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Effects of Copper Sulfate and Arginine Supplements on Performance and Carcass Traits in Broiler Chickens Fed with Canola Meal Based Diet

Research Article

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ABSTRACT

The aim of this study was to evaluate the effects of copper (Cu) sulfate (0, 125 and 250 mg/kg), arginine (Arg) supplements (0, 0.1 and 0.2%) and glucosinolate content on performance and carcass traits in broiler chickens fed with canola meal based diet. During a three-week experimental period (22-42 d), 405 male broilers were used in completely randomized design with a 3 × 3 factorial experiment. Average daily gain and feed conversion ratio were significantly affected (P<0.05) by 250 mg/kg copper treatment. Canola meal treatments with Cu (P<0.01) and addition of 0.2% Arg (P<0.05) significantly increased the proportion of breast muscle. Also, the relative thigh weight was significantly affected by 250 mg/kg copper treatment and 0.2% arginine supplementation (P<0.05). The 0.2% Arg supplementation significantly decreased the proportion of abdominal fat (P<0.01), lung weight (P<0.05) and conversely increased the proportion of duodenum (P<0.05) and jejunum (P<0.01). Cecal relative weight was the lowest (P<0.05) in broilers fed diets based on canola meal treated with 250 mg/kg Cu. In conclusion, the results of this study show that treatments of canola meal with copper sulfate could alleviate adverse effects of glucosinolate on broilers performance. Moreover, these findings suggest that addition of 0.2% Arg able to change energy partitioning toward protein deposition and reduced abdominal fat pads.

KEY WORDS abdominal fat, arginine, broiler, cecal, copper sulfate, duodenum, glucosinolate.

INTRODUCTION

The global production of rapeseed, including canola varieties, ranks second among oilseed crops (United States Department of Agriculture, 2011). The composition of rapeseed has been remarkably altered by plant breeders who have developed new varieties of rapeseed, known as double-zero rapeseed or canola. Canola (canadian oil, low erucic acid) is a type of rapeseed (*Brassica napus*, *Argentinan canola* or *Brassica campestris/rapa*, *Polish canola*), which were bred to have low levels of erucic acid and glucosinolates, less than 2% and 30 µmol/g, respectively (CCAC, 2009). Canola meal (CM) has a lower nutritive value than soybean meal (SBM) because of the higher nonstarch polysaccharide content of CM and also is limited by the presence of several antinutritive factors, such as tannins, glucosinolates, and phytic acid (NRC, 1994). The glucosinolates are major antinutritional factors present in canola meal that limit its inclusion in poultry diets. Glucosinolates are a large group of sulfur-containing secondary plant metabolites which are common to CM and other Brassica. In broiler chickens, feeding a high level of dietary glucosi-

nolates resulted in reduced feed intake (FI), growth rate, and increased mortality (Payvastegan *et al.* 2012). There are several treatments methods and or supplementation was also tried to overcome glucosinolates, which can have deleterious effects on animal health and production and most of them based on the analysis of glucosinolates before feeding (Khajali and Wideman, 2010; Schone *et al.* 1993). One way to neutralize the adverse effects of glucosinolates is through dietary supplementation with copper. Copper is often added to poultry diets at prophylactic concentrations for its growth promoting effects (Payvastegan *et al.* 2012). The copper sulfate supplementation may redirect glucosinolates breakdown products (Tripathi and Mishra, 2007). Pekel and Alp (2011) reported that birds fed the camelina meal, which is a member of the brassica family responded to copper sulfate supplementation with improving live performance and carcass characteristics. Despite the fact that CM is a particularly rich source of sulfur-containing amino acids, the Arg content of CM is approximately two-thirds that of SBM (2.08 *vs*. 3.14% according to NRC, 1994). Broilers are unable to synthesize Arg (Khajali and Wideman, 2010). Substitution of a high proportion of CM protein at the expense of SBM protein in poultry diets may drop dietary Arg level below its requirements and subsequently result in poor performance. Broilers fail to synthesize Arg because they lack the key enzyme carbamoyl phosphate synthase I (EC 6.3.5.5) and have low activities of hepatic arginase (EC 3.5.3.1), 2) and ornithine transcarbamoylase (OTC, EC 4.4.4.17). Therefore, they have an absolute need for Arg and are highly dependent on dietary sources for this amino acid (Khajali and Wideman, 2010). Kidd *et al.* (2001) reported that supplementing broiler diets with 0.2% Arg beyond NRC (1994) requirements even under normal conditions resulted in improved growth performance. The objective of the present study was to evaluate the effects of copper (Cu) sulfate and arginine supplements on performance and carcass traits in broiler chickens fed with canola meal based diet.

