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Original Article

Performance, Fat Digestibility and Blood Chemistry of Broiler Fed Diet Supplemented with Ox Bile Salt

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ABSTRACT

The effects of feeding different levels of ox bile salt (OBS) supplements on broiler performance, digestibility of crud fat and some blood chemical (cholesterol, triglyceride, high density lipoprotein and low density lipoprotein) were investigated. Three hundred and seventy-five, day-old broilers chickens were assessed using a completely randomized design. Five diets, containing different levels of OBS (0.00, 0.15%, 0.30%, 0.45%, and 0.60%) were randomly assigned to 5 replicates each of 15 birds for a 42-d period (7d for adaptation and 35 d for data collection). Feed intake, growth weight, crud fat digestibility and blood metabolites were determined. Supplementation of OBS levels in the diet of the broiler chickens improved ($P< 0.05$) body gain weight, feed conversion rate and crude fat digestibility. OBS had no effects ($P > 0.05$) on the feed intake. Feeding OBS-containing diets caused ($P<0.05$) an increase of content of serum total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) and serum total triglycerides (TG). The animal performance reached its greatest value with simultaneous feeding of 0.45% and 060% OBS. It has been concluded that the dietary supplementation of OBS had positive effects on body gain weight, feed conversion rate, crude fat digestibility, and serum TC, TG, HDL and LDL. Synchronized feeding of the great levels of OBS and fat digestibility (as an energy source) increased utilization efficiency of these byproducts in the performance of broiler chickens.

Keywords: Lipid metabolism (TC, TG, HDL and LDL), growth performance (BGW, FI and FCR), apparent digestibility of fat

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INTRODUCTION

The significant expansion of poultry production, while reducing or stopping the use of antibiotics, calls for the need to find alternative sources to improve the nutritional efficiency and performance of birds. Bile salts function to emulsify lipids and fat soluble to form fat-containing micelles to facilitate the absorption process from the intestinal tract. Bile salts are made of bile acids that are conjugated with glycine or taurine (Marin *et al.*, 2016). They are produced in the liver, directly from cholesterol. Bile salts are important in solubilizing dietary fats in the watery environment of the small intestine. The bile salts differ in their hydrophobic – hydrophilic balance, their hydrophobic bile salts it has a high ability to dissolve fats and to dissolve fats in cell membranes. Once produced, but before being secreted into the gallbladder or digestive system, bile salts are often bonded to glycine or taurine. Glycine is an amino acid, while taurine is derived from the common amino acid cystine. This bonding increases the water solubility of bile salts. (Begley *et al.*, 2005, Reshetnyak, toxins, considering that broiler chicks suffer from a lack of liver VI., 2013, Esteller A., 2008). However, one of the limiting factors is the limited abilities of animals to digest dietary fat and the subsequent consequences on their performance. The increasing interest in finding different ways that may affect the health status of animals and thus improve performance and increase productivity represents an important area in many studies. Perrone *et al.*, (2010) showed that bile salts may protect the intestine from injury or infection. In poultry, digestion of fat is influenced by many factors, such as bile secretion and dietary fat sources (Mossab *et al.*, 2000; Preston *et al.*, 2001; Abudabos, 2014). Alzawqari *et al.*, (2011) concluded that dietary desiccated ox bile supplementation increased in the villus height, crypt depth, villus width and villus apparent surface area in the jejunum and ileum which may improve the fat digestibility. The objective of the present study was to determine the effects of ox bile supplementation on performance, crude fat digestibility and some blood chemistry in broiler chickens.

