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EFFECTS OF GUANIDINOACETIC ACID SUPPLEMENTATION TO BROILER DIETS WITH VARYING ENERGY CONTENT

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Abstract

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An experiment was conducted to determine the response of broiler chickens to guanidinoacetic acid (GAA) added to diets with different energy levels during fattening to 35 days of age. Ross 308 male chicks were allotted to 10 treatments, each consisting of six replicates of 140 birds/pen. Five maize-soyabean meal isonitrogenous diets with decreasing AME_n levels (100, 99, 98, 97 and 96% of requirement) with or without supplements of 0.6 g/kg CreAMINO® containing a minimum of 96% GAA were formulated. The criteria of response were feed intake, body weight gains, feed conversion ratio and carcass, breast meat, leg meat and abdominal fat yields. Supplementation of broiler diets with 0.06% GAA resulted in a significant ($P < 0.05$) decrease in voluntary feed intake. With decreasing AME_n level, voluntary feed intake and consequently protein and amino acid intakes increased which was manifested by improved growth performance ($P < 0.01$). GAA supplements significantly ($P < 0.001$) improved feed conversion ratio and efficiency of AME_n utilization and significantly ($P < 0.01$) increased breast meat yield. With decreasing AME_n level, the effects of GAA supplementation tended to diminish.

Keywords: guanidinoacetic acid, energy, broiler, feed intake, weight gain, feed conversion, carcass yield, breast meat yield

INTRODUCTION

Guanidinoacetic acid (GAA) is the biochemical precursor of creatine, which, in its phosphorylated form, plays an important role as a high-energy carrier in the muscle. GAA is formed from the amino acids glycine and arginine in the kidney or absorbed from the gut. It is transformed to creatine in the liver. Phosphocreatine serves as a dynamic reservoir of high energy phosphate. The phosphocreatine/creatine system buffers ATP/ADP ratio for all energy consuming functions of the cell. The degradation of creatine results in creatinine, which is excreted in the urine. Therefore creatine must be continually replaced from dietary sources or synthesized *de novo* from GAA. In contrast to animal proteins, creatine is not found in plant feedstuffs and may be thus deficient in all-vegetable diets. GAA as a creatine source is more stable and less expensive than creatine itself (Baker, 2009).

The objective of the present experiment was to determine the response of broiler chickens to GAA (trade name CreAMINO®, Evonik Industries) added to all-vegetable diets ranging in AME_n levels from 100% to 96% of requirement.

MATERIAL AND METHODS

The experiment was conducted at the International Poultry Testing Station Ústrašice. The animal procedures were reviewed and approved by the Animal Care Committee of the Mendel University in Brno.

Animals and Procedures

A total of 8400 one-day-old male Ross 308 broiler chicks were randomly assigned to 10 dietary treatments in such a way as to ensure similar mean body weights across treatments. There were six replicates per treatment (140 chicks per pen). Chickens were kept in the windowless house with full climatic control, on deep litter from wood shavings. Each pen was equipped with manually filled tube feeders and nipple drinkers. The stocking density was 17 broilers per square meter. Heating and lighting programmes were in accordance with Ross Broiler Management Manual (2009). On days 10, 24 and 35, the chickens were weighed individually. At the same time, feed consumption per pen was recorded. On the last day of experiment, ten birds of each pen having body weights closest to the pen mean were selected, slaughtered and carcass, breast meat (boneless and without skin), leg meat (without bone, with skin) and abdominal fat yields were determined.

Experimental Diets

Maize-soyabean meal starter (d 1 to 10), grower (d 11 to 24) and finisher (d 25 to 35) basal diets with different levels of metabolizable energy (100, 99, 98, 97 and 96% of AME_n requirement as suggested by Ross Nutrition Supplement, 2009) were formulated. To the basal diets serving as controls (Treatments 1 to 5), 0.6 g/kg of CreAMINO® containing a minimum of 96% GAA was added thus forming five experimental diets (Treatments 6 to 10). The diets for each growing period were formulated to be isonitrogenous. The composition of basal diets having the highest (100%) AME_n level is given in Tab. I. Energy concentrations of other diets were adjusted to the required levels by decreasing the content of soyabean oil and by minor

