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Response of Male Broilers to Different Levels of Food Industries Residual Oil on Serum Lipoproteins, Lipid Peroxidation and Total Antioxidant Status

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Abstract: Three dietary treatments with three replicates containing 0, 2.5 and 5% of Food Industries Residual Oil (FIRO) were fed to 108 one-day old (Ross 308) strain chicks from 1 to 42 days. The chicks were randomly assigned to 9 cages (12 birds per cage). Serum biochemical values containing cholesterol (CHOL), triglyceride (TRIG), HDL, LDL, lipid peroxidation (MDA) and total antioxidant value (TAS) were measured at the end of starter (21d) and grower (42 d) periods. Serum parameters were not affected by dietary treatments at day 21. But results indicate that groups fed containing FIRO levels, increased CHOL, LDL, TRIG at day 42 ($p<0.01$ and $p<0.05$, respectively) and Increased MDA levels as an index of lipid peroxidation and reduced TAS in serum ($p<0.01$). From this study it is recommended that using FIRO in the broiler diets may raise the susceptibility of blood and tissues to free radical oxidative damage.

Key words: Residual oil · Lipoprotein · Lipid peroxidation · Antioxidant · Male broiler

INTRODUCTION

Among the constituents of poultry feed, fats supply concentrated form of energy (2.25 times more energy than carbohydrates and proteins). However, their inclusion as true fat or oil in the ration is limited because of the high risk of rancidity on prolong exposure to air, heat, sunlight and poor storage conditions [1].

In recent years there has been an increased focus on the quality and the composition of fat sources used in the animal feed industry. Also, it is recognized that the type of fat has a large influence on the performance and physiological functions of the animal. For example, the effect of oxidized oil have been examined on diet quality and performance of broilers in the previous studies, today, direct or indirect effects of oxidized oil on animal and human health are being determined. On the other hand, the safety of administering oxidized oil 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 diarrhoea incidence, cause serological and histological changes to blood and tissues such as increase endogenous and exogenous free radicals which can damage structures of lipids, proteins, carbohydrates and nucleic acids by interacting with them and can subsequently produce new free radicals and, in some cases and even the death of birds [2, 3].

Also, among all biomolecules lipids are the most sensitive molecules to free radicals. Double bonds in fatty acids form peroxide products by reacting with free radicals and lipid radicals can be formed subsequently upon removal of electrons. As a result of lipid peroxidation, quite harmful degrading products (namely malondialdehyde (MDA) can be formed in cell membranes. MDA shows both mutagenic and carcinogenic effects by changing membrane properties [4, 5].

Nowadays stabilized increasing of MDA concentration in various diseases such as type1 diabetes; therefore determination of this biomarker has been widely applied as the most common approach for the assessment of lipid peroxidation in biological and medical sciences [6, 7].

On the other hand, living organisms protect themselves from harmful effects of free radicals by antioxidant defense mechanisms. Antioxidants inhibit lipid peroxidation by breaking the chain reactions and scavenging initiating free radicals [8]. Antioxidants are

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classified as enzymatic and non-enzymatic antioxidants. Non-enzymatic antioxidants are α-tocopherol (vitamin E), ß-carotene, vitamin A, ubiquinols, ascorbate, glutathione, melatonin, cysteine, ceruloplasmin, haemoglobin, bilirubin, albumin and some minerals such as zinc, selenium and chromium. Enzymatic antioxidants are superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and catalase (CAT).

In the present study, our objective was to investigate the effects of FIRO on lipoproteins; MDA as well as TAS were measured to shed light on the effects of this type of oil on serum biochemical characteristics in male broilers [9].

MATERIALS AND METHODS

Birds and Diet: One hundred eight, 10-day-old male broiler chicks (Ross308 strain) were randomly assigned to 3 groups consisting of 3 replicates of 12 birds. All chicks were obtained from a poultry-breeding farm. Utmost care was taken to provide equal physical and environmental housing conditions (namely size of units, light, temperature and aeration). All experimental procedures used were in accordance with the published ethical guidelines for the animal use and care. Also, Feed and water were supplied *ad libitum*. Experimental diets, formulated according to [10], included following levels of Food Industries Residual Oil (FIRO): a) control diet (no FIRO), b) 2.5%, c) 5%. Birds were fed with experimental diet for starter (10 -21d) and grower (22- 42 d) periods (Table 1).

