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Effects of dietary energy level and guanidinoacetic acid supplementation on growth performance, carcass quality and intestinal architecture of broilers

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Abstract: Energy, known as the most expensive nutrient in broiler feed, is what strongly adjusts and affects the growth of broilers. Creatine has a key role in cellular energy metabolism and could be synthesised from guanidinoacetic acid (GAA) in the liver; however, its *de novo* synthesis is not able to adequately fulfil the demand of energy metabolism, especially in fast-growing modern broilers. So the aim of the study was to evaluate the efficiency of commercial GAA in energy-reduced broiler diets on performance and intestinal development. Overall, 11 400 day-old Ross 308 chicks were randomly allocated to six dietary treatments with ten replicates in each. Dietary treatments were designed as a 3 × 2 factorial arrangement with three levels of dietary metabolisable energy (AME_n) recommended by Aviagen for Ross 308 broilers (12.55 MJ/kg, 12.97 MJ/kg and 13.38 MJ/kg for starter, grower and finisher, respectively), 0.209 MJ/kg and 0.418 MJ/kg reduced and two levels of GAA (0.00% and 0.06%). There was no significant GAA × AME_n interaction for all performance parameters, carcass traits and jejunal morphological parameters (except for the villus width). Reduction of dietary AME_n (0.209 MJ/kg and/or 0.418 MJ/kg) caused a significant depression in body weight (BW) gain ($P < 0.001$) and feed conversion ratio (FCR) ($P < 0.001$). However, a decreasing AME_n level increased villus height ($P < 0.003$) and villus surface area ($P < 0.03$), while crypt depth and villus width were similar. The GAA improved final BW and FCR by 1.77% and 1.66%, respectively ($P < 0.001$). Birds fed low energy diets supplemented with GAA showed a significant improvement in the performance so that BW and FCR were the same as in the control birds; however, no such positive effects were obtained in jejunal villus development. Hence, it might be concluded that 0.06% GAA supplementation improves BW and FCR and can save at least 0.209 MJ/kg dietary AME_n in broiler diets.

Keywords: broiler; creatine; energy utilisation; jejenum morphologhy; breast meat

The persistent and continuous cost rise of dietary energy sources has forced nutritionists to look for ways to cut down final feed costs. This has occasionally led to the choice of poor-quality energy feed ingredients as one alternative to face the problem. Such practices can result in poor performance related to body weight gain (BWG), feed conversion ratio (FCR) and generally low pro-

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ductivity of the birds (Ahiwe et al. 2018). The second choice is the reduction (alteration) of dietary energy in order to improve the energy efficiency of the bird. However, such a process bears physical and biological restrictions. It is worth mentioning that the current growth rate is considerably high in modern broilers, and this helps to reduce the growing periods. On the other hand, birds on such diets experience limitations in feed intake (FI) as the digestive system development and body growth are not exactly on the same line. Although this importance of higher or lower dietary energy should not be underestimated as it further influences the efficient growth rate, it should be kept in mind that the birds cannot tolerate FI beyond a certain energy level (Dozier et al. 2007; Tallentire et al. 2018). Although the integrity of the gastrointestinal system of a bird depends on several factors, nutrients from diet play an important role in the maintenance of the integrity of the intestinal system (Adedokun and Olejede 2019). Dietary supplementation of guanidinoacetic acid (GAA) as creatine precursor was shown to have sparing effects for arginine (Arg) because of the limited level, especially in starter and grower diets based on maize and soybean meal, which increase Arg availability in broiler chickens (Baker 2009).

Mousavi et al. (2013) reported that supplemental GAA improved energy efficiency by decreasing caloric consumption per kg BWG and additional carcass weight. Indeed, GAA supplementation (0.06%) have been reported to alleviate the effects of reduced dietary metabolisable energy (up to 95% of the Cobb 500 recommendation) on growth performance in broiler chickens (Mousavi et al. 2013).

Although the effects of dietary energy level on gut integrity have been examined in a few studies, their results related to intestinal morphology in broilers were contradictory because it depends on the type of applied regime. For example, increasing FI can adversely affect the intestinal villus length, which can result in shorter villi and deeper crypts (van Beers-Schreurs et al. 1998). Short villus height and large crypts can lead to poor nutrient absorption, increased secretion in the gastrointestinal tract, and lower performance (Xu et al. 2003). Ale Saheb Fosoul et al. (2016; 2018) reported that a reduction in a dietary energy level significantly reduced the height of jejunal villi in broilers.

Most trials examining the effects of dietary GAA have focused on growth performance or meat quality; however, some recent ones have examined the energy and GAA interaction, but no or minimal attention has been given to the effect of dietary GAA on gut integrity. Besides, broiler experiments are run with a relatively small number of birds at university research centres which are not always comparable with a farm with high stocking density and challenges such as litter hygiene that is generally considered high. For example, in order to maintain the needs of the intestinal mucosa, the level of threonine increases in broilers exposed to environments with low hygiene (Corzo et al. 2007). Thus, one can suggest that the effects of dietary GAA on performance and gut integrity can be evaluated better in broilers reared under commercial conditions.

So the present study hypothesised that supplementation of GAA in diets with different energy contents might influence growth performance and intestinal morphology of broiler chickens, either by improving the cellular energy status or by sparing Arg and consequently influencing the gut integrity. Therefore, the present study was conducted to evaluate the interaction effects of GAA and reduced energy levels of broiler diets on growth performance, gut morphology, and processing yields in a commercial-scale research house.

