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The effects of different levels of canola meal and copper on performance, susceptibility to ascites and plasma enzyme activities in broiler chickens

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ABSTRACT

In order to assess the effects of different levels of canola meal (CM at proportion of 0, 10 and 20 %) and copper (fed at three levels of 0, 125 and 250 mg/kg) on performance, susceptibility to ascites syndrome and plasma enzymes activity of broiler chickens, an experiment was performed from d 1 to 21. A total of 495 male broilers (Ross 308) were used in completely randomized design with a 3×3 factorial arrangement with 5 replicates (11 birds per replicate). Feed consumption was not significantly affected by treatments in an experimental period ($P>0.05$). The BW gain and feed conversion ratio was significantly impaired ($P<0.05$) when 20% CM was added in the diets, but supplementation of copper was not significant effects on BW gain and feed conversion ratio ($P>0.05$). Replacing soybean meal by CM significantly increased ($P<0.05$) the proportion of heart weight and right ventricular weight: total ventricular weight ratio ($P<0.05$). However, haemoglobin concentration, packed cell volume and total erythrocyte count were not affected by treatments ($P>0.05$). Concentrations of plasma enzymes and the proportion of liver weight were similar among the treatments ($P>0.05$). This finding indicate that when canola meal used in young broiler diets, the level of this ingredient in the formulation should be considered, because may be impaired young broilers growth performance.

Key words: canola meal, copper, performance, ascites, broilers

INTRODUCTION

Canola is an offspring of rapeseed that was bred to have low levels erucic acid (<2%) in the oil portion and low levels of glucosinolates (<30 µmol/g) in the meal portion [1]. Canola meal (CM) is a widely used protein source with a good balance of amino acids but with a lower amino acid digestibility than soybean meal [1]. The nutritive value of canola meal also is limited by the presence of several antinutritive factors, including tannins, glucosinolates, phytic acid and indigestible nonstarch polysaccharides [2-1]. CM has been associated with reduced feed intake and reduced growth rate in broilers [3]. Min et al. (2009) reported that feed intake and body weight gain declined dramatically with the increasing inclusion of CM during 1 to 18 d [1].

Copper is an essential mineral required for proper bone growth and development as well as enzyme function [4]. Copper is often added to poultry diets at prophylactic concentrations for its growth promoting effects [5]. The most commonly used copper for supplementation in animal diet is inorganic copper in the form of copper sulfate pentahydrate ($CuSO_4.5H_2O$) due to cost and commercial availability [6]. CM contains some secondary plant metabolites called glucosinolates that are common to rapeseed meal and other *Brassica*. Glucosinolates are known for a long to reduce the intake, induce iodine deficiency, hypertrophy of liver and thyroid, decrease growth and production [7]. In general, young animal are more sensitive to glucosinolates than adult and older animals [7].

Various treatments methods and or supplementation were also tired to overcome glucosinolates-associated deleterious effects on animal health and production. Dietary copper supplementation can affect the nutritive value and potential toxic effects of rapeseed meal [7]. The copper sulfate supplementation may redirect glucosinolates breakdown products, may react to form complex with, or to produce secondary breakdown products by rearrangement reactions [7]. Pekel et al. (2009) reported that birds fed the camelina meal, which is a member of the brassica family like canola responded to copper sulfate supplementation with improve live performance and carcass characteristics [8].

Modern strains of broiler are able to achieve market weight in 60% less time than broilers of 40 years ago. Nevertheless, the pulmonary and cardiac capacity of modern broilers is very similar to the old broiler strains, which forces their cardiopulmonary system to work very close to its physiological limit [9]. Pulmonary hypertension occurs whenever the relatively inelastic pulmonary vasculature bed of susceptible birds cannot accommodate rapid increase in cardiac output [10]. Despite the fact that CM is a particularly rich source of sulfur-containing amino acids, the Arg content of canola meal approximately two-thirds that of soy bean meal (2.08 vs. 3.14% according to NRC, 1994) as well as having a lower Arg digestibility compared to soybean meal [11]. Arg is the substrate from which the powerful vasodilator nitric oxide is produced by vascular endothelial cells [10]. Arg was first shown by wideman et al. (1995) to prevent pulmonary hypertension syndrome in broiler challenge with metabolic hypoxemia resulting from fast growth and cool temperature [12]. Birds are unable to synthesize Arg [13]. As a consequence, feeding CM to broiler instead of soybean meal may not provide sufficient Arg to fully support the production of nitric oxide by the pulmonary vascular endothelium [13].