MATERIALS AND METHODS

This study was performed in the research farm of Urmia University of Iran. All methods used in this experiment followed the Federation of Animal Science Societies Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching.

A total of 405, 1-d-old male broiler chicks (Ross 308) obtained from a local hatchery and randomly allocated to 45 floors pens (with 5 replicates and 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). Source of copper was sulfate pentahydrate (Merck Company, Germany) and also synthetic arginine (Merck Company, Germany) was used. 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 and humidity was 50%. Chickens were fed with basal diet from 1 to 21 days of age and the experimental diets were fed from 22 to 42 days of age. The experiment lasted for 20 d (22-42 d), and Continuous light was provided throughout the experiment. Three levels of treated CM with copper sulfate (0, 125 and 250 mg/kg) and arginine (0, 0.1 and 0.2%) in a 3 × 3 factorial design and nine dietary combinations in equinitrogenous and equicaloric diets (Table 1). Analysis of amino acids profile (Table 2) and components of canola meal (Table 3) was done by near-infrared diffuse reflectance (NIR) and total amount of glucosinolates (Table 4) was performed by spectrophotometric analysis (Saini and Wratten, 1987). The basal diets were formulated according to the Ross requirements (Aviagen, 2012) guideline. The nine treatment diets were 1) 0 mg/kg of copper and 0% of Arg , 2) 0 mg/kg of copper and 0.1% of Arg, 3) 0 mg/kg of copper and 0.2% of Arg, 4) 125 mg/kg of copper and 0% of Arg, 5) 125 mg/kg of copper and 0.1% of Arg, 6) 125 mg/kg of copper and 0.2% of Arg, 7) 250 mg/kg of copper and 0% of Arg, 8) 250 mg/kg of copper and 0.1% of Arg, 9) 250 mg/kg of copper and 0.2% of Arg.

Treated canola meal with copper sulfate

For treating canola meal with copper sulfate, spray method was used. At first, canola meal was milled by grinder, then 1.47 and 2.93 g of copper sulfate pentahydrate were completely dissolved in 900 mL of water (per kg of canola meal) and were sprayed uniformly on canola meal. The treated canola meal was heated for 24 h at 60 ˚C until to ensuring constant weight. After complete drying, the canola meal treated with copper was ready to mix with arginine supplementation and another part of the experimental diet (Table 4).

Sample and data collection

Throughout the trials, the feed was weighed when delivered to broilers and mortality was observed daily. Based on the recorded data, feed intake (FI), feed conversion ratio (FCR), body weight (BW) and body weight gain (BWG) were calculated. On day 42 of trial, two birds per pen were randomly selected, allowed to fast for 12 hours and commercially slaughtered for whole carcass analysis.

Table 1 Composition of experimental diets

Ingredients (%)	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
Di-calcium phosphate	2.15	1.34
Calcium carbonate	1.14	1.00
Salt	0.30	0.30
Trace mineral premix[1]	0.25	0.25
Multi vitamin premix[2]	0.25	0.25
Calculated analyses (%)		
Metabolisable energy (kcal/kg)	2900	3000
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
Methionine + cystine	0.90	0.74
Linoleic acid	1.73	3.16

[1] Provided per kg of ration: Copper (cupric sulfate): 10 mg; Iron (ferrous sulfate): 50 mg; Manganese (manganese oxide): 100 mg; Zinc (zinc sulfate): 85 mg; Selenium (sodium selenite): 0.2 mg and Iodine (calcium iodate): 1.0 mg.
[2] Provided per kg of ration: Retinol: 900 IU; Cholecaciferol: 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 and Biotin: 0.1 mg.

