Effect of dietary thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers

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Abstract

A trial was conducted to investigate the effects of dietary vitamin E (E) and thyme oil (TO) supplementation on the growth performance, lipid oxidation, fatty acid concentration of tissues and the serum lipoprotein levels of male broilers. Two-hundred day-old Ross PM3 chickens were assigned to one of five dietary groups (four replicates each). The control group received the basal diet. In addition to the basal diet, the four experimental diets included one of the following: 100 mg vitamin E/kg (E100); 200 mg vitamin E/kg (E200); 100 mg/kg thyme oil (TO100) or 200 mg/kg thyme oil (TO200). Birds that were fed the control, E200 and TO200 diets, exhibited the largest weight gain after a 42-day feeding period. The best feed conversion rate was found in birds that were fed the E200 diet. TBARS values of all of the dietary treatments, except the control, remained unaffected after a 42-day refrigeration period. The addition of thyme oil to the broiler feed led to a significant reduction in the saturated (SFA) and polyunsaturated fatty acid (PUFA) concentrations of the leg and breast tissues. The monounsaturated fatty acid (MUFA) concentrations in these tissues increased. The thyme oil supplementation also led to increased plasma levels of triglycerides, LDL-cholesterol and HDL-cholesterol in broilers. Based on the results of this study, it could be advised to supplement broiler feed with 200 mg/kg of thyme oil as an antioxidant.

Keywords: Thyme oil, broiler, vitamin E, lipid oxidation, fatty acid composition, production performance
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Introduction

The use of antibiotics as growth promoters in chicken feed has led to some unwanted advances in antibiotic resistance in certain bacterial pathogens (Botsoglou & Fletouris, 2001; Madrid *et al*., 2003; Moser *et al*., 2003). Extensive research is thus required in order to find alternative growth promoters. It has been reported that the essential oils extracted from thyme, and in particular the phenolic components (including carcavarol and thymol), were responsible for antioxidant activity observed in lipid systems (Farag *et al*., 1989; Deighton *et al*., 1993). Hertrampf (2001) reported antibacterial, anticocidial, antifungal and antioxidant effects for thyme oil. Furthermore, it was concluded that due to their aromatic characteristic essential oils derived from herbs and spices had the ability to increase feed intake and could thus be successfully used as growth promoters (Hertrampf, 2001). In addition, the supplementation of poultry diets with essential oils led to enhanced weight gain, improved carcass quality and reduced mortality rates (Mandal *et al*., 2000; Hertrampf, 2001; Williams & Losa, 2001). Lee *et al*. (2003) reported considerably increased liver mass in male broilers that were fed diets that had been supplemented with thyme oil. Essential oil supplementation has also been shown to lead to increased concentrations of serum lipoproteins and triglycerides (Sirvydis *et al*., 2003).

The antioxidant effects of vitamin E have been reported in many poultry studies (Bollengier-Lee *et al*., 1998; 1999; Chen *et al*., 1998). Other studies that focused on the composition and antioxidant constituents in thyme leaves, have shown that phenolics, apart for carvacrol and thymol, may be responsible for the antioxidant activity of thyme oil. Phenolics such as caffeic acid, p-cymene-2,3-diol and several other biphenylic as well as flavonoid compounds have been found to exhibit antioxidant activity. These phenolics may even have antioxidant potentials greater than that of α-tocopherol (Schulz & Herrmann, 1980; Miura & Nakatani, 1989).

This study was conducted to describe the effects of the dietary supplementation of thyme oil and vitamin E in male broilers. The effect of supplementation on growth performance of the broilers, the fatty

acid concentrations in their tissues and TBARS (thiobarbituric acid reactive substances) of leg and breast tissues refrigerated at 4 °C were investigated. The influence of dietary thyme oil as well as vitamin E on carcass yield and serum lipoprotein was also determined.

Materials and Methods

A total of 200 day-old male chickens of the commercial strain Ross 308 and a mean mass of 38 ± 1.4 g were randomly allocated to one of five treatments (four replicates each). Each replicate consisted of 10 broiler chickens. Between days 1 and 21 the chickens were fed a starter diet followed by a finisher diet between days 22 and 42 (Table 1). The five dietary treatments consisted of a control (basal diet), basal diet + 100 mg vitamin E/kg, basal diet + 200 mg vitamin E/kg, basal diet + 100 mg thyme oil/kg and basal diet + 200 mg thyme oil/kg.

