Guanidino acetic acid does not provide more than 60% arginine sparing in broilers

Behnam Saremi
CJ Europe GmbH

Background

Guanidino acetic acid (GAA) is synthesized in the kidney using L-arginine-glycine amidinotransferase (AGAT) and glycine and arginine (Arg) as substrate (Brosnan et al., 2009). Then, GAA is methylated to creatine in liver using GAA N-methyltransferase (GAMT). Feeding GAA to human and animals increases creatine in blood and muscle tissues (Ostoic et al., 2013; DeGroot et al., 2018). High creatine in blood has an inhibitory feedback on AGAT thus AGAT is known as a rate limiting enzyme in creatine synthesis (Edison et al., 2007; McGuire et al., 1984; Van Pilsem et al., 1971). Thus, GAA is speculated to have Arg sparing effects. In broilers, GAA is suggested to have either 77% or 149% Arg sparing effect. In this experiment, the Arg sparing effect of GAA is tested by means of using a comparative response test of Arg and GAA, simultaneously.

Methods

A total of 1800 male Ross 308 broilers arrived in the research facility (Poulpharm Bvba, Izegem, Belgium) at the age of zero days. Birds were placed in 120 pens (1m² each). Thirteen treatment groups (Table 1) were randomly allocated to pens (12 pens in basal diet group; 9 pens per treatment for the other treatments; 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. 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 0, 10, 24, and 35). Birds were slaughtered at day 35 (4 animals per pen). Carcass, breast and leg yield were measured. 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. Finisher feed had lower CP content compared with calculated values without a big impact on the amino acid ratios. Data were analysed with R (version 3.2.5.). A quadratic polynomial model was used to compare the birds response to different doses of Arg and GAA below and above the known Arg requirements (Aviagen, 2019). The dosage of GAA to achieve the maximum performance was identified, then the Arg dose needed to reach the same performance was identified and a ratio of the Arg to GAA dose was defined as bio-efficacy in percentage. 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	SID Arg (%)		
				Starter	Grower	Finisher
T01	0.00% Arg (basal diet)	12	15	1.02	0.88	0.75
T02	0.06% Arg	9	15	1.08	0.94	0.81
T03	0.12% Arg	9	15	1.14	1.00	0.87
T04	0.18% Arg	9	15	1.20	1.06	0.93
T05	0.30% Arg	9	15	1.32	1.18	1.05
T06	0.45% Arg	9	15	1.47	1.25	1.20
T07	0.61% Arg	9	15	1.63	1.49	1.36
T08	0.06% GAA	9	15	1.02[1]	0.88[1]	0.75[1]
T09	0.12% GAA	9	15	1.02[1]	0.88[1]	0.75[1]
T10	0.18% GAA	9	15	1.02[1]	0.88[1]	0.75[1]
T11	0.30% GAA	9	15	1.02[1]	0.88[1]	0.75[1]
T12	0.45% GAA	9	15	1.02[1]	0.88[1]	0.75[1]
T13	0.61% GAA	9	15	1.02[1]	0.88[1]	0.75[1]

[1]T08 to T13 have similar SID Arg like the basal diet because GAA would not add SID Arg to the feed itself because of the Arg sparing effect supposed to happen in the chicken body not in the feed.

Table 2. Ingredient composition of the basal diet

Ingredient Name	Starter (0 - 10 days)	Grower (10 - 24 days)	Finisher (24 - 35 days)
Corn	50.00	55.00	69.87
Soybean meal	20.36	14.38	8.86
Rape Seed Meal	8.00	10.00	5.18
Corn starch	6.00		
Corn gluten meal	5.55	5.10	10.00
Corn gluten feed	2.78	6.76	
Soy oil	1.98	4.27	1.34
DCP	1.60	0.95	1.14
Limestone	1.06	0.92	0.96
L - lysine HCl	0.61	0.59	0.62
Broiler premix	0.50	0.50	0.50
Salt	0.37	0.37	0.39
L-methionine	0.37	0.32	0.24
L-threonine	0.29	0.26	0.21
L-glycine	0.27	0.33	0.51
L-isoleucine	0.15	0.16	0.11
L-valine	0.11	0.08	0.06
L-tryptophan	0.02	0.02	0.04

