Guanidino Acetic Acid does not provide energy saving effect in broilers

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Abstract

Guanidino acetic acid (GAA) is sold on the market with arginine and energy sparing effects. The limitations of GAA in arginine sparing is shown already in previous research. Herein, we tested the energy sparing capabilities of GAA. Ross 308 broilers were allocated to five treatments: T1) positive control (normal energy), T2) Negative control 1 (NC1: T1 minus 50 kcal), T3) Negative control 2 (NC2: T1 minus 100 kcal), T4) T2 + 600 gram GAA, T5) T3 + 1200 gram GAA. The body weight (BW), daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) were measured at the end of each growth phase (day 10, 24, and 35). Birds were slaughtered at day 35. Carcass, breast and leg yield were measured (4 animals per pen). Birds of the 600 grams GAA group had a lower BW and BWG compared to the NC1. Adding either 600 or 1200 grams GAA to the feed caused a reduction in feed intake. Adding 600 grams of GAA had a negative impact on live weight and carcass weight of birds compared with the positive control or NC1 and NC2. Data showed that GAA inclusion at 600 or 1200 gram in broiler feed brings either no effect or a negative effect on the performance. Measuring impact of energy on broiler's performance parameters is a factor of the magnitude of the changes in dietary energy. A reduction or increase in feed energy levels less than 125 - 150 kcal AME/kg is a challenge to be measured in broilers even under experimental conditions. In this study, GAA did not bring any extra energy value. Thus, it is not recommended to consider an energy matrix value for GAA.

Background

Guanidino acetic acid (GAA) is a naturally occurring metabolite. For the synthesis of each molecule of GAA, a process which is typically done in the kidney, two amino acids are needed (glycine and arginine). GAA is transferred from the kidney to the liver where it is methylated to creatine, an important molecule in the energy homeostasis in muscle (Brosnan et al., 2009). In the literature, several groups attempted to link GAA to a more efficient energy utilization in broilers (Majdeddin et al., 2019; Ale Saheb Fosoul et al., 2018). It is also claimed that GAA can provide 83000 - 166000 kcal per kg of AME when it is added as a feed additive to a broiler feed. This amount of energy means a reduction of 50 - 100 kcal/kg of feed by adding 600 grams of GAA per metric ton of feed. This is also of commercial interest because a reduction of this amount of energy makes feed cheaper. In other words, it covers the costs of GAA addition to feed. There are also commercial tests done in different operations with a commercial guarantee: reduction of 50 kcal energy and adding GAA to broiler feed is guaranteed not to create any negative impact on broiler performance.

Approximately three quarters of the feed costs are represented by costs for dietary energy. Therefore, an accurate estimate of the energetic value of raw materials is extremely important to reduce the price of poultry feed. Unfortunately, prediction of AME for different raw materials is affected by the inclusion rate of specific raw materials in the feed (Lopez and Leeson, 2008). Moreover, there is an interaction with other raw materials such as fat and oil. There is also a wide variation in AME within and between grain species (Black et al., 2005). For example, the average ileal digestible energy value for different corn samples was 3205 kcal/kg DM with a standard deviation of 488 kcal/kg DM (D'Alfonso, 2005). This high variation will lead to inaccuracy to meet the energy requirement of birds and creates room for false negative results when a reduction in energy content of feed is under investigation.

In this experiment, the energy sparing effect of GAA is tested by means of using enough control groups: one positive control and two negative control groups.

Methods

A total of 1200 male Ross 308 broilers arrived in the research facility (Poulpharm Bvba, Izegem, Belgium) at the age of zero days. Birds were placed in 80 pens (1m² each). Five treatment groups (Table 1) were randomly allocated to pens (16 pens per treatment and 15 birds per pen). The feed was prepared by Research Diet Services BV (RDS) (Table 2 and 3). Feed and water were provided ad libitum to meet the recommendations of Ross 308 except if mentioned in treatment groups. The floor was covered with wood shavings in a thickness of about 5 cm. The body weight (BW), daily weight gain (DWG), daily feed intake (DFI) and feed conversion ratio (FCR) were measured at the end of each growth phase (day 10, 24, and 35). Birds were slaughtered at day 35. Carcass, breast and leg yield were measured (4 animals per pen). Feed samples of the test diets were analysed to determine major nutrients including amino acids which appeared to be at or close to formulated values (Table 4, 5, and 6). Data were analysed with R (version 3.2.5.) using ANOVA ($P < 0.05$). Mortality was analysed using cox proportional hazard models (procedure coxph of the package survival).

