

Effect of dietary inclusion of guanidinoacetic acid on broiler performance

Efecto de la inclusión dietética del ácido guanidinoacético en el desempeño de pollos de engorde

Efeito da inclusão dietética do ácido guanidinoacético no desempenho de frangos de corte

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Abstract

Background: Creatine and its precursor, guanidinoacetic acid (GAA), decrease protein degradation, avoiding amino acid offset to gluconeogenesis. **Objective:** To evaluate the inclusion of GAA in the pre-initial diet (1 to 7 days) on performance, digestibility, and blood parameters of broilers from one to 21 days of age. **Methods:** 252 male broiler chickens aged one day were distributed in a completely randomized design with seven replicates and 12 animals per experimental unit. Treatments consisted of a basal diet, and the basal added with 0.10 and 0.20% GAA. **Results:** From days 1 to 7, a tendency on improved feed conversion (FC) was observed for broilers receiving 0.20% GAA (p<0.10). Between 7 and 14 days, FC improved, as well as a tendency was observed on improved weight gain in animals fed 0.20% GAA. Animals that received 0.20% GAA showed higher blood concentrations of creatine kinase. Blood creatine and blood glucose did not differ between treatments at day six. The assimilation coefficient of crude protein increased during days 3 to 7 for animals receiving 0.20% GAA. On the period between 17 and 21 days, no difference was observed between treatments. Intestinal weight and length did not show significant differences. **Conclusion:** Dietary inclusion of 0.20% GAA during the pre-initial stage could improve weight gain and feed conversion of broilers between 1 and 14 days of age.

Keywords: *additive*; *broilers*; *chicken*; *creatine*; *feed additive*; *guanidinoacetic acid*; *metabolism*; *nutrition*; *organ biometry*; *poultry*.

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Resumen

Antecedentes: La creatina suministra energía y el ácido guanidinoacético (GAA) es su precursor y actúa como sustrato energético, reduciendo la degradación proteica y evitando el desvío de aminoácidos para gluconeogénesis. **Objetivo:** Evaluar el GAA en la dieta de pre-iniciación (1 a 7 dias) sobre el desempeño, la digestibilidad y los parámetros sanguíneos de pollos entre 1 y 21 dias de edad. **Métodos:** Se utilizaron 252 pollitos de engorde machos de un día de edad, distribuidos en un diseño completamente al azar, con siete repeticiones y 12 aves por unidad experimental. Los tratamientos consistieron en: ración basal y ración basal adicionada con 0,10 y 0,20% de GAA. **Resultados:** En el período de 1 a 7 días se observó una tendencia de mejora de la conversión alimenticia (FC) en las aves que recibieron 0,20% de GAA (p<0,10). De 7 a 14 días hubo mejoría en FC y tendencia de mejora en ganancia de peso de los animales que recibieron 0,20% de GAA. Los animales que recibieron 0,20% de GAA presentaron mayor concentración sanguínea de creatina quinasa. La creatinina y la glucosa sanguínea a los seis días no difirieron entre los tratamientos. El coeficiente de metabolización de la proteína bruta aumentó en el período de 3 a 7 días en los animales que recibieron 0,20% de GAA a un nivel de 0,20% de ra presentaron diferencias significativas. **Conclusión:** El GAA a un nivel de 0,20% en la dieta preinicial podria mejorar la ganancia de peso, y la conversión alimenticia en el período de 1 a 14 días de edad en pollos de engorde.