Table 2 Analysis of canola meal amino acid profile

Amino acid	The amount of amino acids based on dry matter (%)
Crude protein	38.53
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

Table 3 Analysis of components of canola meal

Parameter	The amount based on dry matter (%)
Dry matter (%)	92.78
Ether extract (%)	2.70
Crude fiber (%)	10.20
Ash (%)	7.71
Sugar (%)	9.12
Total phosphorus (mg/kg)	9193.00
Phytate phosphorus (mg/kg)	5576.00
Metabolizable energy corrected for nitrogen (kcal/kg)	1916.00

Table 4 Analysis of glucosinolates

Parameter	Glucosinolate content of canola meal (µmol/g meal)[1]	Glucosinolate content of canola meal (µmol/g diet)[2]	The rate of glucosinolate decline in the diet after treatment (%)
Canola meal without treatment	18.34	6.25	-
Canola meal treated with 125 mg/kg copper	16.09	5.49	12.16
Canola meal treated with 250 mg/kg copper	11.78	4.02	35.68

[1] n= 3.
[2] Glucosinolate calculated only on the basis of glucosinolate canola meal diet.

After slaughter, the organs such as breast, thigh, drumstick, abdominal fat, lungs, and intestine were weighed (weight divided by body weight×100).

Statistical analysis

The experiment was conducted using completely randomized design with factorial structure. Data were subjected to ANOVA using the GLM procedure (SAS, 2001) as a 3 × 3 factorial, with the main effects of copper and arginine, and the arginine × copper interaction. The differences between means were considered significant when $P < 0.05$, and when significant main effects were observed, differences between means were determined using the method of Duncan procedure.

RESULTS AND DISCUSSION

Overall performance

The effects of treated canola meal with copper sulfate solution and different levels of supplemental arginine on FI, BW, BWG and FCR are summarized in Table 5. The main effect of BWG and FCR were significantly affected ($P<0.05$) by 250 mg/kg copper treatment. 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 (Payvastegan *et al.* 2012). Various processing techniques were applied to remove glucosinolates in order to minimize their deleterious effects on animals. The Cu-sulfate supplementation may redirect glucosinolates to breakdown products, may react to form complex with, or to produce secondary breakdown products by rearrangement reactions (Tripathi and Mishra, 2007). 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. Schone *et al.* (1993) reported that the deleterious effects of glucosinolates on broiler performance could be normalized by pretreating rapeseed meal with Cu sulfate before feeding. Also, glucosinolates are cleaved after treatment of canola meal with myrosinase or soaked copper sulfate. 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).

Carcass traits

Table 6 shows the effects of treated canola meal with copper sulfate solution and different levels of supplemental arginine on the relative weight of internal organs in broiler chickens fed by canola meal based diet at 42 d of age.

Main effect of carcass yields was significantly increased ($P<0.01$) with supplementation of Cu and 0.2% Arg when added to the diets. An enhancement ($P<0.01$) in breast weight, as percentage of live BW, was observed due to addition of Cu and 0.2% Arg ($P<0.05$) to diets. Table 7 shows the effects of treated canola meal with copper sulfate solution and different levels of supplemental arginine on relative weight of duodenum, jejunum, ileum and cecal in broiler chickens fed by canola meal based diet at 42 d of age.

The use of 0.2% Arg significantly decreased the proportion of abdominal fat ($P<0.01$) and lung weight ($P<0.05$) and conversely linearly increased the main effect of duodenum ($P<0.05$) and jejunum ($P<0.01$). Cecal relative weight was the lowest ($P<0.05$) for birds fed diets based on canola meal treated with 250 mg/kg Cu. The growth stimulation effects of Cu could be attributed to shifting the gastrointestinal microbiota, thereby reducing the susceptibility of birds to disease, reducing intestinal lymphocyte recruitment and infiltration, and thus increasing nutrient absorption (Arias and Koutsos, 2006; Pang *et al.* 2009). Interestingly addition of Cu sulfate could improve intestinal mucosal-morphology, which may contribute in improving nutrient availability and is associated with increasing goblet cell numbers, total goblet cell area, goblet cell mean size, mucosal thickness and a greater number of segmented filamentous bacteria compared with controls (Payvastegan *et al.* 2013). Zhou *et al.* (1994) also demonstrated that Cu might be involved in pituitary growth hormone gene expression and secretion of several neuropeptides in the hypothalamus. Fernandes *et al.* (2009) reported that the average live body weight of broiler chickens at 42 days was not affected by L-Arg supplementation. Tan *et al.* (2006) also found that supplementing broiler chicken diets with 1% L-Arg did not effect on feed intake, body weight and feed conversion ratio. There are contrary reports that suggest Arg supplementation beyond the NRC (1994) recommendations improves broiler productivity (Fernandes *et al.* 2009; Ruiz-Feria, 2009).