MATERIAL AND METHODS

Birds and housing

The research was conducted in the broiler research development centre of Beypilic, one of the biggest broiler integration companies located in the Bolu region of Turkey. A total of 11 400 Ross 308 broiler hatchlings (obtained from Beypilic Co. hatchery, Bolu, Turkey, as hatched), with an average body weight of 41.71 g, were randomly allocated to six experimental groups with ten replicate pens (6.5 × 2 m; covered with wood shavings as a litter material) containing 190 birds each. Birds were raised in an environmentally controlled house for 43 days. Each pen was equipped with an automatic feeder and nipple drinkers. Birds had *ad libitum* access to water and feed during the entire experimental period. All pens were checked daily for general health and mortality. The birds received ND and IBV vaccines on day 13 and the second ND vaccine on day 26 of the trial. The house temperature was

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34 °C on the first day and it gradually decreased to 22 °C after three weeks of age by using the automated heating, cooling and ventilation systems. Relative humidity was kept at 50 ± 5%. The house was artificially ventilated. All experimental procedures and animal care were reviewed and approved by The Animal Ethics Committee of Ankara University (2018-15-91).

Diets and experimental design

The treatments consisted of three levels of dietary metabolisable energy (AME_n) (0 MJ/kg, 0.209 MJ/kg and 0.418 MJ/kg reduced) and two dietary levels of GAA (CreAMINO®; Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany, 0.00% and 0.06%) arranged as a 3 × 2 factorial design. The control diets contained 12.55 MJ/kg, 12.97 MJ/kg and 13.38 MJ/kg for starter, grower and finisher/withdrawal feeds, respectively (Table 1). A decrease in the AME_n was achieved by lowering the amount of dietary soy oil in the feed formulation. All trial diets were maize-soybean meal isonitrogenous diets containing the same amounts of essential amino acids, and the difference was only in dietary energy level and inclusion of GAA. Experimental treatments were as follows: T1 – recommended AME_n MJ/kg; T2 – 0.209 MJ/kg less AME_n than the control; T3 – 0.418 MJ/kg less AME_n than the control; T4 – recommended AME_n MJ/kg + 0.06% GAA; T5 – 0.209 MJ/kg less AME_n than the control + 0.06% GAA; T6 – 0.418 MJ/kg less AME_n than the control + 0.06% GAA. The GAA was administered by adding the feed additive CreAMINO® (> 96% GAA; Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany) on top of the basal diets (600 mg/kg). The GAA content of the experimental feeds was determined according to the ion chromatography method (CRL-FA 2007). Experimental treatments and analysed GAA content (mg/kg) of trial feeds are presented in Table 2. Feeds were produced in the Beypilic feed mill. Starter (0–10 days) feed was in crumble form, and grower and finisher (11–43 days) feeds were in pellet form. Proximate analyses (AOAC 2006; data not shown) were applied for feed ingredients. The mixer was operated for 3.5 min with all ingredients, and then feeds were pelleted in a mill conditioned at 75 °C. Proximate chemical analysis of all trial diets was performed (results included in Table 1).

Data collection and sampling

Birds were weighed at the beginning, on day 10 and 43 in each of the replicates. Feed intake and FCR were also calculated for the same periods. Dead birds were weighed and used to calculate adjusted FCR. At the end of the trial (day 43), four birds per pen, close to the average pen weight, were leg-banded, exsanguinated humanly by cutting the jugular vein, allowed to bleed for approximately 2 min, and then eviscerated. Whole dressed carcass (without abdominal fat and giblets), whole legs (thighs + drumsticks) and breast (pectoralis major and minor muscles) as bone-in and skin-on and also liver were weighed, and yields were expressed as a fraction of the individual live body weight (BW).

For the histological examination, segments of 5 cm long were taken from the midpoint of the jejunum (towards the proximal duodenum) from male broilers (the same birds were used for the carcass quality) and flushed with a saline solution. These segments were fixed in 10% neutral buffered formalin at room temperature for 72 hours. After the routine histological procedures, samples were embedded in paraffin wax. Sections having a thickness of 4 µm were taken on the glass slide using a microtome. Images were taken with a computer-supported imaging system (ImageJ; Laboratory for Optical and Computational Instrumentation, University of Wisconsin-Madison, Madison, WI, USA) connected to a light microscope (Zeiss Axio Lab.A1 Microscope and Zeiss AxioCam ICc 5 Camera; Cacr Zeiss AG, Oberkochen, Germany). The variables measured were villus height and width, villus height to crypt depth ratio and villus surface area. Villus height was measured from the tip of the villus to the junction of the villus and crypts, and the crypt depth was measured from its base up to the region of transition between the crypt and villus. The surface area of the villus was measured using the following formula: $(2\pi) \times (\text{villus width}/2) \times (\text{villus length})/10^6$ (Solis de los Santos et al. 2005).

Statistical analysis

Data for all response variables related to the different phases of the trial were analysed as a completely randomised design with six dietary treatments and ten replicate blocks, using the general ANOVA pro-

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Table 1. Ingredients and nutrient composition of the basal diets through the starter and grower period, g/kg as fed basis[1]

