L-Arginine comparison with Guanidino Acetic Acid (GAA) and their impact on broilers growth and breast myopathy incidence

Behnam Saremi
CJ Europe GmbH

Background

L-Arginine (Arg) is an essential amino acid for chickens due to unavailability of two separate enzymes in urea cycle in the kidney and almost all urea cycle enzymes in the liver (Leeson and Summers, 2001). Thus, providing Arg through feed is essential for the optimum growth in poultry. Arginine can be provided through intact protein or via supplementary L-Arg. However, there are products claiming arginine sparing effects like Guanidino acetic acid (GAA), enzymes, etc.

GAA is the precursor of creatine. Creatine is synthesized via a two steps process involving two enzymes and three amino acids: Arg, glycine, and methionine (Figure 1). In the first step, Arginine: glycine amidinotransferase (AGAT) transfers an amidino group from Arg to the amino group of glycine to produce guanidinoacetate (GAA) and ornithine in kidney. GAA is transferred to liver where GAA methyltransferase (GAMT) employs S-adenosylmethionine (SAM) to methylate GAA, producing creatine and S-adenosylhomocysteine (SAH). Then creatine will migrate to muscle tissue where it is used to store energy in form of creatine phosphate (Brosnan et al. 2009). Typically, creatine satisfaction in muscle will send a negative feedback to AGAT in kidney to reduce its activity and consequently to reduce GAA synthesis. Adding supplementary GAA to feed will reduce AGAT activity in a similar way (Brosnan et al. 2009) thus the Arg, which is used for the synthesis of GAA in kidney could be theoretically spared for other purposes. Despite different claims from GAA suppliers (77% or 149% arginine sparing effect), it remains inconclusive the amount of Arg sparing one can expect from GAA supplementation in feed. Moreover, the accurate dose of GAA to create the AGAT negative feedback stays unclear.

L-Arg and GAA are used in practice to cover Arg requirements of the animals. Thus, understanding the differences between the two products is important. The aim of this study was to determine the effect of increasing the digestible arginine-to-lysine ratio (dArg to dLys) by means of L-Arg or GAA addition to feed on broiler's growth performance, carcass analytics and breast myopathy issues.

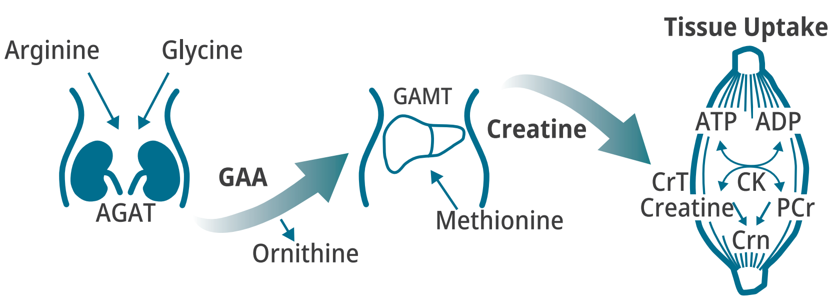

Figure 1. Creatine synthesis pathway (adapted from Ellery et al., 2016)

Methods

A total of 1176 male Ross 308 broiler chickens were allocated (28 birds/pen) to 7 dietary treatments (Table 1 and 2) at random. Each dietary treatment was provided to 6 pen-replicates. Experimental diets were provided in four phases, starter (d 0 to 10), grower 1 (d 10-20), grower 2 (d 20-30) and finisher (d 30-44). Birds and feed were weighed on a pen basis at the start and end of each phase, and FCR was calculated. Four birds/pen were sacrificed on d 28, 35 and 42 to determine the changes in carcass parameters, and foot pad dermatitis (FPD) scores. Breast myopathy (white stripping, wooden breast, and spaghetti muscle scores) dynamics were assessed in slaughtered birds. Protein containing raw materials and final feed were analyzed for major nutrients and amino acids. There was a slight deviation form formulated values in group 1.15 dArg to dLys and 1.25 dArg to dLys specially during the starter phase. Birds received less dArg to dLys and some other nutrients (such as methionine plus cysteine) as it was planned. Consequently, growth parameters were affected, and these effects remained until finisher phase.

Treatment groups were planned as follows: Trt1: A control diet with 1.05 dArg to dLys (without L-Arg supplementation); Three diets supplemented with L-Arg to achieve the dArg to dLys ratios of: Trt 2: 1.15 dArg to dLys ratios, Trt 3: 1.25 dArg to dLys ratios, Trt 4: 1.35 dArg to dLys ratios; Trt 5: A diet with 1.05 dArg to dLys using supplemental L-Arg; Trt 6: A diet with 1.05 dArg to dLys using GAA (77% Arg sparing); Trt 7: A diet with 1.05 dArg to dLys using GAA (149% Arg sparing).
The experimental data was analyzed using Genstat® (19th version). Significant differences were determined using ANOVA. Treatment means were compared using least significant differences (LSD). We also checked for an eventual linear or quadratic response to Arg between the first 4 treatment groups using orthogonal polynomial contrasts. Breast myopathy results were compared using Chi-square test.

