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Effect of Guanidino Acetic Acid Supplementation in Soybean Meal and Canola Meal-Based Diets on Broiler Performance, Carcass Characteristics, Liver Enzymes, and Intestinal Morphology

Research Article

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ABSTRACT

A total of 288 Ross 308 broiler chickens (mixed sex) were used to investigate the effect of supplemental guanidino acetic acid (GAA) to soybean meal (SBM) and canola meal (CM) based diets on growth performance, carcass characteristics, liver enzymes, and morphometric indexes. Birds were randomly assigned to four treatments in a completely randomized design with a 2 × 2 factorial arrangement consisting of 2 types of oil seed meal (SBM and CM) and 2 levels of GAA (0 and 0.6 g/kg) with six replicates in each. The results indicated that birds fed with CM diet had significantly lower feed intake and body weight gain and a worse feed conversion ratio than those fed with SBM diet ($P<0.05$). Supplementing GAA to the CM diet but not to the SBM diet led to increased feed intake so that the value was similar to those birds receiving the SBM diet as a statistical point ($P<0.05$), while the improvement of body weight gain was not achieved completely. Carcass, breast, and thigh yields were statistically lower in birds fed the CM diet than those fed the SBM diet, while the abdominal fat and liver weight were higher ($P<0.05$). Neither type of oil seed meal nor GAA significantly affected the liver enzymes and nitric oxide concentrations, as well as gut morphometric indexes ($P<0.05$). It can be concluded that supplementing 0.6 g/kg GAA to the CM-based diet to some extent improves the growth performance of broiler chickens, and more content may be needed for complete improvement.

KEY WORDS broiler chicken, canola meal, guanidino acetic acid, liver enzyme, soybean meal.

INTRODUCTION

Soybean meal (SBM) is an excellent source of protein with very good protein content, amino acid (AA) composition, digestibility, and low anti-nutritional factors (ANFs) (Thanabalan *et al.* 2021). However, the SBM supply can't adequately keep up with the industrial demand. Thus, the producers are looking to find a reasonable alternative for SBM as a major source of protein for poultry (Tamasgen *et al.* 2021). Canola meal (CM) is one of the most appropriate protein sources for poultry production, which can be an effective candidate for the replacement of SBM due to its high content of crude protein (about 36 to 39%), and a good balance of AAs, especially methionine, cysteine, and histidine (Manyeula *et al.* 2020). Meanwhile, the AA digestibility of CM is about 10% lower than that of SBM. In addition, different ANFs have been reported to be present in this protein source. These ANFs content can reduce the growth performance in poultry by decreasing nutrient digestibility and absorption (Payvastagan *et al.* 2012).

However, replacing high levels of CM with SBM may reduce arginine (Arg) concentration in the diet below the poultry requirement (Izadinia *et al*. 2010). Arginine is an essential substrate for synthesizing vital biochemical substances such as nitric oxide (NO) and guanidino acetic acid (GAA). GAA is the common name of N-(aminoimino-methyl)-glycine, which is methylated by S-adenosyl methionine to creatine and is intrinsically involved in cellular energy metabolism through adenosine triphosphate regeneration. This metabolite is synthesized from glycine (Gly) and Arg by the direct activity of Arg:Gly aminotransferase (AGAT) in the avian kidney and liver (Portocarero and Braun, 2021).

The most important problem dealing with CM is its lowest Arg content rather than SBM (Khajali and Slominski, 2012). Dilger *et al*. (2013) showed that GAA is an efficacious replacement for dietary Arg for broilers. So, this experiment was designed to find whether supplementing GAA to a CM-based diet can improve growth performance originating from Arg deficiency.

MATERIALS AND METHODS

Birds, diets, and rearing management

A total of 288 one-day-old Ross 308 broiler chickens (mixed sex) with an initial average body weight of 40.8 ± 1.1 g were randomly allocated to 24 Pens, 12 birds per each. Birds received four dietary treatments in a completely randomized design with a 2 × 2 factorial arrangement, including 2 types of oil seed meal (SBM and CM) and 2 levels of GAA supplement (zero and 0.6 g/kg).