Body weights of the chickens were recorded on days 1 and 42, and feed intake was measured over this period on day 42. Blood samples were collected from the brachial veins to determine lipoprotein profiles on day 42. Ten birds selected randomly from each treatment were slaughtered (neck cutting) under laboratory conditions. The carcasses were plucked and the heads, necks, shanks as well as feet were removed. The liver, lung and heart were dissected from the viscera. All of the above-mentioned components were weighed individually. Breast, wing and leg muscle samples were also separated according to the WPSA reference cutting method (WPSA, 1984) and individually weighed, and the carcass yield was calculated. The breast and leg meat from different individuals in each specific group were sampled and stored at 4 °C for TBARS analysis, and a sub-sample stored at -20 °C for lipid analysis.

Serum samples from blood were separated by low-speed centrifugation (1500 g for 15 min at 20 ^{o}C). Commercially available kits (Sigma Diagnostics, Taufkirchen, Germany) were used to analyse the serum for total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) on an autoanalyzer. Low density lipoprotein cholesterol (LDL-C) levels were estimated using the Friedewald equation (Fridewald *et al*., 1972). Lipid oxidation was determined at days 1, 3 and 7. Thiobarbituric acid reactive substance values were determined in samples as described by Cherian *et al*. (1996). The TBARS values were expressed as mg malonaldehyde/kg tissue.

Fatty acid analyses were performed at the Biotechnology Application and Research Centre. The preparation and analyses of the fatty acid methyl esters (FAMEs) from all the samples, including feed, were performed according to the method described by Anonymous (2000). One mL of 1.2 M NaOH in 50% aqueous methanol and six glass beads (3 mm diameter) were added to each sample in a screw cap tube. These bottles were then incubated at 100 °C for 30 min in a waterbath. The saponified samples were left to cool for 25 min at room temperature. The samples were then acidified and methylated with the addition of 2 mL of solution composed of 54% 6 N HCI and 46% aqueous methanol, and subsequently incubation at 80 °C for 10 min (in a waterbath). After rapid cooling, the methylated fatty acids (FA) were extracted using 1.25 mL 50% methyl-tert-butyl-ether (MTBE) in hexane. Each sample was mixed for 10 min before the bottom phase was removed with a Pasteur pipette. The top phase was washed with 3 mL 0.3 M NaOH. After mixing for 5 min, the top phase was removed for analysis. Following the base-washing step, the FAMEs were cleaned in anhydrous sodium sulphate and then transferred into a gas chromatography sample vial for analysis. Fatty acid methyl esters were separated using gas chromatography (HP6890, Hewlett Packard, Palo Alto, CA) and a fused silica capillary column (25 m by 0.2 mm) with 5% cross-linked phenylmethyl silicone. The operating parameters for the study were automatically set and controlled by the Sherlock Microbial Identification System [MIS].

The chromatograms with peak retention times and areas were produced on the recording integrator and then electronically transferred to the computer for analysis, storage and report generation. Peak identification and column performance were established using a calibration standard FA mix (Eucary Method 697110) containing C9-C30 saturated fatty acids. Fatty acids were identified on the basis of equivalent chain length data. Fatty acid methyl ester profiles of the tissue were identified by comparing the commercial Eucary database with the MIS software package (MIS ver. No 3.8, Microbial ID, Inc., Newark, Delaware). Individual fatty acid methyl esters were expressed as percentage of all peaks.

Data were tested using analysis of variance (ANOVA) and the statistical package SPSS for Windows (1999), version 10.0. Significant means were subjected to a multiple comparison test (Duncan) at $\alpha = 0.01$ and 0.05 levels (Snedecor & Cochran, 1980).

Table 1 Composition of the basal experimental diets

	Starter	Finisher
Ingredients (%)		
Yellow maize	54.4	61.0
Soyabean meal	32.0	23.6
Fish meal	3.5	3.5
Sunflower meal	2.5	4.0
Limestone	1.0	1.0
Dicalcium phosphate	1.0	1.0
Vitamin premix[1]	0.65	0.65
Mineral premix[2]	0.15	0.15
Salt	0.36	0.36
DL-methionine	0.10	0.60
Lysine	1.30	1.15
Soyabean oil	3.0	3.0
Total	100	100
Calculated analyses		
Crude protein, g/kg	225	210
ME, MJ/kg	12.9	13.6

[1] Supplied the following per kg of vitamin premix: Vitamin A - 2700 IU; cholecalciferol - 94000 IU; vitamin E - 40 mg; menadione - 681 mg; thiamine - 454 mg; riboflavin - 1.5 mg; niacin - 13 mg; pantothenic acid - 3.17 mg; pyridoxine - 908 mg; folic acid - 363 mg; biotin - 30 mg
[2] Supplied the following per kg of mineral premix: Mn - 150 mg; Zn - 120 mg; Fe - 40 mg; Cu - 13 mg; I - 1 mg