MATERIAL AND METHODS

A total of 375 one-day-old male Cobb broiler chicks were randomly assigned to 5 treatments, with 15 per cage and 5 replicate cages per treatment in environmentally-controlled rooms. All birds were provided feed and water ad libitum throughout the experimental period 42 d (7d for adaptation and 35 d for data collection). The birds were fed standard broiler corn-soybean meal based (mash form) formulated to meet nutritional requirements (NRC 1994). The chicks were randomly assigned to one of the five dietary treatments, in a completely randomized design. The dietary treatments were basal diet with ox bile salt (0.00%, 0.15%, 0. 30%, 0. 45% and 0.60%). Fresh ox bile is collected and immediately placed in a refrigerator to prevent

decomposition. When ready for processing, the refrigerated bile is concentrated and dried under a high vacuum at low temperature.

Table 1a: Feed composition

Item	%
Corn	60
Soy bean	27
Fish meal	6
Vegetable Oil	2
Methionine	0.035
Di calcium phosphate	2
Salt	1.62
Limestone	1
Premix	0.3
Determined Analysis	
Moisture	9.5
Crude protein	23.57
Ash	9.77
Ether extract	3.23
Crude fiber	2.67
Nitrogen free extract	50.73
Calcium	1.00
Phosphorus	0.40

Table 1b: premix composition

Item	%
Vitamin A	4000000 IU
Vitamin E	6.666mg
Vitamin D3	833333 IU
Vitamin K	3666mg
Vitamin B1	666 mg
Vitamin B2	1666mg
Vitamin B5	10mg
Vitamin B6	1000mg
Vitamin B12	5 micro. g.
Folic acid	333 mg
Biotin	33333 micro. g.
Choline	166 mg
Methionine	3331333 mg

The ingredients and chemical composition of the basal diet are shown in Tables1a and 1b. All the diets were isocaloric and isonitrogenous, body weight and feed intake were recorded weekly after fasting for 12 h, and these values were used to calculate the body gain weight (BGW), average daily feed intake (FI), and feed conversion ratio (FCR) of the broilers for the course of the experiment. Crude fat of feed and excreta samples were determined using ether extract (EE) method according to AOAC (2006). At 42 days of age, four birds per experimental unit was randomly selected for the collection of 5mL of blood by brachial vein puncture, and the obtained serum was analyzed for total cholesterol total (TC), total triglycerides (TG), high-density lipoprotein (HDL) and low density lipoprotein (LDL) by colorimetric enzymatic method using a commercial kit (Celm), with reading at 505nm in mass spectrometer (Lumeij, 1997). The results were expressed in mg/dL. In order to determine crud fat digestibility, excreta samples were collected every week after day 7. Excreta were placed in plastic bags and stored at -20 ºC. At the end of the collection period, excreta samples were thawed, homogenized, and pre-dried in a forced-ventilation oven at 55 ºC for 72 hours, after which they were ground for subsequent analyses. Dry matter (DM), ether extract (EE) contents were determined according to the methodology recommended by the AOAC (2006) and the apparent coefficients of digestibility were calculated according to the method proposed by Matterson *et al.*, (1965).

Data obtained from the experiment were calculated and expressed as Mean ± SE on all parameters. The results were subjected to statistical analysis of variance (ANOVA) using the general linear model (GLM) procedure of MINITAB (2015) and where significant F value for treatment effect was found, means were compared by Least Significant Difference (LSD). The tests were used to compare treatment means at ($P<0.05$) significant level.

RESULTS

The effects of ox bile salt (OBS) supplementation on performance of broiler chickens are shown in (Table 2). The supplementation of OBS to broiler diets had higher BWG and FCR as compared to the control broiler group. Non-significant differences among the OBS treatments were observed. However, the OBS fed broiler had higher feed intake in general as compared to control broiler but failed to be statistical difference. The presence of OBS in the diets of broiler chicks affects significantly their general performance. Feed intake, was similar among broiler fed diets containing the various levels of OBS (Table 2). Apparent fat digestibility, serum total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) contents of broiler fed various levels of OBS are shown in (Table 3). Supplementation of OBS in broiler diet significantly ($P<0.05$) increased the TC, HDL, LDL and TG among all levels of OBS (0.015, 0.30%, 0.45% and 0.605)

inclusion in broiler diets as compared to control broiler group. No differences were observed between the treatments level of OBS. However, the TG was not increased significantly at 0.015% OBS.