The GAA product in the traditional form of CreAMINO® distributed by Evonik (Nutrition and Care GmbH, Hanau-Wolfgang, Germany) is manufactured by AlzChem and contained at least 96% GAA supplementation was used in this study.

Feed ingredients including corn, SBM, CM, and corn gluten meal were analyzed firstly for determining chemical and amino acid composition using near-infrared spectroscopy (NIR; AminoNIR, Evonik Industries AG, Essen, Germany), and then two diets based on corn-SBM and corn-CM were formulated isocaloric and isonitrogenous by UFFDA software according to the minimum amount of recommended nutrients for Ross 308 broilers (Table 1). The broiler's rearing period consisted of starter (1-10 days), grower (11-24 days), and finisher (25-42 days) periods. The temperature was set at 33 ˚C in the first 3 days and afterward decreased by 0.5 ˚C per day to finally fixed around 22 ˚C.

The relative humidity was 50–60% and the lighting program provided 24 hours of light during the experimental period. Birds reared on deep litter floor pens and had free access to feed as mash form and clean tap water during the entire of experiment.

Growth performance

Broiler chickens were weighed by pen on days 1, 10, 24, and 42, then body weight gain (BWG) was calculated for the starter (1-10 days), grower (11-24 days), and finisher (25-42 days) periods as well as entire of the experiment (1-42 days) by subtracting body weight at the beginning from the end of the period. Feed intake (FI) for each pen was measured by subtracting the feed offered at the beginning of each period from the remaining at the end. The feed conversion ratio (FCR) was calculated by dividing FI to BWG.

Blood parameters

On day 42, blood samples were collected from 2 birds of each pen to evaluate aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and NO concentration. Bleeding was induced in the wing vein using a sterile gauge of 19 needles and normal plastic syringes. A total of 3 mL of blood was collected, poured into sterile glass tubes, and gently mixed. Blood serum samples were immediately transferred to the laboratory for further analysis. The liver enzyme concentrations were measured using traditional kits manufactured by Pars Azmoun Company (Pars Azmoun, Iran), using a photometric spectrometer (UV-Vis model 365 LAMBDA, Perkinelmer, NY, USA) with the emission wavelength specific for each element (Asadi *et al*. 2022). Moreover, NO concentration was determined in serum by the Zelbio Company Kit (Zelbio, Germany and nitric oxide, Sib-Zist, Iran) by ELISA (ELX808. TEX-Bio, BioTek Instruments, Frankfurt, Germany) (Cheragh-Birjandi *et al*. 2020).

Carcass characteristics

At the end of the experiment (day 42), after 6 h of fasting (Li *et al*. 2023), one chicken per pen with body weight as close as possible to the average pen weight was selected for measuring carcass characteristics. The birds were transferred to the slaughterhouse, weighed individually, and then slaughtered according to standard commercial practices. After defeathering and removing offal, cooked carcass, breast, tight, abdominal fat, liver, pancreas, and spleen were weighed. The values were expressed as a percentage of live body weight.

Intestinal morphology

The birds were slaughtered to evaluate carcass characteristics used for measuring morphometric indexes in the jejunum section. To remove intestinal contents, segments of 1.5 cm in length were gently flushed twice with physiological saline solution (1% NaCl), and for fixation of obtained tissues, 10% formalin in 0.1 M phosphate buffer (pH=7.0) was used.