Results and Discussion

The effects of the dietary supplementation of thyme oil and vitamin E on body weight, weight gain, feed intake and feed conversion are presented in Table 2. Differences were significant ($P < 0.01$) between the control, E100 and TO100, and not significant ($P > 0.01$) between the control, E200 and TO200 with respect to weight gain and body weight. The E200 group exhibited the best feed conversion compared to the other groups. Guo *et al*. (2003) showed that addition of vitamin E (100 mg/kg) improved ($P < 0.05$) the growth and feed conversion ratio of broilers compared to those fed the control diet (without vitamin E). Lee *et al*. (2004) reported that thymol did not improve poultry performance. Hertrampf (2001), however, noted that thyme oil supplementation in the drinking water of chickens increased weight gain.

Table 2 The influence of dietary vitamin E (E) (100 and 200 mg/kg) and thyme oil (TO) (100 and 200 mg) supplementation on the performance of broilers

	Control	E100	E200	TO100	TO200	s.e.m.	P
Body weight, g	2316.5[a]	2248.6[c]	2324.3[a]	2303.0[b]	2319.5[a]	0.98	**
Weight gain, g	2278.7[a]	2209.6[c]	2287.3[a]	2265.0[b]	2282.5[a]	1.38	**
Feed intake, g	3900.6[c]	4096.0[a]	3964.4[b]	4031.8[a]	4058.2[a]	12.61	*
FCR	1.75[c]	1.85[a]	1.73[d]	1.78[b]	1.77[b]	0.002	**

[a-d] Means within rows with different superscripts differ at $P < 0.01$ (**) and $P < 0.05$ (*)
FCR - feed conversion ratio (kg feed intake/kg weight gain); s.e.m. – standard error of mean

No significant differences (P > 0.05) with respect to heart, leg and breast weights were detected due to dietary treatments (Table 3). Birds fed the control, E200 and TO200 diets had higher hot carcass weights and wing weights. The group fed the control and E200 diets exhibited significantly (P > 0.01) higher liver weights than the other groups. The carcass yields of the E100 and T100 groups were significantly (P < 0.05) lower than those of the other groups. It was determined that the liver weight of the control group was significantly (P > 0.01) higher than that of the thyme oil groups. It may therefore be concluded that thyme oil supplementation resulted in reduced liver weights. This, however, contradicts the findings of Lee *et al.* (2004) who reported that thymol did not influence liver weight.

Table 3 The effect of dietary vitamin E (E) (100 and 200 mg/kg) and thyme oil (TO) (100 and 200 mg/kg) supplementation on the carcass and organ characteristics of broilers

	Control	E100	E200	TO 100	TO 200	s.e.m.	P
Hot carcass, g	1791 [a]	1714 [b]	1802 [a]	1718 [b]	1780 [a]	2.34	*
Liver, g	43 [a]	39.5 [b]	46 [a]	40 [b]	41 [b]	0.535	**
Heart, g	14.5	12.0	13.5	14.0	14.5	0.34	NS
Wing, g	196 [a]	184 [b]	193 [a]	180 [c]	187 [a]	0.94	**
Leg, g	608	531	541	530	536	2.76	NS
Breast, g	635	577	591	567	582	8.81	NS
Hot carcass yield, %	77 [a]	76 [b]	77.5 [a]	75 [b]	77 [a]	0.45	*

[a-b] Means within rows with different superscripts differ at P < 0.01 (**) and P < 0.05 (*)
NS – not significant; s.e.m. – standard error of mean

Supplementation of the basal diet with 100 and 200 mg/kg vitamin E increased the oxidative stability of the tissue in the present study (Table 4). This result was in agreement with other studies performed on poultry (DeWinne & Dirinck, 1996; Neill *et al.*, 1998; Villar-Patino *et al.*, 2002). Cortinas *et al.* (2005), however, reported that the oxidative stability of meat was not affected by an increase in the dietary α- tocopherol level from 200 to 400 mg/kg.