Palabras clave: *ácido guanidinoacético; aditivo; aditivo alimentario; aves de corral; biometría de órganos; creatina; metabolismo; nutrición; pollos de engorde.*

Resumo

Antecedentes: A creatina é uma substância que fornece energia e o ácido guanidinoacético (GAA) é seu precursor atuando como substrato energético, menor degradação proteica evitando o desvio de aminoácidos para gliconeogênese. **Objetivo:** Avaliar o GAA em dietas pré-iniciais sobre o desempenho, a digestibilidade e parâmetros sanguíneos. **Métodos:** Foram utilizados 252 pintos de corte machos de um a 21 dias de idade distribuído sem delineamento inteiramente casualizado, sete repetições e 12 aves por unidade experimental. Os tratamentos consistiram: ração basal e rações com 0,10 e 0,20% de GAA. **Resultados:** No período de 1 a 7 dias foi observada tendência de melhora da conversão alimentar (FC) (p<0,10) quando as aves receberam 0,20% de GAA. No período de 7 a 14 dias houve melhora na FC e tendência de melhora do ganho de peso dos animais que receberam 0,20% de GAA. Animais que receberam 0,20% de GAA apresentaram concentração sanguínea da enzima creatina quinase maior. A creatinina sanguínea aos seis dias e glicose sanguínea não diferiram entre os tratamentos. O coeficiente de metabolização da proteína bruta foi aumentado no período de 3 a 7 dias para animais que receberam 0,20% de GAA, para o período de 17 a 21 dias não houve diferença entre os tratamentos. Peso e comprimento do intestino não apresentaram diferenças significativas. **Conclusão:** O GAA em nível de 0,20% na dieta pré-inicial melhoram o ganho de peso, e a conversão alimentar no período de 1 a 14 dias de idade em frangos.

Palavras-chave: *ácido guanidinoacético; aditivo; aditivo alimentar; aves; biometria de órgãos; creatina; frango; metabolismo; nutrição.*

Introduction

The poultry industry is in constant growth, increasing the production and commercialization of poultry meat and other products every year. Improvement in genetics, animal welfare, environment, handling, nutrition, hygiene, and equipment consolidates broiler production more and more. Artificial incubation makes it possible to produce a high number of chicks in a short period of time. The incubation period corresponds to about 30% of the cycle of broilers (Goncalves et al., 2013). The initial phase, from 1 to 7 days after hatching, is of great importance for broiler development. The increased metabolic rate during this stage and the high potential for growth can render embryonic resources insufficient, possibly exhausting during the post-hatch period (Yair et al., 2013). Posthatch fasting can affect organ development and body growth, thus jeopardizing performance, primarily due to decreased protein synthesis (Riccardi et al., 2009). Inversely, feeding during the first hours of life can anticipate intestine development and prevent body proteins from being mobilized for energy production.

Among the additives used in feeds is Creatine (CRE), a substance found in products of animal origin since it is mostly stored in muscle. Feeds of plant origin provide insufficient amounts to cover the minimum CRE requirement. Creatine provides temporary energy and transports it between production and consumption sites. It maintains re-synthesis rates of ATP/ADP and plays an essential role in muscle contraction (Wyss e Kaddurah-Daouk, 2000). It is considered chemically unstable and costly. Thus, guanidinoacetic acid (GAA), a CRE precursor, is often used to replace CRE because it is cheaper and more stable (Dilger et al., 2013). Dietary inclusion of GAA helps to save essential amino acids, allowing their use for protein synthesis.

The objective of this study was to evaluate the effects of dietary inclusion of GAA on broiler digestibility, performance and blood parameters.

Materials and Methods

Ethical considerations

The study was approved by the Ethics Committee on Animal Use of Universidade Federal de Goiás (UFG; protocol nº 011/16).

Location and animals

The experiment was conducted at UFG, Goiás, Brazil. A total of 252 one--day old male chicks (Cobb lineage) were used, distributed in a completely randomized design with 3 treatments, 7 replicates, and 12 chickens per replicate (21 experimental units).

Chickens were placed in five floors of steel battery cages (0.77 x 0.74 x 0.23 m), equipped with drinkers, gutter feeders and metal trays to collect excreta. Batteries were kept inside barns, and ventilation was controlled with curtains. Maximum and minimum temperatures were recorded twice a day by thermometers distributed throughout the barn. Incandescent lamps were used to provide luminosity and heating support. Temperatures recorded during the experiment in the periods from 1 to 7, 8 to 14, and 15 to 21 days were, respectively: 24.4 and 33.7; 21.8 and 32.1; 21.5 and 31.8 °C.