Supplementation of OBS significantly ($P<0.05$) improved the digestibility of crude fat in the present study (Table 3). The resulted improvements in fat digestibility were in relation to increased level of the OBS in the diet. No differences were observed for the different levels of OBS however, the highest coefficient of digestibility was with 0.45%. The increase in fat digestibility ranged from 59.36 to 85.60% (0% to 45% OBS in the diet) respectively.

Table 2: Effect of different levels of dietary bile salt on performance of broilers

	Feed Intake	BGW	FCR
Control	83.20±3.67	32.34±1.42 [a]	2.57±0.11 [a]
Bile salt 15%	93.67±5.22	41.45±2.31 [b]	2.25±0.12 [a]
Bile salt 30%	95.44±6.79	44.56±3.12 [b]	2.14±0.15 [b]
Bile salt 45%	97.68±5.77	47.78±2.82 [b]	2.04±0.12 [b]
Bile salt 60%	98.88±5.89	47.99±2.86 [b]	2.06±0.12 [b]
LSD	NS	8.38	0.41

SE standard error, [a,b,c] differ significant at (P<0.05), NS non-significant and LSD least significant difference.

Table 3: Effect of different levels of dietary bile salt on blood chemistry and apparent fat digestibility of broilers

	Fat Digest	TC	TG	HDL	LDL
	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE	Mean ±SE
Control	59.36 ±2.62 [a]	57.75 ±2.55 [a]	41.25 ±1.82 [a]	34.76 ±1.53 [a]	19.98 ±0.88 [a]
Bile salt 15%	77.70 ±4.33 [b]	77.67 ±4.33 [b]	48.67 ±2.71 [a b]	52.54 ±2.93 [b]	30.85 ±1.72 [b]
Bile salt 30%	80.50 ±5.72 [b]	87.63 ±6.23 [b]	52.67 ±3.74 [b]	66.85 ±4.75 [c]	33.67 ±2.39 [b]
Bile salt 45%	85.60 ±5.06 [b]	89.93 ±5.31 [b]	53.70 ±3.17 [b]	65.76 ±3.88 [c]	36.76 ±2.17 [b]
Bile salt 60%	84.98 ±5.06 [b]	88.76 ±5.28 [b]	55.54 ±3.31 [b]	67.54 ±4.03 [c]	35.89 ±2.13 [b]
LSD	15.16	15.87	9.8	11.65	6.26

SE standard error, [a,b,c] differ significant at (P<0.05) and LSD least significant difference.

DISCUSSION

One of the most important basic functions of the liver is the removal of toxins from the body, which may happen when chicken eating feeds deficient in nutrients or on exposed to mycotoxin in the feed. One of the scientific topics that receive special attention is the use of bile acid in feed. It will improve the performance of commercial chicken production.

Bile has a positive effect on digestion and absorption of fats, fat-soluble vitamins A, D, E and K (Maisonnier *et al.*, 2003), as well as eliminating toxins, considering that broiler chicks suffer from a lack of liver and gallbladder functions. Therefore, when the protein and fat content is high in the components of the feed, it may lead to nutritional problems in digestion and absorption, which may