Kidd *et al.* (2001) reported that supplementing broiler diets with 0.2% Arg beyond NRC (1994) requirements even under normal conditions resulted in improved growth performance. Arg is the precursor of several growth factors like putrescine, spermine, and spermidine via the formation of glutamate (Khajali and Wideman, 2010). Arg can also yield increased amounts of proline and hydroxyproline, which are required for the synthesis of connective tissue (Popovic *et al.* 2007). Additionally, the production of insulin-like growth factor (IGF) and the release of anti insulinemic hormones such as glucagon, somatostatin, pancreatic polypeptides, and catecholamines are enhanced by Arg (Barbul, 1986).

Table 5 The effects of treated canola meal with copper sulfate solution and different levels of supplemental arginine on feed intake, body weight, weight gain and feed conversion ratio (FCR) in broiler chickens fed by canola meal based diet at 22-42 d of age

Treatments	Feed intake (g)	Body weight (g)	Weight gain (g)	FCR
		Cu (mg/kg)		
0	2389.35	1930.18^{b}	970.12^{b}	2.55^{a}
125	2330.93	1954.02^{ab}	1035.00^{ab}	2.30^{ab}
250	2412.48	2056.87^{a}	1125.68^{a}	2.18^{b}
SEM	29.73	29.99	40.12	0.09
		Arg (%)		
0	2367.17	1943.00	1008.05	2.41
0.1	2425.35	1963.14	1005.34	2.47
0.2	2340.24	2034.93	1117.41	2.15
SEM	29.73	29.99	40.12	0.09
		Arg × Cu		
0 × 0	2425.37	1865.77	910.76	2.75
0.1 × 0	2391.59	1870.85	873.34	2.74
0.2 × 0	2351.09	2053.94	1126.26	2.15
0 × 125	2337.55	1946.48	1097.32	2.13
0.1 × 125	2346.59	1923.28	948.79	2.53
0.2 × 125	2318.66	1992.31	1058.90	2.25
0 × 250	2348.60	2016.77	1016.08	2.34
0.1 × 250	2537.87	2095.28	1193.88	2.13
0.2 × 250	2350.94	2058.55	1167.06	2.06
SEM	51.46	51.94	69.50	0.16
		P-value		
Cu	0.15	0.01	0.03	0.03
Arg	0.13	0.08	0.09	0.06
Arg × Cu	0.23	0.27	0.08	0.15

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 6 The effects of treated canola meal with copper sulfate solution and different levels of supplemental arginine on relative weight of internal organs[1] in broiler chickens fed by canola meal based diet at 22-42 d of age

Treatments	Carcass yield %	Breast yield %	Thigh yield %	Drumstick %	Abdominal fat %	Lungs %
			Cu (mg/kg)			
0	57.93^{b}	21.27^{b}	11.24^{b}	9.72	1.64	0.53
125	60.48^{a}	22.38^{a}	11.46^{ab}	9.81	1.59	0.48
250	60.70^{a}	22.46^{a}	11.92^{a}	9.84	1.46	0.48
SEM	0.59	0.27	0.19	0.21	0.06	0.01
			Arg (%)			
0	58.43^{b}	21.47^{b}	11.27^{a}	9.65	1.76^{a}	0.53^{a}
0.1	59.66^{ab}	22.14^{ab}	11.37^{ab}	9.70	1.60^{a}	0.49^{ab}
0.2	61.02^{a}	22.52^{a}	11.98^{a}	10.01	1.33^{b}	0.47^{b}
SEM	0.59	0.27	0.19	0.21	0.065	0.01
			Arg × Cu			
0 × 0	57.31	20.79	10.52	9.47	1.92	0.57
0.1 × 0	57.19	21.05	11.55	9.48	1.78	0.57
0.2 × 0	59.28	21.99	11.66	10.21	1.21	0.46
0 × 125	58.69	22.04	11.51	10.01	1.65	0.52
0.1 × 125	60.74	22.43	10.89	9.55	1.66	0.47
0.2 × 125	62.01	22.67	11.97	9.88	1.45	0.46
0 × 250	59.27	21.57	11.79	9.48	1.70	0.50
0.1 × 250	61.06	22.94	11.67	10.09	1.36	0.45
0.2 × 250	61.77	22.88	12.29	9.95	1.34	0.50
SEM	1.03	0.46	0.33	0.37	0.11	0.06
			P-value			
Cu	0.003	0.006	0.054	0.91	0. 17	0.06
Arg	0.015	0.031	0.030	0.45	0.0002	0.04
Arg × Cu	0.83	0.67	0.19	0.53	0.07	0.12

[1] Organ weight / live weight × 100.
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.