Table 1. Treatment groups and their descriptions

Treatments	Description	Replicates	Birds/ replicate	AME kcal/kg		
				Starter	Grower	Finisher
T1	Positive control (normal energy)	16	15	2960	3050	3150
T2	Negative control 1: T1 minus 50 Kcal energy	16	15	2910	3000	3100
T3	Negative control 2: T1 minus 100 Kcal energy	16	15	2860	2950	3050
T4	T2 + 600 grams GAA	16	15	2960[1]	3050[1]	3150[1]
T5	T3 + 1200 grams GAA	16	15	2960[1]	3050[1]	3150[1]

[1]Theoretical AME levels considering 600 grams of GAA provides 50 kcal AME and 1200 grams of GAA provides 100 Kcal AME.

Table 2. Ingredient composition of the positive control (PC) and the negative controls (NC).

Ingredient Name	Starter			Grower			Finisher		
	PC	NC1	NC2	PC	NC1	NC2	PC	NC1	NC2
Corn	39.70	41.00	42.30	38.40	39.70	40.99	40.21	41.51	42.81
Soybean meal	33.24	33.00	32.77	29.16	28.93	28.69	21.70	21.46	21.23
Wheat	20.00	20.00	20.00	25.00	25.00	25.00	30.00	30.00	30.00
Soy oil	2.32	1.25	0.18	3.36	2.29	1.22	3.99	2.91	1.84
DCP	1.63	1.63	1.62	1.08	1.08	1.07	1.15	1.14	1.14
Limestone	1.08	1.08	1.08	0.91	0.92	0.92	0.92	0.93	0.93
Broiler premix	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Salt	0.38	0.38	0.38	0.39	0.39	0.39	0.39	0.40	0.40
L-Methionine	0.37	0.37	0.37	0.32	0.32	0.31	0.30	0.30	0.30
L-Lysine	0.37	0.37	0.38	0.32	0.32	0.33	0.38	0.39	0.39
L-Threonine	0.23	0.23	0.23	0.18	0.18	0.18	0.20	0.20	0.20
L-Arginine	0.12	0.13	0.13	0.09	0.09	0.09	0.16	0.16	0.16
L-Valine	0.04	0.04	0.04	0.01	0.01	0.01	0.04	0.04	0.04
L-Isoleucine	0.03	0.03	0.03	0.01	0.01	0.01	0.06	0.06	0.07
L-Glycine				0.28	0.28	0.28			

PC: Positive control (normal energy and AAs)
NC1: Negative control 1: PC minus 50 Kcal energy
NC2: Negative control 2: PC minus 100 Kcal energy

Table 3. Calculated nutrient composition of the positive control (PC) and negative controls (NC).

Ingredient Name	Starter			Grower			Finisher		
	PC	NC1	NC2	PC	NC1	NC2	PC	NC1	NC2
AME Broiler (kcal/kg)	2960	2910	2860	3050	3000	2950	3150	3100	3050
Crude Protein	22.80	22.80	22.80	21.18	21.18	21.18	18.59	18.59	18.59
Crude Fat	5.27	4.25	3.24	6.29	5.28	4.26	6.97	5.96	4.94
Crude Fiber	2.67	2.69	2.71	2.62	2.64	2.67	2.53	2.55	2.57
Ash	6.40	6.40	6.39	5.53	5.53	5.52	5.25	5.25	5.24
Calcium	0.90	0.90	0.90	0.70	0.70	0.70	0.70	0.70	0.70
Available Phosphorous	0.42	0.42	0.42	0.32	0.32	0.32	0.32	0.32	0.32
Dig. Lysine	1.28	1.28	1.28	1.15	1.15	1.15	1.03	1.03	1.03
Dig. Methionine	0.64	0.63	0.63	0.57	0.57	0.57	0.53	0.53	0.52
Dig Met Plus Cys	0.95	0.95	0.95	0.87	0.87	0.87	0.80	0.80	0.80
Dig. Arginine	1.37	1.37	1.37	1.23	1.23	1.23	1.10	1.10	1.10
Dig. Threonine	0.86	0.86	0.86	0.77	0.77	0.77	0.69	0.69	0.69
Dig. Leucine	1.54	1.54	1.54	1.43	1.44	1.44	1.25	1.25	1.25
Dig. Isoleucine	0.86	0.86	0.86	0.78	0.78	0.78	0.71	0.71	0.71
Dig. Valine	0.96	0.96	0.96	0.87	0.87	0.87	0.78	0.78	0.78
Dig. Tryptophan	0.23	0.23	0.23	0.21	0.21	0.21	0.18	0.18	0.17
Dig. Phenylalanine	0.93	0.93	0.93	0.86	0.86	0.86	0.73	0.73	0.73
Dig. Histidine	0.50	0.50	0.50	0.47	0.47	0.47	0.40	0.40	0.40
Choline	1313	1314	1315	1233	1234	1235	1077	1079	1080
Starch	39.78	40.60	41.42	41.84	42.65	43.47	45.70	46.52	47.34