Feed and water were provided *ad libitum* during the experimental period. Treatments consisted of a basal diet without GAA, and basal with 0.10 or 0.20% GAA. The animals received the dietary treatment until 7 days of age, and the basal diet without GAA until they were 21 days old. The corn-soybean based feed was formulated to meet the minimum nutrient requirements throughout the experimental period, according to Rostagno *et al.* (2011) (Table 1).

Weighing of animals and feed was conducted on days 1, 7, 14, and 21 to calculate performance variables. On day 6, two animals per experimental unit were randomly chosen for blood and intestinal sampling. Chickens were fasted for 2 hours and subsequently dulled in a CO_2 gas chamber.

| | Treatments | | | | | | |
|-----------------------------------|------------|-------------------------|---------|---------|--|--|--|
| Ingredients (%) | | 8 to 21 days(No GAA) | | | | | |
| | 0 | 0.100 | 0.200 | - | | | |
| Corn | 54.866 | 54.866 | 54.866 | 59.242 | | | |
| Soybean | 38.144 | 38.144 | 38.144 | 34.638 | | | |
| Guanidinoacetic acid | 0.000 | 0.100 | 0.200 | - | | | |
| Starch | 0.200 | 0.100 | 0.000 | 0.000 | | | |
| Soybean oil | 2.169 | 2.169 | 2.169 | 2.136 | | | |
| Dicalcium phosphate | 1.909 | 1.909 | 1.909 | 1.509 | | | |
| Chalk | 0.913 | 0.913 | 0.913 | 0.923 | | | |
| Common salt | 0.507 | 0.507 | 0.507 | 0.481 | | | |
| L-Lysine HCL | 0.412 | 0.412 | 0.412 | 0.313 | | | |
| DL-Methionine | 0.361 | 0.361 | 0.361 | 0.288 | | | |
| L-Threonine | 0.115 | 0.115 | 0.115 | 0.065 | | | |
| Premix ¹ | 0.400 | 0.400 | 0.400 | 0.400 | | | |
| TOTAL | 100.000 | 100.000 | 100.000 | 100.000 | | | |
| Calculated composition | | | | | | | |
| Metabolizable energy (kcal/kg) | 2.950 | 2.950 | 2.950 | 3.000 | | | |
| Sodium (%) | 0.220 | 0.220 | 0.220 | 0.210 | | | |
| Calcium (%) | 0.920 | 0.920 | 0.920 | 0.819 | | | |
| Available phosphorus (%) | 0.470 | 0.470 | 0.470 | 0.391 | | | |
| Crude protein (%) | 22.20 | 22.20 | 22.20 | 20.80 | | | |
| Lysine Dig. (%) | 1.310 | 1.310 | 1.310 | 1.174 | | | |
| Met. + Cist. Dig. (%) | 0.944 | 0.944 | 0.944 | 0.846 | | | |
| Threonine Dig. (%) | 0.852 | 0.852 | 0.852 | 0.763 | | | |

Table 1. Centesimal composition of experimental diets containing different levels of guanidinoacetic acid (GAA).

¹Composition per kg of product: vit. A 12,000,000 IU; vit. D3 2,200,000 IU; vit. E 30,000 IU; vit. B1 2,200 mg; vit B2 6,000 mg; vit. B6 3,300 mg; panthothenic acid 13,000 mg; biotin 110 mg; vit. K3 2,500 mg; folic acid 1,000 mg; nicotinic acid 53,000 mg; niacin 25,000 mg; vit. B12 16,000 µg; selenium 0.25 g; antioxidant 120,000 mg, and Enough quantity to (QSP) vehicle 1.000 g; manganese 75,000 mg; iron 20,000 mg; zinc 50,000 mg; copper 4,000 mg; cobalt 200 mg; iodine 1,500 mg, and QSP vehicle 1.000 g.

