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Full Length Research Paper

Effect of inorganic chelate of zinc and restaurant residual oil added to feed mixture on the biochemical traits of thigh muscles in male broilers

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An experiment was planned to study the influence of restaurant residual oil (RRO) and inorganic chelate of Zn (ZnO) on triglyceride (TRG), cholesterol (CHOL) and malondialdehyde (MDA) concentrations of thigh muscles in male broiler chickens. In the present research, three hundred and twenty four 10-day-old male broiler chicks (*Ross 308 strain*) in nine treatments including three levels of experimental oil (0, 2.5 and 5%) and three levels of ZnO (0, 50 and 100 mg/kg of feed) were fed until 42 days. The results showed that using RRO, total biochemical traits (TRG, CHOL and MDA) of muscles increased, MDA ($p < 0.01$), CHOL ($p < 0.01$) and TRG ($p < 0.05$). Also, different levels of zinc oxide supplement significantly decreased the content of MDA and CHOL in thigh ($p < 0.05$), but did not result to a significant alteration in TRG concentration. The interaction effects of RRO and ZnO did not result to a significant change in total biochemical traits of fresh thigh muscles in male broilers. Therefore, the effects of RRO deteriorated the quality of meat by raising the susceptibility of muscles to free radical oxidative damage. Also, the effects of ZnO supplementation improved the quality of meat by reducing the extent of oxidation of muscles.

Key words: Residual oil, zinc, biochemical trait, muscle, broiler.

INTRODUCTION

For quite a number of years, food industries residual oils have been recognized as economical sources of energy in poultry diets. Unfortunately, these types of oils are generally oxidized. Therefore, there has been an increased concentration upon the quality and composition of oil sources used in animal feed production. The type of oil that has a large impact on the performance and cholesterol.

Physiological functions of the animal has been exhibited. Thus, the precaution of managing oxidized oil to diets and their influence on internal organs have become disputable and are the subject of research by various researchers. One of the causes of this controversy is the worry that the lipid oxidation can reduce the nutritive value of a diet, induce depressed growth and digestive disorders incidence and finally, cause serological and histological changes to blood and tissues such as increase internal and external free radicals which can damage the cells biochemical compounds (Izaki et al., 1984; Jakobsen et al., 1993). Many studies showed that poultry meat is sensitive to oxidative deterioration, because the lipids in poultry show a higher amount of unsaturation in the fatty acids of phospholipids when compared with red meat. Moreover, anti peroxidants equilibrium and cholesterol (CHOL) in subcellular membranes are significant factors in the determination of oxidative stability of meats as they cause loss of organoleptic characteristics and therefore restrict their storage-

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Abbreviations: MDA, Malonaldehyde; **CVD,** cardio vascular diseases; **RRO,** restaurant residual oil; **TBA**, thiobarbituric acid; **TCA,** trichloroacetic acid; **BHT,** butylated hydroxytoluene; **PUFA,** polyunsaturated fatty acid; TRG, triglyceride; **CHOL**, cholesterol.

Table 1. Percentage composition diet in the starter period.

Ingredient	Experimental diets		
	T1	T2	T3
Cron	53.9	49.6	49.9
Soybean meal (44% CP)	29.16	29.9	30
Fish meal	4	3	3
Experimental oil[1]	0	2.5	5
Starch	7.7	4.95	3.85
Wheat bran	1.5	5.4	4.75
DL-Methionine	0.1	0.1	0.1
DCP	1.25	1.35	1.3
Oyster	1.3	1.35	1.3
Vitamin permix[2]	0.25	0.25	0.25
Mineral permix[3]	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Coccidiostat	0.05	0.05	0.05
Fine Sand	0.22	1.05	0
Calculated nutrient content			
ME kcal/ kg	2933	2933	2933
Crude protein (%)	20.63	20.63	20.6
Calcium (%)	1.03	1.04	1.04
Available P (%)	0.46	0.46	0.46
ME/CP	142.2	142.2	142.2
Ca/P	2.2	2.2	2.2

