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Dietary Oxidized Poultry Offal Fat: Broiler Performance and Oxidative Stability of Thigh Meat During Chilled Storage[1]

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ABSTRACT

Two experiments were conducted to evaluate the effects of dietary oxidized poultry offal fat on the performance of broilers and on the oxidative stability of dark chicken meat. One hundred and sixty male chicks were fed a corn-soybean meal diet containing 4% fresh or oxidized poultry fat from 10 to 47 days of age. Fresh fat was stored frozen until diets were produced, and oxidized fat was obtained by electrical heating (110 to 120 °C). Birds were slaughtered at 47 days of age, and carcass characteristics were measured. Skinless and deboned thigh meat was stored chilled during 12 days, and samples were periodically collected to assess their quality and oxidative stability. Dietary oxidized fat did not affect bird performance or carcass characteristics. During chilled storage, meat color (L*, a* and b*) was not affected by dietary treatments; however, TBARS (Thiobarbituric Acid Reactive Substances) values were higher (P<0.05) in thigh meat from chickens fed the oxidized fat, indicating that oxidative stability was adversely affected.

INTRODUCTION

Lipid oxidation is considered the main cause of quality damages related to flavor, color, taste, and nutritional composition of meat and meat products (Mielche & Bertelsen, 1994; Gray *et al.*, 1996). Many factors can contribute to the initiation and development of lipid oxidation process in meat, such as fat content and fatty acid profile, degree of processing, storage conditions, and the balance between tissue pro and antioxidants content (Jensen *et al.*, 1997). Poultry meat is notably sensitive to lipid oxidation because of its high content of polyunsaturated fatty acids (Botsoglou *et al.*, 2002), and thigh meat, as compared to breast meat, is particularly vulnerable because of its higher fat content (Jensen *et al.*, 1998). Modern trends lead consumers to avoid foods rich in saturated fatty acids and to increase the demand for higher polyunsaturation, which implies higher susceptibility to oxidation, deterioration and off-flavor development (Buckley *et al.*, 1995).

In meat systems, it is well known that the process of lipid oxidation starts on the highly unsaturated membrane phospholipid fraction immediately after slaughter. At this time, many biochemical changes may occur leading to an unbalance between pro and antioxidant factors, enhancing lipid deterioration (Morrissey *et al.*, 1998). In addition, it is unlikely that the antioxidant defense system is fully functional after slaughter (Buckley *et al.*, 1995).

It was demonstrated in several studies that feeding oxidized diets to broilers resulted in negative effects on bird performance (Cabel *et al.*, 1988; Engberg *et al.*, 1996), on oxidative stability of tissues and membranes (Asghar *et al.*, 1989; Lin *et al.*, 1989a; Jensen *et al.*, 1997;

Grau *et al.*, 2001a) and on shelf-life of meat during storage (Sheehy *et al.*, 1994; Rhee *et al.*, 1996; Sheldon *et al.*, 1997; Grau *et al.*, 2001b).

In this study, broiler chickens were fed diets containing 4% of fresh or oxidized OPF from 10 to 47 days of age and treatment effects were determined on live performance, carcass and meat yield, and on thigh meat oxidative stability during chilled storage.

MATERIAL AND METHODS

Poultry offal fat

A freshly produced batch of poultry offal fat (no antioxidant added) was supplied by a local renderer and immediately sampled for quality and composition analyses. Half of the fat (fresh poultry fat - FPF) was stored frozen (-18 °C) in tightly sealed plastic containers until experimental diets were produced, while the OPF (oxidized poultry fat) was thermally oxidized using an electric fryer (Tedesco, FAO-30, Caxias do Sul, Brazil) for 10 hours daily during 21 days at 110 - 120 °C. During this time, after cooling to room temperature, the fat was exposed to light (2,450 lux) in order to increase oxidative changes. Fat samples were weekly taken to monitor lipid deterioration as determined by specific absorbance in the ultraviolet spectrum (IUPAC, 1979). The basis of this procedure is that the oxidation products of fats and oils (conjugated dienes and trienes) present specific absorption bands at 232 and 270 nm, respectively. Therefore, the determination of absorbance at 232 and 270 nm can reveal the presence or accumulation of secondary products of lipid oxidation, and indicates fat oxidation status (White, 1995).