I: *Composition of diets (as-fed basis)*

Ingredients	Starter (0–10 d)	Grower (11–24 d)	Finisher (25–35 d)
Maize 8%	54.27	58.47	62.93
Soyabean meal, 48%	37.30	32.70	28.41
Soyabean oil	3.79	4.89	4.89
Monocalcium phosphate	1.77	1.57	1.45
Calcium carbonate	1.55	1.29	1.25
Sodium chloride	0.25	0.28	0.28
Sodium bicarbonate	0.17	0.13	0.14
DL-Methionine	0.32	0.24	0.21
L-Lysine.HCl	0.20	0.10	0.11
L-Threonine	0.08	0.03	0.03
Supplementary premix	0.30	0.30	0.30

II: *Nutrient contents of diets (as-fed basis)*

Nutrients	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Level of AME_n (%)	**100**	**99**	**98**	**97**	**96**	**100**	**99**	**98**	**97**	**96**
Starter										
Crude protein (g/kg)[1)]	226.2	226.3	226.4	226.6	226.7	226.2	226.3	226.4	226.6	226.7
AME_n (MJ/kg)[1)]	12.656	12.532	12.405	12.280	12.155	12.656	12.532	12.405	12.280	12.155
Guanidinoacetic acid (mg/kg)[2)]	2	2	2	3	3	555	611	607	595	610
Grower										
Crude protein (g/kg)[1)]	205.8	205.8	205.8	205.8	205.8	205.8	205.8	205.8	205.8	205.8
AME_n (MJ/kg)[1)]	13.180	13.055	12.930	12.804	12.678	13.180	13.055	12.930	12.804	12.678
Guanidinoacetic acid (mg/kg)[2)]	< 1	< 1	< 1	< 1	< 1	554	557	578	628	580
Finisher										
Crude protein (g/kg)[1)]	188.8	188.8	188.8	188.8	188.8	188.8	188.8	188.8	188.8	188.8
AME_n (MJ/kg)[1)]	13.390	13.260	13.140	13.010	12.880	13.390	13.260	13.140	13.010	12.880
Guanidinoacetic acid (mg/kg)[2)]	< 1	< 1	< 1	< 1	< 1	579	549	610	590	564

[1)] Calculated composition
[2)] Analysed composition

changes in maize and soyabean meal so as the diets remained isonitrogenous and contained the same amounts of essential amino acids. Crumbled starter and pelleted grower and finisher diets were supplied *ad libitum*. The diets were analyzed for nitrogen using Dumas procedure and for protein-bound and free amino acids by ion-exchange chromatography as described by Llames and Fontaine (1994). GAA was analyzed as described by Michiels *et al.* (2012 – Tab. II).

Statistical Analysis

Experimental data were analyzed as a completely randomized block design by General Linear Model of Statgraphics Plus 3.1 package, using GAA supplementation and AME_n levels as categorical variables. When the analysis indicated significant ($P < 0.05$) relationship between dependent and predictor variables, the differences between treatment means were assessed by Tukey HSD test. Linear and quadratic models were fitted to data to describe the relationships between variables. Pen was considered the experimental unit except for carcass analysis data, where individual birds were used as experimental units.

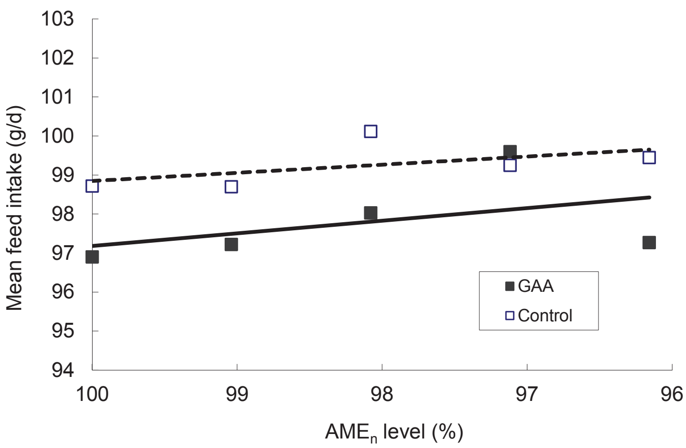
RESULTS AND DISCUSSION

The data on feed intake are summarized in Tab. III. As dietary AME_n density decreased, feed intake in both control and GAA-treated chickens increased (Fig. 1). This response has been frequently observed in *ad libitum*-fed chickens (Leeson *et al.*, 1996). Except for the starting period, the effect of AME_n level on feed intake in the present experiment was rather small and insignificant, possibly due to a narrow range of dietary AME_n concentrations studied. On the other hand, the multiple linear regression