(1) Vitamin content of diets provided per kilogram of diet: vitamins A, D, E and K. (2) Composition of mineral premix provided following the kilogram of premix: Mn, 120.000 mg; Zn, 80.000 mg; Fe, 90.000 mg; Cu, 15.000 mg; I, 1,600 mg; Se, 500 mg and Co, 600 mg 3.

life (Lin et al., 1989; Monahan et al., 1992; Kanner, 1994). The alterations in meat quality due to lipid oxidation are revealed by pernicious changes in the production of poisonous and dangerous compounds like malonaldehyde (MDA) and cholesterol oxides (Gray et al., 1999).

MDA, as lipid peroxidation index, is one of the major causes of quality deterioration in meat and the products made from the meat of those birds; however, the high cholesterol content in broiler meat cause an increase in the risk of cardio vascular diseases (CVD) in human (Lopez-Bote et al., 1997). Meyer et al. (2002) reported the first plan of preventing lipid oxidation as feeding broilers with antioxidants. In the food industry, they can be classified into synthetic and organic antioxidants, for example, trace minerals such as zinc (Zn) has been known to be an essential nutrient for animals for many years. Cunningham-Rundles et al. (1990) show that Zn acts as an antioxidant which reduces the cell membrane damage due to free radicals, which in succession, according to Powell (2000), changes the immunological status of the animal. The mechanism by which Zn applies its antioxidant action is not specified. However, it has been proposed that Zn increases the synthesis of metallo-thionein that controls the Zn pool and consequently, acts as a free radical cleaner (Cunningham-Rundles et al., 1990). Moreover, Zn has an important role in the reduction of MDA levels in broilers serum (Karamouz et al., 2009). In the current research, the effect of the inorganic chelate of Zn (ZnO) and restaurant residual oil (RRO) that was added to the feed mixture on biochemical traits of thigh muscles in male broilers was investigated.

MATERIALS AND METHODS

Three hundred and twenty four 10-day-old male broiler chicks (*Ross 308 strain*) were used in this study. The birds were randomly assigned to 9 treatment groups consisting of 3 replicates of 12 birds each in a 3×3 factorial arrangement of treatments (3 RRO levels and 3 ZnO levels). RRO was used at 0, 2.5 and 5% in diets and ZnO as an inorganic chelate of Zn was used at 0, 50 and 100 mg/kg in diets. Utmost care was taken to provide equal physical and environmental housing conditions (namely size of units, light, temperature and aeration). Feed and water were supplied *ad libitum*. The birds were fed a starter diet for 21 days, followed by a grower diet from 21 to 42 days (Tables 1 and 2). Diets were formulated to meet the requirements of nutrient and energy for broiler chickens on the basis of the nutrients recommended by NRC (1994). The experimental treatments included the following: T_1 = The basal diet (Soybean + corn); T_2 = basal diet + 0% RRO + 50 mg/kg ZnO; T_3 = basal diet + 0% RRO + 100 mg/kg ZnO; T_4 = basal diet + 2.5% RRO + 0 mg/kg ZnO; T_5 = basal diet + 2.5% RRO + 50 mg/kg ZnO; T_6 = basal diet + 2.5% RRO + 100 mg/kg ZnO; T_7 = basal diet + 5% RRO + 0 mg/kg ZnO; T_8 = basal diet + 5% RRO + 50 mg/kg ZnO and T_9 = basal diet + 5% RRO + 100 mg/kg ZnO. To investigate the effect of ZnO and the oxidized oil added to the feed mixture on the biochemical characteristics of thigh muscles, nine birds from each treatment were selected and slaughtered under experimental conditions at the end of the period (42 days). The right side samples of the thigh muscles of the slaughtered birds without the skin were separated immediately. Then, muscle samples were packed in plastic bags and kept in ice during transportation to the processing plant.