Animals and diets – Performance trial

One hundred and sixty male broiler chicks (Cobb, 1-d-old) were raised in floor pens and fed a corn-soybean diet devoid of supplemental fat until 10 days of age. Birds were randomly assigned to the dietary treatments (20 birds per pen), with 4 replications each, and fed a corn-soybean diet formulated to supply the nutritional requirements from 10 to 47 days of age, according to Rostagno *et al.* (2000). Diets were formulated to contain 4% poultry fat (FPF or OPF), and were provided *at libitum* (Table 1).

Feed and birds were weekly weighed to monitor feed intake (FI), live weight (LW), weight gain (WG), and feed efficiency (FE). The performance trial was concluded at 42 days of age; however, the experimental diets were supplied until slaughter.

Table 1. Composition of the experimental diet and calculated nutrients

Ingredients	%
Corn	60.00
Soybean meal (45% CP)	32.00
Poultry offal fat[1]	4.00
Dicalcium phosphate	1.70
Limestone	1.00
Salt	0.500
Vitamin premix[2]	0.300
Mineral premix[3]	0.050
DL-Methionine	0.250
L-Lysine HCl	0.260
Calculated nutrients	
AME, kcal/kg	3,153
Crude protein, %	20.00
Methionine, %	0.553
Met + Cys, %	0.870
Lysine, %	1.240
Calcium, %	0.950
Available Phosphorus, %	0.430

1 - Treatments: fresh (FPF) and oxidized poultry fat (OPF). 2 - Supplying per kilogram of diet: vitamin A, 8,000 UI; vitamin D_3 2,000 UI; vitamin E, 15 UI; vitamin K, 2.0 mg; thiamin, 2.0 mg; riboflavin, 6.0 mg; pyridoxine, 3.0 mg; vitamin B_{12}, 0.012 mg; niacin, 40 mg; folic acid, 1.00 mg; pantothenic acid, 15.00 mg; biotin, 0.06 mg; choline, 340 mg; selenium, 0.3 mg; BHT, 22.5 mg. 3 - Supplying per kilogram of diet: manganese, 75 mg; zinc, 50 mg; iron, 50 mg; cobalt, 8 mg; iodine, 0.75 mg.

Slaughter and meat sampling procedure

At 47 days of age, 15 birds per pen (LW close to the average weight of the pen) were selected and slaughtered under experimental conditions. The carcasses were weighed to evaluate carcass yield, and breast and thighs were manually deboned for breast and thigh muscle yield determination. All the abdominal fat found was separated and weighed. Fifteen thighs from each treatment were randomly taken, packed in plastic bags, and kept in ice during transportation to the processing plant. The thigh samples were packed on polypropylene trays covered with polyvinyl chloride (PVC) film and refrigerated at 4 °C for different periods of up to 12 days.

Meat quality and stability assays

Immediately after slaughter, 3 samples of thigh meat from each treatment (n=3) were deboned and stored frozen for subsequent analyses of moisture, total fat and ash contents (AOCS, 2003), and protein (AOAC, 1995).

Total lipid extraction was carried out in thigh samples from each treatment (n=4) by the dry column methodology, as described by Marmer & Maxwell (1981). Lipid extract was submitted to cold saponification and methylation with BF3 in methanol, according to Metcalfe *et al.* (1966). Fatty acid analyses were conducted in a GC Chrompack CP9002, split

injector at a 1:67 ratio, flame ionization detector, and capillary column of fused silica CP-SIL 88 (50 m, 0.25 mm, and 0.25 m).

