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Effects of Varying Levels of Oxidized Fat and Copper Sulfate on Broiler Performance, Carcass Traits, Blood Metabolites and Immune Cells

Research Article

A. Nobakht[1*], A. Sehati[2] and H. Aghdam Shahryar[2]

[1] Department of Animal Science, Maragheh Branch, Islamic Azad University, Maragheh, Iran
[2] Department of Animal Science, Shabestar Branch, Islamic Azad University, Shabestar, Iran

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*Correspondence E-mail: anobakht20@iau-maragheh.ac.ir

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ABSTRACT

This experiment was conducted to investigate the effects of different levels of oxidized fat (yellow grease) and copper sulfate on performance, carcass traits, blood metabolites and immune cells of broilers. This study was carried out as a 3 × 2 factorial experiment with 3 levels of oxidized fat (0, 2 and 4%) and 2 levels of copper sulfate (0 and 300 mg/kg) in 6 treatments, 3 replicates and 12 birds in each replicate. In this experiment, 216 day-old broiler chicks (Ross 308 strain) were used for 42 days. The results showed that there were significant difference between groups in performance, blood metabolites and immunity parameters ($P<0.05$). The highest amounts of daily feed intake (43.51 g) and the best feed conversion (1.88) were resulted by inclusion 2% of oxidized fat in broiler diets, whereas in these respects they were not significant difference between control group and group with 4% of oxidized fat. The lowest level of triglyceride (34.34 mg/dL) and highest percent of lymphocyte (88.58%) were obtained by the addition of 4% oxidized fat into the broiler diets. The overall results showed that inclusion of 4% oxidized fat into broiler diets compare with the control group do not have desirable effects on their performance and improve the blood metabolites and immunity parameters.

KEY WORDS broiler chicken, carcass traits, oxidized fat, performance.

INTRODUCTION

Many different grades of fat have been used as feed ingredients in the broiler industry. Feed fats provide one essential nutrient (linoleic acid) and dietary ME. Fat produces 2.25 times more energy than carbohydrates and proteins (Pest *et al.* 2002). The most practical method for increasing the energy density of diets in poultry feeding is through the addition of fats and oils. Carcass percentage, weight of proventriculus, pancreas, spleen, heart and abdominal fat pad as a percent of live weight were not affected by using different levels of fish oil. Whereas inclusion of fat at a 4% level, increased the thigh, breast, liver and small intestine weights. An important issue referring to poultry feeding is the supplementation of diets with oxidized fats, namely waste fats of different thermal processes, e.g. frying, and waste products obtained as a result of vegetable oil refining. The safety of administering oxidized fats to diets and their impact on live organisms have become controversial and are the subject of research by numerous authors. One of the reasons of this controversy is the anxiety that the lipid oxidation may decrease the nutritive value of a diet, increase depression and incidence of diarrhoea and cause histological changes to tissues and, in some cases, even the death of birds (Izaki *et al.* 1984; Jakobsen *et al.* 1993). Dietary fat quality not only affects animal growth performance and health (Lin *et al*. 1989; Engberg *et al*. 1996) but also influences the quality of broiler meat and meat products (Lin *et al*. 1989; Asghar *et al*. 1989). Lewis and Wiseman (1977) reported a significant fall in digestibility when the free fatty acid reached 50%. It was showed that rancidity had no effect on the energy content of fats (Hussein and Kratzer, 1982). Rancidity deteriorated the palatability and feed intake (Godber *et al*. 1993). Chicks showed poor growth performance when dietary rice bran was rancid. The addition of 2.5 and 5% of industries residual oil to broiler diets significantly increased the blood levels of cholesterol, low density lipoprotein and triglyceride. Oxidized soybean oil had negative effect on weight gain, feed conversion ratio, liver weight and thiobarbituric acid reactive substance value in liver (Anju *et al*. 2004).

