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Interaction Effects of Zinc Oxide Supplementation and Restaurant Residual Oil on the Biochemical Traits of Fresh Breast Muscle in Male Broilers

[1]*Habib Karamouz,* [2]*Habib Aghdam Shahryar,*
[2]*Jamshid Ghiasi Ghaleh-Kandi and* [2]*Abolfazl Gorbani*

[1]Member of Young Researchers Club,
Islamic Azad University, Shabestar Branch, Shabestar, Iran
[2]Department of Animal Science,
Islamic Azad University Shabestar Branch, Shabestar, Iran

Abstract: 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 fresh breast muscle 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 of biochemical traits (TRG, CHOL and MDA) of breast muscle increased, MDA ($p< 0.01$), CHOL ($p<0.01$) and TRG ($p<0.05$), respectively. Also, different levels of zinc oxide supplement significantly decreased the content of MDA and CHOL in breast muscle ($p<0.05$), but it didn't result in a significant alteration in TRG concentration. The interaction effects of RRO and ZnO didn't result in a significant change in total biochemical traits of fresh breast muscle in male broilers. Therefore, the effects of RRO deteriorated meat quality by raising the susceptibility of muscles to free radical oxidative damage. Also, the effects of ZnO supplementation improved meat quality by reducing the extent of oxidation of muscles.

Key words: Residual oil · Zinc · Biochemical trait · Muscle and broiler

INTRODUCTION

In the last several years, it is recognized that food industries residual oils are the economical sources of energy in the poultry diets. Unfortunately, these types of oils are generally oxidized. Therefore, there has been an increased concentrate upon the quality and the composition of oil sources used in the animal feed production. On the other hand, it is exhibited that the type of oil has a large impact on the performance and physiological functions of the animal. For example, oxidized oils can reduce the nutritive value of a diet, induce depressed growth and digestive disorders incidence and finale, cause serological and histological changes to blood and tissues such as increase internal and external free radicals which can damage of the cells biochemical compounds [1,2]. Scientific researches showed that the alterations in meat quality due to lipid oxidation are revealed by pernicious changes in production poisonous and dangerous compounds like malonaldehyde (MDA) and cholesterol oxides [3]. MDA as lipid peroxidation index is one of the major causes of quality deterioration in meat and products made from the meat of those birds and high cholesterol content in broiler meat increased risk of cardio vascular diseases (CVD) in human [4]. Researchers reported the first plan of prevent lipid oxidation is feeding broilers with antioxidants. In food industry they can be classified into synthetic and organic antioxidants, with the future including, e.g. trace minerals such as Zinc (Zn) has been known to be an essential nutrient for animals for many years [5]. A study shown that Zn acts as antioxidant reducing the cell membrane damage due to free radicals [6], which in succession according to [7], changes the immunological

Corresponding Author: Habib Karamouz, Member of Young Researchers Club, Islamic Azad University, Shabestar Branch, 53815-159 Shabestar, Iran, E-mail: h_karamouz110@yahoo.com.

status of the animal. Moreover, Zn had an important role in reduction of MDA levels in broilers serum [8]. In the current research, interaction effects of inorganic chelate of Zn (ZnO) and restaurant residual oil (RRO) added to feed mixture on biochemical traits of fresh breast muscle in male broilers was investigated.

MATERIALS AND METHODS

Chicks and Diets: Three hundred and twenty four, 10-day-old male broiler chicks (*Ross308 strain*) were used in the 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 until 21 d of age followed by a grower diet from 21 d to 42 d (Table1 and 2). Diets were formulated to meet the requirements for nutrient and energy for broiler chickens on the base of nutrients recommended [9]. To investigate the interaction effects of ZnO and oxidized oil on biochemical characteristics of fresh breast muscle, at the end of period (42 d), nine birds from each treatment were selected and slaughtered under experimental conditions. The right side samples of fresh breast muscles of the slaughtered birds without the skin were separated, immediately. Then, muscle samples packed in plastic bags and kept in ice during transportation to the processing plant.

Samples Procedures: In the current research, restaurant residual oil (RRO) as an oxidized oil was provided by a local restaurant and immediately sampled for quality and composition analyses. The peroxide value was calculated. 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 [10]. Moreover, The thiobarbituric acid (TBA) values were determined for the Malonaldehyde (MDA) formed in fresh muscles. This secondary oxidation product MDA was measured according to the TBA method described by Slavomir *et al.* and Botsoglou *et al.* [11,12]. Also, total cholesterol and triglyceride analyses were conducted in samples from each treatment after lipid extraction, according to Maraschiello. [13].

Table 1: Percentage composition basal diet in starter period

	Experimental diets		
Ingredient	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	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[1]	0.25	0.25	0.25
Mineral permix[2]	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: vitamin A, D, E and K. 2: Composition of mineral premix provided as follows kilogram of premix: Mn, 120.000 mg: Zn, 80.000 mg: Fe, 90.000 mg: Cu, 15.000mg: I, 1,600 mg: Se,500 mg: Co, 600 mg 3 .