Fernandes *et al.* (2009) reported that insulin-like growth factor, stimulated anabolic processes in skeletal muscle such as proliferation and differentiation and aggregating muscle fiber protein. Sklan and Noy (2004) reported that change in the proportion of amino acids and their content in the diet affected amino acid catabolism much more than anabolism of amino acids. Arginine serves as the precursor for nitric oxide (NO) synthesis and, evidently, the lower content of Arg in the canola meal diet resulted in lower plasma NO levels. Plasma NO increased blood flow to the muscles, also increasing hormone reception by tissues and providing more carbohydrate and other nutrients for muscle. In chicks, creatine concentration is related to Arg intake. Creatine, as a precursor of creatine phosphate, has two important roles in normal muscle function; it carries high-energy phosphate from mitochondria to myosin filaments and it acts as a reservoir of high-energy phosphate that can regenerate ATP from ADP. On the other hand, diets with high levels of Arg stimulate the formation of creatine in the muscles of chickens (Chamruspollert *et al.* 2002). Cu supplementation significantly increased carcass yield, which may be related to antimicrobial effects of Cu against the pathogenic bacteria (Pang and Applegate, 2007), which leads to an increase in nutrients absorption that can be used for protein synthesis. Fouad *et al.* (2013) reported that supplementing the diet of meat-type ducks with 1.00% L-Arg significantly decreased the abdominal fat pad percentage by reducing the activities of malate dehydrogenase, glucose-6-phosphate dehydrogenase and fatty acid synthase (FAS) (lipogenic enzymes) in the liver. As well as reported that increasing the dietary L-Arg concentration from 1.49% to 1.79% during the starter phase had no effect on the abdominal fat content of broiler chickens on day 42. Whereas all chickens consumed the same diet without Arg supplementation from 22 to 42 days of age. Therefore, the difference between these two results may be attributed to the timing of the supplementation because the starter phase is associated with a rapid growth rate, but it is not linked to excessive fat deposition in broiler chickens. Fouad and El-Senousey (2012) indicated that the inclusion of arginine in broiler chicken diets at higher levels than NRC (1994) recommendations could reduce carcass fat content. In Japanese quails, Fouad *et al.* (2013) found a significant reduction in the abdominal fat content as a percentage of body weight at 42 days of age after injections of 2.0% arginine. Also reported that dietary arginine inclusion at 0.25% reduced abdominal fat deposition by suppressing hepatic FAS mRNA expression and enhancing carnitine palmitoyl transferase I (CPT1) and L-3-hydroxyacyl-CoA dehydrogenase (L3HADH) mRNA expression in the hearts of broiler chickens (Fouad *et al.* 2013). CPT1 and L3HOAD are recognized as the main enzymes involved with β-oxidation.

In avian species, therefore, dietary L-arginine supplementation inhibits hepatic FAS mRNA expression and improves CPT1 and 3HADH mRNA expression, which causes a reduction in the abdominal fat content by reducing the size of abdominal adipose cells. Sharifi *et al.* (2015) showed that supplementation of the diet with 0.4% arginine in broilers lead to down-regulation of endothelin-1 (ET-1) gene expression in the heart and lung of chickens. ET-1 is the most potent vasoconstrictor substance produced by the cardiovascular system. The pathophysiological role of ET-1 has been proposed in pulmonary hypertension syndrome (PHS) (Sharifi *et al.* 2015). It has been confirmed that high altitude-induced PHS (pulmonary hypertension syndrome) is associated with increased gene expression of ET-1 in the heart and lung of chickens (Sharifi *et al.* 2015). Accordingly, down-regulation of this gene may decelerate development of hypobaric pulmonary hypertension as reported in chickens fed reduced-protein diet supplemented with L-arginine. We thought that reducing the relative weight of the lungs would related to a decrease in pulmonary vascular resistance. The reduction in empty cecal weight with canola meal treated with 250 mg/kg Cu which is in agreement with Newkirk and Classen (2002) results, is likely due to decreased fermentation in the cecum, as soy bean meal contains more fermentable sugars such as stachyose and raffinose than canola meal (Khajali and Slominski, 2012). Another possibility is that canola meal may have some antimicrobial compounds capable of affecting the level of cecal fermentation (Newkirk and Classen, 2002). Pang *et al.* (2009) reported that supplementation of 36.75 mg/kg Cu from Cu sulfate to broiler chicken diets had no influence on intestinal microbiota; however, supplementation with 36.75 mg/kg Cu from Cu-bearing montmorillonite reduced the total viable counts of *Escherichia coli* and *Clostridia* in the small intestine and cecum. Copper is toxic to some species of bacteria such as *Klebsiella aerogenes* (Pang *et al.* 2009), whereas others have developed complex resistance mechanisms. Some experiments have been conducted to determine how high dietary Cu affects microorganisms in swine and poultry. Supplementation of Cu sulphate to pig diets reduces the number of ureolytic bacteria as a group, of which streptococci make up approximately 75%, inhibits the coliforms in the cecum and the colon, and reduces the number of streptococci in fecal samples (Pang *et al.* 2009). Many reports have been presented about the role of arginine on intestinal physiology. L-Arg, like most amino acids, is nutritionally essential for protein deposition. One of the major amino acid induced signaling pathways involved in cell growth, is mammalian target of rapamycin (mTOR). mTOR is a highly conserved serine/threonine protein kinase that controls many aspects of cellular physiology, including protein synthesis.

