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ANIMAL PRODUCTION

Copper sulfate and arginine supplements on performance, thyroid hormones and blood biochemistry of broilers fed with canola meal-based diets

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ABSTRACT: *The present evaluated the effects of copper sulfate solution (CSS) and arginine powder (Arg) supplements on performance, thyroid hormones and blood biochemistry of broiler chickens fed with canola meal (CM)-based diets. The experimental design was completely randomized with a 3 × 3 factorial and 9 treatments, corresponding to 3 levels of CSS (0, 125 and 250 mg/kg) and 3 levels of Arg (0, 0.1 and 0.2%) (n = 45 per treatment). Feeds were offered ad libitum for 21 days, from 22 to 42 days of age. Feed efficiency was significantly affected by the dietary addition of 250 mg/kg CSS and 0.2% Arg, and by the CSS × Arg interaction. CM supplemented with CSS improved the thyroid gland status and increased the plasma levels of triiodothyronine and thyroxine. Birds fed diets supplemented with 0.2% Arg had lower blood glucose level than the other treatments. The addition of 250 mg/kg CSS and 0.2% Arg reduced the stress caused by the rapid growth of broilers, also increasing the overall bird welfare.*
Key words: fee*d supplements, minerals, amino acids, canola-based feed, broilers.*

Avaliação da suplementação com sulfato de cobre e arginina sobre o desempenho, hormônios tireoidianos e bioquímica sanguínea de frangos alimentados com dietas à base de canola

RESUMO: *O objetivo do presente trabalho foi avaliar os efeitos da suplementação com solução de sulfato de cobre (SSC) e arginina em pó (Arg) sobre o desempenho, hormônios tireoidianos e bioquímica sanguínea de frangos de corte alimentados com dietas à base de canola DC. O desenho experimental foi completamente casualizado com fatorial 3 × 3 e nove tratamentos correspondentes a três níveis de inclusão de SSC (0, 125 e 250 mg/kg) e três níveis de Arg (0, 0,1 e 0,2%) (n = 45 para cada tratamento). As rações foram oferecidas ad libitum por 21 dias, de 22 até 42 dias de idade. A eficiência alimentar foi significativamente afetada pela adição de 250 mg/kg de SSC e 0,2% de Arg, assim como pela interação SSC × Arg. A suplementação da DC com SSC melhorou os parâmetros da glândula tireoide e aumentou os níveis plasmáticos de triiodotironina e tiroxina. As aves alimentadas com dietas suplementadas com 0,2% de Arg apresentaram menor nível de glicose sanguínea do que as dos demais tratamentos. A adição de 250 mg/kg de SSC e 0,2% de Arg reduz o estresse causado pelo rápido crescimento dos frangos, além de melhorar as condições gerais de bem estar das aves.*
Palavras-chave: *suplementos alimentares, minerais, aminoácidos, ração à base de canola, frangos de corte.*

INTRODUCTION

Canola, the low glucosinolate and low erucic acid form of rapeseed (*Brassica napus* or *B. rapa*), is an important oilseed crop in several geographic areas in the world (MOUSAVI-AVVAL et al., 2011). The environmental conditions in Iran and other countries including Brazil are ideally suited to the growth of canola, which production is commonly included in crop rotations (MOUSAVI-AVVAL et al., 2011). Canola meal (CM) contains 75% of the equivalent protein content of soybean, being often marketed at values representing 55-65% of the soybean price (BROOKES et al., 2010). However, CM has a lower nutritive value than soybean meal (SBM) because of its higher non-starch polysaccharide content and the presence of several antinutritive factors, such as tannins, glucosinolate, and phytic acid (NEWKIRK & CLASSEN, 2002). The glucosinolates are a large group of sulphur-containing secondary plant metabolites, and one of the most important anti-nutritional compounds of

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CM (ZUKALOVÁ & VASAK, 2002). These anti-nutritional agents are biologically inactive, but their hydrolyzed products have different anti-nutritional effects. In general, reduced feed intake, growth, production, hyperthyroidism, induced hypertrophy in the liver, kidney, and thyroid gland, also alterations in liver enzyme activity are the anti-nutritional effects of glucosinolates in broilers (WOYENGO et al., 2017). Therefore, the analysis of glucosinolates is of prime importance before using CM in feed.

