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The Effect of Glycine and Desiccated Ox Bile Supplementation on Performance, Fat Digestibility, Blood Chemistry and Ileal Digesta Viscosity of Broiler Chickens

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Abstract: In a completely randomized design with a 3*3 factorial arrangement and 4 replicates in each treatment, a study was conducted to evaluate the effect of three levels of Glycine (Gly) (0.00, 0.25 and 0.50%) and three levels of desiccated ox bile (DOB) (0.00, 0.25 and 0.50%) supplementation on performance, fat digestibility, blood chemistry and ileal digesta viscosity of 360 day-old Ross male broiler chickens fed diets containing 5% tallow for a period of 42 days. The isocaloric and isonitrogenous starter and grower diets were fed *ad libitum* to chickens from 7-21 and 22-42 days of age, respectively. Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were measured for starter (7-21d) and grower periods (21-42 days of age). Serum cholesterol (Chol), triglyceride (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and ileal digesta viscosity were measured at 21 and 42 days of age. Chromic oxide at the rate of 3 g/kg was added to experimental diets to determine fat digestibility at 19-21 and 40-42 days of age. FI, BWG and FCR were not affected by dietary treatments during 7-21d of age. Increasing the level of DOB in the diet significantly ($P < 0.05$) increased FI and BWG, and decreased FCR during 22-42 and 7-42d of age. A linear increase in fat digestibility with DOB was observed ($P < 0.05$). No significant difference in blood chemistry among birds fed diet supplemented with Gly during starter (7-21d) and grower (22-42d) periods was seen. However, supplementation of 0.50% DOB significantly ($P < 0.05$) increased Chol, TG, HDL and LDL concentration. Supplementation of Gly with/without DOB in the diets had no effect on the ileal digesta viscosity. The results of this study indicated that supplementation of DOB in the diet significantly ($P < 0.05$) increased performance and measured blood components. Dietary supplementation of Gly and DOB linearly ($P < 0.05$) increased fat digestibility of diets containing 5% tallow.

Key words: Glycine · Desiccated Ox Bile · Tallow · Performance · Blood chemistry · Broiler

INTRODUCTION

Primary bile acids, cholic acid and chenodeoxycholic acid, are a group of water-soluble steroids synthesized from cholesterol in the hepatocytes of the liver. These primary bile acids are then conjugated with glycine or taurine in the small intestine and formed bile salts. The bile salts play a pivotal role in digestion and absorption of dietary fat and other fat-soluble nutrients [1]. The bile salts increase aqueous solubility at acidic pH and resistance to precipitation by Ca^{2+} and renders bile acids impermeable to cell membranes [2, 3]. The bile salts are degraded in the distal ileum by intestinal bacteria and formed secondary bile acids. 90% of these components are reabsorbed in colon via active transport and entered the hepatic circulation. However, deconjugated glycine escape's reabsorption and enters the large intestine [4, 5]. Several studies have reported that crystalline amino acids such as glycine were absorbed very rapidly in the small intestine [6], whereas fat-encapsulated glycine was released slowly along the length of the intestine and therefore, would be more available to bacteria in the distal bowel [7]. Kalmar *et al.* [8] demonstrated that supplementation of dimethylglycine to the broiler diets increased nutrients digestibility. They concluded this effect can be explained by the emulsifying effect of dimethylglycine. Maisonnier *et al.* [9] reported that the addition of bile salts to broiler diets did not affect the small intestinal supernatant viscosity, but an improvement in weight gain was observed.

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Parsaie *et al.* [10] demonstrated that the appropriate supplementation of bile acid to wheat-based diets improved body weight gain and feed consumption of broiler chickens. Reinhart *et al.* [11] also reported that the performance of weaned pigs increased significantly as inclusion level of bile salt in the diet was increased. Early studies showed that the inclusion of desiccated pig bile powder in the diets of early-weaned pigs improved fat digestibility [12]. The present study investigated the effects of glycine (Gly) and desiccated ox bile (DOB) supplementation to broiler chicken diets containing 5% tallow on performance, fat digestibility, blood chemistry and ileal digesta viscosity.

