

Full Length Research Paper

The effects of dietary fat, vitamin E and zinc supplementation on fatty acid composition and oxidative stability of muscle thigh in broilers under heat stress

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The objective of this experiment was to characterize the relationship between dietary fat and antioxidant supplementation on performance, fatty acids profile and lipid oxidation of thigh meat stored under refrigeration in broilers under heat stress. The chicks with a similar body weight were equally assigned to one of the two controlled-environment chambers from 21 to 56 day of age. The birds fed diets as: 1) basal diet supplemented with 5% saturated fatty acid, 2) basal diet supplemented with 5% unsaturated fatty acids (2% canola oil plus 3% fish oil); and 3) The second diet supplemented with antioxidant (100 IU vitamin E and 50 mg/kg zinc); that birds received this 3 diet in two temperature (22 and 32°C). Results showed that feed conversion ratio was not influenced by fat type, but on live body weight and feed Intake had significant effect ($P<0.05$). High environmental temperature showed deleterious effects including: reduction of feed intake, and live body weight and increasing of feed conversion ratio. Proportion of omega-6 to omega-3 (n-6/n-3) of thigh was increased and polyunsaturated fatty acids (PUFA) decreased in heat exposed and tallow-fed chicks. Whereas the proportion of omega-6 to omega-3 (n-6/n-3) of thigh was decreased and polyunsaturated fatty acids (PUFA) increased in chicks fed with canola and fish oils. Fat content and gross energy of thigh in heat exposed and canola and fish oils with antioxidant fed chicks were higher than other treatments. Proportion of omega-6 to omega-3 (n-6/n-3) of thigh was decreased and polyunsaturated fatty acids (PUFA) increased in chicks fed with canola and fish oils. Inclusion of canola and fish oils supplementations increased lipid oxidation of thigh muscle based on TBARA values. Thiobarbituric acid reaction substances (TBARA) values of thigh muscle in chicks fed canola and fish oils reared under heat stress was higher than other treatments. Increasing dietary antioxidants decreased TBARA values of thigh muscle.

Key words: Broilers, heat stress, dietary fat, fatty acids profile, thigh.

INTRODUCTION

Heat stress is of great concern in all types of poultry production. Feed consumption, growth rate, mortality, meat quality and other important traits governing the prosperity of the industry are adversely affected by

severe heat stress. Many authors have studied the inclusion of different fat sources in the broiler's diet (Scaife et al., 1994; Hrdinka et al., 1996; Sanz et al., 1999). However, there are few reports on the effect of increasing levels of dietary PUFA challenged with heat stress on the amount and type of fatty acids deposited in chicken tissues, especially in the edible portions. An increase in the degree of polyunsaturation of meat may enhance the development of organoleptic problems

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(Ajuyah et al., 1993; Gonza'lez-Esquerria and Leeson, 2000; Bou et al., 2001) and lead to an increased susceptibility to lipid oxidation (Klaus et al., 1995; Cortinas et al., 2001; Grau et al., 2001). Supplementation with α -tocopherol has proven to be an effective measure to prevent lipid oxidation (Lin et al., 1989; Ahn et al., 1995; Cortinas et al., 2001) and to improve sensory quality (O'Neill et al., 1998, Bou et al., 2001) of poultry meat. Because α -tocopherol protects PUFA from lipid oxidation, its inclusion in the birds' diet may result in a higher deposition of PUFA in poultry tissues. There is also evidence suggesting that supplemental Zinc can alleviate negative effects of HS in broiler chickens. For example, plasma zinc was greatly reduced and hepatic zinc was found to be more than four times the amount lost from plasma (Klasing, 1984). Supplemental zinc is used in poultry diets and is reported to be of benefit to laying hens during environmental stress (Sahin and Kucuk, 2003). Interactions among minerals and other nutrients e.g., vitamin E are extensive and may be important in the determination of biological availability of other nutrients. Lipid oxidation causes loss of nutritional and sensory values, as well as the formation of potentially toxic compounds that compromise meat quality and reduce its shelflife. One of such product is malondialdehyde (MDA), which has long been considered as an index of oxidative rancidity. Among all the methods proposed for assessing MDA, the 2-thiobarbituric acid (TBA) has been widely adopted as a sensitive assay method for lipid oxidation in animal tissues. The objective of this study was to evaluate the effects of dietary fat, Vitamin E and Zinc on fatty acids profile and lipid oxidation of thigh meat stored under refrigeration in broilers under heat stress conditions.