Table 1 Ingredients and nutrient values of the basal diets

Ingredients	Soybean meal-based diet			Canola meal-based diet		
	Starter	**Growth**	**Finisher**	**Starter**	**Growth**	**Finisher**
Corn (CP=8.5%)	56.23	59.69	62.69	44.19	49.72	54.74
Soybean meal (CP=44%)	34.26	30.38	26.58	-	-	-
Canola meal (CP=35.3%)	-	-	-	38.98	33.64	28.43
Corn gluten meal (CP=57.06%)	3.90	3.90	3.90	8.00	8.00	8.00
Soy oil	1.03	1.86	2.85	4.85	4.96	5.24
Calcium carbonate	1.14	1.05	0.99	0.58	0.57	0.59
Di-calcium phosphate	1.71	1.52	1.41	1.66	1.49	1.39
Common salt	0.39	0.39	0.39	0.35	0.36	0.36
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-methionine	0.31	0.27	0.26	0.12	0.09	0.11
L-lysine hydrochloride	0.37	0.31	0.31	0.65	0.57	0.55
L-threonine	0.11	0.08	0.07	0.07	0.05	0.04
Coccidiostate	0.05	0.05	0.05	0.05	0.05	0.05
Nutrient values (%, unless mentioned)						
ME (kcal/kg)	2880	2980	3080	2880	2980	3080
Crude protein	22.08	20.67	19.25	22.08	20.67	19.25
Lysine	1.382	1.240	1.145	1.382	1.240	1.145
Methionine	0.679	0.613	0.586	0.568	0.518	0.506
Methionine + cystine	1.037	0.952	0.905	1.037	0.952	0.905
Threonine	0.931	0.846	0.779	0.931	0.846	0.779
Arginine	1.36	1.252	1.144	1.140	1.048	0.957
Calcium	0.922	0.836	0.779	0.922	0.836	0.779
Available phosphorus	0.461	0.418	0.389	0.461	0.418	0.389
Sodium	0.173	0.173	0.173	0.173	0.173	0.173

[1] Provides (per kg of diet): vitamin A: 4000000 IU; vitamin D_3: 1800000 IU; vitamin E: 26000 IU; vitamin K_3: 1200 mg; vitamin B_1: 1000 mg; vitamin B_2: 2600 mg; vitamin B_3: 24000 mg; vitamin B_4: 500000 mg; vitamin B_5: 7200 mg; vitamin B_6: 1280 mg; vitamin B_9: 760 mg; vitamin B_{12}: 6.8 mg and vitamin H_2: 72 mg.

[2] Provides (per kg of diet): Copper: 6400 mg; Iodine: 500 mg; Iron: 8000 mg; Manganese: 48000 mg; Selenium: 120 mg and Zinc: 44000 mg.

Then, the samples were processed for 24 hours in a tissue processor and embedded in paraffin. Sections of 5 µm were made and stained with hematoxylin-eosin. Finally, the morphology of the small intestine was examined with an optical microscope (Olympus CX31). A total of ten intact, well-oriented villus–crypt units were selected for each intestinal cross-section villus height was measured from the tip of the villus to the villus crypt junction, and crypt depth was defined as the depth of the invagination between two villus. Villus width was measured at the middle point of the villus. The formula for calculating villus surface area was 2π × (villus width/2) × villus height. The average values for each cross-section were used for data analysis (Ale Saheb Fosoul *et al.* 2018).

Statistical Analyses

This experiment was performed with 4 treatments as a 2 × 2 factorial arrangement (2 types of oil seed meal and 2 levels of GAA) in a completely randomized design (CRD). Before analysis, the data were checked for normal distribution through the UNIVARIATE procedure and Shapiro–Wilk test. The GLM procedure was applied for data analysis using SAS software (SAS, 2003). Tukey test was used to determine significant differences. Differences with P-values less than 0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Neither type of oil seed meal nor GAA had a significant effect on FI in the rearing periods, but their interaction effect was significant, except for the starter period ($P<0.05$, Table 2). The lowest FI in the grower, finisher, and entire experiment period belonged to the CM treatment, and adding GAA led to an increase in it so that the values were similar to the SBM treatment as the statistical point. However, adding GAA to the SBM diet had no significant effect on FI, except for the grower period.