The dietary supplementation with copper sulfate solution (CSS) is one of the most used treatments to overcome the glucosinolates' deleterious effects on animal health and production (AZIMI-YOUVALARI et al., 2017). The CSS supplementation may redirect glucosinolates breakdown products (TRIPATHI & MISHRA, 2007). PEKEL et al. (2009) reported that broilers fed Camelina meal, which is a member of the Brassica family, positively responded to CSS supplementation by improving their performance and carcass characteristics. Copper supplementation also had a positive effect on performance and health status of breeding hens (ATTIA et al., 2011a) and White Pekin male ducks (ATTIA et al., 2012). Even though CM is particularly rich in sulfur-containing amino acids, the arginine (Arg) content of CM is approximately two-thirds of SBM (2.08 vs. 3.14%, according to NRC, 1994). Birds are unable to synthesize Arg (KHAJALI & WIDEMAN, 2010). Therefore, the substitution of a high proportion of CM protein at the expense of SBM protein in poultry diets may drop the dietary Arg level below its requirements, resulting in poor performance. It has been well documented that Arg has large effects on the central nervous system and on hormonal, metabolic, muscular, cardiovascular, pulmonary, and renal functions during rest and exercise (ATTIA et al., 2006; ATTIA et al., 2011b; ZAMPIGA et al., 2019). The potential effects of Arg on broiler's pulmonary hypertension were investigated in previous study, which demonstrated that the supplemention of 1% L-Arg in the diet significantly decreased the incidence of pulmonary hypertension syndrome (ascites) (TAN et al., 2006). Ascites is a condition of fast-growing broiler chickens in which the excess amount of ascitic fluid accumulates in the abdominal cavity (LUGER et al., 2002). There are evidences indicating that the pathogenesis of ascites is multi-factorial, involving genetic, environmental, and immune system components (WIDEMAN et al., 2013), which difficult the assessment of this syndrome. Considering that ascites has become a major concern to the poultry industry around the world, many nutritional, medicinal and management strategies have been proposed to alleviate the problem (ABDULKARIMI et al., 2017). In this context, the present study evaluated

the effects of CSS and arginine powder supplements on performance, thyroid hormones, blood biochemistry and susceptibility to ascites in broiler chickens fed CM-based diets.

MATERIALS AND METHODS

Experimental design and broilers

The experiment was performed in the research farm of Urmia University of Iran. All experimental procedures were approved by the animal Care and Use Committee–Livestock of the Urmia University. A total of 405, 1-day-old male broiler chicks (Ross 308) was obtained from a local hatchery and randomly allocated to 45 floors pens (5 replicates with 9 chicks in each replicate) measuring 1.5 m^2. New wood shavings at a depth of approximately 8 cm were used as bedding material over a concrete floor. The 1-d-old chicks (45 ± 1 g) were weighed individually and allocated to pens so that their initial weights were similar across all pens. All chicks were provided *ad libitum* access to water and their assigned diets (in mash form). Synthetic arginine (174.2 g/mol) was achieved from Merck Millipore, Darmstad, Germany (product number: 101587). The source of CSS was provided as copper sulfate pentahydrate (249.68 g/mol, product number: 102787; Merck Millipore, Darmstad, Germany). The house temperature was maintained at 32 ˚C during the first week of age and a weekly reduction of 3˚C was practiced until a temperature of 25˚C was attained along with a humidity of 50%. Chickens were fed with a basal diet from 1 to 21 days of age, while the experimental diets were fed from 22 to 42 days of age (total experimental period: 21 days). A conventional continuous lighting regimen was provided throughout the experiment (BAYRAM & ÖZKAM, 2010).

The experimental design was completely randomized with a 3 × 3 factorial and 9 treatments, corresponding to 3 levels of CSS (0, 125 and 250 mg/kg) and 3 levels of Arg (0, 0.1 and 0.2%) (n = 45 per treatment) in isonitrogenous and isocaloric diets formulated according to the Ross requirements guideline (NRC, 1994), as indicated in table 1. The levels of CSS and Arg were based on the studies by PAYVASTEGAN et al. (2012) and KHAJALI et al. (2011), respectively. The 9 treatment diets were: 1) 0 mg/kg of CCS and 0% of Arg (control); 2) 0 mg/kg of CSS and 0.1% of Arg, 3) 0 mg/kg of CSS and 0.2% of Arg, 4) 125 mg/kg of CSS and 0% of Arg, 5) 125 mg/kg of CSS and 0.1% of Arg, 6) 125 mg/kg of CSS and 0.2% of Arg, 7) 250 mg/kg of CSS and 0% of Arg, 8) 250 mg/kg of CSS and 0.1% of Arg, 9) 250 mg/kg of CSS and 0.2% of Arg. The amino acids profile and components of CM were determined by near-infrared diffuse reflectance (Table 2).