Table 4 The effect of dietary vitamin E (E) (100 and 200 mg/kg) and thyme oil (TO) (100 and 200 mg/kg) and days of refrigeration on the thiobarbituric acid reactive substance (TBARS) values of leg and breast muscle tissue (mg MDA /kg meat)

Duration of refrigeration	Leg					Breast				
	Control	E100	E200	TO100	TO200	Control	E100	E200	TO100	TO200
1 d	0.99 [b]	0.18 [d]	0.16 [e]	0.24 [c]	0.11 [f]	0.74 [b]	0.09 [f]	0.09 [f]	0.17 [d]	0.10 [e]
3 d	1 [b]	0.14 [e]	0.17 [d]	0.23 [c]	0.12 [f]	0.76 [b]	0.17 [d]	0.09 [f]	0.17 [d]	0.11 [e]
7 d	4.81 [a]	0.15 [e]	0.18 [d]	0.22 [c]	0.12 [f]	4.63 [a]	0.24 [c]	0.12 [e]	0.18 [d]	0.12 [e]
s.e.m.			0.001					0.003		
Diet			**					**		
Days			**					**		
Diet x Day			**					**		

[a-f] Means within rows with no common superscript differ significantly P < 0.01 (**)
MDA - mg malondialdehyde; s.e.m. – standard error of mean

Table 5 The influence of dietary vitamin E (E) (100 and 200 mg/kg) and thyme oil (TO) (100 and 200 mg/kg) on the fatty acid composition of broiler leg and breast tissues

Fatty acids (% of total)														
Fatty acids	Leg							Breast						
	Control	E100	E200	TO100	TO200	s.e.m.	P	Control	E100	E200	TO100	TO200	s.e.m.	P
Myristic	1.67 [a]	1.66 [a]	1.67 [a]	1.61 [b]	1.59 [c]	0.002	**	3.42 [a]	3.41 [a]	3.43 [a]	2.68 [b]	2.25 [c]	0.002	**
Palmitic	18.5	18.5	18.5	18.5	18.5	0.012	NS	16.3 [c]	16.0 [c]	16.0 [c]	17.0 [b]	17.0 [a]	0.007	**
Palmitoleic	2.32 [c]	2.30 [c]	2.31 [c]	3.69 [b]	5.53 [a]	0.003	**	2.20 [c]	2.20 [c]	2.21 [c]	3.33 [b]	5.09 [a]	0.018	**
Stearic	5.11 [a]	5.11 [a]	5.10 [a]	4.58 [b]	3.44 [c]	0.002	**	5.66 [a]	5.64 [a]	5.64 [a]	5.29 [b]	3.31 [c]	0.002	**
Oleic	26.85 [a]	26.87 [a]	26.85 [a]	26.82 [a]	25.42 [b]	0.003	**	26.34 [c]	26.33 [c]	26.34 [c]	27.27 [b]	28.07 [a]	0.005	**
Linoleic	33.74 [c]	33.74 [c]	33.73 [c]	36.70 [b]	37.62 [a]	0.005	**	31.59 [c]	31.60 [c]	31.59 [c]	32.52 [b]	34.56 [a]	0.001	**
Linolenic	3.57 [a]	3.57 [a]	3.57 [a]	1.53 [b]	1.39 [c]	0.002	**	1.45 [a]	1.45 [a]	1.44 [a]	1.22 [c]	1.27 [b]	0.001	**
Arachidonic	5.44 [a]	5.42 [a]	5.43 [a]	2.66 [c]	2.81 [b]	0.002	**	8.08 [a]	8.06 [a]	8.05 [a]	5.84 [b]	4.06 [c]	0.003	**
SFA	25.28 [a]	25.26 [a]	25.27 [a]	24.64 [b]	23.53 [c]	0.004	**	25.11 [a]	25.05 [a]	25.03 [a]	24.94 [b]	22.59 [c]	0.003	**
MUFA	29.17 [c]	29.17 [c]	29.16 [c]	30.51 [b]	30.95 [a]	0.003	**	28.54 [c]	28.53 [c]	28.55 [c]	30.10 [b]	33.16 [a]	0.002	**
PUFA	42.75 [a]	42.73 [a]	42.73 [a]	40.89 [c]	41.82 [b]	0.002	**	41.12 [a]	41.11 [a]	41.08 [a]	39.58 [c]	39.89 [b]	0.002	**
UP	2.80	2.84	2.84	3.96	3.70	0.002	*	5.23	5.31	5.34	5.38	4.36	0.004	NS

[a-d] Means within rows within tissue with different superscripts differ at $P < 0.01$ (**) and $P < 0.05$ (*)
NS – not significant; s.e.m. – standard error of mean
SFA - saturated fatty acid; MUFA - monounsaturated fatty acid; PUFA - polyunsaturated fatty acid; UP - unidentified peaks

With respect to leg tissue, although the addition of vitamin E and thyme oil to broiler diets resulted in a significant ($P < 0.01$) reduction in the TBARS values, the TO200 diet delivered significantly better results than the E200 diet.