The results related to BWG are reported in Table 3. Birds fed with SBM diets had numerically better BWG than those fed with CM diets at the starter period ($P=0.068$). Supplementing GAA to the CM diet led to a significant increase of BWG at the grower period ($P<0.05$), so the value was similar to the SBM diet as a statistical point, while this improvement was not seen for the SBM diet. Birds fed on the SBM diet had significantly higher BWG than those on the CM diet during the finisher period ($P<0.05$), and supplementing GAA did not significantly affect BWG. Birds fed with SBM diets had significantly better BWG than those fed with CM diets during the entire period of the experiment. However, the significant interaction effect type of oil seed meal and GAA level demonstrated that supplementing GAA to the CM diet improved BWG while this effect was not seen for the SBM diet ($P<0.05$).

There was no interaction between the type of oil seed meal and GAA on FCR of the birds. Supplemental GAA also had no significant effect on FCR during all rearing periods ($P<0.05$, Table 4). However, birds fed with SBM diet had significantly better FCR than those fed with CM diet at finisher and the entire experiment ($P<0.05$), while this effect was not seen at starter and grower periods.

The interaction effect between the type of oil seed meal and GAA was not significant for carcass characteristics, except for the liver (Table 5, $P<0.05$). Birds on CM diet had significantly lower carcass and breast but a higher tight relative weight ($P<0.05$). Abdominal fat was significantly greater in broilers fed with the CM diet than those fed with the SBM diet ($P<0.05$). The liver was heavier in birds receiving CM diets than those fed with SBM diets, and supplementing GAA to the SBM diet resulted in increased liver weight ($P<0.05$). Neither type of oil seed meal nor GAA significantly affects the pancreas and spleen percentage.

The effect of the type of oil seed meal and GAA level and their interaction on liver enzyme and NO concentration, except ALT enzyme, was not significant ($P<0.05$; Table 6). Supplementing GAA to the SBM diet decreased ALT, while its supplementation with the CM diet increased ALT concentration ($P<0.05$).

The results of intestinal morphology are reported in Table 7. These results showed that the type of oil meal and GAA supplementation had no significant effect on the morphometric indexes ($P>0.05$). The interaction effect between the type of oil meal and GAA was not significant on the parameters ($P>0.05$). In this experiment, birds that received the CM diet consumed lower feed than those fed with the SBM diet, which is in line with the findings of Taraz *et al.* (2006) who showed that complete replacement of SBM with CM resulted in a decreased FI during the grower and the entire period of the experiment. Slominski and Campbell (1990) also indicated that replacing CM with SBM decreased FI because CM has a higher fiber content and ANFs, but lower Arg than SBM. Arginine is a basic and essential AA for broilers since they lack nearly all the enzymes that are involved in the urea cycle. Hence, Arg deficiency can cause amino acid imbalance and subsequently decrease FI (Khajali and Slominski, 2012). The increase of FI with supplementing GAA to the CM diet shows that GAA can reduce Arg requirement (Portocarero and Braun, 2021; Sharma *et al.* 2022).