III: *Mean feed intake (g/day) of chickens during the experiment*

Treatment	AME_n level	Time interval (days)			
		1–10	11–24	25–35	1–35
Control	100	26.85[a]	85.03[a]	169.00[a]	98.72[a]
	99	26.93[a]	85.82[a]	167.90[a]	98.70[a]
	98	27.67[a]	85.58[a]	170.65[a]	100.12[a]
	97	28.30[a]	85.55[a]	168.57[a]	99.25[a]
	96	28.57[a]	86.20[a]	168.32[a]	99.45[a]
Guanidinoacetic acid	100	27.23[a]	82.33[a]	166.43[a]	96.90[a]
	99	27.48[a]	84.50[a]	164.67[a]	97.22[a]
	98	27.48[a]	84.93[a]	166.55[a]	98.03[a]
	97	28.08[a]	85.00[a]	170.38[a]	99.60[a]
	96	28.25[a]	82.93[a]	165.82[a]	97.27[a]
Pooled SEM		0.440	1.096	1.430	0.890
Significance A[1)]		0.858	0.007	0.025	0.012
Significance B[1)]		0.008	0.364	0.205	0.288

[a] Means within a column sharing a common superscript were not significantly different. Tukey HSD test, $P < 0.05$
[1)] A = GAA vs. control; B = AME_n levels



1: *Effect of GAA supplementation on mean feed intake of chickens at five dietary AME_n levels. Plotted from the equations $y = 120.23 - 0.214x$, $R^2 = 0.02$ (Control) and $y = 129.63 - 0.325x$, $R^2 = 0.04$ (GAA)* analysis relating feed intake (FI, g/d) to AME_n level (MEL, %) and AME_n intake (MEI, kJ/d) for the whole experimental period yielded the following regression equation:

$$FI = 98.43 - 1.0038\ MEL + 0.0769\ MEI$$
$$P < 0.001,\ R^2 = 0.999$$

All the model parameters were highly significant ($P < 0.001$) and the R-squared statistic indicated that there was a close relationship between the variables. This finding suggests that the chickens were able, at least partly, to adjust their feed intake to meet their AME_n needs.

Furthermore, as shown in Fig. 1, the GAA-treated chickens consumed less feed than their control counterparts, the differences being significant in all growing periods except for the first one. This response might have been due to the better energy utilization in chickens receiving the GAA-supplemented diets or a negative effect of GAA on voluntary feed intake. A similar effect was reported by Mousavi *et al.* (2013), even though the differences between GAA-treated and control groups were not significant ($P = 0.09$) in their study. GAA supplements also reduced feed intake in turkeys (Lemme *et al.*, 2010). In contrast, Michiels *et al.* (2012) found slightly higher feed intakes in chickens receiving supplements of 0.6 or 1.2 g/kg GAA as compared with the control birds. No effect of GAA on feed intake of broilers was observed by Ringel *et al.* (2007).

The effect of GAA supplementation on body weights or weight gains was small and, except for the finishing period, the differences as compared to the control group were not significant (Tabs. IV–V). A greater response to GAA was observed at higher AME_n levels. Growth rate increased quadratically with decreasing AME_n level and reached maximum around 97% of AME_n requirement (Fig. 2). Better growth performance at lower AME_n intakes may be explained by higher feed consumption and consequently by higher intake of protein, amino acids and other nutrients. This is evidenced by Fig. 3, showing the relationship between lysine intake and body weight gains for the whole experiment. The response of control chickens to AME_n level tended to be greater than that of GAA-treated chickens, but the difference between the linear slopes fitted to experimental data was not significant ($P = 0.35$).