Throughout the trial, feed was weighted when delivered in the throughs. The European efficiency factor (EEF) and feed efficiency (FE) were calculated for the grower period of the trial (22-42 d). The EEF was calculated according to MARCU et al. (2013), as follows: EEF = (liveability % x live weight kg) x 100 / (d of age x feed conversion ratio). FE was calculated using the following expression: FE = (g of weight gain per g of feed intake) x 100.

Diet preparation

The supplementation of CM with CSS was performed by the spray method according to AZIMI-YOUVALARI et al. (2017). Firstly, CM was milled by grinder, then 1.47 and 2.93 g of CSS was completely dissolved in 900 mL of water. These solutions were sprayed uniformly on 1-kg CM previously milled by a grinder, and the resulting mixtures were heated at 60°C for 24 h until constant weight. After complete drying, CM treated with both levels of CSS was mixed with experimental diets to achieve the required concentrations of 125 and 250 mg/kg diet. After preparation of the

Table 1 - Composition and calculated analysis of experimental diets.

	----------------------------------% inclusion------------------------------------	
Ingredient	Starter (0-21 d)	Grower (21-42 d)
Corn	57	55.34
Soybean meal	38	0
Canola meal	0	34.10
Corn Gluten meal	0	3.53
Soy oil	0.63	3.80
Dicalcium phosphate	2.15	1.34
Calcium carbonate	1.14	1.00
Salt	0.30	0.30
Trace mineral supplement [1]	0.25	0.25
Multi vitamin supplement [2]	0.25	0.25
Calculated analysis	--%--	
Crude protein	22	20
Fiber	2.74	5.06
Calcium	1.01	0.91
Available phosphorus	0.45	0.35
Arginine	1.54	1.07
Lysine	1.35	1.05
Arginine to lysine ratio	1.14	1.01
Methionine + cysteine	0.90	0.74
Linoleic acid	1.73	3.16
Metabolizable energy (Kcal/Kg)	2,900	3,000

[1]Provided per kg of ration: copper: 10 mg (Cupric sulphate); iron: 50 mg (ferrous sulphate), manganese: 100 mg (manganese oxide); zinc: 85 mg (zinc sulphate); selenium: 0.2 mg (sodium selenite); iodine: 1.0 mg (calcium iodate).

[2]Provided per kg of ration: retinol: 900 IU; cholecalciferol: 2000 IU; tocopherol: 18.0 IU; menadione: 2.0 mg; thiamine: 1.8 mg; riboflavin: 6.6 mg; pyridoxine: 3.0 mg; cyanocobalamin: 0.015 mg; niacin: 30 mg; pantothenic acid: 10 mg; folic acid: 1.25 mg; choline: 500 mg; biotin: 0.1 mg.

Table 2 - Composition and amino acid profile of the canola meal used in the experimental diets.

Parameter	% [1]
Composition	
Dry matter (%)	92.78
Crude protein	38.53
Ether extract (%)	2.70
Crude fiber (%)	10.20
Ash (%)	7.71
Sugar (%)	9.12
Total phosphorus (mg/kg)	9,193
Phytate phosphorus (mg/kg)	5,576
Metabolizable energy corrected by nitrogen (kcal/kg)	1,916
----------Amino acid profile----------	
Methionine	0.72
Cysteine	1.04
Sulfur amino acid	1.74
Lysine	1.82
Threonine	1.49
Tryptophan	0.53
Arginine	2.46
Isoleucine	1.49
Leucine	2.56
Valine	1.85
Histidine	1.01
Phenylalanine	1.52

[1]Values are based on the dry matter.

experimental diets, a 1-kg sample was collected from each treatment and analyzed to determine the total amount of glucosinolates in CM before and after supplementation with CSS (Table 3) by spectrophotometric analysis (SAINI & WRATTEN, 1987). The total erucic acid content of the CM used in this study was 0.20%.