Blood sampling was performed by femoral vein puncture and 2 mL blood was stored in vacutainer tubes with sodium fluoride as anticoagulant and kept in Styrofoam boxes filled with ice. At the UFG's Departamento de Patología Clínica, tubes without anticoagulant were kept resting for 2 hours, then centrifuged and the resulting serum was used to determine creatinine and creatinine-kinase levels. Tubes containing anticoagulant went through the same centrifugation process, with plasma collected to determine blood glucose levels. Determinations

were carried out using specific commercial kits in an automated biochemical analyser (CM 200, Wiener Lab Group®). Sampled intestines were weighted, and their lengths measured with a measuring tape.

Two metabolism assays were conducted: one from the 3rd to the 7th day and the other from the 17th to the 21st day of age. The nutrient assimilation coefficient in feeds was determined from total excreta, following Sakomura and Rostagno (2007) methods. Excreta were collected and kept frozen until further analysis. At the end of the sampling period, excreta were thawed, homogenized, and 400 g of sample were dried in a forced-air oven at ± 55 °C for 72 hours. Then, samples were weighed and ground in a Willey mill for analysis. The analysis was carried out in the Laboratório de Nutrição Animal at UFG. Dry matter, crude protein, and ether extract from excreta and feed samples were determined according to the methods described by Silva and Queiroz (2009).

Statistical analysis

Data were analysed using the R software. An ANOVA was performed, and averages were compared using the Tukey test. Adopting α =0.05, and considering a tendency when α was between 0.05 and 0.10.

Results

Performance

Supplementing GAA in broiler feed during the pre-initial period from 1 to 7 days did not affect weight gain and food consumption (p>0.05) (Table 2). However, we observed a tendency of improvement for feed conversion (p<0.08) in animals that were fed 0.20% GAA compared to the other treatments.

From 1 to 14 days, animals that received the diet containing 0.20% GAA until 7 days of age showed a better feed conversion at 14 days (p<0.05) compared to animals that received the remaining treatments. The 0.20% GAA broiler chickens also showed a tendency (p<0.07) of a greater weight gain in this same period, which explains the improved feed conversion results. Regarding the use of GAA in experimental feeds at the pre-initial period, no residual effect was verified (p>0.05) on broiler performance until 21 days of age.

Creatinine, creatine kinase (CK), and blood glucose

The concentration of CK in the blood of animals that received 0.20% GAA was significantly higher (p<0.01) when compared to those that received other treatments (Table 3).

Table 2. Effects of dietary inclusion of guanidinoacetic acid (GAA) during the first 7 days of age on broiler performance from 1 to 7, 1 to 14, and 1 to 21 days of age.

| Variable — | GAA (%) | | | n valua | | Standard |
|-------------|---------|--------|--------------|---------|--------|-------------|
| | 0.00 | 0.10 | 0.20 | p-value | CV (%) | error |
| | | | 1 to 7 days | | | |
| WG (kg) | 0.165 | 0.168 | 0.172 | 0.3586 | 5.9 | ± 0.003 |
| FI (kg) | 0.131 | 0.137 | 0.131 | 0.4524 | 7.6 | ± 0.003 |
| FCR (kg/kg) | 0.798 | 0.820 | 0.761 | 0.0718 | 5.6 | ± 0.017 |
| | | | 1 to 14 days | | | |
| WG (kg) | 0.421 | 0.441 | 0.452 | 0.0638 | 5.3 | ± 0.008 |
| FI (kg) | 0.490 | 0.513 | 0.506 | 0.3232 | 5.5 | ± 0.010 |
| FCR (kg/kg) | 1.164a | 1.161a | 1.120b | 0.0138 | 2.4 | ± 0.010 |
| | | | 1 to 21 days | | | |
| WG (kg) | 0.925 | 0.909 | 0.912 | 0.5224 | 2.9 | ± 0.010 |
| FI (kg) | 1.160 | 1.169 | 1.150 | 0.7153 | 3.6 | ±0.015 |
| FCR (kg/kg) | 1.286 | 1.286 | 1.266 | 0.4406 | 2.4 | ±0.012 |