MATERIALS AND METHODS

Experimental design, animals, housing and diets

Two hundred forty female broiler chicks from Arbor Acres strain were used to evaluate the effects of experimental diets and different temperatures on fatty acids profile and lipid oxidation of thigh meat. Two factors of temperature and fat type (2x3) were considered in a completely randomized design (CRD) to form 6 separate treatments. Each treatment involved 4 replications and 10 birds and it lasted for 6 weeks. Experimental diets were included: 1) basal diet supplemented with 5% saturated fatty acids, 2) basal diet supplemented with 5% unsaturated fatty acids (2% of canola oil plus 3% of fish oil); 3) basal diet supplemented with 5% unsaturated fatty acids (2% of canola oil plus 3% of fish oil) and antioxidant (100 IU vitamin E and 50 mg/kg zinc); that birds received this 3 diets in two temperatures (22 and 32°C). Table 1 shows the ingredients and chemical analyses of the starter and grower diets fed to broiler chickens. The chicks were placed in floor pens equipped with stainless steel feeders, and automatic water drinkers. Lighting was continuous and water and feed were provided *ad libitum* for the whole experimental period. Fatty acid profiles of supplemented fats and experimental diets are shown in Table 3. The weight of birds was recorded at 21 day of age. At the end of the experimental

period, weights and feed intakes per pen were recorded. The chickens were killed on day 56 of the experiment.

Fatty acid content

Fatty acid content of feeds was determined following the methodology described by Metcalf et al. (1996). The fatty acid (FA) content was determined using a gas chromatograph (HP68908, Unicam 4600, USA) equipped with a flame ionization detector and an HP 19091 to 136 capillary column (30 m, 0.22 mm internal diameter) with a film thickness (0.25 μ m) of stationary phase. Helium was used as gas carrier. Oven temperature was programmed as follows: from 140 to 160°C at 1.50°C/min; from 160 to 180°C at 20°C/min; and from 180 to 190°C at 20°C/min. The other chromatographic conditions were: injector and detector temperatures, 280°C; sample volume injected, 1 μ L. Fatty acids were identified by matching their retention times with those of their relative standards, as well as by mass spectrometry (HP5973), in which 8 is for each peak. Fatty acid composition of diets is shown in Table 3.

Analysis of meat cholesterol, gross energy and percentage fat

Details of the dissection of the carcasses to recover the thigh are described elsewhere (Aletor et al., 2000). Total lipids were extracted from freeze dried and finely milled animal tissues using a mixture of n-hexane and isopropanol (3:2, vol/vol; Hara and Radin, 1978) at ambient temperature over 24 h. Lipids of the extracts were thereafter dissolved in the aqueous phase of the test reagent with Triton X-100 (De Hoff et al., 1978; Eder and Kirchgessner, 1994). Cholesterol concentrations in the extracts were assayed enzymatically using reagent kit¹. Crude fat were determined as outlined by AOAC (1990). Gross energy determined with an gallenkamp bomb calorimeter as outlined by AOAC (1995).

Lipid oxidation

Storage

The samples were stored at 4°C. For every sampling time (0, 3, and 6 day), 3 pieces of chicken thigh of each treatment were taken randomly for analyses and the remaining samples were kept in storage at 4°C.

Concentration of tissue malondialdehyde

The extent of lipid oxidation was determined by measuring the TBAR substances at 1, 3, and 6 day of storage and was expressed as micrograms of MDA per gram of muscle using the procedure described by Salih et al. (1987). Ten grams of ground meat was homogenized with 35 mL of 3.86% perchloric acid in an Ultra-Turrax at 21, 280 g for 1 min. Butylated hydroxyanisole was added before homogenization at a level of 125 g/mg of fat. The blended sample was filtered through Whatman number 2V filter (Whatman International Ltd., Maidstone, UK) into 50-mL Erlenmeyer flasks. Five milliliters of the filtrate was mixed with 5 mL of 0.02 TBAR in distilled water in capped test tubes. Tubes were incubated at room temperature in the dark for 15 to 17 h or heated in boiling water for 30 min. The absorbance was determined at 531 nm against a blank containing 5 mL of distilled water and 5 mL of 0.02 thiobarbituric

¹ PARS AZMUN

Table 1. Ingredients and chemical analyses of the starter and grower diets.