Birds on the CM diet had lower body weight gain than those on the SBM diet which is in line with the findings of Gopinger *et al.* (2014) who observed similar results. They attributed this decrease to the presence of glucosinolates, tannins, sinapin, and other ANFs in CM. In addition, the decrease observed in BWG could be partly related to the lysine: Arg imbalance in diets with high CM levels (Taraz *et al.* 2006). In agreement with the results of this study, Dilger *et al.* (2013) showed that adding GAA to Arg-deficient diets increased body weight, which could be due to the compensation of Arg deficiency by GAA. Cengiz and Kucukersan (2010) stated that feeding broilers according to the NRC recommendations results in higher BWG than the lower levels of Arg. Other researchers also showed that Arg-deficient diets lead to a decrease in BWG (Xu *et al.* 2018; Castro *et al.* 2019). Of course, it should be considered that improving BWG by supplementing GAA to the CM diet in this experiment occurred with an increase in FI.
The results of this study indicated that the birds that received SBM-based diets showed better FCR than CM diets. An increase in FCR in chickens fed CM may be due to the presence of ANFs and a decrease in the protein digestibility (Khajali and Slominski, 2012; Gopinger *et al.* 2014), or high fiber, lignin, and polyphenols contents (Gopinger *et al.* 2014) which results in decreased FI. The high level of fiber in CM containing diet can reduce protein and energy digestibility (Kocher *et al.* 2000; Yadav *et al.* 2022). It is believed that GAA supplementation provides more energy by increasing creatine synthesis in liver and muscle tissues which leads to increase the production of ATP from ADP (Michiels *et al.* 2012). It has also been reported that GAA can improve the performance of broilers by increasing muscle creatine stores (as a source of cellular energy), and supplying Arg for protein synthesis and cell proliferation (Portocarero and Braun, 2021).

Table 2 The effect of type of oil seed meal and guanidino acetic acid supplementation on feed intake of broilers (g)

Type of oil seed meal	GAA level	Starter period (1-10 d)	Grower period (11-24 d)	Finisher period (25-42 d)	Entire of experiment (1-42 d)
SBM		222.2	1027.9	3022.0	4272.1
CM		219.7	1022.2	2979.0	4220.9
P-value		0.705	0.630	0.294	0.292
SEM		4.61	8.30	28.21	40.95
	0	223.1	1033.2	2967.0	4223.3
	0.6 g/kg	218.8	1016.9	3034.0	4267.9
P-value		0.517	0.181	0.109	0.339
SEM		4.61	8.30	28.21	40.95
Interaction effect (type of oil seed meal×GAA level)					
SBM	0	228.4	1058.4^{a}	3055.4^{a}	4342.2^{a}
CM	0	217.8	1008.0^{b}	2878.7^{b}	4104.4^{b}
SBM	0.6 g/kg	216.0	997.4^{b}	2988.6^{a}	4202.0^{ab}
CM	0.6 g/kg	221.6	1036.4^{ab}	3079.3^{a}	4337.3^{a}
P-value		0.327	0.001	0.003	< 0.001
SEM		6.52	11.74	39.89	57.91

GAA: guanidino acetic acid; SBM: soybean meal and CM: canola meal.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 3 The effect of type of oil seed meal and guanidino acetic acid supplementation on body weight gain of broilers (g)

Type of oil meal	GAA level	Starter period (1-10 d)	Grower period (11-24 d)	Finisher period (25-42 d)	Entire of experiment (1-42 d)
SBM		174.3	638.6	1766.7^{a}	2579.5^{a}
CM		160.3	631.2	1530.7^{b}	2321.8^{b}
P-value		0.068	0.741	<0.001	< 0.001
SEM^{5}		5.13	15.65	25.65	29.82
	0	169.9	627.9	1653.2	2451.1
	0.6 g/kg	164.7	641.8	1644.1	2450.3
P-value		0.485	0.537	0.803	0.981
SEM		5.13	15.65	25.65	29.82
Interaction effect (type of oil seed meal×GAA level)					
SBM	0	177.6	662.8^{a}	1780.5	2620.9^{a}
CM	0	162.1	593.1^{b}	1526.0	2281.3^{b}
SBM	0.6 g/kg	170.9	614.4^{ab}	1752.8	2538.2^{a}
CM	0.6 g/kg	158.5	669.2^{a}	1535.3	2362.3^{b}
P-value		0.830	0.011	0.615	0.028
SEM		7.25	22.13	36.28	42.17

GAA: guanidino acetic acid; SBM: soybean meal and CM: canola meal.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The current study revealed that supplementation of GAA to the CM-based diet to some extent could improve growth performance, while it could not in SBM-based diet. In agreement with our results, DeGroot *et al.* (2019) showed that broilers' BWG and FCR improved when GAA was supplemented to an Arg deficient diet at 0.12%, but not at 0.06%, and the improvement was not seen when GAA was added to an Arg adequate diet at each levels of 0.06, 0.12, and 0.18%.