Table 1: Planned treatment (Trt) groups and calculated levels of arginine to lysine in different growth phases

Trt	Description	Summary description	dArg to dLys*			
			Starter D0-10	Grower 1 D10-20	Grower 2 D20-30	Finisher D30-44
1	Control (Con, no L-Arg added, dArg to dLys = 1.05)	Control	1.06	1.06	1.07	1.08
2	Con + L-Arg (dArg to dLys = 1.15)	1.15 dArg to dLys	1.15	1.15	1.15	1.15
3	Con + L-Arg (dArg to dLys = 1.25)	1.25 dArg to dLys	1.25	1.25	1.25	1.25
4	Con + L-Arg (dArg to dLys = 1.35)	1.35 dArg to dLys	1.35	1.35	1.35	1.35
5	Con + 0.0469% L-Arg	1.05 dArg to dLys	1.06	1.06	1.07	1.08
6	Con + 0.06% GAA (77% dArg, 83000 Kcal/Kg AMEn)	GAA=77%	1.06	1.06	1.07	1.08
7	Con + 0.06% GAA (149% dArg, 83000 Kcal/Kg AMEn)	GAA=149%	1.06	1.06	1.07	1.08

*Digestible amino acid evaluation was based on the standardized amino acids.

Table 2: Feed formulation of seven dietary treatment groups.

	1.05 Arg/Lys (no suppl Arg)	1.15 Arg/Lys	1.25 Arg/Lys	1.35 Arg/Lys	1.05 Arg/Lys (with suppl Arg)	1.05 Arg/Lys (with GAA 77%)	1.05 Arg/Lys (with GAA 149%)
LIMESTONE	1.183	1.183	1.183	1.183	1.190	1.191	1.205
CORN GLUTEN MEAL (60% CP)							2.134
SOYBEAN OIL	2.114	2.114	2.114	2.114	1.805	0.973	0.355
MONOCALCIUM PHOSPHATE	0.561	0.561	0.561	0.561	0.576	0.574	0.594
SALT	0.263	0.263	0.263	0.263	0.229	0.228	0.403
LYSINE HCL (79%)	0.288	0.288	0.288	0.288	0.341	0.342	0.258
L-METHIONINE (99%)	0.267	0.267	0.267	0.267	0.279	0.277	0.089
L-THREONINE (98%)	0.066	0.066	0.066	0.066	0.088	0.087	0.087
L-TRYPTOPHAN (98%)							0.011
L-VALINE (99%)					0.027	0.026	0.022
P premix Sacox	0.580	0.580	0.580	0.580	0.580	0.580	0.580
NSP enzyme GLU-XYL	0.250	0.250	0.250	0.250	0.250	0.250	0.250
SODIUM BICARBONATE	0.100	0.100	0.100	0.100	0.150	0.151	0.299
Broiler premix	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Phytase	0.500	0.500	0.500	0.500	0.500	0.500	0.500
Corn	36.534	36.424	36.302	36.180	36.341	37.062	40.558
SBM	31.795	31.795	31.795	31.795	29.887	29.747	27.056
Wheat	25.000	25.000	25.000	25.000	27.211	27.450	25.000
L-ARGININE	-	0.110	0.232	0.354	0.047	-	-
GAA - 77% Arg / 83k AMEn	-	-	-	-	-	0.060	-
GAA - 149% / 83k AMEn	-	-	-	-	-	-	0.060

Results and Discussion

Overall, birds were healthy with a low mortality rate and without any issue with FPD score. Performance was similar among groups (Table 3) except for Trt 2 and 3 with a major difference in growth because of mixing issues and lack of methionine plus cysteine, arginine and lysine in these feeds specially during the starter phase. This lack was compensated for FCR until end of the finisher phase but growth of birds could not be compensated although in the post starter phases adequate nutrients were supplied.