Ingredients	Starter			Grower			Finisher			Withdrawal		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Corn	461.74	472.81	485.02	492.26	503.32	514.36	546.43	557.70	568.98	586.64	597.92	609.19
Soybean meal (46%)	287.33	286.82	292.07	237.53	237.02	236.51	184.78	184.37	183.96	153.29	152.88	152.47
Full fat soybean (34.5%)	156.87	155.31	145.81	160.69	159.15	157.61	154.38	152.51	150.62	152.95	151.08	149.20
Sunflower meal (36.5%)	30.00	30.00	30.00	40	40	40	40.00	40.00	40.00	40.00	40.00	40.00
Soy oil	25.00	16.00	8.00	35	26	17	42.00	33.00	24.00	36.00	27.00	18.00
Dicalcium phosphate	18.59	18.56	18.55	15.88	15.85	15.82	14.24	14.20	14.17	13.79	13.76	13.73
Limestone	6.30	6.32	6.34	5.61	5.63	5.65	5.12	5.15	5.17	5.03	5.05	5.07
$NaHCO_3$	2.10	2.10	2.10	2	2	2	2.20	2.20	2.20	2.20	2.20	2.20
Salt	2.50	2.50	2.50	2.5	2.5	2.5	2.50	2.50	2.50	2.50	2.50	2.50
Lysine HCL (78%)	1.80	1.83	1.87	1.33	1.36	1.39	1.44	1.47	1.51	1.63	1.66	1.70
DL-methionie	3.75	3.74	3.73	3.21	3.19	3.18	2.75	2.74	2.73	2.55	2.53	2.52
Choline (75%)	0.42	0.41	0.41	0.39	0.38	0.38	0.41	0.41	0.41	0.42	0.42	0.42
Vitamin premix[2]	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mineral premix[3]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Anticoccidial	0.60	0.60	0.60	0.60	0.60	0.60	0.75	0.75	0.75	0.00	0.00	0.00
Total	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000	1 000
Calculated and analysed contents[4]												
Gross energy (MJ/kg)	17.80	17.54	17.20	18.26	17.99	17.67	18.59	18.25	17.96	18.53	18.22	17.94
ME (MJ/kg)	12.55	12.34	12.13	12.97	12.76	12.55	13.38	13.17	12.97	13.38	13.17	12.97
Crude protein (%)	23.0 (23.27)	23.0 (23.32)	23.0 (23.47)	21.5 (21.74)	21.5 (21.85)	21.5 (21.65)	19.5 (19.87)	19.5 (19.81)	19.5 (19.99)	18.3 (18.52)	18.3 (18.56)	18.3 (18.79)
Crude fiber (%)	3.41 (3.23)	3.42 (3.33)	3.41 (3.31)	3.47 (3.54)	3.48 (3.35)	3.49 (3.40)	3.63 (3.39)	3.64 (3.52)	3.66 (3.62)	3.61 (3.44)	3.63 (3.33)	3.64 (3.50)
Crude ash (%)	6.66 (6.46)	6.66 (6.34)	6.66 (6.52)	6.12 (5.98)	6.12 (5.89)	6.12 (6.01)	5.7 (5.51)	5.7 (5.61)	5.7 (5.45)	5.49 (5.19)	5.49 (5.05)	5.49 (5.27)
Crude fat (%)	7.31 (7.08)	6.43 (6.52)	5.51 (5.44)	8.44 (8.23)	7.56 (7.39)	6.68 (6.79)	9.47 (9.25)	8.59 (8.08)	7.7 (7.47)	8.97 (8.57)	8.09 (7.82)	7.2 (7.31)
Ca (%)	0.91	0.91	0.91	0.81	0.81	0.81	0.74	0.74	0.74	0.72	0.72	0.72
Total P (%)	0.73	0.73	0.73	0.67	0.67	0.67	0.64	0.64	0.64	0.62	0.52	0.62
Available P (%)	0.455	0.455	0.455	0.405	0.405	0.405	0.370	0.370	0.370	0.360	0.360	0.360
Na (%)	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17	0.17
Cl (%)	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19	0.19

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Table 1 to be continued

Ingredients	Starter			Grower			Finisher			Withdrawal		
	T1	T2	T3	T1	T2	T3	T1	T2	T3	T1	T2	T3
Methionine (%)	0.71	0.71	0.71	0.64	0.64	0.64	0.57	0.57	0.57	0.53	0.53	0.53
Methionine + Cystine (%)	1.08	1.08	1.08	0.99	0.99	0.99	0.90	0.90	0.90	0.85	0.85	0.85
Lysine (%)	1.44	1.44	1.44	1.29	1.29	1.29	1.15	1.15	1.15	1.08	1.08	1.08

T1 = recommended AME_n (nitrogen corrected metabolisable energy) MJ/kg; T2 = 0.209 MJ/kg less AME_n than the control; T3 = 0.418 MJ/kg less AME_n than the control

[1]CreAMINO (GAA) was supplemented to basal diets to obtain T4, T5 and T6

[2,3]Supplied the following per kg of complete feed: 4.13 mg retinyl acetate; 125 µg cholecalciferol; 100 mg DL-α-tocopheryl acetate; 3.5 mg menadione; 3.5 mg thiamine; 9 mg riboflavin; 20 mg calcium D-pantothenate; 65 mg niacin; 0.02 mg vitamin B_{12}; 2.2 mg folic acid; 4.5 mg pyridoxine; 0.22 mg biotin; 120 mg manganese (Mn oxide); 1.5 mg iodine (Ca iodate); 25 mg iron (Fe sulphate); 16 mg copper (Cu sulphate); 110 mg zinc (Zn oxide); 0.3 mg selenium (Na selenite)

[4]Values given inside the parentheses indicate analysed results, and values outside the parentheses refer to calculated values

cedure of SAS/STAT® v9.2 software (SAS Institute, Inc., Cary, NC, USA). Main effects and interactions between the main effects were calculated in a 2 × 3 factorial arrangement in a completely randomised design. When significant differences ($P < 0.05$) were found between groups, means were separated using the Tukey HSD test. The chi-square test assessed mortality results.