Treatment	Starter			Grower		
	Percentage of DM			Percentage of DM		
	O	A	T	O	A	T
Ingredients						
Ground corn	54.75	54.75	54	61.73	61.73	61
Soybean meal	37.28	37.28	37.28	30.25	30.25	30.25
Fish oil	4.51	4.51	-	4.65	4.65	-
Tallow	-	-	5.26	-	-	5.38
Dicalcium phosphate	1.37	1.37	1.37	1.33	1.33	1.33
Sodium chloride	0.1	0.1	0.1	0.2	0.2	0.02
Limestone ground	0.53	0.53	0.53	0.53	0.53	0.53
Calcium carbonate	0.1	0.1	0.1	0.16	0.16	0.16
DL-methionine	0.24	0.24	0.24	0.25	0.25	0.25
Lysine	-	-	-	0.12	0.12	0.12
Cavilamycin	0.1	0.1	0.1	0.1	0.1	0.1
Ca- propionate	0.1	0.1	0.1	0.1	0.1	0.1
Vitamin E IU	-	100	-	-	100	-
Zinc mg/Kg	-	50	-	-	50	-
Clinao	0.1	0.1	0.1	0.1	0.1	0.1
Toxin	0.3	0.3	0.3	-	-	-
Vitamin premix*	0.27	0.27	0.27	0.27	0.27	0.27
Trace mineral premix**	0.16	0.16	0.16	0.16	0.16	0.16
DM%	89.65	89.65	89.63	89.68	89.68	89.68
Chemical analyses						
ME, kcal/kg	3100	3100	3100	3200	3200	3200
Cp	23	23	23	20.03	20.03	20.03
Crude fat	7.04	7.04	7.04	6.35	6.35	6.35

* premix supplied for 2 kilogram: vitamin A, 15000 IU; cholecalciferol, 3 IU, vitamin E 15 IU; menadione, 2.5 mg; vitamin B1, 1 mg; vitamin B2, 10mg; niacin, 70mg; d-pantothenic acid, 20 mg; vitamin B12, 4 mg; folic acid, 2 mg; biotin, 0.1 mg. ** premix supplied for 2 kg; Mn, 80 mg; Fe, 25 mg; Zn, 50 mg; Cu, 7 mg; Iodine, 0.3 mg; Se, 0.15 mg; Choline chloride, 350 mg.

acid solutions.

Statistical analyses

Data for chickens were analyzed with analysis of variance (ANOVA). Performance parameters were analyzed for each pen as a replication. The general linear model (GLM) of the Statistical Analysis System (SAS Institute, 1999) was conducted for the statistical processing of data. Differences were considered significant at $P < 0.05$ and means were compared by Duncan test. Interactions were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Broiler growth Performance

The effects of dietary fat and temperature on the performance of broilers have been shown in Table 2. Feed to gain ratio was not influenced by fat type.

However, high environmental temperature reduced the feed intake, body weight and increased feed to gain ratio in broiler ($P < 0.05$). This result showed that diet contained saturated fat had a significant effect on feed intake and body weight, although the same result on feed to gain was not convenient ($P \leq 0.05$). It might be due to high energy level of tallow in comparison with fish oil. Addition of zinc and vitamin E to the unsaturated oil ration increased in body weight of broilers though it was less than body weight of tallow ration. Interaction of fat type and temperature had a significant effect on broilers performance ($P < 0.05$). High environmental temperature had deleterious effects on performance including: feed intake and live weight. Not only feed intake and live weight showed reduction, but also increase in feed to gain ratio increased in broilers at high environmental temperature (Donkoh et al., 1989). This negative effect of heat stress on growth rate and production is speculated to be due to reduced feed intake (Hurwitz et al., 1980).

Table 2. Effect of temperature, fat type and supplemental antioxidant on feed intake, feed conversion and body weight at whole experimental period.

Variable	Feed intake(g)	Feed conversion	Body weight(g)
Temperature			
Heat stress(H)	4976 ^b	1.96 ^a	2539 ^b
Normal(C)	6329 ^a	1.89 ^b	3349 ^a
P	0.02	0.03	0.02
Fat type			
O	5423 ^b	1.93	2810 ^b
T	5929 ^a	1.92	3088 ^a
A	5662 ^{ab}	1.93	2934 ^{ab}
P	0.03	n.s	0.027
Interaction (Temp × Fat type)			
TH	5297 ^c	1.95 ^a	2717 ^b
TC	6539 ^a	1.89 ^b	3460 ^a
OH	4682 ^d	1.97 ^a	2377 ^c
OC	6131 ^b	1.89 ^b	3244 ^a
AH	4947 ^{cd}	1.96 ^a	2525 ^{bc}
AC	6301 ^{ab}	1.89 ^b	3334 ^a
P	0.02	0.03	0.024
SEM	192	0.23	88