MATERIALS AND METHODS

Experimental Birds, Diets, and Housing: One day-old male broiler chicks (Ross 308) were purchased from a local hatchery. The chicks were housed in floor pens (1×1 m) containing pine shavings throughout the trail. A total of 360 chicks were randomly divided into 9 treatments (with four replicates each) under a completely randomized design with a factorial arrangement of three levels of glycine (Gly) (0.00, 0.25 and 0.50 %) and three levels of desiccated ox bile (DOB) (0.00, 0.25 and 0.50%) supplementation. The experimental diets were given to chickens for the periods of 7-21 (starter) and 22-42 days of age (grower). The mash form diets were isocalric and isonitrogenous and formulated to contain 5% of tallow and meet the nutrient requirements of broilers based on NRC 1994 [13] (Table 1). Birds were exposed under continuous light and had free access to feed and water through the experiment.

Supplements Preparation: Glycine amino acid (Gly) was obtained from Evonic Degussa Corporation, Tehran, Iran and fresh ox bile was collected from local slaughterhouse. The ox bile was obtained from the gall bladder of the oxen killed freshly and the homogenates were filtered through a coarse nylon mesh and transferred to closed containers at -20°C immediately to prevent decomposition [14]. The DOB product was obtained by concentrated and dried fresh ox bile under a high vacuum at low temperature 60°C [15]. Color and some typical analysis characteristics are presented in (Table 2). DOB free contaminants (*E. coli* and *salmonella*) as powder form, at the rate of 0.00, 0.25, and 0.50% was added to the diets.

Performance Measurements: The body weight gain (BWG) and feed intake (FI) of each group of birds were determined 4 h after feed removal and feed conversion ratio (FCR) was calculated during 7-21, 22-42 and 7-42 day of age. Daily mortalities were recorded and used to correct performance criteria.

Table 1: Percentage diet composition and calculated nutrient content

Ingredient (%)	Starter diet (7-21 days)	Grower diet (22-42 days)
Corn	49.27	53.06
Soybean meal (44%)	37.63	28.45
Wheat Bran	4.30	10.32
Dicalcium phosphate	1.47	1.01
Limestone	1.42	1.31
Vit. & Min. premix[1]	0.50	0.50
Salt	0.43	0.30
Tallow	5.00	5.00
DL-Methionine	0.15	0.05
Total	100.00	100.00
Calculated nutrient content		
ME (kcal/kg)	2950	2950
Crude Protein (%)	21.20	18.44
Crude Fat (%)	7.32	7.57
Crude Fiber (%)	4.25	4.35
Calcium (%)	0.92	0.83
Available phosphorus (%)	0.42	0.32
Sodium (%)	0.18	0.14
Arginine (%)	1.42	1.21
Lysine (%)	1.17	0.97
Methionine + cystine (%)	0.83	0.66
Tryptophan (%)	0.32	0.30

[1]Supplied per kilogram of diet: vitamin A, 10000 IU; vitamin D_3, 9790 IU; B_{12}, 20 µg; riboflavin, 4.4 mg; calcium pantotenate, 40 mg; niacin, 22 mg; choline, 840 mg; biotin,30 µg; thiamine, 4 mg; zinc sulphate, 60 mg; manganese oxide, 60 mg.

Table 2: Typical analysis characteristics of Desiccated Ox Bile (DOB)

Color powder	Brownish to yellow
Color in solution	Yellow-beige
Solubility (%)	100.0
pH	5.5-7.5
Total ash (%)	15.0
Water (%)	5.0

Fat Digestibility: The chromic oxide marker method of Scott *et al.* [16] was used to measure fat digestibility. Chromic oxide was supplemented at 3 g/kg of the diets, and digestibility was determined for 3 consecutive days of 19-21 and 40-42 days of age. Chromic oxide-marked feeds were fed to chickens during 2-day adaptation periods (days 17-18, 38-39) and during three days collection periods (days 19-21 and 40- 42). At 19-21 and 40-42 days of age, excreta samples were collected twice daily (6.00 and 18.00) from all chickens in 4 pens per treatment. Contaminants such as feathers and scales were removed carefully and then excreta samples were stored in -20°C for further analysis. Fat content of the diet and excreta samples were determined by Soxhlet extraction (Soxtec system HT 1043 Extraction unit) according to the standard procedure of analysis [17]. Chromic oxide was determined using the method of Fenton and Fenton [18].