In this study, carcass yield and breast relative weight were lower in CM-based diet than SBM-based diet while the thigh relative weight was vice versa which is similar to the findings of Khajali and Slominski (2012). They explained that this alteration in carcass yield is related to the lower content of Arg in CM, because Arg is the precursor of several growth factors and amino acids that are required for the synthesis of connective tissues. Similarly, Jiao *et al.* (2010) showed that an Arg-deficient diet resulted in lower muscle weight but higher abdominal fat yield compared with the standard diet. They found that Arg supplementation enhanced breast and thigh muscle growth but decreased abdominal fat yield. Some research showed that CM at moderate levels (less than 18% of diet) had no adverse effect on carcass and breast meat yield in turkeys (Mikulski *et al.* 2012) and broiler chickens (Amerah *et al.* 2015).

Table 4 The effect of type of oil seed meal and GAA supplementation on the feed conversion ratio of broilers

Type of oil meal	GAA level	Starter period (1-10 d)	Grower period (11-24 d)	Finisher period (25-42 d)	Entire of experiment (1-42 d)
SBM		1.29	1.63	1.72^{b}	1.66^{b}
CM		1.38	1.63	1.95^{a}	1.82^{a}
P-value		0.139	0.061	< 0.001	< 0.001
SEM		0.040	0.267	0.028	0.013
	0	1.32	1.67	1.81	1.73
	0.6 g/kg	1.34	1.59	1.86	1.75
P-value		0.644	0.2146	0.199	0.360
SEM		0.040	0.267	0.028	0.013
Interaction effect (type of oil seed meal×GAA level)					
SBM	0	1.29	1.62	1.72	1.66
CM	0	1.35	1.71	1.89	1.80
SBM	0.6 g/kg	1.29	1.62	1.71	1.65
CM	0.6 g/kg	1.40	1.55	2.01	1.84
P-value		0.615	0.9388	0.089	0.308
SEM		0.057	0.378	0.039	0.019

GAA: guanidino acetic acid; SBM: soybean meal and CM: canola meal.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 The effect of type of oil seed meal and guanidino acetic acid supplementation on carcass characteristics and organ weight of broilers

Type of oil seed meal	GAA level	Carcass (% of live body weight)	Breast	Thigh	Abdominal fat	Liver	Pancreas	Spleen
			(% of carcass weight)					
SBM		68.62^{a}	41.22^{a}	27.25^{b}	1.91^{b}	2.64^{b}	0.33	0.17
CM		65.05^{b}	39.59^{b}	27.97^{a}	2.29^{a}	3.17^{a}	0.36	0.19
P-value		< 0.001	0.035	0.005	0.044	0.003	0.476	0.258
SEM		0.368	0.511	0.163	0.125	0.111	0.016	0.013
	0	66.84	40.52	27.59	2.27	2.82	0.33	0.17
	0.6 g/kg	66.83	40.28	27.63	1.92	2.99	0.36	0.18
P-value		0.989	0.745	0.875	0.058	0.305	0.334	0.574
SEM		0.368	0.511	0.163	0.125	0.111	0.016	0.013
Interaction effect (type of oil seed meal×GAA level)								
SBM	0	68.60	41.99	27.21	1.96	2.37^{b}	0.29	0.17
CM	0	65.07	39.04	27.98	2.59	3.27^{a}	0.36	0.17
SBM	0.6 g/kg	68.64	40.44	27.29	1.85	2.91^{ab}	0.37	0.16
CM	0.6 g/kg	65.02	40.12	27.97	1.98	3.06^{a}	0.35	0.20
P-value		0.938	0.083	0.842	0.176	0.029	0.189	0.377
SEM		0.520	0.723	0.231	0.177	0.157	0.023	0.018