Hence, the present study was designed to 1) compare the effect of dietary CM and soybean meal on bird performance, 2) to determine effects of copper sulfate supplementation on broiler performance, 3) to evaluate whether such effects can be normalized with the addition of prophylactic levels (125 & 250 mg/kg) of copper, and 4) to study any effects of substitution of CM meal instead of soybean meal on susceptibility to ascites in young broilers chickens.

MATERIALS AND METHODS

The experiment was conducted in the experimental facility of Urmia University in Urmia (Iran). The Conditions and standards of care used in this study were approved by the committee of Urmia University**.**

Dietary Treatments & Animal Husbandry

A total of 495 1-d-old male broiler chicks (Ross 308) obtained from a local hatchery and randomly allotted to 1 of 45 floors pens (with 5 replicates and 11 chicks in each replicate) measuring 1.5 m^2. New wood shavings at a depth of approximately 10 cm were used as bedding material over a concrete floor. The 1-d-old Chicks were weighed individually and allocated to pens so that their initial weights were similar across all pens. Three levels of CM (0, 10 and 20%) were used with three levels of copper (0, 125 and 250 mg/kg) in a factorial design of 3×3 in 9 dietary combinations in equinitrogenous and equicaloric diets (Table 1). The basal diets were formulated according the Ross requirements (Aviagen Company) guideline. The 9 treatment diets were 1) 0% CM diet with 0 mg/kg of copper, 2) 0% CM diet with 125 mg/kg of copper, 3) 0% CM diet with 250 mg/kg copper, 4) 10% CM diet with 0 mg/kg of copper, 5) 10% CM diet with 125 mg/kg of copper, 6) 10% CM diet with 250 mg/kg of copper, 7) 20% CM diet with 0 mg/kg of copper, 8) 20% CM diet with 125 mg/kg of copper, 9) 20% CM diet with 125 mg/kg of copper. All chicks were provided ad libitum access to water and their assigned diets (in mash form). The copper was source of copper sulfate pentahydrate (Merck Company, Germany) and after calculating the purity was added to basal diets. Birds were vaccinated against Newcastle disease on 10, 20 and 30 days. The house temperature was maintained at 32^{o}C during the first week of age and a weekly reduction of 3^{o}C was practiced until a temperature of 25^{o}C was attained. The experiment lasted for 21 d, and 24 h of light was provided throughout the experiment.

Measurements

At 7, 14, and 21 d of age all of the birds in each pen were weighed in groups and feed intake per pen was measured. Feed intake, body weight gain and feed: gain were calculated for the 1 to 21 d period. Birds that were removed or died during the experiments were weighed and used to adjust the feed: gain. At the end of experiment, blood sample were collected from one bird per replicate (5 birds per treatment) by neck slitting; 2.5 mL of blood were collected into tubes containing heparin and another 2.5 mL were collected into a hypodermic syringe. The blood in the heparinaized tubes was used to determination of packed cell volume (PCV), total erythrocyte count (TEC) and haemoglobin (Hb). The remaining blood samples were centrifuged at 2500 × g for 10 min. The obtained plasma samples were kept in −20°C for later analyses. Plasma concentrations of Alanine aminotransferase (ALT) and Aspartate aminiotransferase (AST) measured by AutoAnalyzer (Alyson 300, UK) with using commercial kits (Pars Azmun Company, Iran). After birds were killed liver and heart removed, and weighed. The ventricles were also dissected and weighed to calculate the right ventricular weight: total ventricular weight ratio (RV:TV). Total glucosinolates and erucic acid contents of the CM which used in this study were 24.6 $\mu mol.g^{-1}$ and 0.25 %, respectively.