Blood Chemistry: On day 21 and 42, one bird from each replicate pen was selected randomly, marked, weighted and wing vein blood samples were obtained and immediately withdrawn into a syringe. The syringes were allowed to clot for 2 h at 37°C, and then serum was decanted and stored at -20°C for further analyses [19]. Serum samples were thawed and Serum Cholesterol (Chol), Triglyceride (TG), High density lipoprotein (HDL) and Low density lipoprotein (LDL) were determined using an autoanalyzer (Autolab, BT 3500, Autoanalyzaer Medical System, Rome, Italy).

Ileal Digesta Viscosity: At 21 and 42 days of age, one bird per replicate was selected randomly, marked, weighed, and euthanized by cervical dislocation. The ileum (defined as the midway between Meckel's diverticulum and the ileo-cecal junction) was dissected aseptically, and the digesta contents were collected and pooled by replicate as described by Lazaro *et al.* [20]. The digesta was homogenized, and 2 Eppendorf tubes were filled (1.5 g of sample) and centrifuged (12,000 × g, 3 min). The viscosity (in centipoises, cP) of a 0.5 mL aliquot obtained from the supernatant solution was determined at 21 and 42 days of age with a digital viscosimeter (model DV-II, Brookfield Engineering Laboratories Inc., Stoughton, MA) at 37°C. Each sample was read twice at the speed of 12 rpm, and the average value was used for the statistical analysis.

Statistical Analysis: The data were subjected to ANOVA using the GLM procedure of SAS software [21] as a 3×3 factorial arrangement of treatments that included dietary supplementation of Gly and DOB level as the main effects and their respective interactions. The treatment means were separated by Tukey's test [22] at $P < 0.05$ statistical level.

RESULTS AND DISCUSSION

Performance: The effects of Gly and DOB supplementation on performance of broiler chickens are shown in (Table 3). The supplementation of DOB to broiler diets had no significant effects on FI, BWG and FCR during 7-21 days of age. The DOB-fed broilers had higher feed intake as compared to control broilers during days 22-42 and 7-42. However, the difference among these treatments was not significant. Higher levels of DOB improved BWG and FCR during days 22-42 and 7-42. The supplementation of Gly to broiler diets had no effect on FI, BWG and FCR during 7-21, 22-42 and 7-42 days of age. The results of this study are in agreement with those of others. Reinhart *et al.* [11] observed that performance of pigs weaned at 21 days of age increased as dietary bile salt increased. Maisonnier *et al.* [9] reported that the addition of bile salts to diets increased weight gain.

Table 3: Effect of supplemental Glycine (Gly) and Desiccated ox bile (DOB) levels on performance of male broiler chickens during 7 to 42 days of age

	Feed intake			Body weight gain			Feed conversion ratio (g:g)		
	7-21	22-42	7-42	7-21	22-42	7-42	7-21	22-42	7-42
Main effects	(g/bird/days)			(g/bird/days)			(days)		
Gly (%)									
0.00	52.5	118.0	90.2	29.0	47.3	38.8	1.83	2.57	2.36
0.25	51.8	118.1	89.9	29.4	47.3	38.6	1.77	2.53	2.35
0.50	53.0	120.0	91.5	30.0	48.2	39.2	1.77	2.54	2.36
DOB (%)									
0.00	51.7	106.6^b	83.2^b	29.6	36.4^c	31.9^c	1.75	2.94^a	2.61^a
0.25	52.8	122.8^a	93.0^a	30.1	50.5^b	40.8^b	1.76	2.43^b	2.28^b
0.50	52.8	126.7^a	95.3^a	28.6	55.9^a	43.9^a	1.87	2.26^c	2.17^c
±SEM	1.20	2.39	1.76	1.07	1.27	0.90	0.08	0.06	0.04
Source of variance					Probability				
Gly (%)	0.506	0.526	0.498	0.494	0.605	0.686	0.511	0.746	0.902
DOB (%)	0.467	0.0001	0.0001	0.199	0.0001	0.0001	0.145	0.0001	0.0001
Gly × DOB	0.435	0.330	0.366	0.055	0.150	0.222	0.034	0.541	0.754