WG = weight gain; FI = Feed Intake; FCR = Feed Conversion Rate; CV = coefficient of variation; (a, b) averages with different letters within the same row differ from each other through the Tukey test (p<0.05).

| Variable — | GAA (%) | | | | | Standard |
|-------------------------------|---------|---------|---------|---------|--------|----------|
| | 0.00 | 0.10 | 0.20 | p-value | CV (%) | error |
| CK (IU/L) | 167.85b | 112.85b | 247.43a | < 0.001 | 46.0 | ±2.167 |
| Creatinine (mg/dL) | 0.250 | 0.264 | 0.282 | 0.5509 | 29.6 | ±0.021 |
| Glucose plasma (mg/ dL) | 245.68 | 255.68 | 255.58 | 0.5032 | 10.2 | ±6.876 |

Table 3. Serum parameters in broilers on the sixth day of age receiving different levels of guanidinoacetic acid (GAA).

CK = creatine kinase; CV = coefficient of variation; (a, b) averages with different letters within the same row differ from each other through the Tukey test (p<0.05).

Blood creatinine values at six days of age presented no difference (p>0.05) between animals that received treatments with GAA inclusion. Blood glucose concentration also did not show any differences (p>0.05) between treatments.

Nutrient assimilation

The dry matter (DMAC), crude protein (CPAC), and ether extract (EEAC) assimilation coefficients in the periods ranging from 3 to 7 and 17 to 21 days are shown in table 4. In the period from 3 to 7 days, no difference was observed (p>0.05) forboth DMAC and EEAC. A significant

difference was found for CPAC (P=0.0306), which was higher when animals received 0.20% GAA but did not differ when compared to the group that received 0.10% GAA. No difference (p>0.05) was found between treatments for the period from 17 to 21 days of age. Providing feeds with GAA inclusion during the pre-initial period did not influence the assimilation coefficient in the period from 17 to 21 days.

Intestine weight and length

No difference was found between treatments for intestine weight and length (p>0.05; table 5).

Table 4. Assimilation coefficients (%) of dry matter (DMAC), crude protein (CPAC), and ether extract (EEAC) of feeds with different levels of guanidinoacetic acid (GAA) for broiler chickens.

| Variable (%) | GAA (%) | | | p-value | CV (%) | Standard error |
|--------------|---------|----------|---------------|---------|--------|----------------|
| | 0.00 | 0.10 | 0.20 | p value | | Stanuaru crior |
| | | | 3 to 7 days | | | |
| DMAC | 68.629 | 68.603 | 68.097 | 0.8451 | 2.8 | ±0.727 |
| CPAC | 56.177b | 59.561ab | 60.797a | 0.0306 | 5.2 | ±1.159 |
| EEAC | 86.280 | 85.092 | 85.092 | 0.2385 | 2.0 | ± 0.666 |
| | | | 17 to 21 days | | | |
| DMAC | 68.008 | 68.847 | 68.152 | 0.4964 | 2.0 | ±0.525 |
| CPAC | 49.454 | 50.502 | 51.879 | 0.1842 | 4.6 | ± 0.891 |
| EEAC | 84.301 | 83.919 | 83.585 | 0.5120 | 1.3 | ± 0.430 |

CV=coefficient of variation; (a, b) averages with different letters within the same row differ from each other through the Tukey test (p<0.05).