Table 7 The effects of treated canola meal with copper sulfate solution and different levels of supplemental arginine on relative weight of duodenum, jejunum, ileum and cecal[1] in broiler chickens fed by canola meal based diet at 22-42 d of age

Treatments	Duodenum	Jejenum	Ileum	Cecal
		Cu (mg/kg)		
0	0.72	2.14	0.97	0.26[a]
125	0.70	2.12	0.97	0.24[b]
250	0.69	2.08	0.96	0.22[b]
SEM	0.01	0.02	0.02	0.01
		Arg (%)		
0	0.68[b]	2.07[b]	0.97	0.24
0.1	0.70[ab]	2.09[b]	0.95	0.24
0.2	0.73[a]	2.18[a]	0.98	0.23
SEM	0.01	0.02	0.02	0.01
		Arg × Cu		
0 × 0	0.71	2.11	0.92	0.27
0.1 × 0	0.71	2.04	0.99	0.27
0.2 × 0	0.75	2.26	0.99	0.22
0 × 125	0.65	2.05	0.95	0.24
0.1 × 125	0.69	2.15	0.93	0.22
0.2 × 125	0.75	2.14	1.02	0.26
0 × 250	0.68	2.05	1.01	0.22
0.1 × 250	0.69	2.07	0.94	0.22
0.2 × 250	0.69	2.13	0.92	0.21
SEM	0.02	0.04	0.03	0.01
		P-value		
Cu	0.12	0.29	0.90	0.03
Arg	0.02	0.009	0.65	0.59
Arg × Cu	0.35	0.12	0.10	0.17

[1] Organ weight / live weight × 100.
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.

mTOR is a common signaling complex that mediates the cellular anabolic responses to insulin and amino acids. Some *in vivo* studies in rat or porcine IEC showed that arginine and leucine stimulate downstream targets of mTOR, namely, ribosomal protein S6 kinase 1 (S6K1) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) (Khajali and Wideman, 2010; Ban *et al.* 2004).

Yuan *et al.* (2015) reports addition of 100, 200, or 400 micromol L-Arg to culture medium increased protein synthesis and reduced protein degradation in chicken intestinal epithelial cells, also analysis of the cell cycle distribution in chicken IEC using flow cytometry revealed that L-Arg treatment for 4 d increased the cells in S and G2/M phase to a significant extent and decreased cell numbers in G0/G1 phase. It has been shown that NO benefits the antioxidant system by eliminating reactive oxygen species such as superoxide and hydrogen peroxide and protects cells from apoptosis (Khajali *et al.* 2011).

It is possible that antioxidative effect of NO produced by dietary supplement of Arg increases the growth of epithelial cells in the intestine and improves nutrient assimilation. Khajali *et al.* (2013) demonstrated that supplementation of broiler diets with 1% arginine, despite any change in performance, enhanced the length and width of villus in the jejunum.

CONCLUSION

In conclusion, the results of this study show that treatments of canola meal with copper sulfate could alleviate adverse effects of glucosinolate on broilers performance. Moreover, these findings suggest that addition of 0.2% Arg able to change energy partitioning toward protein deposition and reduced abdominal fat pads.

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