Means with no common superscripts differ significantly ($P < 0.05$).

Table 4: Effect of supplemental Glycine (Gly) and Desiccated ox bile (DOB) levels on fat digestibility of male broiler chickens during 7 to 42 days of age

		(Periods (day))	
		19-21	40-42
Main effects		---------Fat digestibility (%)----------	
Gly (%)			
0.00		64.80^{b}	70.51^{b}
0.25		61.95^{b}	74.35^{a}
0.50		69.92^{a}	75.18^{a}
DOB%			
0.00		53.51^{c}	55.93^{c}
0.25		69.00^{b}	79.36^{b}
0.50		74.20^{a}	84.74^{a}
±SEM		2.07	1.56
Interaction effects			
Gly ×	Bile		
0.00	0.00	46.76^{e}	56.36^{c}
0.00	0.25	75.37^{ab}	74.60^{b}
0.00	0.50	72.28^{ab}	80.56^{ab}
0.25	0.00	52.58^{de}	57.22^{c}
0.25	0.25	63.36^{bc}	81.37^{ab}
0.25	0.50	69.93^{abc}	84.45^{ab}
0.50	0.00	61.20^{cd}	54.22^{c}
0.50	0.25	68.18^{bc}	82.10^{ab}
0.50	0.50	80.37^{a}	89.21^{a}
Source of variance		Probability	
Gly (%)	0.0006	0.004	
DOB (%)	0.0001	0.0001	
Gly × DOB	0.0006	0.016	

Means with no common superscripts differ significantly ($P < 0.05$).

Parsaie *et al.* [10] also reported significant increase in FI and BWG of broilers fed a diet supplemented with bile acid. Addition of 0.2% colic acid to broiler diet improved performance [23]. Studies showed that the addition of bile acid to trout rainbow fish diet had a positive effect on growth performance and nutrient utilization [24, 25]. The significant improvement observed in BWG of DOB-fed broilers during 22-42 days of age is related to higher FI at this period in addition to higher fat digestibility seen in both starter and grower periods (Table 4). The interaction between DOB and Gly was not significant for FI and BWG during the experiment. However, the interaction between DOB and Gly was significant for FCR during 7-21 days of age. Studies showed that dietary bile salts increase the digestibility of saturated fats with long chain fatty acids [26, 27].