PC: Positive control (normal energy and AAs)
NC1: Negative control 1: PC minus 50 Kcal energy
NC2: Negative control 2: PC minus 100 Kcal energy

Table 4. Analysed nutrient composition of the treatment groups during starter phase.

	T1	T2	T3	T4	T5
Lysine	1.43	1.42	1.43	1.47	1.43
Methionine	0.60	0.61	0.62	0.64	0.69
Cystine	0.42	0.36	0.36	0.38	0.35
Aspartic acid	2.16	2.11	2.17	2.22	2.13
Threonine	0.96	0.99	0.99	1.04	1.03
Serine	1.02	1.03	1.01	1.06	1.02
Glutamic acid	4.25	4.21	4.28	4.38	4.23
Proline	1.27	1.29	1.25	1.40	1.28
Glycine	0.90	0.88	0.88	0.92	0.88
Alanine	1.01	0.98	0.90	0.98	0.95
Valine	1.05	1.01	1.05	1.08	1.04
Isoleucine	0.98	0.97	0.95	0.96	0.93
Leucine	1.74	1.75	1.67	1.71	1.62
Tyrosine	0.73	0.71	0.66	0.68	0.69
Phenylalanine	1.06	1.03	1.00	1.05	1.00
Histidine	0.56	0.55	0.57	0.57	0.55
Arginine	1.54	1.50	1.51	1.47	1.48
Sum amino acids	21.68	21.40	21.30	22.01	21.30

T1: Positive control (normal energy), T2: Negative control 1: T1 minus 50 Kcal energy, T3: Negative control 2: T1 minus 100 Kcal energy, T4: T2 + 600 grams GAA, T5: T3 + 1200 grams GAA.

Table 5. Analysed nutrient composition of the treatment groups during grower phase.

	T1	T2	T3	T4	T5
Lysine	1.29	1.31	1.23	1.28	1.28
Methionine	0.56	0.57	0.53	0.56	0.55
Cystine	0.36	0.36	0.34	0.36	0.36
Aspartic acid	1.98	1.94	1.89	1.99	2.00
Threonine	0.88	0.91	0.86	0.90	0.90
Serine	0.93	0.98	0.93	0.99	0.98
Glutamic acid	4.14	4.08	3.95	4.10	4.09
Proline	1.28	1.30	1.30	1.31	1.34
Glycine	1.09	1.11	1.06	1.08	1.09
Alanine	0.89	0.95	0.93	0.95	0.94
Valine	0.97	0.96	0.96	0.97	0.99
Isoleucine	0.85	0.87	0.86	0.87	0.89
Leucine	1.58	1.62	1.61	1.62	1.64
Tyrosine	0.63	0.68	0.66	0.68	0.69
Phenylalanine	1.00	0.98	0.95	0.98	1.01
Histidine	0.54	0.52	0.52	0.52	0.53
Arginine	1.35	1.32	1.33	1.35	1.35
Sum amino acids	20.32	20.46	19.91	20.51	20.63

T1: Positive control (normal energy), T2: Negative control 1: T1 minus 50 Kcal energy, T3: Negative control 2: T1 minus 100 Kcal energy, T4: T2 + 600 grams GAA, T5: T3 + 1200 grams GAA.

Table 6. Analysed nutrient composition of the treatment groups during finisher phase.