Table 2:Percentage composition basal diet in grower period

	Experimental diets		
Ingredient	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[1]	0.25	0.25	0.25
Mineral permix[2]	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: vitamin A, D, E and K. 2: Composition of mineral premix provided as follows kilogram of premix: Mn, 120.000 mg: Zn, 80.000 mg: Fe, 90.000 mg: Cu, 15.000mg: I, 1,600 mg: Se, 500 mg: Co, 600 mg 3.

Table 3: Fatty acids composition and peroxide value of experimental oil

Fatty acids	(% 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})
1-Trans 9- Octadecenoic- acid (Elaidic).
2-Trans Isomers of Octadecadienoic acid

Statistical Analyses: Data obtained were subjected to a one-way analysis of variance using the General Linear Models (GLM), procedure of SAS User's guide [14]. Analysis of variance according to the model,

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

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 experimental error

Values of different parameters were expressed as the mean ± standard deviation (X±SD). When significant difference among means was found, means were separated using Duncan's multiple range tests[14].

RESULTS AND DISCUSSION

The Independent and interaction effects of RRO and ZnO on MDA, CHOL and TRG of fresh breast muscles are presented in Tables 4. The MDA, CHOL and TRG values in fresh breast 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 indicate that biochemical traits in the examined muscles were affected by the feeding manner of birds. Igene *et al.* [15] and Kanner *et al.* [16] reported in their researches that free iron content from heame pigment of muscle is a significant catalyst of lipid oxidation which alters among muscles in a bird. 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 and led to the increase of the oxidative

Table 4: Biochemical traits of fresh breast 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)		1.01± 0.11[c]	41.65± 5.01[c]	65.23± 7.54[c]
2.5 %		1.37± 0.13[b]	57.87± 6.23[b]	70.65± 8.02[b]
5%		1.62± 0.16[a]	70.27± 8.82[a]	74.31± 8.09[a]
Supplementary ZnO effect				
0 mg /kg (control)		0.97± 0.08[a]	44.04± 4.08[a]	55.93± 4.44
50 mg /kg		0.81± 0.07[b]	40.03± 4.13[b]	56.23± 5.61
100 mg /kg		0.61± 0.06[c]	38.21± 3.92[c]	55.06± 7.03
Interaction				
Supplementary RRO effect	Supplementary ZnO effect			
0 %	0 mg /kg	1.21± 0.17	42.12± 5.55	68.04± 9.62
	50 mg /kg	1.25± 0.12	41.09± 5.08	69.36±10.12
	100 mg /kg	1.20± 0.10	43.97± 4.34	67.44± 8.89
2.5 %	0 mg /kg	1.34± 0.19	51.43± 7.11	73.74± 7.94
	50 mg /kg	1.23± 0.18	52.18± 4.91	74.34± 8.09
	100 mg /kg	1.17± 0.17	47.94± 5.22	72.12±10.01
5%	0 mg /kg	1.31± 0.20	60.67± 8.83	70.98± 7.64
	50 mg /kg	1.27± 0.21	57.96± 7.70	72.33± 8.30
	100 mg /kg	1.24± 0.23	56.04± 7.85	72.66± 8.99
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 ($P<0.05$) and ($P< 0.001$). Experimental treatments, included following: T_1= The basal diet (Soybean + Corn), T_2= The basal diet + 0% RRO + 50 mg/kg ZnO, T_3= The basal diet + 0% RRO + 100 mg/kg ZnO, T_4= The basal diet + 2.5% RRO+ 0 mg/kg ZnO, T_5= The basal diet + 2.5% RRO+50 mg/kg ZnO,T_6= The basal diet + 2.5% RRO+100 mg/kg ZnO, T_7= The basal diet + 5% RRO+0 mg/kg ZnO, T_8= The basal diet + 5% RRO+50 mg/kg ZnO and T_9= The basal diet + 5% RRO+100 mg/kg ZnO.

damage risk in the muscles. These results were in accordance with the findings of Engberg *et al.* [17], in which the amount of oxidation in animals' meat increased with the usage of oxidized fat. Also, Karamouz *et al.* [18] reported that preserving broilers meat (for two months) fed with oxidized oil, would increase the risk of the oxidation of fat, especially thigh and breast muscles. On the other hand, Jensen *et al.*[19] 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. Furthermore, in other researcher conducted by Karamouz *et al.* [20], on the blood serum of the broiler chickens it was revealed that the oxidized oil decreased the concentration of serum's total anti-oxidants and increased MDA. They also have reported that these kinds 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 [21]. In the present study, different levels of zinc oxide supplement significantly decreased the content of MDA and cholesterol in breast 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 [22]. In a research conducted by Karamouz *et al.*[8] 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 superoxide (O-) into H_2O_2 and Di-Oxygen [23]. In the current research, the interaction effects of RRO and ZnO didn't result in a significant alteration in total biochemical traits of fresh breast and thigh muscles in male broilers.

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