Fat Digestibility: The effect of different levels of Gly and DOB on fat digestibility is presented in (Table 4). Fat digestibility was significantly affected by Gly and DOB supplementation ($P < 0.05$) and significant interaction between Gly and DOB was observed ($P < 0.05$). Although the difference between control and 0.25% Gly diets was not significant, the supplementation of 0.5% Gly to broiler diets significantly increased fat digestibility during 19-21 days of age. The fat digestibility was significantly higher in Gly supplemented diets fed to broilers as compared with control broilers during 40-42 days of age. As dietary DOB increased, fat digestibility during 19-21 and 40-42 days of age increased. The interaction between DOB and Gly was significant for fat digestibility. The increase in fat digestibility ranged from 53.51% (0.00% DOB in the diet) to 84.74% (0.50% DOB in the diet) and from 64.80% (0.00% Gly in the diet) to 75.18% (0.50% Gly in the diet). These results of this study are in agreement with those of Fedde *et al.* [28], who reported a linearly increase in fat digestibility. When dietary ox bile level increased in the diet, fat digestibility increased. They showed that the apparent fat absorption of beef tallow increased from 53% to 80%, when 0.5% or more of dietary ox bile was added to the diets of chickens. Kalmar *et al.* [8] reported the supplementation of dimethylglycine to the broiler diets increased nutrients digestibility. They concluded this may be due to the emulsifying effect of dimethylglycine. The improvement of digestibility coefficients of nutrients by addition of an emulsifier to the diet occurs because non-fat nutrients become less insulated by fat droplets and hence more nutrients would be available for digestive enzymes and the absorptive brush border of the small intestine. Orban and harmon, [12] also demonstrated that the supplementation of various levels of pig bile to the diets of early-weaned pigs improved digestion and utilization of dietary fat linearly. The supplementation of bile salt to broiler diets significantly increased fat digestibility at 14 days of age as measured with ileal digesta ($P < 0.001$) or Excreta methods [29]. Maisonnier *et al.* [9] reported that addition of bile salt to broiler diet had a positive effect on lipid digestibility. It has been suggested that the observed improvement in fat digestibility by addition of bile salt to diets might be due to insufficient bile salt secretion by the animal or replenishment of the active catabolism of bile salts by the intestinal microflora. This detergent property of bile also confers potent antimicrobial activity [30]. Kocsar *et al.* [27] emphasized the important role of bile acids in the defense mechanism of the microorganism

Table 5: Effect of supplemental Glycine (Gly) and Desiccated ox bile (DOB) levels on Blood Chemistry of male broiler chickens at 21 and 42 days of age

	(Periods (day))							
	21				42			
Item	Chol	TG	HDL	LDL	Chol	TG	HDL	LDL
Main effects	(mg/ dl)							
Gly (%)								
0.00	115.56	65.33	65.0	35.11	75.78	41.78	53.67	24.78
0.25	114.78	66.22	64.00	36.00	76.56	43.44	48.22	23.56
0.50	108.33	60.89	62.33	36.22	77.22	43.33	49.11	27.11
DOB (%)								
0.00	93.22^{c}	57.56	54.00^{b}	29.11^{b}	61.00^{b}	31.56^{b}	37.11^{b}	17.78^{b}
0.25	118.22^{b}	58.33	67.67^{a}	38.00^{a}	79.00^{ab}	46.22^{ab}	50.89^{ab}	24.67^{ab}
0.50	127.22^{a}	76.56	69.67^{a}	40.22^{a}	89.56^{a}	50.78^{a}	63.89^{a}	33.00^{a}
±SEM	4.00	9.303	4.042	2.998	8.939	9.034	8.391	5.437
Source of variance				Probability				
Gly (%)	0.078	0.757	0.721	0.892	0.981	0.969	0.701	0.722
DOB (%)	0.0001	0.036	0.0003	0.0006	0.004	0.045	0.006	0.011
Gly × DOB	0.076	0.125	0.741	0.133	0.018	0.098	0.524	0.057

Means with no common superscripts differ significantly ($P < 0.05$).

Chol, cholesterol; TG, triglyceride; HDL, high density lipoprotein; LDL, low density lipoprotein.

against bacterial endotoxins. Young *et al.* [31] also demonstrated that addition of antibiotics to diets of chicks appeared to increase their fat utilization, probably by reducing intestinal microbial populations and thereby reducing their rate of bile salt catabolism. Another possibility is the added advantage of dietary bile salt in enhancing the digestibility of saturated fats with long chain fatty acids [26, 30].