Table 3. Calculated nutrient composition of the basal diet

Nutrient %	Starter (0 - 10 days)	Grower (10 - 24 days)	Finisher (24 - 35 days)
AMEn Broiler (kcal/kg)	2960	3050	3150
Crude Protein	21.43	20.12	18.11
Crude Fat	5.49	8.12	5.56
Crude Fiber	3.32	3.70	2.89
Ash	6.15	5.41	4.95
Calcium	0.90	0.70	0.70
Available Phosphorous	0.42	0.32	0.32
Dig. Lysine	1.28	1.15	1.03
Dig. Methionine	0.65	0.59	0.52
Dig Met Plus Cys	0.95	0.87	0.80
Dig. Arginine	1.02	0.88	0.75
Dig. Threonine	0.86	0.77	0.69
Dig. Leucine	1.63	1.48	1.70
Dig. Isoleucine	0.86	0.78	0.71
Dig. Valine	0.96	0.87	0.78
Dig. Tryptophan	0.20	0.18	0.16
Dig. Phenylalanine	0.86	0.78	0.75
Dig. Histidine	0.47	0.44	0.39
Choline	1439	1492	1030
Starch	34.63	38.07	46.94

Results

On average, broilers needed less Arg to achieve the maximum response created using GAA (GAA bio efficacy was 59.56%). Bio-efficacy of GAA was determined to be approximately 46, 77, and 55% for BW at the end of starter, grower, and finisher phases, respectively (Table 4). Similarly, bio-efficacy of GAA compared with Arg for DWG was 46, 84, 44 and 57% during the starter, grower, finisher or during the whole growth period, respectively (Table 4). GAA had a negative impact on BW and DWG at higher doses when adding more than 0.18% GAA to an Arg deficient feed (Figure 1 and 2).

Table 4. Efficacy of GAA compared with Arg for BW and DWG estimated using a quadratic polynomial model

	Max performance	Dose to achieve the GAA max performance	% Arg/GAA[1]
BW at Day 10			
Arg	295	0.07	Ref.
GAA	275	0.16	46
BW at Day 24			
Arg	1343	0.2	Ref.
GAA	1269	0.26	77
BW at Day 35			
Arg	2325	0.16	Ref.
GAA	2109	0.28	55
DWG (Day 0 - 10)			
Arg	25.3	0.07	Ref.
GAA	23.3	0.16	46
DWG (Day 10 - 24)			
Arg	74.8	0.23	Ref.
GAA	71.1	0.27	84
DWG (Day 24 - 35)			
Arg	89.8	0.14	Ref.
GAA	77.1	0.32	44
DWG (Day 0 - 35)			
Arg	65.2	0.16	Ref.
GAA	59	0.29	57

[1]The dosage of GAA to achieve the maximum performance was identified, then the Arg dose needed to reach the same performance was identified and a ratio of the Arg to GAA dose was defined as bio-efficacy in percentage.

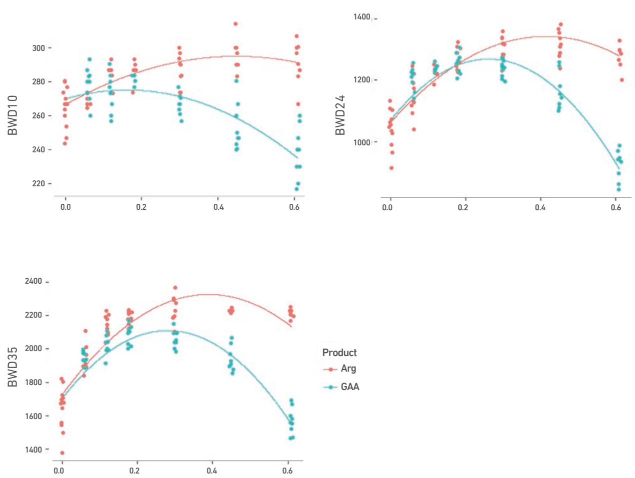

Figure 1. Efficacy of GAA compared with Arg for BW estimated using a quadratic polynomial model