Blood biochemistry analysis

At the end of experiment (broilers at 42 days of age), blood samples were collected from two birds per replicate (10 animals per treatment) by neck slitting. An aliquot of 2.5 mL of blood was placed in tubes containing heparin, while another 2.5 mL were collected into a hypodermic syringe. Plasma separation was carried out according to JAHANPOUR et al. (2013), and the obtained plasma samples were stored at −20 °C until analysis. Analyses of glutamic-oxaloacetic transaminase (SGOT), lactate dehydrogenase (LDH), creatine kinase, triglycerides, cholesterol HDL, LDL and VLDL were performed in heparinized plasma using diagnostic kits (Pars Azmun Company, Iran) and an automated biochemical analyzer (Alyson 300, UK) according to the manufacturer's instructions. Plasma triiodothyronine (T3) and thyroxine (T4) concentrations were determined by enzyme-linked immunosorbent assay kits (Pishtaz Teb, Tehran, Iran). The intra-assay coefficient variations (CV) of the T3 and T4 assay were 3.8 and 5.1%, respectively, and inter-assay CV of the T3 and T4 were 6.1 and 6.6%, respectively.

Statistical analysis

Data on performance and serum biochemistry parameters from the treatment groups were subjected to one-way ANOVA as a 3 x 3 factorial using the General Linear Model

Table 3 - Mean concentrations (n = 3) of glucosinolate in the canola meal (CM) and in the experimental diets supplemented with copper sulphate solution (CSS).

CSS supplementation (mg/kg)	Glucosinolate in CM (µmol/g)	Glucosinolate in the diet (µmol/g) [1]	Glucosinolate reduction (%) [2]
0 (control)	18.3	6.25	-
125	16.1	5.49	12.2
250	11.8	4.02	35.7

[1]Calculated on the basis of glucosinolate level in canola meal diet.
[2]Values are related to the levels of glucosinolate in the diet before and after CSS supplementation.

procedures of SAS® (SAS, 2004), considering the main effects of CSS and Arg, and the CSS × Arg interaction. Significant means among variables were separated using the Tukey's least significance test. Statistical significance was accepted at $P < 0.05$.

Data (cage means) were subjected to ANOVA (Snedecor and Cochran, 1967) as a 2 x 3 factorial using the General Linear Models procedure in the SAS® software (SAS Institute, 1992). Variable means for treatments showing significant differences in the ANOVA were compared using the Fisher's protected least significant difference procedure.

RESULTS AND DISCUSSION

The effects of treated CM with CSS and different levels of supplemental Arg on EEF and FE in grower period of the trial are summarized in table 4. EEF was not affected ($P > 0.05$) by CSS, Arg and CSS x Arg interaction. However, the interactions between 125 or 250 mg/kg CSS and 0 or 0.2 % Arg levels, as well as between no CSS addition (0 mg/kg) and 0.2 % Arg, resulted in increased ($P < 0.05$) FE values compared with the CSS controls (no addition) and 0 or 0.1 % Arg levels. The broiler chick's nutritional requirement for copper is approximately 8 mg/kg (NRC, 1994). Copper is usually fed commercially at much higher pharmacological levels (100 to 300 mg/kg) because of its growth promoting properties, which is caused by its antibacterial properties (ATTIA et al., 2011a; 2012; AZIMI-YOUVALARI et al., 2017). Supplementation with metal salts have been applied to remove glucosinolates, to minimize their deleterious effects on animals (SOETAN & OYEWOLE, 2009).

The Cu-sulfate supplementation may redirect glucosinolates to breakdown products, react to form complex with, or produce secondary breakdown products by rearrangement reactions (TRIPATHI & MISHRA, 2007). This is in agreement with the increased FE observed in the group supplemented with 125 or 250 mg/kg CSS and 0.2 % Arg. However, PAYVASTEGAN et al. (2013) reported no significant effects of copper supplementation on feed intake, body weight gain and feed conversion ratio. Dietary Cu supplementation can affect the nutritive value and potential toxic effects of rapeseed meal. AZIMI-YOUVALARI et al. (2017) reported that the deleterious effects of glucosinolates on broiler performance could be normalized by pretreating rapeseed meal containing high levels of glucosinolates with Cu sulfate before feeding. Also, glucosinolates are cleaved after treatment of CM with myrosinase or soaked CSS (SCHÖNE et al., 1993). It has been reported that supplementing 200 mg/kg of Cu in the form of Cu sulfate improves performance in broilers (SKIRVAN et al., 2000).

Table 5 shows the effects of treated CM with CSS and different levels of supplemental arginine on thyroid hormones and thyroid glands in broiler chickens fed by CM based diet at 42 d of age. Plasma T3 and T4 concentrations increased in the group receiving CM with 250 mg/kg CSS ($P < 0.05$), when compared with the control group. The relative weight of thyroid gland was reduced by treated CM with 125 or 250 mg/kg CSS. The relative weight of thyroid gland and plasma T3 and T4 concentrations were not influenced by Arg supplementation and Cu×Arg interaction ($P > 0.05$). Thyroid hormones are good indicators of susceptible broilers to ascites (HASSANZADEH

Table 4 - Effects of processed canola meal supplemented with copper sulphate solution (CSS) and arginine on European efficiency factor (EEF) and feed efficiency (FE) of broiler chickens during grower period of the trial (22-42 d).