GAA: guanidino acetic acid; SBM: soybean meal and CM: canola meal.
The means within the same column with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Liver relative weight was greater in birds fed with the CM-based diet than those fed with the SBM-based diet. Similarly, Goudarzi *et al.* (2017) reported that liver weight in laying hens increased by substitution of SBM with CM. GAA supplementation to the CM or SBM-based diet in the current experiment did not affect relative weights of carcass and internal organ. In consistence with our results, Kodambashi Emami *et al.* (2017) reported that GAA supplementation of diets did not affect carcass and spleen in broilers reared under normal or subnormal temperature. Zarghi *et al.* (2020), and Mousavi *et al.* (2013), reported that dietary GAA supplementation does not affect carcass traits.

According to the findings of Michiels *et al.* (2012), GAA supplementation in diets of broiler chickens enhanced breast meat without affecting carcass yield.

The concentrations of liver enzymes and NO were not affected by replacing SBM with CM. In agreement with these results, other researchers reported that feeding Japanese quails with diets containing 0, 10, 20, and 30% CM (Raei *et al.* 2020), or broiler chickens with 0, 10, and 20% CM (Payvastagan *et al.* 2012) did not affect on the concentration of AST, ALT, and ALP. Disetlhe *et al.* (2018) found no differences between liver enzymes of broilers fed with SBM or CM-based diet, even though the birds receiving CM diet had a higher level of AST.

Table 6 The effect of type of oil seed meal and guanidino acetic acid supplementation on liver enzyme and nitric oxide concentration in broilers

Type of oil seed meal	GAA level	Variable			
		AST (u/L)	ALT (u/L)	ALP (u/L)	NO (nmol/L)
SBM		368.7	37.5	2535.8	105.6
CM		367.6	29.7	1705.1	124.1
P-value		0.981	0.227	0.136	0.365
SEM		32.17	4.44	378.27	14.17
	0	371.2	33.9	1972.8	118.3
	0.6 g/kg	365.1	33.2	2268.0	111.3
P-value		0.895	0.908	0.587	0.730
SEM		32.17	4.44	378.27	14.17
Interaction effect (type of oil seed meal×GAA level)					
SBM	0	378.0	45.7^{a}	2207.3	103.2
CM	0	364.3	22.2^{b}	1738.3	133.5
SBM	0.6 g/kg	356.3	29.3^{b}	2864.2	107.9
CM	0.6 g/kg	359.3	37.2^{ab}	1671.8	114.7
P-value		0.785	0.021	0.507	0.565
SEM		45.49	6.28	534.95	20.04

AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; NO: nitric oxide; GAA: guanidino acetic acid; SBM: soybean meal and CM: canola meal.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 7 The effect of type of oil seed meal and guanidino acetic acid supplementation on morphometric indexes of broilers

Type of oil meal	GAA level	Villus height (µm)	Villus width (µm)	Crypt depth (µm)	Villus height/crypt depth	Villus surface area (mm^2)
SBM		1384.8	136.8	184.0	8.51	0.571
CM		1467.7	143.7	193.3	8.87	0.649
P-value		0.307	0.669	0.824	0.785	0.478
SEM		55.93	12.35	28.94	0.923	0.076
	0	1392.1	127.1	172.8	8.68	0.543
	0.6 g/kg	1460.4	153.5	204.5	8.69	0.677
P-value		0.398	0.146	0.448	0.993	0.227
SEM		55.93	12.35	28.94	0.923	0.076
Interaction effect (type of oil seed meal×GAA level)						
SBM	0	1344.8	128.3	182.1	8.04	0.547
CM	0	1439.5	125.9	163.5	9.32	0.539
SBM	0.6 g/kg	1424.8	145.5	185.9	8.97	0.595
CM	0.6 g/kg	1496.0	161.6	223.0	8.41	0.759
P-value		0.883	0.603	0.504	0.902	0.433
SEM		79.10	17.46	40.93	1.306	0.108