Copper (Cu) is a component of various intracellular and extracellular enzymes such as cytochrome oxidase, lysyl oxidase, ceruloplasmin and superoxide dismutase (Klasing, 1998). Supplementation of Cu at the level of 125-250 ppm improved growth rate and feed conversion ratio in broilers (Baker *et al*. 1991; Paik, 2001) and pigs (Roof and Mahan, 1982; Cromwell *et al*. 1989).

Pesti and Bakalli (1996) reported that 125-250 ppm of Cu from copper sulfate penthahydrate and citrate copper supplementation reduced cholesterol levels of serum and breast muscle Higher concentrations of Cu significantly decreased weight gains and insignificantly reduced mortality of chickens (from 2.6 to 0.6-1.3%). Feed conversion was worse in Cu added treatments). Total lipid and cholesterol concentration in breast muscles of chickens was significantly lower than in control chickens (Skrivan *et al*. 2002). However, methionine Cu linearly increased serum cholesterol level but decreased serum triglycerides level (Chowdhury *et al*. 2004).

The present study was planned to determine the effects of different levels of oxidized fat (OF; yellow grease) and copper sulfate (CS) on growth performance, slaughter data (dressing, gizzard, breast, thigh and liver percentages), blood metabolites and immune cells of broilers.

MATERIALS AND METHODS

This study was carried out as a 3 × 2 factorial experiment with 3 levels of oxidized fat (0, 2 and 4%) and 2 levels of copper sulfate (0 and 300 mg/kg) in 6 treatments, 3 replicates and 12 birds in each replicate. In this experiment, 216 day-old broiler chicks (Ross 308 strain; male and female) were used for 42 days in floor pens. The diets were formulated (Table 1) to meet the requirements of broiler chicks as established by the NRC (1994). The diets and water were provided *ad libitum*. The lighting program during the experimental period consisted of a period of 23 hours of light and 1 hour of darkness. Environmental temperature was gradually decreased from 33 ˚C to 25 ˚C on day 21 and was then kept constant. Body weight, feed intake and feed conversion were determined weekly and calculated on a per bird basis. Mortality was also recorded. At 42 days of age, two birds from each replicate (totally six birds from each treatment) were randomly chosen based on the average weight of the group and after 12 h feed withdrawal, sacrificed and measured the percents of dressing yield, gizzard, thigh, breast and liver. Dressing yield was calculated by dividing eviscerated weight by live weight. The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (2005). Means were compared using the Duncan multiple range test. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

The effects of different levels of OF and CS and interactions of these treatments on broiler performance are summarized in Table 2. The highest amount of daily weight gain (43.51 g) and the best feed conversion (1.88) were resulted by inclusion 2% of OF into broiler diet, however they were not significantly different between the control group and the 4% of OF treatment. The addition of OF numerically increased feed intake. Inclusion of 300 mg/kg CS in broiler diets and interaction of OF × CS did not significantly affect the performance of broilers. The effects of different levels of OF and CS and interaction of OF × CS on carcass parts of broilers are shown in Table 3. Adding different levels of OF and CS and interaction OF × CS had no significant effects on carcass traits of broilers ($P>0.05$).

The effects of different levels of OF and CS and their interaction on blood biochemical parameters and immune cells are summarized in Table 4. Including different levels of OF significantly affected the level of triglyceride and lymphocyte percent ($P<0.05$). Inclusion OF to the diet of broilers significantly reduced blood triglyceride levels and increased the percentage of lymphocyte cells, therefore the lowest level of triglyceride (34.34 mg/dL) and highest percentage of lymphocyte cells (88.58%) resulted by adding 4% of OF. Inclusion CS and interaction OF × CS showed no significant effects on blood biochemical and immunity cells of broilers.

Addition of OF at 4% in comparison to the control group had no significant effect on performance of broilers. However numerically the highest amount of daily weight gain and feed conversion were resulted from group 2 with 2% OF and 4% OF compare to 2%. Four percent of OF significantly decreased the amount of daily weight gain, and increased feed conversion.