CSS (mg/kg)	Arginine (%)	EEF	FE (%)
---Mean effects---			
0	0	246	80.9
125	0	248	83.8
250	0	267	85.4
0	0	244	82.1
0	0.1	252	81.0
0	0.2	264	87.1
---Interaction effects---			
0	0	236	76.9^{b}
0	0.1	239	78.3^{b}
0	0.2	262	87.5^{a}
125	0	240	83.3^{a}
125	0.1	251	82.0^{ab}
125	0.2	253	85.0^{a}
250	0	257	86.0^{a}
250	0.1	266	82.6^{ab}
250	0.2	277	87.6^{a}
SEM	24.2	1.84	
---Source of variation---		---Probability---	
CSS	0.520	0.010	
Arginine	0.590	0.006	
CSS x Arginine	0.95	0.009	

$^{a-b}$In a same column, means with different superscript letters differ significantly (P < 0.05).

et al., 1997), and the increase in plasma T3 and T4 concentration may also improve the body weight gain of broilers (VARMAGHANY et al., 2021); although, these parameters were not evaluated in the present study. Accordingly, T3 and T4 can stimulate the transcription of growth hormone mRNA and growth hormone synthesis in the pituitary (YEN, 2001). There are many studies reporting on the abnormalities in thyroid function and induced thyroid hypertrophy in birds fed CM (TRIPATHI & MISHRA, 2007; PAYVASTEGAN et al., 2013). BAO et al. (1995) reported that isothiocyanate compete with the iodine in the microvilli of the thyroid gland for binding to the tyrosine ring and synthesis of thyroid hormones was decreased. Decreased levels of thyroid hormones stimulate the secretion of TSH from the pituitary gland.

Long-term stimulation of the thyroid gland by TSH is associated with enlargement of this gland and the development of hypertrophy and hyperplasia of the thyroid follicular cells (KERO et al., 2007). PAYVASTEGAN et al. (2017) observed that follicle numbers, epithelial thickness and follicle diameters increased proportionally with increase in CM inclusions from 0 to 30%, indicating hypertrophy and hyperplasia of thyroid cells. Thus, the observed increase in thyroid weights may be partially attributed to hyperplasia and hypertrophy of thyroid cells. SCHÖNE et al. (1988) reported that the addition of excess copper would inactivate the compounds derived from glucosinolates degradation, thus reducing the growth inhibitory effects of these compounds. In our study, lower relative weights of thyroid were observed in birds receiving 125 or 250 g of CSS, which also contained decreased levels glycosinalates in the feed. In animal diets, the negative effects of glucosinolates depend on the level and composition of glucosinolates and

Table 5 - Effects of processed canola meal supplemented with copper sulphate solution (CSS) and arginine on thyroid hormones (T3 and T4) and thyroid relative weight of broiler chickens during grower period of the trial (22-42 d).

CSS (mg/kg)	Arginine (%)	T4 (ng/mL)	T3 (ng/mL)	Relative weight (mg/kg body weight)
Mean effects				
0	0	3.44 [b]	7.46 [b]	96.2 [a]
125	0	3.56 [ab]	7.61 [ab]	89.7 [b]
250	0	3.81 [a]	8.19 [a]	85.5 [b]
0	0	3.58	7.70	93.9
0	0.1	3.61	7.87	89.4
0	0.2	3.72	7.68	88.2
Interaction effects				
0	0	3.43	8.10	97.5
0	0.1	3.43	7.18	97.2
0	0.2	3.45	7.10	94.0
125	0	3.43	7.10	97.5
125	0.1	3.73	7.98	86.0
125	0.2	3.83	7.74	85.8
250	0	3.89	7.90	86.6
250	0.1	3.67	8.46	85.1
250	0.2	3.87	8.20	84.8
SEM		0.103	0.340	3.00
Source of variation		Probability		
CSS		0.0004	0.031	0.0004
Arginine		0.260	0.747	0.066
CSS x Arginine		0.111	0.065	0.305

[a-b]In a same column, means with different superscript letters differ significantly ($P < 0.05$).

their degradation products, and the tolerance for glucosinolates is different among animal species (TRIPATHI & MISHRA, 2007). The tolerance level (μmol/ g diet) of glucosinolates in poultry is 5.4 mol (TRIPATHI & MISHRA, 2007).