RESULTS AND DISCUSSION

This study was designed with the aim of investigating the effect of GAA supplementation and dietary energy interaction on broiler performance under the influence of commercial conditions. Performance data related to 0–10, 11–43 and 0–43 day periods are presented in Table 3. There were no significant interactions between GAA and dietary energy level on various performance parameters. Neither dietary energy level nor GAA and their interaction had a significant effect on mortality (Table 3). During the starter period, FI and FCR were negatively affected only by dietary energy levels, when 0.418 MJ/kg reduced energy significantly impaired FI ($P < 0.001$) and FCR ($P < 0.01$), while BWG was not affected. However, after the starter period, a reduction of dietary AME_n, at both levels, significantly decreased BWG, so that birds fed T2 and T3 diets showed lower BWG in the 11–43 day period, and also when looking at the whole period of the trial (Table 3) compared with the control ($P < 0.001$).

Reducing energy by 0.209 MJ/kg and 0.418 MJ/kg (T2 and T3) caused significant impairment in FCR compared with T1 diet ($P < 0.05$; Table 3). During the whole period, in spite of consuming almost the same amount of feed, the birds that received a low energy diet gained less body weight compared with birds fed on T1 diet, i.e. resulting in poorer FCR ($P < 0.001$, Table 3). Our findings are consistent with reports related to the energy and FCR relationship (Hu et al. 2019). On the other hand, contrary to the growth performance results of the present study, Sharma et al. (2018) reported that reducing dietary energy from 13.59 MJ/kg to 12.76 MJ/kg (–0.836 MJ/kg) in 14–34 days boosted WG without negative impact on FCR. Although it is well known that the low level of energy negatively influences the growth performance, the response of growing broilers to energy density is variable and

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Table 2. Experimental treatments and analysed guanidinoacetic acid (GAA) content (mg/kg) of dietary treatments[1]

Treatments	Experimental treatments		Starter	Grower	Finisher
	energy level	GAA			
T1	normal AME_n	no	< 20	< 20	< 20
T2	0.209 MJ/kg low AME_n	no	< 20	< 20	< 20
T3	0.418 MJ/kg low AME_n	no	< 20	< 20	< 20
T4	normal AME_n	yes	591	595	571
T5	0.209 MJ/kg low AME_n	yes	583	617	646
T6	0.418 MJ/kg low AME_n	yes	555	600	562

AME_n = nitrogen corrected metabolisable energy; T1 = recommended AME_n MJ/kg; T2 = 0.209 MJ/kg less AME_n than the control; T3 = 0.418 MJ/kg less AME_n than the control; T4 = recommended AME_n MJ/kg + 0.06% GAA; T5 = 0.209 MJ/kg less AME_n than the control + 0.06% GAA; T6 = 0.418 MJ/kg less AME_n than the control + 0.06% GAA

[1]No analysis was performed in withdrawal feeds because of three days introduction to save the cost of analysis. CreAMINO® consists of 96% GAA, so the expected GAA content in the feed is 576 mg/kg

may depend on several factors such as bird gender, breed, age, etc., including amino acid density, fat type and inclusion level as well as fibre type and content of diets (Classen 2017). In the present study the energy concentration of the diets was reduced by mainly decreasing the fat level, trying to keep

Table 3. Effects of dietary energy level and guanidinoacetic acid supplementation (GAA) on body weight gain (BWG), feed intake (FI) and whole period mortality of broilers

Treatments	Initial weight (g)	BWG (g)			FI (g)			FCR			Mortality (%) 0–43 days
		0–10 days	11–43 days	0–43 days	0–10 days	11–43 days	0–43 days	0–10 days	11–43 days	0–43 days	
T1	41.83	270.00	2 568.50	2 838.50	321.37	4 330.09	4 648.76	1.190	1.686	1.638	3.00
T2	41.77	267.28	2 544.35	2 811.63	325.84	4 336.72	4 659.13	1.220	1.705	1.658	3.63
T3	41.66	271.99	2 522.82	2 794.81	330.41	4 302.33	4 630.70	1.215	1.706	1.657	2.68
T4	41.73	270.25	2 637.31	2 907.56	323.04	4 326.38	4 646.83	1.196	1.641	1.599	3.11
T5	41.73	270.90	2 583.34	2 854.24	323.02	4 323.92	4 644.45	1.193	1.674	1.627	2.95
T6	41.58	266.50	2 560.79	2 827.30	329.43	4 338.17	4 664.86	1.238	1.694	1.650	3.26
AME_n main effects											
Normal AME_n	41.77	270.14	2 608.98^{a}	2 879.12^{a}	322.351^{b}	4 327.91	4 647.63	1.193^{b}	1.659^{b}	1.614^{b}	3.05
–0.209 MJ/kg AME_n	41.74	269.09	2 563.84^{b}	2 832.93^{b}	324.431^{ab}	4 330.32	4 651.79	1.206^{ab}	1.689^{a}	1.642^{a}	3.29
–0.418 MJ/kg AME_n	41.62	269.247	2 541.81^{b}	2 811.06^{b}	329.918^{a}	4 320.25	4 647.78	1.226^{a}	1.700^{a}	1.653^{a}	2.97
GAA main effects											
0.00%	41.75	269.73	2 542.64	2 812.37	326.372	4 322.27	4 645.91	1.210	1.700	1.652	3.11
0.06%	41.68	269.216	2 593.81	2 863.03	325.163	4 329.49	4 652.05	1.208	1.669	1.625	3.11
SEM	0.521	2.373	17.392	19.631	2.582	17.792	25.970	0.011	0.009	0.008	0.451
***P*-values**											
AME_n	0.756	0.698	0.001	0.001	0.001	0.69	0.997	0.01	0.001	0.001	0.869
GAA	0.565	0.862	0.001	0.001	0.465	0.487	0.537	0.81	0.001	0.001	0.875
AME_n × GAA	–	0.091	0.264	0.245	0.353	0.38	0.381	0.10	0.12	0.09	0.781