Samples Procedures: Fatty acids composition and peroxidation values of FIRO (Table 2) were determined according to AOAC [11]. At days 21 and 42, 2 chicks from each pen were selected for blood parameters. Blood samples were collected from the vena axillaries. Then, Samples were centrifuged at 3000×rpm for 10 min and sera were collected. The analysis of serum CHOL, HDL-C, TRG levels were measured on biochemical autoanalyzer (Alcyon abbot-300, USA) by using commercially available kits. Also, LDL-C level was estimated by Friedewald *et al.* equation. The levels of MDA were measured in the serum with the tiobarbituric acid reaction by the method of

Table 1: Composition of the experimental diet in starter and grower periods

	Experimental diets					
	Starter [1]			Grower		
Ingredient	T1	T2	T3	T1	T2	T3
Cron	54.00	49.70	50.00	57.00	53.97	54.00
Soybean	29.16	29.90	30.00	27.00	27.00	27.00
Fish meal	4.00	3.00	3.00	1.50	1.50	1.50
FIRO[2]	0.00	2.50	5.00	0.00	2.50	5.00
Starch	7.70	4.95	3.85	8.48	5.28	0.33
Wheat bran	1.50	5.40	4.75	1.78	3.50	3.43
DL Methionine	0.00	0.00	0.00	0.10	0.10	0.10
DCP	1.25	1.35	1.30	1.39	1.35	1.30
Oyster	1.30	1.35	1.30	1.55	1.50	1.40
Vitamin[3]	0.25	0.25	0.25	0.25	0.25	0.25
Mineral[4]	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Coccidiostat	0.05	0.05	0.05	0.05	0.05	0.05
Fine Sand	0.22	1.05	0.00	0.40	2.50	5.14
Calculated nutrient content						
ME kcal/ kg	2933.60	2933.60	2933.35	2952.52	2952.52	2952.52
Crude protein (%)	20.63	20.63	20.63	18.45	18.45	18.45
Calcium (%)	1.04	1.04	1.04	1.08	1.08	1.08
Available P (%)	0.46	0.46	0.46	0.41	0.41	0.41
ME/CP	142.20	142.20	142.20	160.02	160.02	160.02
Ca/P	2.25	2.25	2.25	2.40	2.40	2.40

1- Starter period (10-21d) and Grower period (22-42d). 2- FIRO = Food Industries Residual Oil.

3.Vitamin content of diets provided per kilogram of diet: vitamin A, D, E and K. 4- Composition of mineral premix provided as fllows kilogram of premix: Mn, 120.000 mg: Zn, 80.000 mg Fe, 90.000 mg: Cu, 15.000 mg: I, 1, 6000 mg: Se, 500 mg: Co, 600 mg 5. T1= basal diet, T2= basal diet + 2.5% FIRO, T3= basal diet+ 5%FIRO.

Table 2: Fatty acids composition and peroxidation value of FIRO

Fatty Acid	Percent
(C_{16}:0)	20.80
(C_{18}:0)	11.13
(C_{18}:1t[1])	34.50
(C_{18}:1)	22.00
(C_{18}:2t)	1.70
(C_{18}:2)	9.20
(C_{18}:3)	0.30
(C_{20}:2)	0.20
Peroxide Value (mEq O_2 kg^{-1})	36.00

1-Trans fatty acid

Mihara *et al.* [12]. Total Antioxidant Status (TAS) Randox (SAO as described in the Randox Laboratories manual) which measured the capacity of the serum to neutralise the oxidative action of free radicals.

Statistical Analyses: The data collected were subjected to an analysis of variance and any significant differences were determined. When the ANOVA revealed significant differences, Duncan's multiple range tests was performed to establish where such means differed. All the data were analyzed by ANOVA using the general linear model (GLM) procedures of the SAS Institute [13].

RESULTS AND DISSCUTION

The levels of serum lipids (CHOL, TRG, HDL-C and LDL-C), lipid peroxidation (MDA) and TAS are depicted in Tables 3 and 4. At day 21, serum parameters were not influenced by different levels of FIRO in diet. But with increasing FIRO additives, the levels of serum lipids such as CHOL and LDL raised earlier. Also, the levels of all this parameters were significantly affected by levels of FIRO in dietary groups at day 42 ($p<0.01$ and $p<0.05$, respectively). Result show that in 5% and 2.5% FIRO groups an increase in CHOL, TRG, LDL-C and decrease HDL as compare with control group, possibly because of high levels of trans fatty acids in FIRO. The presence of trans fatty acids in FIRO raise the question of their safety as feed additives. Moreover, they are metabolized more like saturated than like the cis-unsaturated ones and may have profound significance on the molecular packing in membranes [14].