cause diarrhea, indigestion, and a high level of toxins in the body which leads to poor growth and productivity. In this study, supplementation of OBS improved (P <0.05) performance of the broiler, these results are in agreement with those observed by (De Oliveira *et al.*, (2019) concluded that the inclusion of emulsifier improved the growth performance. Bontempo *et al.*, (2018) stated that the feeding a synthetic emulsifier may have a beneficial effect on growth performance. The heaviest weight of the birds was observed with 60% of OBS supplementation. However, no statistically significant differences observed between the levels of OBS added in the diets. Wenqing Lai, (2018) noted that bile acids led to a significant increase in the average daily weight gain of birds however, the beneficial of feeding broiler with OBS was in agreement with the work of Maisonnier *et al.*, (2003), Parsaie *et al.*, (2007), Cho *et al.*,(2012), Wang *et al.*, (2016) and Ahmed *et al.*, (2020). In this study showed a decrease in feed conversion ratio between bile salt supplemented feed and control feed. Al-Zawqari *et al.*, (2010) stated that the feed conversion ratio improved in broiler feed with bile salt additives. This effect was similar to some studies that reported that bile salts enhanced growth performance and improved feed conversion ratio (Lee *et al.*, 2004, Wenqing Lai, 2018, Samuel *et al.*, 2020). Whereas, supplementation of bile acids did not affected daily feed consumption. Several studies have shown that the use of bile salts in diets showed no effects on the amount of feed consumption (Diego-Cabero *et al.*, 2015 Piekarski *et al.*, 2016) On the other hand, D Pantaya,(2020), reported that the bile acids decreased feed intake. The performance improvement of the chickens explained by the fact that bile has a role in dissolving and breaking down compounds, and thus it can act as a detergent, which is similar to an antibiotic action against bacteria and their toxins (Begley *et al.*, 2005). The energy gained from the improvement in the fat digestibility may explain the improvement in BWG and FCR. Fat digestibility increased with the bile salt supplementation in chicken feed (Reinhart *et al.*, 1988). This comes as confirmation that adding bile salts in chicken feed helps improve absorption and breakdown of fats. (Orban and Harmon (2000), Guerreiro Neto 2011, Zenga, 2012; Zaefarian *et al.*, 2015; M.H. Alzawqari1, 2016 and Wenqing Lai,(2018).

Guerreiro Neto (2011) indicated in his study that emulsifiers when added to poultry feed improve fat digestion and increase the productivity and secretion of pancreatic lipase. Gomez and Polin (1976) showed that adding bile salts works to break down both short and long chain fatty acids. It has been proven in different studies that taurine conjugated with bile promotes lipolysis and fatty acid formation, leads to a significant improvement in fat metabolism and enables the body to gain energy necessary for vital processes (Lee, DN et al, 2004). When taurine added at 0.15%, it was found that the metabolic energy increased (Zheng *et al.*, 2015). The OBS supplementation in broiler feed increased fat digestibility, which play an

important source of energy. When enough energy is available to the bird, this will lead to a decrease in the amount of feed consumption, and improvement in body gain weight and feed conversion ratio. The highest level of OBS used in this study was 0.60%. Serum TC, TG, LDL and HDL concentrations are diagnostic markers in lipid metabolism. The results of this study were consistent with the results of Alzawqari *et al.*, (2016). Bontempo *et al.*, (2018) concluded that feeding synthetic emulsifier may have a beneficial effect on lipid metabolism (cholesterol and HDL). Fatty acids via LDL or chylomicron stored to adipose tissue as triglyceride (Marin *et al*,. 2016). The serum TG play an important effect on adipose tissue, lipid deposition and yolk formation. In this study the TG increased by as much 27% over the non OBS feed control group. Serum HDL enhances the absorption of cholesterol from peripheral tissues of the animals. It facilitates the transport of cholesterol to the liver for breakdown and analysis (Miller and Miller, 1975). Bile salt will increase the content of serum TC, TG, LDL and HDL. Fat absorption of fat increases the energy metabolism which will improve the growth performance of the bird.

CONCLUSION

It is concluded that the addition of bile salts had a positive effect on growth performance and fat digestibility. But feed consumption was not affected. The study reveals that bile salt fed broiler had a positive effect on blood serum (TC, TG, HDL and LDL). Update information that supplementation of bile acid in diets could potentially useful for future improvement in broilers production. Further studies should focus on the mechanism of the bile salt as feed additive on performance and liver function.

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