Feed conversion ratio (Tab. VI) was not significantly affected by dietary AME_n level, but it responded positively to GAA supplementation. Significant differences were found in the finishing period and for the whole experiment, but numerical improvements were also observed in the starting and growing periods. This is in line with the report by Michiels *et al.* (2012) who concluded that the GAA effect was most apparent in the finisher period, when weight gains were maximized. There are other studies demonstrating a positive effect of GAA on feed conversion (Lemme *et al.*, 2007; Ringel *et al.*, 2008). Similarly to growth rate, the effect of GAA on FCR diminished with decreasing AME_n level (Fig. 4), but the slope of regression line was significantly different from zero only in the control group. In contrast to FCR, efficiency of AME_n conversion was significantly improved by both GAA supplementation and AME_n level. The effect was most apparent in the finishing period and at the highest AME_n intake (Tab. VII, Fig. 5). In both groups, energy utilization significantly increased with decreasing AME_n level (and consequently increasing feed and amino acid intakes). These results suggest that the ratio of amino acids to AME_n in the present diets might have been underestimated.

Of the carcass analysis parameters, only breast meat yield was significantly affected by

IV: *Mean body weights of chickens (g) at 1, 10, 24 and 35 days of age*

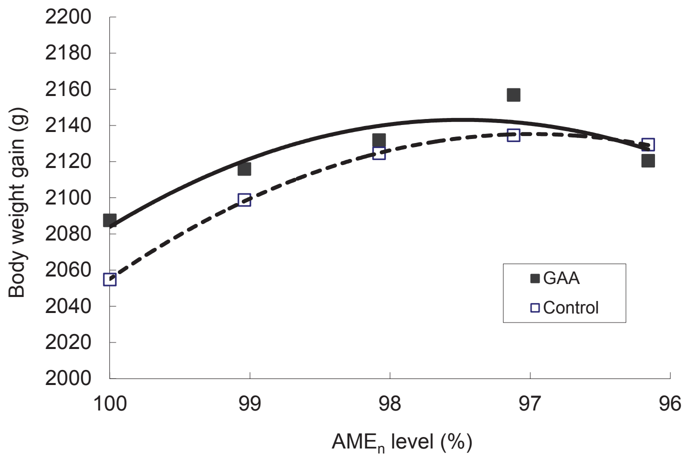
Treatment	AME_n level	1 d	10 d	24 d	35 d
Control	100	42.4^{a}	262^{a}	1089^{a}	2097^{a}
	99	42.5^{a}	266^{a}	1101^{a}	2141^{ab}
	98	42.4^{a}	270^{a}	1118^{a}	2167^{ab}
	97	42.5^{a}	277^{a}	1125^{a}	2177^{ab}
	96	42.4^{a}	276^{a}	1119^{a}	2172^{ab}
Guanidinoacetic acid	100	42.5^{a}	270^{a}	1083^{a}	2130^{ab}
	99	42.4^{a}	276^{a}	1100^{a}	2159^{ab}
	98	42.4^{a}	275^{a}	1114^{a}	2174^{ab}
	97	42.3^{a}	276^{a}	1123^{a}	2199^{b}
	96	42.3^{a}	275^{a}	1101^{a}	2163^{ab}
Pooled SEM		0.17	5.14	14.74	18.26
Significance A[1)]		0.536	0.197	0.500	0.220
Significance B[1)]		0.947	0.300	0.093	0.002

[a,b] Means within a column not sharing a common superscript were significantly different. Tukey HSD test, $P < 0.05$
[1)]A = GAA vs. control; B = AME_n levels

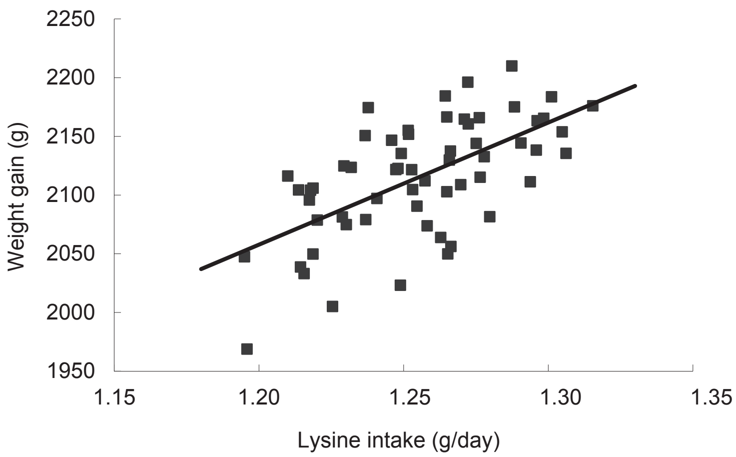
V: *Mean body weight gains of chickens (g)*