Our data confirmed that achieving 105% dArg to dLys ratios using supplementary Arg had a beneficial effect on FCR although birds had similar body weight gain like the group provided with 105% dArg to dLys ratios though crude protein (Table 3). From a pure performance perspective birds of Trt 5, 6 and 7 did not exhibited any differences. However, 17% of breast fillets derived from birds of Trt 7 presented severe cases of breast myopathies which was four times higher than Trt 5 and 6 (Table 4). Low dietary Arg contents has been linked to breast myopathy issues (Zampiga et al. 2019). Trt 7 was a group where we applied 149% dArg and 83000 Kcal per Kg AMEn for GAA to achieve 105% dArg to dLys ratios in the feed. Thus, Trt 7 received a very low level of dArg to dLys through feed (0.96) thus it was a good candidate to present signs Arg deficiency and severe cases of breast myopathy.

Table 3: Performance results of birds in the whole experiment.

Trt		Analysed Arg:Lys				D0 - 44				
		St	Gr1	Gr2	Fi	BWG (g)	FI (g)	FCR (g/g)	FCR 3500	Mort (%)
1	Control	1.03	1.01	1.02	1.05	3690[bc]	5683[b]	1.540[b]	1.502	3.6
2	1.15 dArg:Lys	0.92	1.09	1.07	1.09	3615[ab]	5372[a]	1.486[a]	1.463	3.0
3	1.25 dArg:Lys	1.04	1.18	1.19	1.19	3578[a]	5403[a]	1.510[ab]	1.494	3.0
4	1.35 dArg:Lys	1.20	1.24	1.23	1.27	3685[bc]	5496[a]	1.491[a]	1.454	3.6
5	1.05 dArg:Lys	1.01	1.03	1.05	1.05	3785[cd]	5741[b]	1.517[ab]	1.460	4.8
6	GAA=77%	1.00	1.00	0.98	1.02	3814[d]	5767[b]	1.512[ab]	1.449	4.2
7	GAA=149%	0.96	0.96	0.96	0.96	3784[cd]	5726[b]	1.514[ab]	1.457	4.8
	LSD					106.3	187	0.032	0.040	4.7
	P-value					<0.001	<0.001	0.039	0.07	0.97
1,2,3,4	*P-value (linear)*					0.28	0.007	0.004	0.06	0.88
1,2,3,4	*P-value (quadratic)*					0.10	0.040	0.28	0.92	0.72

LSD: least significant difference
a,b,c,d Values without a common superscript within a column differ significantly (p≤ 0.0).
Birds per replicate (pen): 16

Table 4: Breast myopathy results of birds slaughtered at day 42.

Trt		Analysed Arg:Lys			Nail scratches			White striping		
		St	Gr1	Gr2	0	1	2	0	1	2
1	Control	1.03	1.01	1.02	87.5	12.5	0.0	95.8	4.2	0.0
2	1.15 dArg:Lys	0.92	1.09	1.07	91.7	8.3	0.0	91.7	8.3	0.0
3	1.25 dArg:Lys	1.04	1.18	1.19	83.3	16.7	0.0	95.8	4.2	0.0
4	1.35 dArg:Lys	1.20	1.24	1.23	91.7	8.3	0.0	100.0	0.0	0.0
5	1.05 dArg:Lys	1.01	1.03	1.05	66.7	33.3	0.0	95.8	4.2	0.0
6	GAA=77%	1.00	1.00	0.98	79.2	20.8	0.0	100.0	0.0	0.0
7	GAA=149%	0.96	0.96	0.96	83.3	16.7	0.0	95.8	4.2	0.0
P-value (chi-square)					0.22	0.22	*	0.69	0.69	*

* Results could not be analyzed statistically.
Results expressed in % of animals per score.

Trt		Analysed Arg:Lys			Wooden breast			Spaghetti meat		
		St	Gr1	Gr2	0	1	2	0	1	2
1	Control	1.03	1.01	1.02	75.0	20.8	4.2	100.0	0.0	0.0
2	1.15 dArg:Lys	0.92	1.09	1.07	79.2	20.8	0.0	100.0	0.0	0.0
3	1.25 dArg:Lys	1.04	1.18	1.19	83.3	16.7	0.0	100.0	0.0	0.0
4	1.35 dArg:Lys	1.20	1.24	1.23	58.3	37.5	4.2	91.7	8.3	0.0
5	1.05 dArg:Lys	1.01	1.03	1.05	62.5	33.3	4.2	95.8	4.2	0.0
6	GAA=77%	1.00	1.00	0.98	62.5	33.3	4.2	95.8	4.2	0.0
7	GAA=149%	0.96	0.96	0.96	58.3	25.0	16.7	95.8	0.0	4.2
P-value (chi-square)					0.72	0.59	0.57	0.59	0.50	0.41

* Results could not be analyzed statistically.
Results expressed in % of animals per score.