Total cholesterol analyses were conducted in thigh meat samples from each treatment (n=4) after lipid extraction, according to Folch *et al.* (1957) and Csallany and Ayaz (1976). Total cholesterol was quantified using the method described by Chen & Chen (1994) and Vicente (2003). HPLC analyses were carried out using a liquid chromatograph equipment (TSP, USA), with a P-4000 quaternary pump, an AS-3000 auto-sampler, and a UV-2000 variable ultraviolet detector operating simultaneously at 206 and 233 nm. Samples were injected into the HPLC using hexane/2-propanol (97:3 v/v) as the mobile phase at 1.0 ml min^{-1} flow rate. A CN-bonded stainless steel CPS type column (Thermo Hypersil, UK) with 25 cm x 0.4 cm (spherical particles of 5 mm) was used to perform HPLC separations.

TBARS (Thiobarbituric Acid Reactive Substances)

Meat stability during chilled storage was evaluated by quantification of malonaldehyde on days 1, 4, 8, and 12 of chilled storage. TBARS were determined in quadruplicate in samples from each treatment (n=3), according to Tarladgis *et al.* (1960). Absorbance was measured at 532 nm using a Shimadzu UV-1203 Spectrophotometer (Shimadzu, Japan). TBARS were expressed in mg malonaldehyde (MAD) per kilogram of sample using a standard curve (concentration range of 0 to 10 nmol/l) built with 1,1,3,3-tetraethoxypropane (TEP).

Color

Raw meat color measurements were performed using a Minolta Chroma Meter 200b (Minolta Corp., USA). Color L* (lightness), a* (redness), and b* (yellowness) values were determined in triplicates on the dorsal surface of 3 samples per treatment on days 1, 4, 8, and 12.

pH

Determination of pH was performed in triplicate in 3 samples per treatment at 24 hours *post mortem* after homogenization of equal parts of ground meat (10 grams) and distilled water (10 ml), according to the AOAC (1995).

Statistical analysis

In order to analyze the effects of the experimental treatments (dietary utilization of FPF or OPF) on broiler performance, carcass characteristics, and meat and oil composition, means were compared by t-test using General Linear Model Procedure of SAS. In the thigh meat stability assay, the experimental factors (treatment: fresh or oxidized fat, and storage time: 1, 4, 8 and 12 days) and the interaction between those factors were studied in a completely randomized design by General Linear Model Procedure of SAS statistical package (SAS, 1996), using TBARS as the response variable.

RESULTS AND DISCUSSION

Oxidation process of POF

Tables 2 and 3 show the composition and quality parameters of fresh (FPF) and oxidized (OPF) poultry offal fat added to chicken diets. As expected, FPF presented low specific absorbance values (2.86 and 0.32 at 232 and 270 nm, respectively) indicating freshness by exhibiting small concentrations of secondary oxidation compounds. OPF showed higher peroxide (38.73 *vs.* 2.83 meq O_2/kg) and higher specific absorbance values as compared to FPF (10.53 *vs.* 2.86 at 232 nm and 1.97 *vs.* 0.32 at 270 nm). These results demonstrate strong lipid deterioration by the increase and accumulation of primary (hydroperoxides) and secondary oxidation products (dienes and trienes) in the fat during the oxidation process.

Table 2 - Characterization of experimental poultry offal fats before production of experimental diets (n=2, duplicate).

Analysis	Units	FPF[1]	OPF[2]
Moisture	%	0.53	0.48
Total lipids	%	99.05	99.72
Acid Value	mg NaOH/g	1.22	1.73
Peroxide Value	meq O_2/kg	2.83	38.73
Specific Absorbance			
232 nm		2.86	10.53
270 nm		0.32	1.97

1 - Fresh poultry fat. 2 - Oxidized poultry fat.

Table 3 - Fatty acid composition of fresh (FPF) and oxidized poultry fat (OPF) before production of experimental diets (n=1, in duplicate).