AME_n = nitrogen corrected metabolisable energy; FCR = feed conversion ratio; T1 = recommended AME_n MJ/kg; T2 = 0.209 MJ/kg less AME_n than the control; T3 = 0.418 MJ/kg less AME_n than the control; T4 = recommended AME_n MJ/kg + 0.06% GAA; T5 = 0.209 MJ/kg less AME_n than the control + 0.06% GAA; T6 = 0.418 MJ/kg less AME_n than the control + 0.06% GAA

[a,b]Means within the same column without common superscripts are significantly different ($P < 0.05$)

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other nutrients similar to the control diet (Table 1). As indicated in Table 1 and 2, the crude nutrient and GAA analyses confirmed the aim to reduce energy stepwise and in each feeding phase, and insofar the dietary objective was reached. So the response of broilers to both levels of reduced energy concentration in the present study was found as expected.

The results in Table 3 show that FI in birds fed T3 diet during the starter period (0–10 days) was higher ($P < 0.001$) than that of the control birds that received normal dietary energy. This indicates that if the energy level is not dramatically low, physical and biological conditions of the body do not hinder the increase of FI at early ages. Heger et al. (2014) showed that broiler chickens increase their feed consumption by almost 6.4% at 0–10 days when fed a diet containing 3% less energy than the control group (12.27 MJ/kg vs 12.64 MJ/kg, respectively). At the same time, these researchers found no effect of dietary energy reduction on FI in the post starter periods of the trial. These results are consistent with the present study. However, birds that received the 0.209 MJ/kg reduced diet could not compensate BW and FCR during the post starter phase and entire period because they could not increase FI, which is in line with Dozier et al. (2007) and Tallentire et al. (2018), who mentioned that broilers cannot maintain their energy balance by adjusting their FI in case of decreasing dietary energy concentration. The response of broilers to dietary energy has been shown to be affected by physical limits in the digestive tract capacity (Tallentire et al. 2018). These findings are also in line with Heger et al. (2014) and Hu et al. (2019), who found no significant difference in FI after the starter phase when the birds were fed low energy diet compared with control birds (12.64 MJ/kg and 13.38 MJ/kg AME_n, respectively). However, reports and research on the influence of dietary energy intake on FI in broilers have been conflicting. More work is needed to establish if genetic selection for growth has resulted in today's broiler being less sensitive to the classical appetite control mechanisms. The results of Hu et al. (2019) study showed that feeding a high energy diet to broiler chickens inhibited appetite and central adenosine monophosphate-activated protein kinase signalling. In contrast, a diet with lower energy (12.13 MJ/kg) activated central adenosine monophosphate-activated protein kinase and appetite.

The GAA supplementation of all three diets in the present study significantly improved ($P < 0.001$) BWG and FCR in the 11–43 day period and throughout the whole period of the study (Table 3). FCR at the end of the trial (43 days) was found to be 1.625 and 1.652 ($P < 0.001$) for birds receiving feeds with and without GAA, respectively (Table 3).

Mousavi et al. (2013) reported that GAA improved the energy utilisation efficiency by lowering the calorie intake per kg of BWG and carcass weight by 2.9% and 3.7%, respectively.

Hu et al. (2019) recently showed that a diet containing 12.13 MJ/kg AME_n reduced average daily weight gain and increased FCR compared with those having normal (13.38 MJ/kg) or high energy (14.64 MJ/kg) at the end of 21 days. The present study indicates that although dietary GAA has no significant effect on FI, it resulted in a remarkable ($P < 0.001$) improvement in BWG (excluding the starter period) and FCR (Table 3). The results related to FI are in line with observations by European Food Safety Authority (EFSA 2009), Michiels et al. (2012) and Nasiroleslami et al. (2018), who found no effect of GAA on FI. Some reports showed that dietary GAA supplementation in broilers significantly reduced FCR and indicated improvements up to five points in FCR (Lemme et al. 2007a; Mousavi et al. 2013). On the other hand, research has also shown that with the same inclusion level of GAA (0.06%), FCR improved up to seven points compared with the control with no GAA in vegetable-based diet (Ringel et al. 2007). GAA improves FCR without a significant change in FI by improving the cell energy metabolism through increasing the muscle creatine content (Lemme et al. 2007a; Ringel et al. 2007), and some metabolites related to the energy metabolism, such as phosphocreatine and ATP. It has been reported that dietary inclusion of 0.06% GAA significantly increases the ATP level in breast meat from 3.10 µmol/g to 4.30 µmol/g compared with an unsupplemented control group (Lemme et al. 2007a); such changes might improve the utilisation of nutrients for muscle accretion and growth (Lemme et al. 2007b). Other than supporting cell-mediated energy metabolism by *de novo* synthesis of endogenous creatine, GAA has other modes of action such as increasing plasma insulin-like growth factor-I (Michiels et al. 2012) and serving as a direct or indirect (via creatine-induced) pro- or antioxidant (Nasiroleslami et al. 2018), which can be referred to as one of the possible mechanisms be-

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hind the relationship between the inclusion of GAA and improvement in growth performance and carcass yield in the present trial.