Treatment	AME_n level	Time interval (days)			
		1–10	11–24	25–35	1–35
Control	100	220[a]	827[ab]	1008[a]	2055[a]
	99	223[a]	836[ab]	1040[a]	2099[ab]
	98	227[a]	849[b]	1049[a]	2125[bc]
	97	235[a]	848[b]	1052[a]	2135[bc]
	96	234[a]	843[b]	1053[a]	2129[bc]
Guanidinoacetic acid	100	228[a]	812[a]	1047[a]	2088[ab]
	99	233[a]	825[ab]	1058[a]	2116[bc]
	98	233[a]	839[b]	1060[a]	2132[bc]
	97	233[a]	847[b]	1076[a]	2157[c]
	96	232[a]	826[ab]	1062[a]	2121[bc]
Pooled SEM		5.1	5.5	16.2	18.4
Significance A[1)]		0.189	0.211	0.047	0.218
Significance B[1)]		0.284	0.223	0.197	0.002

[a,b,c] Means within a column not sharing a common superscript were significantly different. Tukey HSD test, $P < 0.05$
[1)] A = GAA vs. control; B = AME_n levels



2: *Effect of GAA supplementation on body weight gains of chickens at five dietary AME_n levels. Plotted from the equations $y = -81313.6 + 1720.72x - 8.870x^2$, $R^2 = 0.31$ (Control) and $y = -86092.8 + 1810.31x - 9.285x^2$, $R^2 = 0.22$ (GAA)*

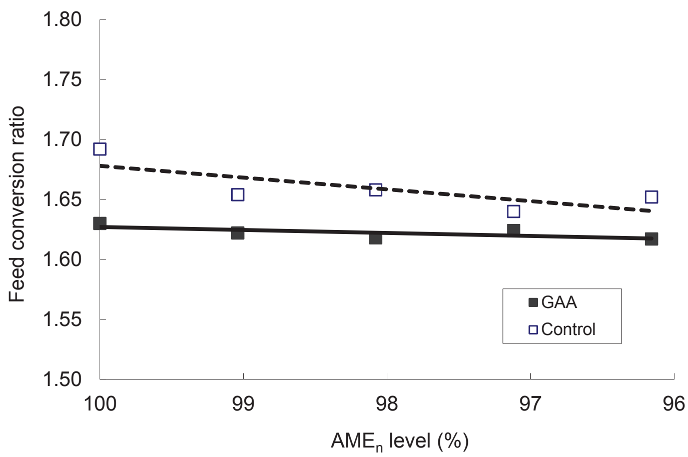


3: *Effect of lysine intake on weight gains of chickens. Each point represents one pen. Plotted from the equation $y = 809.9 + 1039.9x$, $P < 0.001$, $R^2 = 0.38$*

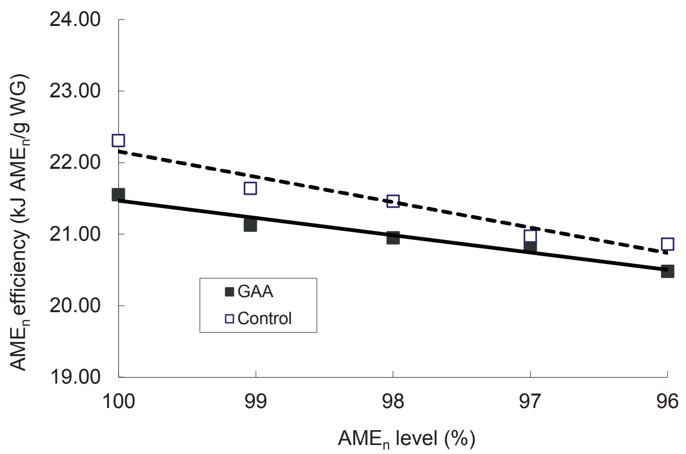
VI: *Feed conversion ratios of chickens during the experiment*