Statistical Analysis
The experiment was conducted using completely randomized design with factorial structure. Data were subjected to ANOVA using the GLM procedure (SAS , version 9.1) as a 3×3 factorial, with the main effects of CM and copper, and the CM×copper interaction. Significant means among variables were separated by Duncan's multiple range test at 5% level of significance.

RESULTS AND DISCUSSION

Body weight gain, feed consumption and feed conversion results of study are summarized in Table 2. Canola meal may be viewed as an economically viable alternative to soybean meal in poultry diets. Feed intake was not significantly affected by levels of CM ($P > 0.05$). Probably Lack of effect of CM on feed intake has attributed the cause of the low glucosinolate of CM that used in this study. Leeson et al. (1987) found that even complete replacement of soybean meal (100%) with CM (<30µmol/g glucosinolate) did not affect on feed intake of broilers and laying hens [14].

Body weight gain and feed conversion ratio were impaired ($P<0.05$) by the addition of CM at the 20% of diet. Baloch et al. (2003) reported that the inclusion of extracted dehulled canola rapeseed up to 20% of the diet had no adverse effect on the performance of chicks [15]. In present study, when dietary CM was increased to 20%, it resulted in reduced performance during 1 to 21 d, which showing the negative effect in younger birds. Mushtaq et al. (2007) reported that body weight gain was significantly reduced by feeding diets with 30% CM only during the starter phase, and was unaffected during 1 to 42 d [3]. Reduced performances with high levels of rapeseed meal in young broilers were also reported by other researchers [16-17].

It was noted that diets containing 20% CM had 4.14 $\mu mol.g^{-1}$of glucosionolates, while the diets containing 10% CM had 2.07 $\mu mol.g^{-1}$of glucosinolates. In general, believed that glucosinolates in poultry diets must be less than 2.5 $\mu mol.g^{-1}$ [3].Thus significant reduction in performance of broilers during 1 to 21 d may be result of high glucosinolate content of experimental diets. In general young animal are more sensitive to glucosinolates than adult and older animals [7]. The tolerance to glucosinolates in younger birds is less, which impairs thyroid functions [3]. As the birds grow, the thyroid develops and mature birds can tolerate a fairly high amount of glucosinolates [3]. On the other hand, Over 70% of canola phosphorus in phytic acid form [3]. The poor growth performance in younger birds in the present study may be due to high phytate content of CM. Phytate has the ability to chelate cations such as iron, sodium, sulfur, calcium, zinc, copper as well as nitrogen and amino acids [18]. Phytate is also known to inhibit a number of digestive enzymes, such as pepsin and trypsin [19]. Additionally, Digestive enzyme activities in the pancreas and intestinal contents increase with age [20]. Therefore the adverse effect of CM at the level of 20% on performance in young broilers may be due to inadequate development of enzyme activities in contrast to mature birds [3]. In addition, High crude fiber content of CM compared whit soybean meal may be negatively affected AME_n value for broiler chickens [21]. Unfavorable effect of CM on growth performance of broilers can attributed to the lysine-arginine imbalance in higher levels of CM in diets [22].

The broiler chick's nutritional requirement for copper is approximately 8 mg/kg [2]. 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 [23]. In current study, there were no significant effects of copper supplementation on feed intake, body weight gain and feed conversion ratio ($P > 0.05$).

The lack of a growth enhancing effect of extra copper in the present study may be related to environmental sanitary conditions of the experimental facility and minimal environmental challenges during the experimental period. The increase of copper concentration may affect performance under some conditions such as in stressful environments [24]. Arias and Koutsos (2006) reported that supplementing the broiler diet with extra copper (188 ppm) was more effective under immune-challenging conditions [25]. Therefore, the extent of microbial challenge may influence the response of broilers dietary copper supplementation. More importantly, another possible explanation for the lack of significant effect of extra copper in this study may be related to short term supplementation of copper [26]. Ewing et al. (1998) reported that extra copper (63 0r 125 mg/kg) significantly improved broiler chick performance at 35 or 42 d; however, it did not have a significant effect at 18 or 21 d [23].