Table 1 The ingredients and nutrients composition of starter and grower diets of broilers

Feeding periods	1-21 days			22-42 days		
Diets	Control group	2% OF	4% OF	Control group	2% OF	4% OF
Ingredients (% in diets)						
Yellow corn	61.87	55.94	50.70	66.22	60.42	55.28
Soybean meal	34.14	36.25	37.06	29.80	31	31.52
Fish meal	0.48	0	0	0.55	0.55	0.55
Oxidized fat	0	2	4	0	2	4
Inert	0	2.22	4.64	0	2.52	5.13
Oyster shell	1.32	1.32	1.31	1.31	1.31	1.31
Dicalcium phosphate	1.30	1.37	1.38	1.30	1.37	1.38
Salt	0.27	0.28	0.28	0.3	0.3	0.3
Vitamin premix[1]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[2]	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.12	0.12	0.13	0.02	0.03	0.03
Calculated composition						
Metabolisable energy (kcal/kg)	2900	2900	2900	2900	2900	2900
Crude protein (%)	20.84	20.84	20.84	19.34	19.34	19.34
Calcium (%)	0.91	0.91	0.91	0.85	0.85	0.85
Available phosphorous (%)	0.41	0.41	0.41	0.37	0.37	0.37
Sodium (%)	0.14	0.14	0.14	0.15	0.15	0.15
Linoleic acid (%)	1.5	1.68	1.86	1.58	1.77	1.94
Lysine (%)	1.17	1.19	1.21	1.0۶	1.0۸	1.1
Methionine + Cysteine (%)	0.87	0.87	0.87	0.68	0.68	0.68
Thryptophan (%)	0.28	0.27	0.27	0.25	0.25	0.25

[1] Vitamin premix per kg of diet: vitamin A (retinol): 2.7 mg; vitamin D_3 (cholecalciferol): 0.05 mg; vitamin E (tocopheryl acetate): 18 mg; vitamin K_3: 2 mg; B_1 (thiamine): 1.8 mg; B_2 (riboflavin): 6.6 mg; B_5 (panthothenic acid): 10 mg; B_6 (pyridoxine): 3 mg; cyanocobalamin: 0.015 mg; niacin: 30 mg; biotin: 0.1 mg; folic acid: 1 mg; choline chloride: 250 mg and antioxidant: 100 mg.

[2] Mineral premix per kg of diet: Fe (FeSO4.7H2O, 20.09% Fe): 50 mg; Mn (MnSO4.H2O, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu (CuSO4.5H2O): 10 mg; I (K_1, 58% I): 1mg and Se (NaSeO3, 45.56% Se): 0.2 mg.

* 300 mg/kg of diets added as copper sulfate to experimental diets.

Table 2 The effects of different levels of oxidized fat and copper sulfate on broiler performance (1-42 days)

Performance	Feed intake (g)	Weight gain (g)	Feed conversion (g: g)
Supplements			
Control group	79.32	40.75^{ab}	1.95^{ab}
2% oxidized fat	81.54	43.51^{a}	1.88^{b}
4% oxidized fat	81.16	39.45^{b}	2.07^{a}
SEM	0.9	1.15	0.05
Without copper sulfate	80.98	41.11	1.99
300 mg/kg copper sulfate	80.36	41.37	1.95
SEM	0.73	0.94	0.04
Without oxidized fat × without copper sulfate	80.59	39.59	2.04
Without oxidized fat × 300 mg/kg Copper sulfate	78.05	41.93	1.87
2% oxidized fat × without copper sulfate	81.39	43.56	1.88
2% Oxidized fat × 300 mg/kg Copper sulfate	81.70	43.47	1.88
4% oxidized fat × no copper sulfate	80.98	40.19	2.04
4% oxidized fat × 300 mg/kg copper sulfate	81.35	38.73	2.1
SEM	1.27	1.62	0.08

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error.