Carcass parameters were also similar between treatments (Table 5) with the exception of Trt 1 being behind in terms of carcass percentage. Both GAA groups were also presenting a lower "a" colour value (redness) as compared with the Arg group (Trt 5). A low redness or being pink and pale is in line with a higher incidence of breast myopathies issues in GAA groups.

Table 5: Carcass parameters of birds slaughtered at day 42.

Trt		Analysed Arg:Lys				Carcass (%)[1]	Leg (%)[1]	Abd gat (%)[1]	Breast (%)[1]	Hunter colour values		
		St	Gr1	Gr2	Fi					L*	a*	b*
1	Control	1.03	1.01	1.02	1.05	66.2 ^{a}	20.6 ^{ab}	0.45	20.2	53.4	4.3 ^{a}	20.1
2	1.15 dArg:Lys	0.92	1.09	1.07	1.09	67.8 ^{b}	20.9 ^{abc}	0.49	21.0	53.8	4.1 ^{a}	19.5
3	1.25 dArg:Lys	1.04	1.18	1.19	1.19	67.7 ^{ab}	21.2 ^{cd}	0.49	20.2	54.6	4.5 ^{ab}	19.3
4	1.35 dArg:Lys	1.20	1.24	1.23	1.27	68.5 ^{b}	21.7 ^{d}	0.46	20.3	56.2	4.1 ^{a}	19.8
5	1.05 dArg:Lys	1.01	1.03	1.05	1.05	68.8 ^{b}	21.1 ^{bc}	0.53	21.3	53.7	5.0 ^{b}	20.2
6	GAA=77%	1.00	1.00	0.98	1.02	67.7 ^{b}	20.7 ^{abc}	0.51	21.0	55.2	4.1 ^{a}	19.7
7	GAA=149%	0.96	0.96	0.96	0.96	67.6 ^{ab}	20.4 ^{a}	0.54	21.8	55.3	4.6 ^{ab}	20.7
	LSD					1.50	0.62	0.13	1.21	2.64	0.56	1.33
	P-value					0.042	0.002	0.76	0.07	0.29	0.016	0.42
1,2,3,4	*P-value (linear)*					0.009	0.004	0.68	0.69	0.06	0.84	0.44
1,2,3,4	*P-value (quadratic)*					0.87	0.08	0.49	0.22	0.13	0.93	0.47

LSD: least significant difference
a,b,c Values without a common superscript within a column differ significantly (p≤ 0.0).
[1] Percentage of live weight

Conclusions

Myopathy scores were in general low in the present study, even in the control treatment. However, the treatment group which was supplemented with GAA considering 149% dArg content for GAA resulted in much higher percentage of birds with a severe breast myopathy cases (Wooden breast) compared to any other treatments at day 42. Thus, 0.06% GAA at 149% dArg sparing resulted in appearance of severe breast myopathy cases (Wooden breast). It is interesting to mention that in the other 6 treatment groups, breast muscles were quite normal and had a very high meat quality (exception was the case of GAA 149% group). Thus, arginine deficiency can lead to appearance of breast myopathy issues.

Moreover, 0.047% L-Arg could be replaced by 0.06% GAA (at 77% arginine sparing) without any detrimental effect on performance, litter and footpad quality, carcass yields and score for myopathies. The commercial consequences of a higher volume demand for GAA (600 grams per ton) vs. L-Arg (470 grams per ton) thus creation of extra logistics and product expenses should be taken into consideration.

Extra dArg to dLys (especially 1.15 and 1.35) resulted in a better feed efficiency in male broilers from day 0-44 of age and higher carcass yield at day 42. However, the treatment with 1.35 dArg to dLys resulted in higher feed costs.

Products with Arg sparing effects might not cause a performance issue but can eliminate beyond performance benefits of arginine.

REFERENCES

1. Brosnan JT, Enoka P, Wijekoon P, Warford-Woolgar L, Trottier NL, Brosnan ME, Brunton JA, et al. Creatine synthesis is a major metabolic process in neonatal piglets and has important implications for amino acid metabolism and methyl balance. J. Nut. 2009; 139:1292–1297.
2. Ellery SJ, Dickinson H, McKenzie M, Walker DW. Dietary interventions designed to protect the perinatal brain from hypoxic-ischemic encephalopathy – Creatine prophylaxis and the need for multi-organ protection. Neurochemistry International. 2016; 95:15-23.
3. Leeson S, Summers J.D. Scott's Nutrition of the Chicken. 2001. Publ. Univ. Books, Guelph, Ontario Canada.
4. Zampiga M, Soglia F, Petracci M, Meluzzi A, Sirri F. Effect of different arginine-to-lysine ratios in broiler chicken diets on the occurrence of breast myopathies and meat quality attributes. Poult. Sci. 2019; 98:1707-1714.