   |                |      |   |
| SEM±    | 0.33 |   | 0.41          | 0.27 |   | 0.17           | 0.21 |   |                   |      |   |                |      |   |
| P       | ns   | ns | ns            | ns   |   | ns             | ns   |   |                   |      |   |                |      |   |

*Breast quarter, leg quarter thigh and drumstick were expressed as percentage of the carcass weight. *P<0.05; **P<0.01; ns, not significant.

Table 9. Effect of treatments on abdominal fat, liver and gizzard.

| Diet | CreAmino, % | Energy, kcal/kg | Fat³, % | Liver⁴, % | Gizzard⁵, % |
|------|-------------|-----------------|---------|----------|-------------|
| 1    | 0           | 3075            | 2.18    | 3.27     | 4.27        |
| 2    | 0           | 3100            | 2.30    | 3.13     | 4.16        |
| 3    | 0           | 3125            | 1.99    | 2.95     | 3.88        |
| 4    | 0           | 3150            | 2.32    | 2.94     | 3.57        |
| 5    | 0.6         | 3075            | 2.26    | 2.92     | 3.76        |
| 6    | 0.6         | 3100            | 1.83    | 2.85     | 4.40        |
| 7    | 0.6         | 3125            | 1.94    | 2.76     | 4.00        |
| 8    | 0.6         | 3150            | 1.85    | 2.80     | 3.90        |
| SEM± |             |                 | 0.25    | 0.12     | 0.19        |
| P    | ns          | ns              | ns      | ns       | ns          |

Energy

| Energy | SEM± | P  | Energy: cubic | SEM± | P  | Energy: linear | SEM± | P  | Energy: quadratic | SEM± | P  | Energy: cubic | SEM± | P  |
|--------|------|----|---------------|------|----|----------------|------|----|-------------------|------|----|----------------|------|----|
| 2925   |      |    |               |      |    |                |      |    |                   |      |    |                |      |    |
| 2950   |      |    |               |      |    |                |      |    |                   |      |    |                |      |    |
| 2975   |      |    |               |      |    |                |      |    |                   |      |    |                |      |    |
| 3000   |      |    |               |      |    |                |      |    |                   |      |    |                |      |    |
| SEM±   | 0.17 |    | 0.27          | 0.13 |    |                |      |    |                   |      |    |                |      |    |
| P      | ns   |    | ns            | ns   |    | ns             | ns   |    |                   |      |    |                |      |    |

*Fat, liver and gizzard percentages were expressed as percentage of the carcass weight. *P<0.05; **P<0.01; ns, not significant.
Conclusions

Guanidino acetic acid improved FCR for each growing period and for the cumulative period for broilers. On the other hand, dietary ME level could be adjusted in small increments to lower the cost of feed without losing performance. Based on that, it is recommended to lower the ME level by 50 kcal/kg for each growing period and supplement diets with CreAM.

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