When considering carcass parameters and liver percentage of BW at day 43 (Table 4), the effect of dietary AME_n × GAA interaction and the effect of AME_n as the main effect were not significant for these parameters. The main effect of dietary GAA was not significant for the percentage of carcass parts, but carcass yield was significantly ($P < 0.01$) improved almost by 1% when dietary GAA was used (Table 4), so that dietary supplementation of GAA to diets containing normal AME_n and a diet with 0.418 MJ/kg lower AME_n significantly improved carcass yield. The results regarding the effect of GAA on carcass parameters are contradictory. For example, in a study, birds receiving a diet containing GAA showed higher breast meat yield (30.6%) compared with the control (29.4%) (Michiels et al. 2012). Mousavi et al. (2013) found that carcass traits were not affected by dietary GAA inclusion (0% and 0.06%), energy levels and their interactions. More recently, the Arg-sparing effect of GAA was speculated by DeGroot et al. (2019) even when supplemented to a diet with adequate Arg. This might allow Arg to be used for functions other than creatine synthesis, most notably muscle growth (Edwards et al. 1958). In the present study, diets were sufficient in Arg content (over 1.40%, 1.30% and 1.19% digestible lysine for starter, grower and finisher, respectively) to meet the recommended Aviagen level for Ross 308 broilers, which may support the hypothesis of DeGroot et al. (2019) on Arg-sparing, and help to explain the significant increase in carcass yield (Table 4).

Table 4. Effects of dietary energy level and guanidinoacetic acid (GAA) supplementation on carcass parameters and liver [% of body weight (BW)] of broilers[1]

Treatments	Carcass yield (%)[1]	Whole leg yield (%)	Breast yield (%)	Liver (%) of live BW
T1	73.53	19.46	34.47	1.879
T2	73.92	20.19	33.81	1.834
T3	73.95	19.71	34.71	1.895
T4	74.39	20.16	34.58	1.812
T5	74.29	20.10	34.55	1.911
T6	74.99	20.01	34.91	1.867
AME_n main effects				
Normal AME_n	73.98	19.82	34.53	1.844
–0.209 MJ/kg AME_n	74.11	20.14	34.19	1.875
–0.418 MJ/kg AME_n	74.47	19.86	34.81	1.881
GAA main effects				
0.00%	73.80[b]	19.78	34.33	1.869
0.06%	74.54[a]	20.09	34.67	1.863
SEM	0.432	0.291	0.331	0.043
***P*-values**				
AME_n	0.41	0.48	0.14	0.69
GAA	0.01	0.31	0.23	0.75
AME_n × GAA	0.55	0.33	0.55	0.27

AME_n = nitrogen corrected metabolisable energy; T1 = recommended AME_n MJ/kg; T2 = 0.209 MJ/kg less AME_n than the control; T3 = 0.418 MJ/kg less AME_n than the control; T4 = recommended AME_n MJ/kg + 0.06% GAA; T5 = 0.209 MJ/kg less AME_n than the control + 0.06% GAA; T6 = 0.418 MJ/kg less AME_n than the control + 0.06% GAA

[1]Carcass yield represents the yield of bled, defeathered, and eviscerated chickens after removing the head and feet

[a,b]Means within the same column without common superscripts are significantly different ($P < 0.05$)

The effects of dietary AME_n and GAA levels and their interactions on jejunal morphology are presented in Table 5. As for the interaction effects, only the villus width was significantly affected by dietary treatment; when dietary AME_n decreased to 0.209 MJ/kg compared with the control, the villus width was decreased ($P < 0.01$) from 187.51 µm to 164.20 µm. Villus width ($P < 0.01$) and villus surface area ($P < 0.03$) decreased in birds fed T2 diet (Table 5), but interestingly, there was no significant difference between T1 and T3 in the villus surface area (Table 5). Also, birds fed T3 diet had a significantly higher ($P < 0.003$) villus height compared with the control diet with normal AME_n (1 167.85 µm and 1 025.33 µm, respectively). In the present study, the improvement in jejunal villus by reduction of dietary energy might be based on the reason that broilers receiving the reduced energy diet may have increased their absorptive surface area to some extent as a compensatory mechanism to overcome reduced energy intake. Another possible mechanism behind this improvement may be attributed to the importance of protein and amino acids in epithelial cell proliferation of the intestine, as explained by Ale Saheb Fosoul et al. (2016), who reported that the effects of protein on the intestinal morphometric properties are more important than energy. Because the dietary energy level is reduced, the ratio of protein and amino

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Table 5. Effects of dietary energy level and guanidinoacetic acid (GAA) supplementation on jejunal villus morphology of broilers[1]

Treatments	Villus height (μm)	Crypt depth (μm)	Villus length to crypt depth	Villus width (μm)	Villus surface area (mm^2)
T1	1 022.32	186.81	5.70	203.42[a]	0.633
T2	1 060.21	183.27	6.04	162.35[c]	0.542
T3	1 156.13	194.06	6.08	185.87[ab]	0.676
T4	1 028.77	172.17	6.17	168.94[bc]	0.537
T5	1 031.74	181.10	5.89	166.06[bc]	0.532
T6	1 179.56	198.13	6.16	157.38[c]	0.578
AME_n main effects					
Normal AME_n	1 025.33[b]	179.98	5.92	187.51[a]	0.585[ab]
–0.209 MJ/kg AME_n	1 045.97[b]	182.19	5.97	164.20[b]	0.537[b]
–0.418 MJ/kg AME_n	1 167.85[a]	196.09	6.12	171.63[ab]	0.627[a]
GAA main effects					
0.00	1 079.55	188.05	5.94	183.03	0.617
0.06 %	1 082.25	184.30	6.07	163.69	0.550
SEM	44.142	8.809	0.332	7.588	0.033
***P*-values**					
AME_n	0.003	0.09	0.45	0.01	0.03
GAA	0.98	0.41	0.73	0.005	0.03
AME_n × GAA	0.81	0.43	0.64	0.03	0.29