Blood Chemistry: Serum total Cholesterol, HDL- and LDL-cholesterol and triglycerides contents of broilers fed diets containing various levels of Gly and DOB are shown in (Table 5). No significant differences in blood chemistry such as Cholesterol (Cho), Triglyceride (TG), High Density Lipoproteins (HDL) and Low Density Lipoproteins (LDL) among birds fed diet supplemented with different levels of Gly during starter (21days) and grower (42 days) periods was observed. The total Cholesterol, HDL-and LDL-cholesterol and TG concentrations were not affected by the addition of Gly in the diets. Supplementation of the diets with 0.25% DOB significantly increased total cholesterol, HDL- and LDL-cholesterol concentrations as measured at 21 days of age. Total cholesterol concentration was significantly higher in 0.50% DOB fed broilers as compared to 0.25% DOB-fed broilers. However, the HDL- and LDL-cholesterol concentrations were not increased as dietary DOB increased from 0.25 to 0.50%. The 0.50% DOB-fed broilers had the higher total cholesterol, TG, HDL and LDL concentrations at 42 days of age. The concentrations of these parameters were intermediate in broilers fed 0.25% DOB. Similarly, Ide *et al.* [32] reported that supplementation of Gly to the free fiber rat diet did not affect the serum lipids contents. Sugiyama *et al.* [33] also demonstrated that the addition of 5% Gly in rat cholesterol-enriched diet did not change the serum cholesterol concentration. In previous study, Hegsted *et al.* [34] reported that the addition of cholic acid to a diet containing 0.8% of cholesterol caused a small increase in the serum cholesterol content of young chickens. Edwards *et al.* [35] showed that supplementation of 0.10% of lithocholic acid to hen diet had increased serum cholesterol level. Crespo and Esteve-Garcia [36] also observed that total cholesterol content of tallow-fed broilers was higher as compared to those fed sunflower and linseed oils. It has been shown that higher serum cholesterol content increased bile secretion and thus increased the digestion and absorption of fat [37]. The low bile acid secretion can limit the digestion and absorption of fats particularly those enrich in the long chain saturated fatty acids [38]. The present results revealed that fat digestibility for 0.50% DOB was greater ($P < 0.05$) than those of the other treatments. These results might be expectable since more bile salt concentration is present in the small intestine of broiler chickens.

Table 6: Effect of supplemental Glycine (Gly) and Desiccated ox bile (DOB) levels on ileal digesta viscosity of male broiler chickens during 7 to 42 days of age

	(Periods (day))	
	21	42
Main effects	Viscosity (cPs[1])	
Gly (%)		
0.00	1.16	1.19
0.25	0.98	1.16
0.50	1.16	1.17
DOB%		
0.00	1.17	1.21
0.25	1.05	1.19
0.50	1.08	1.11
±SEM	0.13	0.15
Source of variance	Probability	
Gly (%)	0.18	0.95
DOB (%)	0.55	0.71
Gly × DOB	0.22	0.47

Means with no common superscripts differ significantly (P < 0.05).

[1]cps = centipoises

Ileal Digesta Viscosity: The effect of different levels of Gly and DOB on ileal digesta viscosity is given in Table 6. The supplementation of Gly and DOB to broiler diets had no significant effect on ileal digesta viscosity at 21 and 42 days of age. The interaction between DOB and Gly was not significant for ileal digesta viscosity too. Similar results were obtained by Maisonnier *et al.* [9]. They reported that the addition of bile salts in the diets did not affect the small intestinal supernatant viscosity. There is no evidence to determine the effect of supplemented Gly on ileal digesta viscosity. However, more research might be needed to test the effect of DOB and Gly in more viscose diets (e.g. wheat-soybean meal based diet) than that of corn-soybean meal based diets.

CONCLUSION

The results of this study indicate that supplementation of DOB into the diet significantly (P < 0.05) increased performance and blood chemistry. However, supplementation of Gly with/without DOB linearly (P < 0.05) increased fat digestibility of the diets containing 5% tallow. Spite no significant difference for blood chemistry among birds fed diets supplemented with Gly at starter (21 days of age) and grower (42 days of age) periods, DOB supplementation significantly (P < 0.05) increased Cholesterol, TG, HDL and LDL concentration. Furthermore, supplementation of Gly and DOB did not affect the ileal digesta viscosity. More research is needed to clarify if addition of DOB and Gly can change the measured parameters in other based diets?

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