The effects of CM and copper on PVC, Hb, TEC, heart weight and RV:TV are shown in Table 3. PVC, Hb and TEC of birds were not significantly affected by dietary treatments, while heart weight (as proportion of body weight) and RV:TV significantly increased (P<0.05) as a result of substituting CM for soybean meal.

In avian species, Arg is an essential amino acid, because birds lack the enzyme carbamyl phosphate synthetase I, which aids in the conversion of ornithine to citrulline and thus Arg [10]. For this reason, Arg concentrations in birds are correlated only with dietary intake. Arg content of in canola meal as well as Arg digestibility is lower than soybean meal [2-11]. Substitution of a high proportion of CM instead of soybean meal in poultry diets may drop the dietary Arg level below its requirements [11]. Thus, if CM is included at high levels in a broiler diet, it is possible that dietary Arg content may not adequate to fully support the production of nitric oxide by avian macrophages and the pulmonary vascular endothelium [27]. Impaired endothelial nitric oxide production contributes to the increased vascular resistance leading to pulmonary hypertension [10].

Table1. Composition of basal diets and calculated and determined nutrient analysis (as-fed basis)

Ingredient (%)	0 % Canola meal	10 % Canola meal	20 % Canola meal
Corn	55	52.8	52. 6
Soybean meal	35.5	26.85	16.05
Canola meal	0	10	20
Corn Gluten meal	1.77	3	3.57
Soy oil	1.78	2.2	2.7
Dicalcium phosphate	2.80	2.7	2.7
Calcium carbonate	0.94	0.9	0.78
Salt	0.36	0.36	0.36
Trace mineral supplement[a]	0.25	0.25	0.25
Multi vitamin supplement[b]	0.25	0.25	0.25
L-Lysine HCL	0.3	0.3	0.3
DL- Methionine	0.33	0.3	0.33
Sand	0.72	0.09	0.11
Calculated analyses (%)			
ME (kcal/kg)	2950	2950	2950
CP	22	22	22
CF	2.62	3.36	4.05
Calcium	1.06	1.06	1.06
Available P	0.54	0.54	0.54
Arg	1.48	1.42	1.29
Lys	1.43	1.43	1.43
Met+Cys	1.07	1.07	1.07
Determined analyses			
Total glucosinolates (µmol/g)	0	2.07	4.14
Erucic acid (%)	0	0.025	0.05

[a] Provided per kg of ration; copper 10 mg (Cupric sulfate), iron 50 mg (ferrous sulfate), manganese 100 mg (manganese oxide), 85 mg zinc (zinc sulfate), selenium 0.2 mg (sodium selenite) and iodine 1.0 mg (calcium iodate).[b] 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 500mg and biotin 0.1 mg.

Newkirk and classen (2002) reported the ascetic mortality of broiler chickens increased from 1.9 to 9.6 %, when soybean meal was replaced with CM [28]. These researchers did not explain the reason for the high rate of ascites mortality induced by CM, but Khajali et al. (2011) suggested that lower nitric oxide synthesis probably due to lower nitric oxide synthesis [13]. Khajali et al. (2011) and Izadinia et al. (2010) demonstrated that substitution of CM for soybean meal caused by significant reduction in plasma nitric oxide concentration [11-13]. These authors also showed addition of CM to broiler diets increased heart weight, RV: TV and ascites mortality [11-13]. Newkirk and classen (2002) indicated that feeding CM resulted in a linear increase in heart weight as proportion of body weight [28]. If the values of RV: TV greater than 25% is considered as pulmonary hypertension [29]. In the current study values of RV: TV was lower than 25% for all treatments. Additionally there were not any effects on PVC, Hb and TEC, ascites and total mortality (did not show) of birds. Substitution CM for soybean meal did not have a significant effect on induction of ascites syndrome. Nevertheless, Based on the results of this study, it seems that utilization of higher levels of CM in broiler diets, feeding it for long period of time and feeding under the ambient temperature may be increase the susceptibility to ascites syndrome.