a, b, c, d: mean values within a row with no common superscript differ significantly ($P < 0.05$). ¹O = basal diet + Unsaturated fatty acid; A = basal diet + 100 IU/ kg vitamin E and 50 mg/kg zinc; T= basal diet + saturated fatty acid; OH: high temperature and canola oil plus fish oil diet, OC: normal temperature and canola oil plus fish oil diet; AH: high temperature and Antioxidant diet; AC: normal temperature and Antioxidant diet; TH: high temperature and Tallow diet; TC: normal temperature and Tallow diet.

Fatty acids composition

Fatty acid profiles of diet fed chickens are shown in Table 3. The effects of treatments on fatty acid composition thigh muscles are shown in Table 5. Monounsaturated fatty acids (MUFA) and unsaturated fatty acids (UFA) and proportion of UFA/SFA decreased in heat exposed chicks. Proportion of omega-6 to omega-3 (n-6/n-3) of thigh was increased and polyunsaturated fatty acids (PUFA) decreased in heat exposed and tallow-fed chicks. Whereas proportion of omega-6 to omega-3 (n-6/n-3) of thigh was decreased and polyunsaturated fatty acids (PUFA) increased in chicks fed with canola and fish oils. This result was similar with the result of Sonaya (1988) that showed heat stress causing increased PUFA diet Supplemented whit antioxidant causing decreased Docosa hexaenoic acid (DHA), PUFA and USFA, n-3, n-6 and USFA/ SFA. This result was similar with Barja et al. (1996).

Tissue cholesterol, gross energy and percentage fat

Fat contents were higher in chickens reared under heat stress that this subject led to the increased gross energy of meat. This result is similar to that of EL- Hussein and

Cregur (1980) and the increased gross energy of meat was repugnant with the result of EL- Hussein and Cregur (1980). The diet supplemented with antioxidant caused the increase in fat percentage and gross energy. However, none of the treatments was influenced by meat cholesterol (Table 4).

Concentration of tissue malondialdehyde

Higher MDA was observed on OH treatment (Table 4). The supplemented diet with antioxidant caused the decrease in meat MDA at day 0, 3 and 6, and also caused that of the slaughter. Heat stress caused the increase in radical free, while the increased radical free caused the increase in oxidation lipid and digestion protein (Edens and Siegel, 1975). Also, heat stress caused collision balance in blood PH (Richards, 1970). Furthermore, it increased expulsion zinc (post 2003) and decreased absorption of vitamin E, which was the ingredient causing the increased effect of destroyer free radical on cell well (Howlinder and Rose, 1989). Altan et al. (2003) reported that heat stress caused collision balance in blood antioxidant, in that this subject increased oxidation lipids in meat. Then, of OH treatment, the maximum MDA meat was observed in OC treatment

Table 3. Fatty acid composition in experimental diets (mg/g fat).

Fatty acids ²	Experimental Diets ¹		
	O	T	A
16:00	26.6	26	36
16:01	6	6.4	6.22
18:00	6.6	5.3	6.5
18:01	38	42	37.9
18:02	15	11.6	12.9
18:03	1.57	0.58	1.4
EPA	1.53	0.09	1.52
DHA	1.16	0.047	1.09
SFA	33	32	33
UNFA	44.24	49.13	43.3
PUFA	19.73	17.2	13
UFA	63.97	62	61.5
omega-3	4.6	1.25	4
omega-6	15.2	11.81	13
n6/n3	3.27	9.61	3
UFA/SFA	1.95	1.95	1.84

^{a b c d}, values that do not have common superscripts are significantly different (P < 0.05). ¹O = basal diet + Unsaturated fatty acid, A = basal diet + 100 IU/ kg vitamin E and 50 mg/kg zinc T= basal diet + saturated fatty acid; ²SFA: saturated fatty acids; UFA unsaturated fatty acids; MUFA monounsaturated fatty acids; and PUFA polyunsaturated fatty acids; n6/n3 = perportion of omega-6 to omega-3.

Table 4. Effect of temperatures and fat type on meat MDA, percentage fat, gross energy and cholesterol meat.