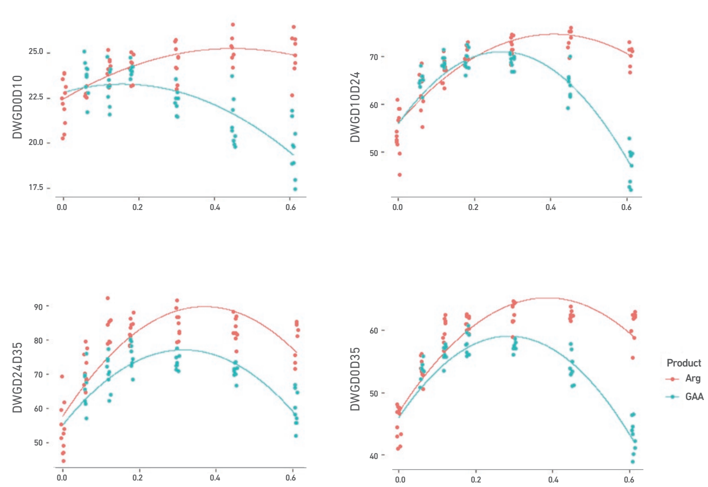

Figure 2. Efficacy of GAA compared with Arg for DWG estimated using a quadratic polynomial model

Feed intake was increased as a response to Arg or GAA addition ($P < 0.05$; Figure 3). However, GAA had a negative impact on feed intake in an age dependent manner. Bio-efficacy of GAA compared with Arg during the grower, finisher or during the whole growth period was 73, 39, or 44%, respectively (Table 5). During the starter phase, GAA created only a negative impact on feed intake thus

Arg bio-efficacy determined to be infinite. Feed conversion ratio was improved by both Arg and GAA. However, GAA had a detrimental impact on FCR at doses higher than 0.18% (Figure 4). Efficacy of GAA vs. Arg was defined equal to 50, 102, 61, and 78% during the starter, grower, and finisher phases and during the whole growth period, respectively (Table 5).
Mortality was reduced by the first dose of Arg ($P < 0.05$) contrary to the first dose of GAA ($P>0.05$). No difference in mortality was observed between next consecutive doses of Arg and GAA. Overall, mortality rate was low (Data not shown).

Table 5. Efficacy of GAA compared with Arg for Daily Feed intake (DFI) and FCR estimated using a quadratic polynomial model

	Max performance	Dose to achieve the GAA max performance	% Arg/GAA[1]
DFI (Day 0 - 10)			
Arg	26.3	0.3	Ref.
GAA	26.3	0.0	Infinite
DFI (Day 10 - 24)			
Arg	95.7	0.2	Ref.
GAA	93.0	0.2	73.0
DFI (Day 24 - 35)			
Arg	176.9	0.1	Ref.
GAA	160.5	0.3	39.0
DFI (Day 0 - 35)			
Arg	101.1	0.1	Ref.
GAA	94.4	0.3	44.0
FCR (Day 0 - 10)			
Arg	1.034	0.17	Ref.
GAA	1.084	0.34	50.0
FCR (Day 10 - 24)			
Arg	1.261	0.32	Ref.
GAA	1.279	0.32	102.0
FCR (Day 24 - 35)			
Arg	1.902	0.22	Ref.
GAA	2.025	0.36	61.0
FCR (Day 0 - 35)			
Arg	1.527	0.26	Ref.
GAA	1.576	0.33	78.0

[1]The dosage of GAA to achieve the maximum performance was identified, then the Arg dose needed to reach the same performance was identified and a ratio of the Arg to GAA dose was defined as bio-efficacy in percentage.

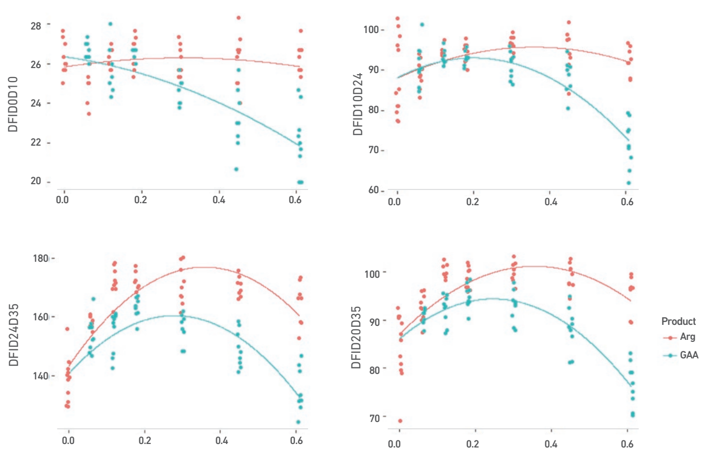

Figure 3. Efficacy of GAA compared with Arg for DFI estimated using a quadratic polynomial model