| Variable — | GAA (%) | | | | | Standard |
|-----------------|---------|--------|--------|---------|--------|----------|
| | 0.00 | 0.10 | 0.20 | p-value | CV (%) | error |
| Total I.W. (cm) | 16.167 | 16.426 | 16.000 | 0.885 | 13.8 | ±0.6081 |
| S.I.L. (cm) | 81.071 | 84.000 | 80.538 | 0.2075 | 6.2 | ±1.4225 |
| L.I.L. (cm) | 11.214 | 10.454 | 10.750 | 0.2335 | 10.1 | ±0.3151 |
| Total I.L. (cm) | 92.285 | 94.750 | 91.692 | 0.4077 | 6.4 | ±1.6536 |

Table 5. Intestine weight and length in broiler chickens receiving feeds with different levels of guanidinoacetic acid (GAA) from 1 to 7 days of age.

Total I.W. = Total intestine weight; Total I.L. = Total intestine length; S.I.L. = Small intestine length; L.I.L. = Large intestine length; CV = coefficient of variation.

Discussion

Performance

We included GAA in broiler chicken diets to improve performance during the first days of life. GAA produces creatine without consuming essential amino acids in the process, which can then be used by the organism to perform other functions, such as protein synthesis and to increase muscle mass. Thus, 0.20% GAA inclusion showed potential for improvement of poultry performance in the period from 1 to 7 days and had a positive effect on feed conversion from 1 to 14 days.

The first seven days of life are determinant for the optimum development of broilers. Therefore, good results should be pursued during this period, as losses are not recovered with compensatory growth until the end of the cycle. This fact can explain the improvement in feed conversion in animals that received a diet containing GAA during the first 7 days of life, which was reflected in the period from 1 to 14 days. In a study by Bryant-Angeloni (2010), the inclusion of GAA in arginine-deficient diets yielded no improvement on weight gain but had the opposite effect on feed conversion. On the other hand, when GAA was added to diets containing adequate arginine concentration, improvement was observed in both weight gain and feed conversion. These results confirm that dietary inclusion of GAA can reduce arginine use, thus improving animal performance. This was also observed by Lemme *et al.* (2007), who included GAA in male broiler diet between 1 and 41 days of age and found improved feed conversion as well as increased creatine, phosphocreatine, and muscle ATP content. Therefore, energy availability likely increased, affecting the metabolic use of nutrients for growth. Thus, efficient stocking makes energy available in the short term to other metabolic pathways, such as protein synthesis. The improvement in feed conversion can be a reflection of these effects.

Inclusion of 0.20% GAA in broiler preinitial diets proved efficient for improving feed conversion until 14 days of age. Furthermore, performance improvement can be linked to the fact that GAA inclusion saves amino acids for endogenous synthesis of CRE so they can be used for protein synthesis and muscle growth instead. Additionally, GAA inclusion makes CRE levels increase, as GAA is a direct precursor of CRE, and the energy available for metabolic processes and performance improvement consequently increases.

Supplementing diets with GAA brings better results during the final rearing period of broilers. Michiels *et al.* (2012) reported that GAA supplementation was beneficial in the final period when growth rates are the highest, showcasing the stage when there is better gain:feed relationship. Thus, satisfactory results can be obtained when GAA is supplemented during the final period, considering that greater muscle growth occurs at that stage and more ATP is needed.

In the present study, no residual effect of performance improvement at 21 days was observed. However, the time between birth and adaptation to the experiment was short, in such a way that broilers did not go through a considerable fasting period. Our results suggest that late adaptation, in which the time for chick arrival to the farm enables a longer fast, the response to GAA could be more favorable, which would help to save gluconeogenic precursors.

Creatinine, creatine kinase (CK) and blood glucose

A higher concentration of CK could represent more intense muscle growth (Lorsheitter *et al.*, 2015). Thus, higher CK at the sixth day of life for broilers receiving 0.20% GAA could explain the tendency for improvement in feed conversion from 1 to 7 days, confirming that higher CK blood concentration can be related to more intense muscle growth in this phase.