During oxidation process of fats and high fat ingredients, thiobarbituric acid reactive substance values increase (Waheed *et al.* 2004) which might have negative impact on chicks growth and feed conversion. These results are in line with the previous findings (Bartov and Bornstein, 1972; Takigawa and Ohyama, 1983; Miyzawa and Knobb, 1986). Lower weight gain of birds fed 4% OF was generally similarly to earlier reports (Cabel and Waldroup, 1988; Lin *et al.* 1989; Engberg *et al.* 1996; Wang *et al.* 1997). Chae *et al.* (2002) also reported lesser weight gain in chicks fed rancid rice polish compared to the chicks fed fresh rice polish. This might be due to destruction of fat-soluble vitamins in OF that leads to reduced availability of nutrients as well as immunity, and consequently depressed growth performance (Lin *et al.* 1989; Cheeke, 1991). Finally according Anju *et al.* (2004) addition of 2% oxidized soybean oil in broiler diets had negative effects on weight gain and feed conversion.

Table 3 The effects of different levels of oxidized fat and copper sulfate on broiler carcass parts (%)

Performance	Carcass	Gizzard	Breast	Thigh	Liver
Supplements					
Control group	74.49	2.89	34.25	25.96	2.91
2% oxidized fat	72.63	2.68	33.07	25.99	3.12
4% oxidized fat	71.96	2.71	33.56	25.34	3.06
SEM	0.88	0.12	0.61	0.4	0.12
Without copper sulfate	72.30	2.85	33.69	25.55	3.06
300 mg/kg copper sulfate	72.42	2.69	33.57	25.98	2.99
SEM	0.72	0.1	0.5	0.33	0.1
Without oxidized fat × without copper sulfate	72.26	2.91	33.79	25.96	3.01
Without oxidized fat × 300 mg/kg Copper sulfate	72.72	2.87	34.71	25.96	2.81
2% oxidized fat × without copper sulfate	72.60	2.81	34.20	25.51	3.06
2% Oxidized fat × 300 mg/kg Copper sulfate	72.67	2.56	31.95	26.47	3.18
4% oxidized fat × No copper sulfate	72.05	2.79	33.08	25.19	3.11
4% oxidized fat × 300 mg/kg copper sulfate	71.88	2.64	34.05	25.50	3
SEM	1.24	0.17	0.86	0.56	0.17

Table 4 The effects of different levels of oxidized fat and copper sulfate on blood metabolites and immunity parameters of broilers

Performance	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Albumin (mg/dL)	Total protein (mg/dL)	Uric acid (mg/dL)	Heterophil (%)	Lymphocyte (%)	Heterophil / Lymphocyte
Supplements								
Control group	172.04	60.7[a]	2.15	4.48	2.89	15.58	82.5[ab]	0.20
2% oxidized fat	135.14	34.62[b]	2.41	3.15	3.23	15.34	78.92[b]	0.19
4% oxidized fat	174.14	34.34[b]	2.04	3.40	3.09	10.08	88.58[a]	0.12
SEM	17.29	7.85	0.22	0.62	0.41	2.15	2.05	0.03
No copper sulfate	166.91	44.41	2.26	4.02	3.53	14.56	84.28	0.18
300 mg/kg copper sulfate	153.98	42.38	2.14	3.33	2.61	12.78	82.39	0.16
SEM	14.12	6.41	0.18	0.51	0.33	1.75	1.67	0.20
No oxidized fat × no copper sulfate	182.84	65.79	2.14	5.38	3.40	16.17	82.44	0.80
No oxidized fat × 300 mg/kg Copper sulfate	161.25	55.62	2.17	3.58	2.39	15	82.67	0.23
2% oxidized fat × no copper sulfate	119.85	36.74	2.47	3	2.48	18.34	80.17	0.15
2% Oxidized fat × 300 mg/kg Copper sulfate	150.44	32.50	2.35	3.29	2.98	12.34	77.67	0.10
4% oxidized fat × no copper sulfate	198.04	30.70	2.17	3.69	2.71	9.17	90.34	0.13
4% oxidized fat × 300 mg/kg copper sulfate	157.04	39.02	1.91	3.11	2.47	11	86.84	0.13
SEM	24.45	11.09	0.32	0.87	0.58	8.6	2.89	0.04

The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error.