Fatty acids (% of total fat)	FPF	OPF
C14:0	-	0.58
C16:0	24.91	30.37
C16:1	9.55	9.39
C18:0	5.84	6.16
C18:1	38.24	38.39
C18:2	19.53	14.71
C18:3	0.63	0.39
C20:4	1.29	-

In agreement with previous results (Sheehy *et al.,* 1994; Engberg *et al.*, 1996), the process of lipid

oxidation in this study resulted in changes in the fatty acid profile, as shown on Table 3. The oxidation process resulted in an artificially increased proportion of saturated fatty acids in OPF (particularly palmitic acid $C_{16:0}$), whereas the proportion of polyunsaturated fatty acids (particularly linoleic $C_{18:2}$, linolenic $C_{18:3}$ and arachidonic $C_{20:4}$) decreased as compared to FPF. These changes result in modifications in the saturated: unsaturated fatty acid ratio of OPF, as the loss of double bonds from unsaturated fatty acids caused by the addition of oxygen to the molecule leads to the identification of those fatty acids as less unsaturated.As polyunsaturated fatty acids are highly-sensitive to oxidation, the contents of linoleic ($C_{18:2}$) and linolenic acids ($C_{18:3}$) in OPF were 25% and 38% reduced (Table 3) and associated with the formation of diene and triene compounds found in UV analysis. According to Shahidi and Wanasundara (2002), during oxidation, lipids containing methylene-interrupted dienes or polyenes present a shift in the double-bond position due to isomerization and conjugate formation resulting in conjugated dienes (from linoleic acid) that exhibit intense absorption at 234 nm; similarly, conjugated trienes (from linolenic acid) absorbs at 268 nm.

Performance trial

Live weight at 42 days of age, feed intake, and daily weight gain were not affected by dietary treatments, as shown on Table 4. However, feed conversion ratio was statistically lower (P<0.05) in birds fed OPF. This may be explained by the trend of reduced feed consumption associated to a similar weight gain found for OPF as compared to FPF.

Table 4 - Performance evaluation of broilers fed experimental diets from 10 to 42 days of age (n=160).

Variable	FPF	OPF	CV[1] (%)
Live weight (kg)	2.701	2.690	2.29
Feed intake (kg)	4.358	4.262	2.87
Feed conversion ratio	1.792[a]	1.759[b]	0.87
Daily weight gain (kg)	0.076	0.076	2.37

a,b - Average values in the same line with no common superscript differ (P<0.05) using t test. 1 - Coefficient of variation.

In general, the concern about feeding oxidized fats or any other non-stabilized feed ingredient is their negative impact on performance and on meat quality caused by the reduction of α-tocopherol concentration in muscular tissue. As a consequence, the susceptibility of muscles to cholesterol oxidation, color deterioration, warmed-over flavor, and lipid oxidation increases dramatically (Erickson, 2002; Eitenmiller & Lee, 2004). Some studies describe reduced body and carcass weights associated to the consumption of oxidized fat, and suggest that oxidation is responsible for degradation of fat-soluble vitamins and carotenoids, impairing the nutritional value of the oil and, consequently, the efficiency of nutrient utilization (Lin *et al.*, 1989b; Sheehy *et al.*, 1994; Engberg *et al.*, 1996).

Particularly in this study, the energy value of the OPF diet was expected to be lower as compared to the FPF diet, considering that it was previously shown that OPF metabolizable energy resulted in less 1,500 kcal/kg than FPF (Racanicci *et al.*, 2004). Apparently, the detrimental composition and poor quality aspects found in the studied OPF was not sufficient to impair performance when 4% fat was added to the diet. These results are not consistent with studies carried out with broilers described in literature (Cabel *et al.*, 1988; Engberg *et al.*, 1996), and could be attributed to the low number of replicates used in the performance trial, which were insufficient to detect small differences between treatments.

Carcass characteristics

Birds fed diets containing 4% OPF from 10 to 47 days of age had similar live weight at slaughter (3.048 kg *vs.* 3.047 kg) and carcass weight (2.167 kg *vs.* 2.163 kg) as compared to birds fed FPF (Table 5).

Table 5 - Carcass characteristics at slaughter (47 days of age) and fat deposition (n=120).

	FPF	OPF	CV[1] (%)
Live weight (kg)	3.047	3.048	5,17
Carcass weight (kg)	2.163	2.167	6.03
Carcass yield (%)	70.95	71.11	2.59
Breast yield (%)	35.26	34.85	4.55
Breast meat yield (%)	27.16	26.57	6.53
Leg yield (%)	32.23	31.76	3.98
Thigh meat yield (%)	13.30[a]	12.78[b]	6.77
Abdominal fat (g)	42.01	43.43	31.42
Abdominal fat (%)	1.94	2.00	30.65

a,b - Average values in the same column with no common superscript differ *(P<0.05)* using t test. 1 - Coeficient of variation.