Creatinine is a product of creatine (CRE) breakdown from muscles. Consequently, blood creatinine levels are closely related to muscle CRE concentration. Creatinine is filtered in the kidneys and excreted in urine; kidney malfunction compromises creatinine filtration decreasing creatinine excretion and increasing its blood concentration. According to Barbosa et al. (2011), CRE is excreted through the kidneys before being converted into creatinine. This metabolite exists at low concentrations in poultry serum, usually lower than the minimum level detected by laboratory tests. Normal creatinine levels in poultry are constant, between 0.1 and 0.4 mg/dL (Saukas, 1993). Thus, the creatinine levels observed in the present study are considered normal.

To determine muscle activity, Lemme et al. (2007) established CRE, creatinine, and

concentrations in han the minimum y tests. Normal constant, between 1993). Thus, the *Intestine weight and length*

According to Viola *et al.* (2008), increase in intestinal mass and length is related to increased villi size and crypt depth. Some ingredients used in broiler diets can have trophic and

guanidinoacetic acid directly from the breast muscle in broilers that received GAA from 1 to 41 days of age. At 41 days of age, muscle CRE content increased about 14% when GAA supplement was raised up to 0.06% in the diet, suggesting that dietary GAA is a strong source of CRE. Furthermore, the number of high-energy molecules increased with GAA supplementation 1-hour *post-mortem*, suggesting a more efficient energy stocking due to GAA inclusion, which is available in the short term to metabolic functions such as protein synthesis.

Glucose blood concentration in healthy chickens varies from 200 to 500 mg/dL. Chickens that undergo short fast periods keep normal glucose levels through hepatic gluconeogenesis. In longer periods, however, healthy chickens up to 8 days of age do not reduce glucose use as mammals do (Schmidt *et al.*, 2007). In the present study, blood glucose levels were kept in normal levels for healthy chickens.

Nutrient assimilation

The crude protein assimilation coefficient (CPAC), which increased in animals that received 0.20% GAA in the feed during the period between days 3 and 7, demonstrates an improvement in feed protein usage, reflecting on performance, as can be seen in Table 2. This is noticed when observing feed conversion alone from days 1 to 7, and feed conversion and weight gain from days 1 to 14 immediately afterward. The improvement in CPAC increased amino acid availability, caused by GAA inclusion, which might have favored the use of the amino acids from protein deposition, thus demonstrating higher muscle growth, which is also justified by higher blood concentration of CK. Muscle growth is related to the improved tendency of weight gain and feed conversion observed during the pre-initial period

enterocyte-stimulating effects causing an increase in intestinal mass and improvement of nutrient-absorbing capacity. In the present study, GAA inclusion apparently did not alter intestinal morphology. The GAA did not show activities that stimulate intestinal mass growth during the pre-initial rearing period.

Studying the effects of GAA inclusion is necessary, mostly because GAA methylation to CRE consumes many methyl groups from methionine. According to Michiels et al. (2012), dietary GAA supplementation increases the demand for methylation. In methylation reactions, homocysteine is produced (Deminice et al., 2009). The great demand for methylation during CRE production causes an accumulation of homocysteine in the blood, as well as a deficiency of methionine, choline, betaine, folic acid, and vitamin B12, increasing the need of these nutrients in the diet. High plasma concentration of homocysteine can cause damage to cell functioning, besides being related to cardiovascular disease (Setoue et al., 2008).

In conclusion, inclusion of 0.20% GAA in preinitial diets of broiler chickens could improve weight gain and feed conversion between 1 and 14 days of age.

Declarations

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Author contributions

Kamilla M Borges, Alessandra G Mascarenhas and Heloisa H de C Mello: conceived and designed the experiments. Kamilla M Borges and Hyara P F Xavier: performed the experiments. Emmanuel Arnhold: analyzed the data. Alessandra G Mascarenhas, Heloisa H de C Mello and Marcos B Café: contributed reagents, materials, and analytical tools. Kamilla M Borges and Helder F de Oliveira: wrote the paper. Helder F de Oliveira: edited the manuscript.

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