Treatment	AME_n level	Time interval (days)			
		1–10	11–24	25–35	1–35
Control	100	1.101[a]	1.443[a]	2.034[a]	1.692[a]
	99	1.091[a]	1.437[a]	1.957[ab]	1.654[ab]
	98	1.098[a]	1.427[a]	1.974[ab]	1.658[ab]
	97	1.086[a]	1.414[a]	1.956[ab]	1.640[ab]
	96	1.103[a]	1.431[a]	1.960[ab]	1.652[ab]
Guanidinoacetic acid	100	1.077[a]	1.417[a]	1.926[ab]	1.630[c]
	99	1.063[a]	1.438[a]	1.895[b]	1.622[c]
	98	1.063[a]	1.415[a]	1.905[ab]	1.618[c]
	97	1.085[a]	1.403[a]	1.921[ab]	1.624[c]
	96	1.098[a]	1.403[a]	1.904[ab]	1.617[c]
Pooled SEM		0.021	0.022	0.029	0.012
Significance A[1)]		0.160	0.252	< 0.001	< 0.001
Significance B[1)]		0.795	0.670	0.352	0.122

[a,b,c] Means within a column not sharing a common superscript were significantly different. Tukey HSD test, $P < 0.05$
[1)] A = GAA vs. control; B = AME_n levels



4: *Effect of GAA supplementation on feed conversion ratio of chickens at five dietary AME_n levels. Plotted from the equations $y = 0.686 + 0.0099x$, $R^2 = 0.17$ (Control) and $y = 1.379 + 0.0025x$, $R^2 = 0.02$ (GAA)*

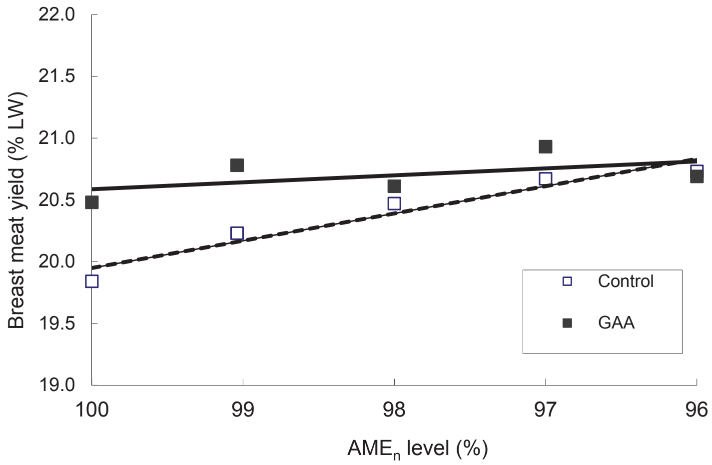


5: *Effect of GAA supplementation on AME_n efficiency in chickens at five dietary ME levels. Plotted from the equations $y = -14.82 + 0.370x$, $R^2 = 0.62$ (Control) and $y = -3.878 + 0.253x$, $R^2 = 0.53$ (GAA)*

VII: *Energy efficiency (kJ AME_n/g WG) in chickens during the experiment*

Treatment	AME_n level	Time interval (days)			
		1–10	11–24	25–35	1–35
Control	100	15.47[a]	19.00[a]	24.70[a]	22.31[a]
	99	15.18[a]	18.81[a]	23.57[ab]	21.64[ab]
	98	15.13[a]	18.48[a]	23.54[ab]	21.46[bcd]
	97	14.80[a]	18.13[a]	22.96[b]	20.97[bcde]
	96	14.90[a]	18.16[a]	22.68[b]	20.86[cde]
GAA	100	15.15[a]	18.70[a]	23.45[ab]	21.55[bc]
	99	14.79[a]	18.74[a]	22.72[b]	21.13[bcde]
	98	14.65[a]	18.34[a]	22.72[b]	20.95[bcde]
	97	14.81[a]	17.99[a]	22.67[b]	20.83[de]
	96	14.83[a]	17.82[a]	22.14[b]	20.48[e]
Pooled SEM		0.289	0.280	0.330	0.153
Significance A[1)]		0.165	0.260	< 0.001	< 0.001
Significance B[1)]		0.405	0.004	< 0.001	< 0.001