Our results corresponded with several reports clearly demonstrate that modest intake of trans fatty acids can deleteriously affect lipoproteins by increasing LDL, decreasing HDL with along an increase in cholesteryl ester transfer protein activity, i.e., enhance transfer of cholesteryl ester from HDL to LDL [15].

Result show that with usage 5 and 2.5% FIRO in experimental diet caused significant increases in serum MDA and a decreases in TAS at days 21 and 42 ($p< 0.01$). These results were in accordance with the reports of Karamouz [3] and Kazuaki *et al.* [16]. They reported that feeding oxidized fat decreased serum total antioxidant status and increased serum and meat MDA in broilers.

Table 3: Serum lipids[1] of male broilers fed different levels of FIRO (X±SD)

	Measurement							
	Cholesterol		Triglyceride		HDL		LDL	
Diet	21 d	42 d	21 d	42 d	21 d	42 d	21 d	42 d
0.00% FIRO	119±5.02	129.77±4.32^{b}	68±2.34	77.83±3.61^{b}	33±1.05	25.05±1.09^{a}	94±2.32	89.15±2.23^{b}
2.5% FIRO	131±6.13	144.66±5.87^{a}	67±3.78	85.44±4.56^{ab}	31±1.02	21.88±1.10^{ab}	117±4.44	105.68±5.43^{a}
5% FIRO	132± 6.92	153.68±6.04^{a}	70± 3.93	91.05± 5.23^{a}	29± 0.95	20.01± 0.97^{b}	121±6.98	115.4±7.89^{a}
P value	NS	**	NS	*	NS	*	NS	**

[1]n = 6 samples within each treatment group.
NS=non significant, * = ($p < 0.05$), ** = ($p < 0.01$)
[a,b] Means within columns with no common superscript differ significantly ($P < 0.05$), ($p < 0.01$).

Table 4: The levels of MDA and total antioxidant status[1] of male broilers fed different levels of FIRO (X±SD)

	Measurement			
	MDA		TAS	
Diet	21 d	42 d	21d	42 d
0.00% FIRO	2.01±0.16	2.51±0.11^{c}	0.88±0.05	1.21±0.09^{a}
FIRO 2.5%	2.11±0.19	4.42±1.03^{b}	0.91±0.09	0.78±0.04^{b}
FIRO 5%	2.21±0.23	5.68± 1.07^{a}	0.90±0.85	0.69±0.02^{b}
P value	NS	**	NS	**

[1]n = 6 samples within each treatment group.
NS=non significant, * = ($p < 0.05$), ** = ($p < 0.01$)
[a,b] Means within columns with no common superscript differ significantly ($P < 0.05$), ($p < 0.01$).

In other study, also they reported that decrease of TAS may be considered as a weak protective mechanism against oxidative stress. Also, reported that chicks fed on oxidized oil had extremely low plasma and tissue alpha tocopherol concentrations as a non-enzymatic antioxidant. This result indicated that a significant inverse correlation was observed between the log of plasma alpha tocopherol concentration and the MDA concentration in plasma [17].

In general, the oxidative stress is manifested primarily via alterations of antioxidant enzyme activities and the reductions of some non-enzymatic antioxidants such as the vitamins A, C, E and some minerals [18]. Antioxidant enzyme as an example superoxide dismutase is involved in the antioxidant defense system in a first attempt to control and eliminate the toxic reactive oxygen species (ROS) [19]. According Amstad *et al.* [20] the decrease of the activities of antioxidant enzymes could have a negative impact on cellular resistance against the oxidant induced damage of cell genome and cell killing. On the other hand, Speranza *et al.* [21] and Popova *et al.* [22] reported that the CAT and GSH-PX were important for adaptation of cells to oxidative stress and preserved cells via degradation of the reactive hydrogen peroxide.

Based on our results and literature data, we suggest that usage FIRO in the broiler diets may raise the susceptibility of blood and tissues to free radical oxidative damage.

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