Quality control of the experimental oil

In the current research, RRO, as an oxidized oil, was provided by a local restaurant and was immediately sampled for quality and composition analyses. At the first onset, the experimental oil sample (5 g) was weighed into a 250 ml flask, and then 30 ml acetic acid-chloroform (3:2) solution was added and blended energetically until the experimental oil sample was dissolved. This was followed by an addition of 0.5 ml saturated potassium iodide (KI) solution. After 1 min, 30 ml distilled water was added and the solution was titrated using sodium thiosulfate (0.01 N). After the deterioration of the yellowish color, 0.5 ml of 1% soluble starch indicator was added and titration was continued until the color changed. At the titration time, the solution was shaken vigorously. Finally, the iodine was released from the chloroform layer (Narwar, 1996; Horwitz, 2002). The peroxide value (Table 3) was calculated using:

$$\text{Peroxide value (meq 02/kg)} = \frac{\text{Sodium thiosulfate (ml)} \times 0.01}{\text{Weight of sample (g)}} \times 100$$

Also, the composition of the fatty acids of lipids is determined by the separation of the methyl esters of the fatty acids, using gas chromatography (AOAC, 1999).

Table 2. Percentage composition diet in the grower period.

Ingredient	Experimental diets		
	T1	T2	T3
Cron	57	53.97	54
Soybean	27	27	27
Fish meal	1.5	1.5	1.5
Residual oil	0	2.5	5
Starch	8.48	5.28	0.33
Wheat bran	1.78	3.5	3.43
DL-Methionine	0.1	0.1	0.1
DCP	1.39	1.35	1.3
Oyster	1.55	1.5	1.4
Vitamin permix[2]	0.25	0.25	0.25
Mineral permix[3]	0.25	0.25	0.25
Salt	0.25	0.25	0.25
Coccidiostat	0.05	0.05	0.05
Sand	0.4	2.5	5.14
Calculated nutrient content			
ME kcal/ kg	2950	2950	2950
Crude protein (%)	18.44	18.44	18.45
Calcium (%)	1.01	1.02	1.01
Available P (%)	0.41	0.41	0.41
ME/CP	160	160	160
Ca/P	2.4	2.4	2.4

(1) Vitamin content of diets provided per kilogram of diet: vitamins A, D, E and K. (2) Composition of mineral premix provided following the kilogram of premix: Mn, 120.000 mg; Zn, 80.000 mg; Fe, 90.000 mg; Cu, 15.000 mg; I, 1,600 mg; Se, 500 mg and Co, 600 mg 3.

Table 3. Fatty acids composition and peroxide value of the experimental oil.

Fatty acids	Percentage of total fat (%)
(c_{16}:0)	20.8
(c_{18}:0)	11.13
(c_{18}:1t[1])	34.5
(c_{18}:1)	22
(c_{18}:2t[2])	1.7
(c_{18}:2)	9.2
(c_{18}:3)	0.3
(c_{20}:2)	0.2
Peroxide value (meq O_2 kg^{-1})	36

[1]Trans 9-octadecenoic-acid (elaidic); [2]Trans isomers of octadecadienoic acid.

Determination of the biochemical characteristics of fresh meat samples

The thiobarbituric acid (TBA) values were determined for the MDA formed in fresh muscles. This secondary oxidation product (MDA) was measured according to the TBA method described by Slavomir et al. (2003) and Botsoglou et al. (1994). With the use of spectro photometry and emphasis on some modifications, samples were homogenized (polytron hemogenizer, PCU, Switzerland). Subsequently, 10 ml of the 10% trichloroacetic acid (TCA) and 500 ppm of butylated hydroxytoluene (BHT) were added to 2 g of the homogenized sample in the test tube. The sample was heated for 30 min at 95°C in water bath. After cooling at room temperature, the sample was centrifuged at 3000 g for 10 min, and then 2 ml of the thiobarbituric acid (0.02 mol/l), dissolved in acetic acid, were added to 2 ml of the supernatant and was heated for 30 min at 95°C in water bath. After it was cooled under tap water and submitted to the conventional spectrophotometery (Shimadzu, Model UV-160A, Tokyo, Japan), the absorbance at 532 nm was measured. Also, total cholesterol and triglyceride (TRG) analyses were conducted in samples from each treatment after lipid extraction, according to Maraschiello (1998).