[a,b,c,d,e] Means within a column not sharing a common superscript were significantly different. Tukey HSD test, $P < 0.05$
[1)]A = GAA vs. control; B = AME_n levels



6: *Effect of GAA supplementation on breast meat yield of chickens at five dietary AME_n levels. Plotted from the equations $y = 42.90 - 0.230x$, $R^2 = 0.05$ (Control) and $y = 26.33 - 0.058x$, $R^2 = 0.04$ (GAA)*

GAA supplementation (Tab. VIII). However, the magnitude of response was small. On average, the difference between control and GAA-treated groups was 0.4% (20.4 vs. 20.7%). Ringel *et al.* (2007) and Michiels *et al.* (2012) reported a more pronounced effect of GAA on breast meat yield, which tended to increase with increasing dietary GAA level (Ringel *et al.*, 2007). In contrast, no effect of GAA on carcass parameters was found in the study by Mousavi *et al.* (2013). As dietary concentration of AME_n in the present experiment decreased, the percentage of breast meat increased, particularly in the control birds (Fig. 6). The response pattern coincided with that of body weight which corroborates the well-known fact that breast meat yield is closely related to body or carcass weight (Ross 308 Broiler Performance Objectives, 2012).

The mechanism by which GAA affects broiler performance is not fully understood. Guanidinoacetic acid is a natural precursor of creatine, which is involved in cell energy metabolism, particularly in tissues with high and varying energy demand such as skeletal muscle (Michiels *et al.*, 2012). In fast-growing animals fed all-vegetable diets lacking creatine, GAA may restore creatine reserves, thus improving tissue energy metabolism and body growth. Another mode of action of GAA may be associated with amino acid metabolism. In vivo, GAA is synthesized from glycine and arginine and GAA supplementation may thus spare arginine, one of the potentially limiting amino acids in low-protein broiler diets (Baker, 2009). Indeed, Dilger *et al.* (2013) showed that GAA supplementation markedly improved growth of chickens fed arginine-deficient casein-based

VIII: *Results of carcass analysis*

Treatment	AME_n level	n	Body weight (g)	Carcass yield (% LW)	Breast meat yield (% LW)	Leg meat yield (% LW)	Abdominal fat yield (% LW)
Control	100	60	2188.0[a]	68.43[a]	19.84[a]	17.69[a]	1.35[a]
	99	60	2183.3[a]	68.86[ab]	20.23[bc]	17.95[a]	1.31[a]
	98	60	2200.8[ab]	68.81[ab]	20.47[de]	17.76[a]	1.24[a]
	97	60	2238.5[bcd]	69.26[b]	20.67[ef]	17.84[a]	1.37[a]
	96	60	2216.2[abc]	69.11[ab]	20.73[ef]	18.04[a]	1.36[a]
Guanidinoacetic acid	100	60	2215.8[abc]	68.54[a]	20.48[b]	17.73[a]	1.38[a]
	99	60	2244.5[cd]	69.05[ab]	20.78[cd]	18.00[a]	1.30[a]
	98	60	2263.0[d]	69.27[b]	20.61[de]	17.99[a]	1.31[a]
	97	60	2194.8[a]	69.05[ab]	20.93[ef]	17.68[a]	1.34[a]
	96	60	2219.2[abc]	68.85[ab]	20.69[f]	17.92[a]	1.33[a]
Pooled SEM			9.13	0.158	0.243	0.136	0.044
Significance A[1)]			0.002	0.564	0.003	0.920	0.760
Significance B[1)]			0.037	< 0.001	0.002	0.154	0.340

[a,b,c,d] Means within a column not sharing a common superscript were significantly different. Tukey HSD test, $P < 0.05$
[1)] A = GAA vs. control; B = AME_n levels

diets. In practical-type diets with moderate arginine deficiency, there was no growth response to GAA, but feed conversion was significantly improved. In accordance with the present study, these results suggest that feed conversion ratio is a more sensitive indicator of nutritient adequacy than growth rate in broilers.

CONCLUSIONS

Supplementation of broiler diets with guanidinoacetic acid decreases voluntary feed intake, improved efficiency of energy utilization and increased breast meat yield.

With slightly decreasing AME_n level of isonitrogenous diets, voluntary feed intake increased and, as a result of higher amino acid intake, breast meat yield increased.

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