	T1	T2	T3	T4	T5
Lysine	1.13	1.14	1.14	1.15	1.12
Methionine	0.50	0.49	0.57	0.51	0.50
Cystine	0.32	0.31	0.3	0.31	0.31
Aspartic acid	1.62	1.64	1.57	1.66	1.61
Threonine	0.79	0.80	0.82	0.81	0.79
Serine	0.81	0.85	0.81	0.85	0.83
Glutamic acid	3.71	3.69	3.61	3.70	3.64
Proline	1.08	1.21	1.18	1.19	1.16
Glycine	0.74	0.73	0.72	0.73	0.72
Alanine	0.82	0.83	0.80	0.82	0.82
Valine	0.89	0.88	0.88	0.89	0.87
Isoleucine	0.78	0.79	0.78	0.78	0.77
Leucine	1.38	1.43	1.38	1.40	1.39
Tyrosine	0.53	0.59	0.56	0.57	0.56
Phenylalanine	0.82	0.87	0.81	0.85	0.84
Histidine	0.46	0.47	0.46	0.47	0.47
Arginine	1.18	1.25	1.23	1.24	1.22
Sum amino acids	17.56	17.97	17.62	17.93	17.62

T1: Positive control (normal energy), T2: Negative control 1: T1 minus 50 Kcal energy, T3: Negative control 2: T1 minus 100 Kcal energy, T4: T2 + 600 grams GAA, T5: T3 + 1200 grams GAA.

Results

Mortality was not affected by any of the treatment groups. BW at day 0, 10, and 24 was also not affected by treatment groups. On day 35, birds of the 600 grams GAA group (-50kcal + 600 grams GAA) had a lower BW compared to the NC1 (-50 kcal) ($P < 0.05$, Table 7). BWG had a similar response to treatments (Data not shown).
In general, feed intake was not affected by 50 or 100 kcal/kg reduction in dietary energy (Table 8). In the starter phase, adding 1200 grams GAA per metric ton of a feed containing -100 kcal/kg energy caused a reduction in feed intake. In the finisher phase and during day 0-35, a similar reduction in feed intake happened when birds were fed with a diet lacking 50 kcal/kg energy and supplemented with 600 grams of GAA per metric ton of feed compared to the reference diet (-50 kcal/kg).
FCR was not affected by treatments during the grower, finisher, or the whole growth period. During the starter period, 100 kcal reduction of energy caused an increase in FCR, but the other groups stayed irresponsive (Table 9).
Slaughter data (Table 10) demonstrated that breast meat weight does not respond to any of the treatment groups. Reduction of energy by 50 or 100 kcal did not impact live weight of slaughtered birds or their carcass weight. Adding 600 grams of GAA per metric ton to a diet lacking 50 kcal of energy had a negative impact on live weight and carcass weight of birds compared with the positive control or the diets containing -50 or -100 kcal energy. Leg weight was increased when diet had a lack of energy (-50 kcal) although -100 kcal did not create a similar response. Adding 600 grams of GAA per metric ton to a diet lacking 50 kcal energy reduced the leg weight to a greater extend.

Table 7. Mean body weight of birds at the end of starter, grower, and finisher phase.

Treatments	Day 0				Day 10			
	LSM	SE	P-value	Sign. diff.	LSM	SE	P-value	Sign. diff.
T1: control diet	41.7	0.2	Ref.	a	302.2	2.9	Ref.	a
T2: T1 - 50 Kcal energy	41.7	0.2	0.989	a	303.3	2.9	0.789	a
T3: T1 - 100 Kcal energy	41.7	0.2	0.991	a	302.1	2.9	0.976	a
T4: T2 + 600g GAA	41.8	0.2	0.648	a	304.2	2.9	0.629	a
T5: T3 + 1200g GAA	41.7	0.2	0.989	a	301.2	2.9	0.810	a
Treatments	**Day 24**				**Day 35**			
	LSM	SE	P-value	Sign. diff.	LSM	SE	P-value	Sign. diff.
T1: control diet	1355	10	Ref.	a	2396	29	Ref.	ab
T2: T1 - 50 Kcal energy	1374	10	0.198	a	2460	29	0.121	a
T3: T1 - 100 Kcal energy	1358	10	0.839	a	2412	29	0.695	ab
T4: T2 + 600g GAA	1359	10	0.779	a	2369	29	0.515	b
T5: T3 + 1200g GAA	1358	10	0.874	a	2384	29	0.772	ab

P value shows a difference to control group.
Significant difference (sign. diff.) is a multicompanies between treatment groups.

Table 8. Average daily feed intake of birds at the end of starter, grower, and finisher phase.