There were not significant differences between the experimental group in feed intake Dietary fat may increase palatability and increase broiler feed consumption. Diaz (1977) reported non-significant difference in feed intake among broiler chicks fed on ration having oxidized fat/oils with or without added antioxidants which supports the present findings. L'Estrange *et al.* (1966) reported no differences in feed efficiency in broilers fed oxidized beef tallow compared with the control. Adding 300 mg/kg CS had no significant effects on performance of broilers.This findings is in contrast with results of Skrivan *et al.* (2002) who reported that inclusion 126 mg/kg Cu in broiler diets was worsened feed conversion. Inclusion of OF, CS and the interaction of OF × CS had no significant effect on carcass traits of broilers. However higher liver weight were found in treatments including OF. Results of dressing percentage are quite comparable to those reported earlier (Diaz, 1977; Hussein and Kartzer, 1982). Liver weight of chicks in the present study is in agreement with L'Estrange and Carpenter (1966), who observed significantly higher liver weight in birds consuming oxidized fat compared to fresh fat. Increased weight of liver may be due to the accumulation of dietary oxidative products (Cherian *et al.* 1996). Oxidized oil raises the levels of aldehyde and other oxidized metabolites (Wang *et al*. 1997), thiobarbituric acid reactive substance values (Waheed *et al.* 2004). Miyzawa *et al*. (1986) stated that oxidized oil stimulated the liver lipid peroxidation in guinea pigs. Our results about effects of CS on carcass traits is in agreement with findings of Zhang *et al.* (2009) who reported that adding Cu up to 300 mg/kg of broiler diets had no significant effects on their carcass traits. Adding OF had significant effects on reducing blood triglyceride and increasing the percent of lymphocytes. This type of dietary fat may not provide enough energy for metabolic processes, therefore, stored energy sources in the body, such as triglycerides can be used to provide enough energy for metabolic and production. For these reasons, blood levels of triglycerides in treatments containing OF have been dropped. The addition of 2.5 and 5% of industrial residual oil to broiler diets significantly increased the blood levels of cholesterol, low density lipoprotein and triglycerides. Decrease in the percentage of lymphocytes in the group with 2% OF may be associated with nutritional stress due to intake of OF. Dibner *et al*. (1996) reported that oxidant stress resulted in lower concentrations of immunoglobulin in intestinal tissue of broilers. However, CS and the interaction of OF × CS had no significant effects on blood biochemical and immunity cells. Nevertheless, Vogt *et al*. (1981) reported that the antimicrobial actions of Cu have growth stimulating action. Bakalli *et al*. (1995) reported that Cu supplementation reduced plasma triglycerides, which is not corroborated by other investigators (Konjufva *et al*. 1997; Paik *et al*. 1999). In Cu supplemented birds, Cu plays a major role as cofactor in hematogenesis (Chiou *et al*. 1999). Cu is one of the most critical trace elements in livestock because it is necessary for hemoglobin formation, iron absorption from the gastrointestinal tract and iron mobilization from tissue reserves (Mpofu *et al*. 1999).

CONCLUSION

According to this experiment, the overall results indicated that the addition of oxidized fat up to 4% to broiler diets did not significantly affect broiler performance, carcass traits, blood chemistry and immunity cells, however the best performance was obtained by addition of 2% oxidized fat. Adding 300 mg/kg copper sulfate and interaction oxidized fat × copper sulfate had no significant effects on performance, carcass traits, blood metabolites and immune cells of broilers.

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