Statistical analysis

Data were subjected to a one-way analysis of variance using the general linear models (GLM) and the procedure of the Statistical Analysis System (SAS) user's guide (2000). The result of the analysis of variance according to the model is:

$$Y_{ijk} = \mu + \alpha i + \beta_j + (a\,\beta)_{ij} + e_{ijk}$$

Where, Y_{ijk} = All dependent variable; μ = overall mean; αi = the fixed effect of RRO levels ($_i$ = 1, 2, 3); β_j = the fixed effect of ZnO levels ($_j$ = 1, 2, 3); e_{ijk} = the effect of the experimental error
Values of different parameters were expressed as the mean ± standard deviation (X±SD). When significant difference among the means was found, means were separated using Duncan's multiple range tests.

RESULTS AND DISCUSSION

The independent and interaction effects of RRO and ZnO on MDA, CHOL and TRG of thigh muscles are presented in Tables 4. The MDA, CHOL and TRG values in thigh muscles were higher in RRO when compared to control groups [MDA ($p< 0.01$), CHOL ($p<0.01$) and TRG ($p<0.05$), respectively]. Therefore, the analyses performed indicated that the biochemical traits in the examined muscles were affected by the feeding manner of birds. Moreover, as it was reported in another research, due to the lesser amounts of polyunsaturated fatty acid (PUFA) in the breast muscle, the MDA concentration was low, which was the opposite of the thigh muscle (Karpinska et al., 2004; Ikeme, 1990).

Igene et al. (1985) and Kanner et al. (1988) reported in their researches that the free iron content from heme pigment of the muscle is a significant catalyst of lipid oxidation which alters muscles in a bird. This theorem is very important in thigh muscle because the thigh muscle has high free iron. Furthermore, related to the results gained from the mentioned study, it could be claimed that the experimental oil significantly increased the amount of MDA and challenged the anti-oxidant defense system which led to the increase of the oxidative damage risk in the muscles. These results were in accordance with the findings of Engberg et al. (1996) in which the amount of

Table 4. Biochemical traits of fresh thigh muscles from broiler chickens fed diets containing supplementary RRO and ZnO.

		MDA (mg/g)	Cholesterol (mg/100g)	Triglyceride (mg/100g)
Supplementary RRO effect				
0 % (control)		1.72± 0.12 [c]	80.57± 12.37 [c]	101.91±17.12 [c]
2.5 %		2.89± 0.25 [b]	88.43± 13.49 [b]	109.22±17.87 [b]
5%		3.01± 0.26 [a]	93.28± 13.70 [a]	113.17±19.23 [a]
Supplementary ZnO effect				
0 mg /kg (control)		2.02± 0.31 [a]	86.26± 14.44 [a]	100.03±16.70 [a]
50 mg /kg		1.94± 0.26 [b]	82.43± 14.82 [b]	99.98± 16.56 [b]
100 mg /kg		1.81± 0.022 [c]	80.10± 15.56 [c]	101.23±17.03 [c]
Interaction				
Supplementary RRO effect	**Supplementary ZnO effect**			
0 %	0 mg /kg	1.81±0.12	84.15±7.44	92.02±14.21
	50 mg /kg	1.77±0.11	83.43±6.78	93.10±12.32
	100 mg /kg	1.71±0.14	81.01±9.43	89.18±17.34
2.5 %	0 mg /kg	1.94±0.15	87.11±11.12	90.44±16.53
	50 mg /kg	1.91±0.17	86.12±10.10	91.01±14.33
	100 mg /kg	1.88±0.18	86.01±13.40	88.15±15.54
5%	0 mg /kg	2.20±0.21	90.05±16.23	92.11±18.11
	50 mg /kg	2.10±0.20	89.93±9.55	94.35±18.34
	100 mg /kg	2.03±0.18	88.51±12.41	90.91±19.02
Statistical significance				
Supplementary RRO		**	**	*
Supplementary ZnO		*	*	NS
Interaction		NS	NS	NS