Treatments	Day 0-10				Day 10-24			
	LSM	SE	P-value	Sign. diff.	LSM	SE	P-value	Sign. diff.
T1: control diet	27.7	0.3	Ref.	ab	98.5	0.9	Ref.	a
T2: T1 - 50 Kcal energy	28.2	0.3	0.348	ab	100.5	0.9	0.095	a
T3: T1 - 100 Kcal energy	28.7	0.3	0.051	a	100.4	0.9	0.119	a
T4: T2 + 600g GAA	27.8	0.3	0.967	ab	98.9	0.9	0.733	a
T5: T3 + 1200g GAA	27.7	0.3	0.885	b	99.7	0.9	0.301	a
Treatments	**Day 24-35**				**Day 0-35**			
	LSM	SE	P-value	Sign. diff.	LSM	SE	P-value	Sign. diff.
T1: control diet	164.4	2.4	Ref.	ab	98.6	1	Ref.	ab
T2: T1 - 50 Kcal energy	168.8	2.4	0.191	a	100.7	1	0.168	a
T3: T1 - 100 Kcal energy	167.8	2.4	0.312	a	100.7	1	0.155	a
T4: T2 + 600g GAA	159.7	2.4	0.164	b	97.0	1	0.285	b
T5: T3 + 1200g GAA	164.4	2.4	0.997	ab	99.0	1	0.785	ab

P value shows a difference to control group.
Significant difference (sign. diff.) is a multicompanies between treatment groups.

Table 9. Feed conversion ratio of birds at the end of starter, grower, and finisher phase.

Treatments	Day 0-10				Day 10-24			
	LSM	SE	P-value	Sign. diff.	LSM	SE	P-value	Sign. diff.
T1: control diet	1.07	0.01	Ref.	b	1.32	0.01	Ref.	a
T2: T1 - 50 Kcal energy	1.08	0.01	0.172	b	1.32	0.01	0.601	a
T3: T1 - 100 Kcal energy	1.11	0.01	<0.001	a	1.33	0.01	0.184	a
T4: T2 + 600g GAA	1.07	0.01	0.7	b	1.32	0.01	0.892	a
T5: T3 + 1200g GAA	1.08	0.01	0.157	b	1.32	0.01	0.681	a
Treatments	**Day 24-35**				**Day 0-35**			
	LSM	SE	P-value	Sign. diff.	LSM	SE	P-value	Sign. diff.
T1: control diet	1.75	0.03	Ref.	a	1.48	0.01	Ref.	a
T2: T1 - 50 Kcal energy	1.72	0.03	0.441	a	1.47	0.01	0.666	a
T3: T1 - 100 Kcal energy	1.76	0.03	0.928	a	1.49	0.01	0.309	a
T4: T2 + 600g GAA	1.77	0.03	0.676	a	1.48	0.01	0.866	a
T5: T3 + 1200g GAA	1.77	0.03	0.701	a	1.49	0.01	0.485	a

P value shows a difference to control group.
Significant difference (sign. diff.) is a multicompanies between treatment groups.

Table 10. Slaughter data (in kg) of birds at the end of finisher phase

Treatments	Live weight				Carcass weight			
	LSM	SE	P-value	Sign. diff.	LSM	SE	P-value	Sign. diff.
T1: control diet	2438	18	Ref.	ab	1848	16	Ref.	a
T2: T1 - 50 Kcal energy	2476	18	0.138	a	1882	16	0.143	a
T3: T1 - 100 Kcal energy	2439	18	0.956	ab	1848	16	0.991	a
T4: T2 + 600g GAA	2366	18	0.006	c	1800	16	0.038	b
T5: T3 + 1200g GAA	2408	18	0.246	bc	1842	16	0.798	ab
Treatments	**Breast weight**				**Leg weight**			
	LSM	SE	P-value	Sign. diff.	LSM	SE	P-value	Sign. diff.
T1: control diet	521	8	Ref.	a	671	7	Ref.	bc
T2: T1 - 50 Kcal energy	523	8	0.865	a	692	7	0.030	a
T3: T1 - 100 Kcal energy	527	8	0.531	a	677	7	0.531	ab
T4: T2 + 600g GAA	512	8	0.440	a	654	7	0.089	c
T5: T3 + 1200g GAA	533	7	0.246	a	671	7	0.997	bc

P value shows a difference to control group.
Significant difference (sign. diff.) is a multicompanies between treatment groups.

Discussion

Broilers response to different levels of energy is highly dependent on the magnitude of a higher or a lower content of energy. Recent literature has used 125 to 150 kcal / kg feed of lower or higher energy to see a response to energy (Barekatain et al. 2021; Maharjan et al. 2020). Others have gone one step further and used true metabolized energy (TME) and measured the TME of their raw materials to make sure that they will see a response to energy by means of increasing their accuracy (Naranjo, 2018). Lower magnitudes (25 - 50 kcal AME) have often led to a conclusion that birds do not respond to different energy levels (Dozier and Gehring, 2014). Herein, we also did not observe a clear response of broiler to -50 or -100 kcal AME in their feed as compared to the positive control group except for FCR in the starter phase causing a higher FCR; an effect which was not seen in grower and finisher phase.