Treatment	Cholesterol meat	Gross energy	Fat content	MDA (µg/g)		
	Mg/dl	Kcal/kg	%	0 day	3 day	6 day
TH	0.172 ^{ab}	5202 ^d	9.15 ^{ab}	0.15 ^d	1 ^d	1.7 ^d
TC	0.171 ^{ab}	5502 ^b	7.53 ^c	0.27 ^{cd}	1.4 ^c	1.9 ^d
OH	0.145 ^b	5238 ^b	9.01 ^b	0.64 ^a	2.55 ^a	5.94 ^a
OC	0.19 ^a	5033 ^d	6.98 ^c	0.49 ^b	2 ^b	4.78 ^b
AH	0.17 ^b	5597 ^a	9.40 ^a	0.35 ^c	2.07 ^b	3.25 ^c
AC	0.18 ^a	5312 ^c	8.94 ^b	0.19 ^d	1.60 ^c	2.48 ^c
P	0.04	0.023	0.027	0.035	0.025	0.04
SEM	0.013	53	0.24	0.048	0.37	0.22

a, b, c, d: mean values within a row with no common superscript differ significantly (P < 0.05). OH: high temperature and canola oil plus fish oil diet, OC: normal temperature and canola oil plus fish oil diet, AH: high temperature and Antioxidant diet, AC: normal temperature and antioxidant diet. TH: high temperature and tallow diet, TC: normal temperature and tallow diet.

after which it was observed in AH treatment. However, the supplemented diet with antioxidant caused the decreased effect of the destroyer heat stress and the decreased free radical that was produced.

Conclusions

In conclusion, Inclusion of canola and fish oils supplementations increased lipid oxidation of thigh

muscle based on TBARA values. TBARA values of thigh muscle in chicks fed canola and fish oils reared under heat stress was higher than other treatments. Increasing dietary antioxidants decreased TBARA values of thigh muscle.

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Table 5. Effect of temperatures and diet fat type on fatty acid composition of thigh (mg/g fat).

Treatment	USFA/SFA	n-6/n-3	n-6	n-3	USFA	PUFA	MUFA	SFA	DHA	EPA	18:03	18:02	18:01	18:00	16:01	16:00
TH	1.9a	10.7a	11.1d	1e	60.8c	12.1d	48a	31	0.06c	0.08c	0.48d	11d	42a	3.5b	6.4ab	27.4a
TC	1.9a	8.5 ^b	12.4c	1.4d	63b	13.8c	49.6a	32.6	0.03c	0.1c	0.7c	12.3c	42.9a	7a	6.5ab	26.5ab
OH	1.88 ^b	3.38 ^c	15.12 ^a	4.47 ^b	62.32 ^b	19.45 ^a	42.86 ^b	33.19	1.15 ^a	1.49 ^b	1.5a	15a	37c	6.5a	5.4c	26b
OC	2.02 ^a	3.17 ^c	15.30 ^a	4.83 ^a	65.63 ^a	20 ^a	45.63 ^a	32.49	1.18 ^a	1.59 ^a	1.6a	15a	39b	6.7a	6.3ab	25c
AH	1.78 ^c	3 ^c	12.84 ^b	4.28 ^c	60.03 ^c	17.01 ^b	43.02 ^b	33.70	1.09 ^b	1.53 ^b	1.4b	13bc	37c	6.8a	5.7bc	26b
AC	1.90 ^b	3.09 ^c	13.20 ^b	4.32 ^c	63.02 ^b	17.39 ^b	45.62 ^a	33.19	1.11 ^b	1.52 ^b	1.4b	13b	38.8b	6a	6.7a	26b
SEM	0.025	0.047	0.3	0.06	0.56	0.35	0.40	0.24	0.01	0.012	0.09	0.31	0.47	0.26	0.12	0.20

a, b, c, d: mean values within a row with no common superscript differ significantly (P<0.05). ¹OH: high temperature and canola oil plus fish oil diet, OC: normal temperature and canola oil plus fish oil diet, AH: high temperature and Antioxidant diet, AC: normal temperature and antioxidant diet, TH: high temperature and Tallow diet, TC: normal temperature and tallow die, DHA= docosa hexaenoic acid ; EPA = eicosa pantadocanoic acid ; SFA = saturated fatty acids; UFA= unsaturated fatty acids; MUFA = monounsaturated fatty acids; and PUFA = polyunsaturated fatty acids; n6/n3 = proportion of omega-6 to omega-3.

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