The effects of the level of CM and copper on liver weight and plasma enzymes concentration are shown in Table 4. Liver weight (as proportion of body weight) was not significantly increased by replacing CM and supplementation copper to diets (P>0.05). Increasing the liver weight in chicks fed on rapeseed meal as a consequence of the toxic effects of the hydrolytic products of glucosinolates, have been reported by others researchers [30-31]. As the canola meal utilized in this study contained low content of glucosinolates (20.7µmol.g^{-1}), did not significantly affect on liver weight.

Table2. Effects of dietary canola meal and copper supplementation on feed intake, body weight gain and feed conversion ratio in broiler chickens [a]

Item	*Feed intake (g)*	*Body weight gain (g)*	*Feed conversion ratio (g/g)*
CM			
0%	883.47	667.60^{a}	1.32^{b}
10%	882.60	666.47^{a}	1.33^{b}
20%	893.00	642.87^{b}	1.39^{a}
SEM	5.397	4.35	0.011
Cu			
0 mg/kg	884.00	655.27	1.35
125 mg/kg	885.73	657.93	1.35
250mg/kg	889.23	663.73	1.34
SEM	5.397	4.35	0.011
CM×Cu			
0 % - 0 mg/kg	876.40	668.80	1.31
0 % - 125 mg/kg	877.40	650.60	1.35
0 % - 250 mg/kg	896.60	683.40	1.31
10 % - 0 mg/kg	883/40	663.20	1.33
10 % - 125 mg/kg	884.40	666.40	1.33
10 % - 250 mg/kg	880.00	669.80	1.32
20 % - 0 mg/kg	892.20	633.80	1.41
20 % - 125 mg/kg	895.40	656.80	1.36
20 % - 250 mg/kg	891.40	638.80	1.40
SEM	16.192	13.03	0.034
	P-value		
CM	0.686	0.043	0.031
Cu	0.919	0.721	0.962
CM×Cu	0.923	0.371	0.776

Means in the same row with different letters (a and b) are significantly different (P<0.05).
[a] Eeach mean represents values from 5 replicates.

Table3. Effects of dietary canola meal and copper supplementation on heart weight, right ventricular weight: total ventricular weight ratio (RV/TV), haemoglobin concentration, packed cell volume and total erythrocyte count in broiler chickens [a]

Item	*RBC ($\times 10^6 \mu l$)*	*PCV (%)*	*Hb (g/dl)*	*RV/TV*	*Heart (%)*
CM					
0%	2.63	24.94	8.37	21.68^{b}	0.506^{b}
10%	2.67	25.01	8.38	21.57^{b}	0.514^{b}
20%	2.65	25.11	8.36	22.12^{a}	0.551^{a}
SEM	0.054	0.133	0.044	0.088	0.014
Cu					
0 mg/kg	2.69	24.90	8.39	21.72	0.528
125 mg/kg	2.67	25.01	8.38	21.90	0.519
250mg/kg	2.59	25.16	8.34	21.76	0.525
SEM	0.054	0.133	0.044	0.088	0.014
CM×Cu					
0 % - 0 mg/kg	2.53	24.86	8.40	21.63	0.512
0 % - 125 mg/kg	2.68	25.00	8.28	21.69	0.502
0 % - 250 mg/kg	2.65	24.96	8.44	21.72	0.504
10 % - 0 mg/kg	2.75	24.80	8.40	21.45	0.508
10 % - 125 mg/kg	2.64	25.00	8.55	21.70	0.532
10 % - 250 mg/kg	2.64	25.24	8.18	21.57	0.504
20 % - 0 mg/kg	2.78	25.04	8.36	22.08	0.564
20 % - 125 mg/kg	2.69	25.02	8.32	22.30	0.524
20 % - 250 mg/kg	2.49	25.28	8.41	22.00	0.566
SEM	0.163	0.398	0.132	0.262	0.021
		P-value			
CM	0.924	0.867	0.992	0.032	0.026
Cu	0.748	0.725	0.922	0.685	0.875
CM×Cu	0.752	0.989	0.373	0.973	0.483

Means in the same row with different letters (a and b) are significantly different (P<0.05).
[a] Eeach mean represents values from 5 replicates.