GAA: guanidino acetic acid; SBM: soybean meal and CM: canola meal.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

They discussed that these enzymes elevated in the blood when hepatocytes were damaged by toxins and their metabolites. Thus, it can be concluded that CM did not cause any damage to the liver tissues or other tissues containing these enzymes, indicating that the birds were healthy status during the CM feeding experiment. Despite our expectation in decreasing NO concentration in the CM-based diet, we observed no difference between the treatments. Izadinia *et al.* (2010) reported that replacing CM with SBM at fifty percent had no significant effect on blood NO concentration of broilers reared in cold temperature while it was significantly lower when compelety replaced.

A significant decrease in NO concentration was observed by Khajali and Slominski (2012) when SBM was replaced completely with CM in broilers grown at high altitude and supplementing the CM-based diet with L-Arg increased the NO level above the amount observed in SBM-based diet. Khakran *et al.* (2018) indicated that supplementing GAA to the SBM-based diet had no significant effect on NO concentration in laying hens. On the other hand, Raei *et al.* (2020) showed that NO concentration increased linearly in laying Japanese quails when GAA was supplemented with the SBM-based diet at 0, 0.6, 1.2, and 1.6 g/kg. A decrease in NO concentration in broilers exposed to acute lactic acidosis has been reported by Boroumandnia *et al.* (2021) when birds .received the SBM-based diet supplemented with GAA at 1.2 g/kg GAA. Some evidence indicated supplementing GAA to SBM-based diet in laying hens (Khakran *et al.* 2018), and also in corn-SBM-based diet with or without poultry by-product meal in broiler chickens (Cordova-Noboa *et al.* 2018) had no significant effect on the liver enzymes. Boroumandnia *et al.* (2021) supplemented GAA to the SBM-based diet at 0, 0.6, 1.2, 1.8, 2.4, and 3 g/kg in broiler chickens exposed to lactic acidosis and found increased ALT at 1.8 g/kg supplementation, while no differences were observed between supplemented diets and control for AST and ALP enzymes. Hence, it seems that the effect of GAA on liver enzymes and NO concentration depends on diet composition and rearing conditions.

Since CM has lower Arg content than SBM and Tan *et al.* (2010) in an *in vitro* study demonstrated that this indispensable AA is effective against enterocyte damage, we expected to see some beneficial effects by supplementing GAA to the CM-based diet. However, according to the current results, it seems that CM did not alter the integrity of the intestinal mucosa and it is possible to replace it with SBM at high levels without detrimental effects on the gastrointestinal tract. Similarly, Figueiredo *et al.* (2003) found no difference between broilers fed CM at the levels of 0, 10, 20, 30, and 40% for villus height while Gopinger *et al.* (2014) reported a quadratic response with a maximum at 23.6% CM. Kodambashi Emami *et al.* (2017) showed that GAA supplementation had no significant effect on jejunum morphology of broilers raised at normal temperature while jejunal villus surface area improved in birds raised under cold stress at 1.2 g/kg GAA, but not by 0.6 g/kg supplementation. Ahmadipour *et al.* (2018) found increased villus height, villus width, and villus surface area in the various sections of the small intestine of broilers by GAA supplementation above 0.5 g/kg.

CONCLUSION

Broilers fed with CM based diet had lower FI and BWG, and worse FCR than birds fed with SBM based diet. Supplementing GAA at 0.6 g/kg to the CM-based diet resulted in increasing FI and subsequently improving BWG and FCR. However, this improvement is lesser than the values compared to the SBM diet. No detrimental effect was seen on liver enzymes and morphometric indexes by CM-based diet, hence it seems that the reduction of growth performance could be related to the lesser Agr in CM than SBM. So, it seems that GAA should be supplemented to the diets containing CM at values more than 0.6 g/kg or the Arg content of the diet should be considered.

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