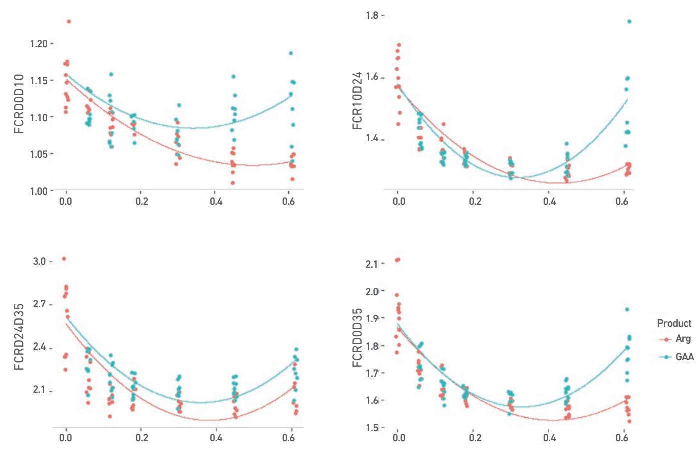

Figure 4. Efficacy of GAA compared with Arg for FCR estimated using a quadratic polynomial model

At day 35, maximum slaughter performances (live weight, carcass weight, breast weight and leg weight) were also achieved with much less Arg compared with GAA (56, 53, 51, and 56%, respectively) (Table 6, Figure 5).

Table 6. Efficacy of GAA compared with Arg for slaughter parameters at days 35 estimated using a quadratic polynomial model

Kg	Max performance	Dose to achieve the GAA max performance	% Arg/GAA[1]
Live weight			
Arg	2321	0.16	Ref.
GAA	2113	0.29	56
Carcass weight			
Arg	1765	0.15	Ref.
GAA	1572	0.28	53
Breast weight			
Arg	457	0.15	Ref.
GAA	388	0.3	51
Leg weight			
Arg	658	0.15	Ref.
GAA	597	0.27	56

[1]The dosage of GAA to achieve the maximum performance was identified, then the Arg dose needed to reach the same performance was identified and a ratio of the Arg to GAA dose was defined as bio-efficacy in percentage.

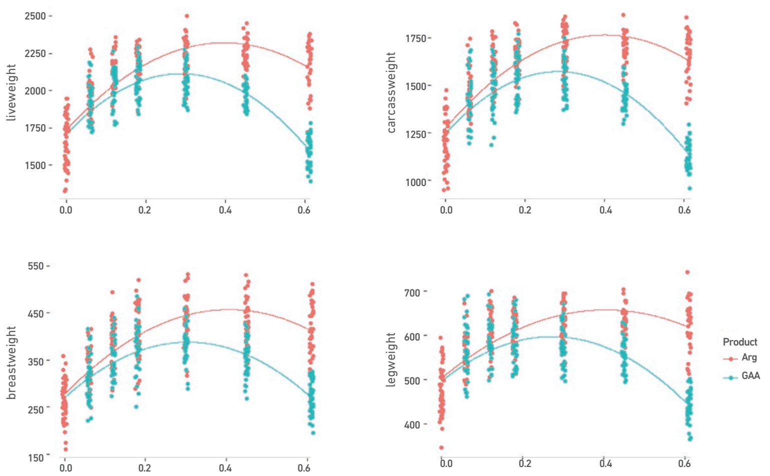

Figure 5. Efficacy of GAA compared with Arg for slaughter parameters estimated using a quadratic polynomial model. Live weight at slaughter (top left), the carcass weight (top right), the breast weight (below left) and the leg weight (below right).