AME_n = nitrogen corrected metabolisable energy; T1 = recommended AME_n MJ/kg; T2 = 0.209 MJ/kg less AME_n than the control; T3 = 0.418 MJ/kg less AME_n than the control; T4 = recommended AME_n MJ/kg + 0.06% GAA; T5 = 0.209 MJ/kg less AME_n than the control + 0.06% GAA; T6 = 0.418 MJ/kg less AME_n than the control + 0.06% GAA

[a–c]Means within the same column without common superscripts are significantly different ($P < 0.05$)

acid to dietary ME increases. In the case of the present study, the lysine to ME ratio, for example, rose to 0.118 (% of MJ ME) from 0.114 (T3 vs T1, respectively). Such increases in the amino acid ratio could help to improve the internal development of the birds receiving reduced energy diets.

Adding dietary GAA had no significant effect on villus height, crypt depth and villus length to crypt depth ratio, but villus width (183.03 μm vs 163.69 μm; $P < 0.005$) and villus surface area (0.617 μm vs 0.550 μm; $P < 0.03$) were significantly lower with dietary inclusion of GAA (Table 5). Ale Saheb Fosoul et al. (2018) showed that a reduction in the energy content of the diet decreased jejunal villus height and crypt depth. Although the mode of action for GAA on intestinal morphology has been proposed by some recent papers (Ahmadipour et al. 2018; Ren et al. 2018), information and data about the issue especially related to GAA and energy interactions are very limited in poultry, and most of the studies has addressed the role of Arg in relation to gut morphology and function. In the present study, birds fed the diet containing GAA had lower villus width and surface area. The effect of dietary GAA on gut integrity can mainly be attributed to the sparing of Arg, which plays an important role as a source of ornithine and precursor of nitric oxide for gut integrity because creatine is synthesised de novo from Arg and glycine. Arginine itself also has an important contribution to the gut mucosa being involved in the maintenance of intestinal enterocytes. In adult rats, 40% of luminal Arg absorbed by the intestinal mucosa is catabolised in a single pass, and the remaining 60% of the absorbed Arg enters the intestinal venous blood intact (Windmueller and Spaeth 1976). So it can be speculated that any reason restricting the use of Arg as an ornithine source or precursor of nitric oxides, such as creatine deficiency in fast-growing broilers fed all-vegetable diets, may lead to weakening the mucosal integrity of the intestine.

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However, the results of the present study do not confirm the above-mentioned possible role of GAA in Arg sparing. In the present study the level of dietary Arg in both normal and reduced AME_n diets, 1.41% and 1.45% respectively, met the recommended level by Aviagen for Ross 308 broilers. So we can assume that the level of Arg was good enough in the diets with or without GAA supplemented to fulfil all metabolic roles, including gut maintenance. This finding is in contrast with some latest reports which showed that GAA supplementation caused better development of the small intestine, particularly greater height and width of intestinal villi (Ahmadipour et al. 2018; Ren et al. 2018).

CONCLUSION

Dietary energy content influences the intake of all other nutrients. However, broilers regulate their FI to adjust for a wide range of diets, with different energy levels at various ages and in response to different factors, including dietary energy. However, any change in FI in relation to dietary energy is affected by the amount of feed in the gut and feed form (as physical limitations) as well as other physiological limitations (Tallentire et al. 2018). Overall, it can be concluded that a reduction of dietary energy in diets (especially 0.418 MJ/kg) significantly impaired the growth performance while adding 0.06% GAA to a diet containing 0.209 MJ/kg less AME_n significantly improved the growth performance (BWG and FCR) of broilers. More studies must be run on different levels of dietary metabolic energy with and without GAA in broilers in order to eliminate contradictory results as far as possible.

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Conflict of interest

The authors declare no conflict of interest.

REFERENCES

Adedokun SA, Olojede OC. Optimizing gastrointestinal integrity in poultry: The role of nutrients and feed additives. Front Vet Sci. 2019 Jan 31;5: 11 p.

Ahiwe EU, Omede AA, Abdallh MB, Iji PA. Managing dietary energy intake by broiler chickens to reduce production costs and improve product quality. In: Yucel B, Taskin T, editors. Animal husbandry and nutrition. London: IntechOpen; 2018 Nov 5. p. 115-45.

Ahmadipour B, Khajali F, Sharifi MR. Effect of guanidinoacetic acid supplementation on growth performance and gut morphology in broiler chickens. J Poult Sci. 2018 Jan;6(1):19-24.

Ale Saheb Fosoul SS, Toghyani M, Gheisari A, Tabeidiyan SA, Mohammadrezaei M, Azarfar A. Performance, immunity, and physiological responses of broilers to dietary energy and protein sequential variations. Poult Sci. 2016 Sep 1;95(9):2068-80.

Ale Saheb Fosoul SS, Azarfar A, Gheisari A, Khosravinia H. Energy utilisation of broiler chickens in response to guanidinoacetic acid supplementation in diets with various energy contents. Br J Nutr. 2018 Jul;120(2):131-40.

AOAC – Association of Official Analytic Chemists. Official methods of analysis. 18th ed. Arlington, USA: Association of Official Analytical Chemists; 2006.

Baker DH. Advances in protein–amino acid nutrition of poultry. Amino Acids. 2009 May 1;37(1):29-41.

Classen HL. Diet energy and feed intake in chickens. Anim Feed Sci Technol. 2017 Nov 1;233:13-21.

Corzo A, Kidd MT, Dozier WA, Pharr GT, Kotsos EA. Dietary threonine needs for growth and immunity of broilers raised under different litter conditions. J Appl Poult Res. 2007 Dec 1;16(4):574-82.