Mean ± Standard deviation, NS = Not significant (P > 0.05), * = P < 0.05 and ** = P < 0.001
Means with different superscripts within the same column and for the same parameter are significant (P<0.05).

oxidation in animals' meat increased with the usage of oxidized fat. On the other hand, Jensen et al. (1997) reported that the amount of anti-oxidants, such as *α*-tocopherol, in birds' muscles was significantly decreased due to their being fed with oxidized oil. One of the reasons may be the destruction of *α*-tocopherol in the gastrointestinal tract by free radicals from the oxidized oil. They resumed their report by asserting that preserving meat, especially thigh, would increase the risk of fat oxidation. In addition, in another research conducted by Karamouz et al. (2009), on the blood serum of the broiler chickens, it was revealed that the oxidized oil decreased the concentration of the serum's total anti-oxidants and increased MDA. They also reported that these kind of oil increase the amount of cholesterol, LDL and triglyceride in the serum. The results gained encourage the idea that all oxidative parameters form a negative correlation with the alpha tocopherol content of the meat (Grau et al., 2001). In the present study, different levels of zinc oxide supplement significantly decreased the content of MDA and cholesterol in thigh muscles (p<0.05), which might be due to the zinc element that can act as an anti-oxidant. Besides, other researchers have reported that zinc supplementation led to a significant increase in the

amount of selenium (as an anti-oxidant) in meat and serum (Bou et al., 2005). Generally, zinc's anti-oxidant mechanism is divided into two: (a) Chronic effects and (b) acute effects. Chronic effect indicates the gradual activity of zinc in the tissue which leads to the stimulation of anti-oxidant compounds such as metallothioneins. In fact, metallothioneins take the responsibility for providing the necessary amount of zinc in the process of oxidation and banding the redox-active transition metals like iron and cupper as well as preventing them from producing free radicals. Acute effect includes the protection of the sulfhydryl group in proteins or it contributes to the decrease of the formation of hydroxyl radicals (OH°) from hydrogen peroxide (H_20_2). This operation takes place as a result of the completion among zinc and other redox-active transition metals like iron and cupper in banding sulfhydryl groups (Powell et al., 2000). In a research conducted by Karamouz et al. (2009), it was confirmed that different levels of zinc oxide supplement leads to the decrease of MDA content as well as the increase of all present anti-oxidants in the broiler chickens' serum. Also, it has been proved that organic and inorganic zinc supplements in the ration of the laying chickens decreased the MDA concentration due to the role of zinc in the formation of Cu/Zn-SOD enzyme (which decreases lipid peroxidation). The mentioned enzyme transforms the superoxide (O-) into H_2O_2 and di-oxygen (Robinson, 1998). In the current research, the interaction effects of RRO and ZnO did not result in a significant alteration in total biochemical traits of and thigh muscles in male broilers.

Conclusion

The use of ZnO in feed mixtures, given to broiler chickens from day 10 of their life, increased the oxidative stability of fats and decreased the CHOL content in thigh muscles. Moreover, it was better at a higher level of ZnO (100 mg/kg diet). Also, significant depression of the fat oxidative stability was achieved with the addition of RRO to the broiler chicken feeds, and it was worse at a higher level of RRO (5%). Finally, it is concluded that the usage of RRO in the broilers diet may raise the susceptibility of tissues to free radical oxidative damage. Therefore, the quality of oil used in feed is important.

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