Discussion

GAA is a pro-oxidant, a methyl group scavenger, and a precursor of creatine which its application as a feed additive needs to be carefully monitored because its efficacy is highly depending on availability of methyl donors (EFSA, 2016). There is also a minimum inclusion rate needed for its efficacy (600 grams per ton of feed) and a maximum inclusion rate is recommended for safety reasons (1200 grams per ton of feed). Herein, we observed a clear negative impact of GAA on performance parameters with a dose higher than 0.18% when GAA is added to an Arg deficient diet. What would be the consequences of adding GAA to an Arg adequate diet stays to be elucidated.

Using a semi-purified broiler feed, Dilger et al. (2013) attempted to define the efficiency of GAA. Adding 0.06, 0.12, 0.39, 0.78% GAA to an Arg deficient diet (0.88% Arg) could not match the performance results (212 vs 145 grams weight gain; 8 to 17 days post hatch) with the deficient diet supplemented with 1% Arg (source of L-Arg was not mentioned: unknown purity). Dilger et al. (2013) in an additional experiment using a semi-purified diet, compared the efficacy of GAA with Arg using an exponential response model. However, in this model in both groups the response was created with graded levels of Arg in two different basal diets: with or without GAA inclusion (0.12% vs 0%). Dilger et al. (2013) concluded that GAA is an efficacious Arg source under Arg deficient conditions because there was a difference between the two groups when less than 0.4% L-Arg was supplemented to the deficient diet (0.88% Arg) and no response was found when more than 0.4% L-Arg was supplemented to the basal diet. According to the current broiler Arg requirements (1.37% SID basis; Aviagen, 2019), 0.88% Arg is considered a severely deficient diet. Nevertheless, a quantitative efficacy number was not provided. Herein, we defined the efficacy of GAA and compared it with Arg. On average, GAA could be replaced with 59.56% Arg to achieve a similar maximum performance. During the starter phase, one need to be more careful with GAA because of a linear negative impact of GAA on feed intake and a less efficiency of GAA (47.33%) in young birds.

Conclusions

Herein, complications attached with the use of GAA is clearly demonstrated. It is not clear how much Arg sparing effect one can expect depending on animal age and physiological condition. Currently, it is two sized solutions (77% and 149% Arg sparing) for all animal species at different ages. According to our findings, GAA can be easily replaced with 59.56% L-Arg in broiler chicken. GAA is not recommended to be used in young chickens because GAA linearly caused a reduction in their feed intake. The current recommended 77% or 149% Arg efficacy for GAA is rejected.

REFERENCES

1. DeGroot, A. A., U. Braun, and R. N. Dilger. 2018. Efficacy of guanidinoacetic acid on growth and muscle energy metabolism in broiler chicks receiving arginine-deficient diets. Poult. Sci. 97: 890–900.
2. Dilger, R. N., K. Bryant-Angeoni, R. L. Payne, A. Lemme, and C. M. Parsons. 2013. Dietary guanidino acetic acid is an efficacious replacement for arginine for young chicks. Poult. Sci. 92: 171–177.
3. Edison, E. E., M. E. Brosnan, C. Meyer, et al. 2007. Creatine synthesis: production of guanidinoacetate by the rat and human kidney in vivo. Am J Physiol Renal Physiol. 293: F1799-804.
4. EFSA (European Food Safety Authority). 2016. Safety and efficacy of guanidinoacetic acid for chickens for fattening, breeder hens and roosters, and pigs. The EFSA Journal:14(2):4394.
5. McGuire, D. M., M. D. Gribss, J. F. Van Pilsum, et al. 1984. Repression of rat kidney L-arginine:glycine amidinotransferase synthesis by creatine at a pretranslational level. J Biol Chem. 259: 12034-8.
6. Ostojic, S. M., B., Niess, M., Stojanovic, and M. Obrenovic. 2013. Creatine Metabolism and Safety Profiles after Six-Week Oral Guanidinoacetic Acid Administration in Healthy Humans. Int. J. Med. Sci. 10(2):141-147.
7. Van Pilsum J. F. 1971. Evidence for a dual role of creatine in the regulation of kidney transamidinase activities in the rat. J Nutr. 101:1085-92.