Breast yield (35.26 *vs.* 34.85% for FPF and OPF, respectively), leg yield (32.23 *vs.* 31.76% for FPF and OPF, respectively), and breast meat yield (27.16 *vs.* 26.57% for FPF and OPF, respectively) were also not influenced by the oxidized diets. However, thigh meat yield (Table 5) was depressed by the utilization of OPF in the diet, and resulted in significantly (P<0.05) lower values (13.30 vs. 12.78% for FPF and OPF, respectively).

Chemical analysis of thigh meat

Composition of skinless thigh meat samples showed

higher total fat content and lower moisture, protein, and ash contents as compared to USDA National Nutrient Database for Standard Reference, Release 18, (2005), and are shown in Table 6. In this study, feeding FPF or OPF to broilers from 10 to 47 days showed no effect on thigh meat moisture, protein, fat, or ash contents.

Table 6 - Averages of thigh meat composition and pH (n=3, in duplicate).

Analysis	Units	FPF	OPF	CV[1] (%)
Moisture	%	70.77	70.69	0.24
Protein	%	17.97	17.83	1.10
Total lipids	%	9.53	9.34	1.97
Ash	%	0.82	0.83	6.40
pH[2]		6.17	6.16	173

1 - Coeficient of variation. 2 - Determined at 24h after slaughter.

Fatty acid profile of raw thigh meat (Table 7) showed similar trends as compared to previous studies (Asghar *et al.*, 1989; Lin *et al.*, 1989b), and confirmed that 80% of total fatty acids were represented by C_{16} (palmitic and palmitoleic acids) and C_{18} (stearic, oleic, and linoleic acids). Fatty acid profile of thigh meat from broilers fed OPF reflected the composition of OPF samples. The concentration of linoleic acid ($C_{18:2}$) in thigh meat was statistically lower (P<0.05) as a consequence of the lower concentration of linoleic acid found in OPF samples (Table 3), which resulted from the advanced oxidation process, showed by the extremely high content of diene compounds (Table 2) originated mainly from linoleic acid.

Table 7 - Fatty acid composition and total cholesterol content (mg/100 g of meat) analyzed in thigh meat samples (n=3, in duplicate).

Fatty acids (% of total fat)	FPF	OPF	CV[1] (%)
C16:0	23.42	23.28	2.5
C16:1	3.84[b]	4.74[a]	7.8
C18:0	10.05[b]	11.05[a]	1.9
C18:1	30.75	29.70	3.4
C18:2	20.60[a]	19.80[b]	3.0
C20:4	4.64[b]	5.75[a]	10.4
Total Cholesterol	52.26[b]	77.73[a]	21.66

a,b -Average values in the same line with no common superscript differ (P<0.05) using t test. 1 - Coeficient of Variation.

Total cholesterol content of thigh meat samples of broilers fed OPF diets was statistically higher (P<0.05) as compared to FPF muscles. Opposite results were found by Grau *et al.* (2001b), who did not detect differences in total cholesterol content in the meat from broilers fed diets containing fresh or oxidized sunflower oil, although the degree of lipid oxidation was reportedly low as compared to this study.

Chilled Assay

The degree of lipid oxidation on thigh meat of broilers fed FPF or OPF is presented on Figure 1. During 12 days of chilled storage, TBARS values ranged between 0.397 and 0.536 mg malonaldehyde (MDA)/kg sample in FPF meat, and between 0.546 and 0.666 mg MDA/kg sample in OPF meat. OPF thigh meat showed statistically higher (P<0.05) concentration of secondary lipid oxidation products, as demonstrated by TBARS values evaluated on days 1, 4, 8, and 12 of chilled storage.