GAA is suggested to improve energy homeostasis in muscle tissue because of the data showing extra creatine phosphate and free creatine in muscle tissue (DeGroot et al., 2019). This is often misinterpreted giving GAA a huge energy value (83000 to 166000 kcal/kg). There is controversy in the literature looking at energy saving effect of GAA. On one hand, a negative impact of GAA on growth parameters is observed specially under deficient or sufficient methionine levels (Majdeddin et al., 2019). On the other hand, a positive impact of GAA is concluded when it is added on top of an energy deficient feed (Ale Saheb Fosoul et al., 2018). Herein, addition of GAA to an energy deficient diet had no positive impact on performance parameters.

Conclusions

Measuring impact of energy on broiler's performance parameters is a factor of the magnitude of the changes in dietary energy. A reduction or increase in feed energy levels less than 125 - 150 kcal AME/kg is a challenge to be measured in broilers even under experimental conditions.
In this study, GAA did not bring any extra energy value. Thus, it is not recommended to consider an energy matrix value for GAA.

REFERENCES

1. Ale Saheb Fosoul, S., Azarfar, A., Gheisari, A., and H. Khosravinia. 2018. Energy utilisation of broiler chickens in response to guanidinoacetic acid supplementation in diets with various energy contents. British Journal of Nutrition, 120 (2): 131-140.
2. Barekatain, R., Romero, L. F. Sorbara, J. O. B., and A. J. Cowieson. 2021. Balanced nutrient density for broiler chickens using a range of digestible lysine-to-metabolizable energy ratios and nutrient density: Growth performance, nutrient utilisation and apparent metabolizable energy. Animal Nutrition. doi.org/10.1016/j.aninu.2020.12.003.
3. Black, J. L., Hughes, R. J., Nielsen, S. G., Tredrea, A. M., MacAlpine, R., and R. J. van Barneveld. 2005. The energy value of cereal grains, particularly wheat and sorghum, for poultry. Proceedings of Australian Poultry Science Symposium. 17: 21-29.
4. Brosnan, J. T., Wijekoon, E. P., Warford-Woolgar, L., Trottier, N. L., Brosnan, M. E., Brunton, J. A., and R. F. P., Bertolo. 2009. Creatine synthesis is a major metabolic process in neonatal piglets and has important implications for amino acid metabolism and methyl balance. The Journal of Nutrition. 139: 1292–1297.
5. D'Alfonso, T. H. 2005. Sources of variance of energy digestibility in corn-soy poultry diets and the effect on performance: Starch, protein, oil and fiber. Krmiva. 47: 83-86.
6. DeGroot, A. A., Braun, U., and R. N., Dilger. 2019. Guanidinoacetic acid is efficacious in improving growth performance and muscle energy homeostasis in broiler chicks fed arginine-deficient or arginine-adequate diets. Poultry Science. 98(7): 2896–2905.
7. Dozier, W. A., and C. K., Gehring. 2014. Growth performance of Hubbard × Cobb 500 and Ross × Ross 708 male broilers fed diets varying in apparent metabolizable energy from 14 to 28 days of age. Journal of Applied Poultry Research. 23(3): 494-500.
8. Lopez G., and S., Leeson. 2008. Assessment of the nitrogen correction factor in evaluating metabolizable energy of corn and soybean meal in diets for broilers. Poultry Science. 87(2): 298-306.
9. Maharjan P., Mullenix, G., Hilton, K., Caldas, J., Beitia, A., Weil, J., Suesuttajit, N., Kalinowski, A., Yacoubi, N., Naranjo, V., England, J., and C., Coon. 2020. Effect of digestible amino acids to energy ratios on performance and yield of two broiler lines housed in different grow-out environmental temperatures. Poultry Science. 2020. 99(12): 6884-6898.
10. Majdeddin, M., Golian, A., Kermanshahi, H., Michiels, J., and S. De Smet. 2019. Effects of methionine and guanidinoacetic acid supplementation on performance and energy metabolites in breast muscle of male broiler chickens fed corn-soybean diets. British Poultry Science. 60(5): 554-563.
11. Naranjo, V. D. 2018. Response of modern male broiler to different levels of energy and amino acids: Effects on growth and carcass parameters. XXXIV Curso de especialización FEDNA.