Table 6 presents the effects of processed CM with CSS and different levels of supplemental Arg on blood biochemistry parameters. On day 42, dietary treatments did not affect urea, SGOT, LDH, creatine kinase, triglyceride, cholesterol, HDL, LDL and VLDL concentrations ($P > 0.05$), whereas plasma glucose concentrations decreased with increasing Arg levels ($P < 0.05$). Glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in plasma. Furthermore, previous studies reported that ascitic broilers show greater concentration of glucose in the liver (KAMELY et al., 2016) and plasma (DANESHYAR et al., 2009) due to an increase in gluconeogenesis level during the development of broilers ascites. Arg has been documented to be the precursor of nitric oxide (ATTIA et al., 2006; 2011b). Nitric oxide improves the responsiveness to insulin hormone and increases the tendency to respond for insulin receptors. Moreover, increased insulin receptor sensitivity led to decreased blood glucose levels.

In conclusion, results of this trial indicated that the addition of 250 mg/kg CSS and 0.2% Arg reduces the stress caused by the rapid growth of broilers, also increasing the overall bird welfare. In addition, treatments of CM with CSS could alleviate the adverse effects of glucosinolates on broilers performance. These findings are in line with international scientific efforts aiming to increase the potential applications of CM protein in poultry diets.

Table 6 - Effects of processed canola meal supplemented with copper sulphate solution (CSS) and arginine on blood biochemical parameters of broiler chickens during grower period of the trial (22-42 d).

CSS (mg/kg)	Arginine (%)	Glucose (mg/dL)	Urea (mg/dL)	SGOT (U/L)	LDH (U/L)	Creatine kinase (U/L)	Triglyceride (mg/dL)	Cholesterol (mg/dL)	HDL[1] (mg/dL)	LDL[2] (mg/dL)	VLDL[3] (mg/dL)
----------Mean effects----------											
0	0	220	1.99	180	246	4,605	72.0	131	72.5	44.3	14.4
125	0	219	1.95	196	260	4,624	70.7	130	73.0	42.9	14.1
250	0	226	1.82	194	268	4,704	73.0	129	73.2	40.7	14.6
0	0	226^{a}	2.09	182	264	4,490	72.0	131	72.5	43.7	14.4
0	0.1	223^{ab}	1.98	190	258	4,665	72.8	128	71.7	41.8	14.6
0	0.2	217^{b}	1.92	192	252	4,778	70.9	131	74.5	42.4	14.2
----------Interaction effects----------											
0	0	228	2.11	170	270	4,298	73.4	134	74.8	44.3	14.7
0	0.1	218	1.99	178	235	4,681	74.0	127	70.6	41.4	14.8
0	0.2	214	1.85	173	231	4,836	68.6	133	72.2	47.1	13.7
125	0	219	2.09	187	244	4,353	68.4	129	69.8	45.3	13.7
125	0.1	227	1.89	198	273	4,853	69.8	131	73.6	43.6	14.0
125	0.2	213	1.95	203	264	4,666	73.8	130	75.6	39.6	14.8
250	0	230	2.02	188	276	4,819	74.2	129	72.8	41.4	14.8
250	0.1	224	1.94	195	267	4,460	74.6	126	71.0	40.3	14.9
250	0.2	224	1.80	200	262	4,832	70.2	130	75.8	40.6	14.0
SEM		4.17	0.14	11.3	12.19	175	2.03	1.68	1.94	2.46	0.40
Source of variation		----------Probability----------									
CSS		0.152	0.283	0.082	0.071	0.764	0.380	0.162	0.907	0.223	0.384
Arginine		0.030	0.149	0.504	0.530	0.139	0.510	0.068	0.204	0.632	0.511
CSS x Arginine		0.17	0.11	0.97	0.09	0.11	0.07	0.08	0.17	0.34	0.06

[a-b]In a same column, means with different superscript letters differ significantly ($P < 0.05$).

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DECLARATION OF CONFLICT OF INTEREST

We have no conflict of interest to declare.

AUTHORS' CONTRIBUTIONS

Conceptualization: SAY, PF, PBK, SP, AS and CAFO. Data acquisition: SAY, PF, PBK and SP. Design of methodology and data analysis: SAY, PF, PBK, SP, AS and CAFO. SAY, PF, PBK, SP, AS and CAFO prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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Erratum

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