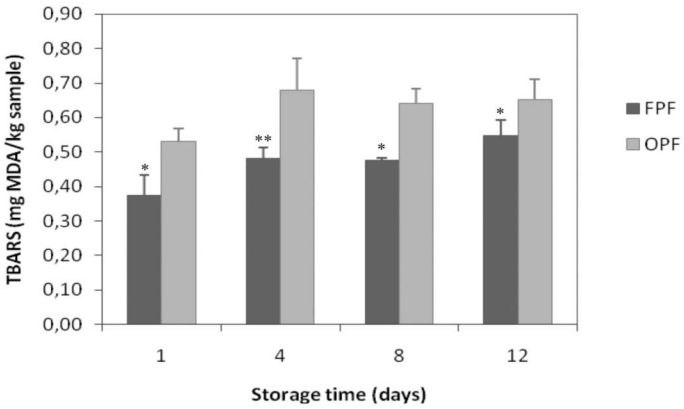

Figure 1 - Progression of lipid oxidation measured in TBARS values in thigh meat samples (n=3) stored chilled. Standard deviations are represented by *bars*, and significant differences within dietary treatments (*FPF:* fresh poultry fat diet, *OPF:* oxidized poultry fat diet) are indicated by *asterisks* (* P<0.05; ** P<0.001).

The results found in this study demonstrate increased susceptibility of thigh meat of broilers fed OPF diets to lipid oxidation, as previously established (Asghar *et al.*, 1989; Lin *et al.*, 1989a; Galvin *et al.*, 1997; Jensen *et al.*, 1997). In earlier studies, Sheehy *et al.* (1993; 1994) correlated this increased susceptibility with the reduction of α-tocopherol concentration in the plasma, and thigh and breast muscles of broilers fed diets with heat-oxidized oils. This occurs because oxidized molecules seems to be absorbed in small intestine as a consequence of consumption of oxidized diets, and initiate a cascade of subsequent oxidation reactions that result in increased oxidation (Suomela *et al.*, 2005) and the depletion of natural antioxidant protection.

Color is the main quality attribute used by the consumers to classify acceptable or rejected stored food products, including poultry meat. Discoloration of meat is directly related to the concentration of α-tocopherol in the muscle (Eitenmiller & Lee, 2004), the

Table 8 - Means and standard deviation of L*, a* and b* measured in experimental thigh meat samples during chilled storage (n=3, in triplicate).

Days of storage	FPF[1]			OPF[2]		
	L*	a*	b*	L*	a*	b*
1	55.40 ± 0.52	4.02 ± 1.14	4.91 ± 1.77	56.74 ± 3.73	5.49 ± 0.54	4.87 ± 1.80
4	53.60 ± 1.34	5.03 ± 1.01	8.70 ± 1.22	55.34 ± 2.03	4.12 ± 1.43	5.92 ± 1.45
8	53.90 ± 2.65	2.98 ± 1.04	6.62 ± 1.37	54.45 ± 2.47	3.63 ± 1.39	7.00 ± 0.60
12	53.30 ± 2.38	5.85 ± 2.83	5.22 ± 0.37	53.75 ± 2.03	4.70 ± 2.33	5.75 ± 1.44
Averages	54.05 ± 0.90	4.53 ± 1.24	5.92 ± 1.73	54.90 ± 1.29	4.41 ± 0.80	5.84 ± 0.88

1 - FPF = diet containing fresh poultry fat. 2 - OPF = diet containing oxidized poultry fat.

degree of oxidation of blood pigments, and the efficiency of enzymatic systems to reduce metamyoglobin, according to Jensen *et al.* (1998). In this study, thigh meat samples from both treatments (FPF and OPF) showed high L* values (Table 8), and can be considered lighter than normal (L* > 53), according to Qiao *et al.* (2001). This thigh paleness found in the present experiment is probably due to the leakage of pigments from the meat to ice slush during transportation. However, no significant effect was verified when comparing thigh meat from FPF or OPF treatments.

CONCLUSIONS

Feeding diets containing oxidized poultry offal fat did not adversely affect performance or carcass characteristics of broilers; however, fatty acid composition and, consequently, lipid stability of thigh muscle was severely impaired.

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