Adjustment to fluctuations in copper supply is achieved predominantly by hepatic storage and biliary copper secretion, but apportionment of copper trapped by the liver varies widely between species. Namely, those faced with endemic risks of copper deficiency (such as ruminants) avidly store excess copper, while species at no risk (such as poultry) mainly excrete excess copper via the bile and maintain low liver copper levels [32]. So the birds have less risk of liver damage than other species related to excess copper intake.

The concentration of plasma enzymes of broiler chickens was unaffected by CM and copper addition (P>0.05). Some researchers showed the changes in liver enzyme activity of blood [31], but some others did not observe [33]. For instance, Kloss et al. (1994) did not find any impact from feeding glucosinolates–extracted crambe meal on AST, GGT, lipase and amylase [34].

Levels of plasma AST, ALT and GGT were positively correlated with increased copper intake and indicative of hepatic damage starting with 300 mg Cu/head/day in a goat kids [35]. Thompson and told (1974) reported that serum AST activity begins to rise during the prehaemolytic period [36]. So it can be conclude that supplementation of copper-salt up to 250 mg/kg could not able to produce any dystrophy in hepatic or others tissues containing these enzymes and signified that the birds were apparently healthy during the feeding trail.

In this study, the interaction between canola meal and copper levels showed insignificant effects on performance criteria, ascites susceptibility and plasma enzymes concentration.

Table4. Effects of dietary canola meal and copper supplementation on liver weight and plasma enzymes oncentration in broiler chickens [a]

Item	*Liver*	*ALT (U/L)*	*AST (U/L)*	*GGT (U/L)*
CM				
0%	2.67	4.25	202.5	19.55
10%	2.63	4.27	205.0	21.00
20%	2.69	4.45	199.2	20.80
SEM	0.027	0.101	3.80	0.33
Cu				
0 mg/kg	2.65	4.35	200.1	21.49
125 mg/kg	2.65	4.23	199.7	20.20
250mg/kg	2.69	4.39	206.8	19.67
SEM	0.027	0.101	3.80	0.33
CM×Cu				
0 % - 0 mg/kg	2.68	4.29	192.6	20.66
0 % - 125 mg/kg	2.58	4.17	196.0	18.80
0 % - 250 mg/kg	2.75	4.30	219.0	19.20
10 % - 0 mg/kg	2.61	4.26	202.4	21.80
10 % - 125 mg/kg	2.65	4.12	204.2	21.00
10 % - 250 mg/kg	2.64	4.43	208.2	20.20
20 % - 0 mg/kg	2.66	4.48	205.4	22.00
20 % - 125 mg/kg	2.71	4.43	199.0	20.80
20 % - 250 mg/kg	2.70	4.44	193.0	19.60
SEM	0.08	0.303	11.39	1
		P-value		
CM	0.554	0.643	0.751	0.166
Cu	0.626	0.795	0.586	0.081
CM×Cu	0.615	0.987	0.297	0.930

Means in the same row with different letters (a and b) are significantly different (P<0.05).
[a] Eeach mean represents values from 5 replicates.

CONCLUSION

In conclusion, the CM could be used up to 10% of the broiler diets during the starter phases and higher proportions of CM may be impaired performance. Addition of copper is not able to recover the negative effects of dietary glucosinolates on growth performance. In present study, there were no effects on susceptibility to ascites by Substitution of CM instead of soy meal, but higher levels of CM in the diet probably increased the incidence of chronic heart failure in broiler chickens.

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