CRL-FA – Community Reference Laboratory for Feed Additives. CRL evaluation report on guanidinoacetic acid CreAmino™. Parma (Italy): European Food Safety Authority; 2007 Aug 29. 8 p. Report No.: D08/FSQ/CVH/DG/D (2007) 19586.

DeGroot AA, Braun U, Dilger RN. Guanidinoacetic acid is efficacious in improving growth performance and muscle energy homeostasis in broiler chicks fed arginine-deficient or arginine-adequate diets. Poult Sci. 2019;98(7):2896-905.

Dozier WA 3rd, Corzo A, Kidd MT, Branton SL. Dietary apparent metabolizable energy and amino acid density effects on growth and carcass traits of heavy broilers. J Appl Poult Res. 2007 Jul 1;16(2):192-205.

Edwards HM Jr, Young RJ, Gillis MB. Studies on arginine deficiency in chicks. J Nutr. 1958 Feb 1;64(2):271-9.

EFSA – European Food Safety Authority. Scientific opinion of the panel on additives and products or substances used

https://doi.org/10.17221/11/2021-CJAS

in animal feed on a request from the European Commission on the safety and efficacy of guanidinoacetic acid as feed additive for chickens for fattening. EFSA J. 2009 Mar;988: 30 p.

Heger J, Zelenka J, Machander V, de la Cruz C, Lestak M, Hampel D. Effects of guanidinoacetic acid supplementation to broiler diets with varying energy content. Acta Univ Agric Silvic Mendel Brun. 2014 Aug 6;62(3):477-85.

Hu X, Wang Y, Sheikhahmadi A, Li X, Buyse J, Lin H, Song Z. Effects of dietary energy level on appetite and central adenosine monophosphate-activated protein kinase (AMPK) in broilers. J Anim Sci. 2019 Nov 4;97(11):4488-95.

Lemme A, Ringel J, Sterk A, Young JF. Supplemental guanidinoacetic acid affects energy metabolism of broilers. In: Proceedings of the 16th European Symposium on Poultry Nutrition; 2007 Aug 26-30; Strasbourg (France): European Federation of WPSA Branches; 2007a. p. 339-42.

Lemme A, Ringel J, Rostagno HS, Redshaw MS. Supplemental guanidinoacetic acid improved feed conversion, weight gain, and breast meat yield in male and female broilers. In: Proceedings of the 16th European Symposium on Poultry Nutrition; 2007 Aug 26-30; Strasbourg (France): European Federation of WPSA Branches; 2007b. p. 26-30.

Michiels J, Maertens L, Buyse J, Lemme A, Rademacher M, Dierick NA, De Smet S. Supplementation of guanidinoacetic acid to broiler diets: Effects on performance, carcass characteristics, meat quality, and energy metabolism. Poult Sci. 2012 Feb 1;91(2):402-12.

Mousavi SN, Afsar A, Lotfollahian H. Effects of guanidinoacetic acid supplementation to broiler diets with varying energy contents. J Appl Poult Res. 2013 Mar 1;22(1):47-54.

Nasiroleslami M, Torki M, Saki AA, Abdolmohammadi AR. Effects of dietary guanidinoacetic acid and betaine supplementation on performance, blood biochemical parameters and antioxidant status of broilers subjected to cold stress. J Appl Poult Res. 2018 Jan 1;46(1):1016-22.

Ren QC, Xuan JJ, Yan XC, Hu ZZ, Wang F. Effects of dietary supplementation of guanidinoacetic acid on growth performance, thigh meat quality and development of small intestine in Partridge-Shank broilers. J Agric Sci. 2018 Nov;156(9):1130-7.

Ringel J, Lemme A, Knox A, McNab J, Redshaw MS. Effects of graded levels of creatine and guanidinoacetic acid in vegetable-based diets on performance and biochemical parameters in muscle tissue. In: Proceedings of the 16th European Symposium on Poultry Nutrition; 2007 Aug 26-30; Strasbourg (France): European Federation of WPSA Branches; 2007. p. 387-90.

Sharma NK, Choct M, Toghyani M, Laurenson YC, Girish CK, Swick RA. Dietary energy, digestible lysine, and available phosphorus levels affect growth performance, carcass traits, and amino acid digestibility of broilers. Poult Sci. 2018 Apr 1;97(4):1189-98.

Solis de los Santos F, Farnell MB, Tellez G, Balog JM, Anthony NB, Torres-Rodriguez A, Donoghue AM. Effect of prebiotic on gut development and ascites incidence of broilers reared in a hypoxic environment. Poult Sci. 2005 Jul 1;84(7):1092-100.

Tallentire CW, Leinonen I, Kyriazakis I. Artificial selection for improved energy efficiency is reaching its limits in broiler chickens. Sci Rep. 2018 Jan 18;8(1):1-10.

van Beers-Schreurs HMG, Nabuurs MJA, Vellenga L, Kalsbeek-van der Valk HJ, Wensing T, Breukink HJ. Weaning and the weanling diet influence the villous height and crypt depth in the small intestine of pigs and alter the concentrations of short-chain fatty acids in the large intestine and blood. J Nutr. 1998 Jun 1;128(6):947-53.

Windmueller HG, Spaeth AE. Metabolism of absorbed aspartate asparagine, and arginine by rat small intestine in vivo. Arch Biochem Bio Phys. 1976 Aug 1;175(2):670-6.

Xu ZR, Hu CH, Xia MS, Zhan XA, Wang MQ. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult Sci. 2003 Jun 1;82(6):1030-6.

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