The interaction between Diet x Days was significant ($P < 0.01$) with respect to the TBARS values of leg and breast tissues. With respect to the TBARS values, the difference between the TO200 and E200 diets was not significant in breast tissue stored for seven days. Schulz & Herrmann (1980) as well as Miura & Nakatani (1989) reported that thymol exhibited a higher antioxidant activity than α-tocopherol. Extracts of rosemary and sage, as well as other herbs from the *Labiatae* family (such as thyme) have also exhibited substantial antioxidant activity in lard (Economou *et al*., 1991; Schwartz *et al*., 1996). Studies on the stabilizing activity of thyme oil in lipid systems have shown that the phenolic components (carvacrol and especially thymol) were primarily responsible for its antioxidant activity (Farag *et al*., 1989; Deighton *et al*., 1993).

The fatty acid compositions of the leg and breast tissues of broilers were significantly altered by dietary thyme oil supplementation (Table 5). The supplementation of thyme oil in the diet significantly decreased the SFA content of the leg and breast tissues. The myristic, palmitic and stearic acid concentrations of leg and breast tissues from the TO100 and TO200 groups were significantly lower than those of tissues from the control group. Youdim & Deans (2000) also found that the palmitic and stearic acid content of a rat's brain fed thyme oil was lower than that from the control group.

The proportion of MUFA in the leg and breast tissue lipids was significantly increased ($P < 0.01$) by dietary thyme oil supplementation. The addition of thyme oil increased the oleic and palmitoleic acid content of the tissues. Youdim & Deans (2000) also noted that thyme oil increased the concentration of oleic and palmitoleic acid level in rat brains. However, Lee *et al*. (2003) showed that oleic acid levels of the adipose tissue of broilers decreased when fed a diet containing thymol.

The effects of the dietary supplementation of thyme oil and vitamin E on the total polyunsaturated fatty acid (PUFA) composition of the tissues are shown in Table 6. In comparison to the control group, the PUFA concentration of leg and breast tissues was reduced ($P < 0.01$) by dietary thyme oil supplementation. The linoleic acid concentration, however, increased with dietary thyme oil supplementation. The linolenic and arachidonic acid concentrations ($P < 0.01$) decreased. Lee *et al* (2003) also found that the linoleic acid levels increased in adipose tissue with dietary thymol supplementation. Youdim & Deans (2000) investigated the effect of thyme oil on the fatty acid composition of rat brains and also found that the arachidonic acid level decreased with the addition of dietary thyme oil.

Table 6 The influence of dietary vitamin E (E) (100 and 200 mg/kg) and thyme oil (TO) (100 and 200 mg/kg) supplementation on serum lipoprotein concentrations (mg/dL)

Parameters	Control	E100	E200	TO100	TO200	s.e.m.	P
Total cholesterol	166 [b]	155 [c]	172 [a]	166 [b]	171 [a]	0.835	**
Triglyceride	91 [d]	79 [e]	97 [c]	122 [b]	135 [a]	0.435	**
HDL-C	91 [e]	101 [c]	115 [a]	110 [b]	97 [d]	0.235	**
LDL-C	52 [c]	49.7 [d]	58 [b]	61.5 [a]	62.5 [a]	0.267	**

[a-d] Means within rows with different superscripts differ at $P < 0.01$ (**); s.e.m. – standard error of mean
HDL-C - high density lipoprotein cholesterol; LDL-C - low density lipoprotein cholesterol

Total cholesterol (TC) concentrations and HDL-C reached a maximum in broilers consuming the E200 and TO200 (Table 6) diets. Triglyceride and LDL-C levels decreased ($P < 0.01$) whilst HDL-C levels increased when the E100 diet was fed. Thyme oil also significantly increased the triglyceride, HDL-C and LDL-C concentrations. Triglyceride, total cholesterol and LDL-C reached a maximum in broilers fed the TO200 diet. Lee *et al*. (2004), however, reported that thyme oil supplementation resulted in a decrease in the total cholesterol and triglyceride levels, but increased HDL-C. Similarly, Case *et al*. (1995) found that

thymol supplementation of Leghorn chickens led to a reduction in serum cholesterol. In contrast to Case *et al*. (1995), the results of the present study failed to exhibit that any of the supplements had hypocholesterolaemic effects.

Conclusions

The beneficial effects of dietary thyme oil and vitamin E supplementation on broiler performance were not evident in this study. The effect of the dietary supplementation of thyme oil and vitamin E on body weight, weight gain and feed conversion did not differ significantly between the E200 and TO200 diets. The TBARS values of the tissues were significantly lowered by thyme oil and vitamin E supplementation. The proportion of MUFA in the leg and breast muscles of broilers increased with increasing dietary thyme oil supplementation, whilst the proportions of SFA and PUFA decreased.

It was thus concluded that the antioxidant ability of thyme oil exceeded that of vitamin E in leg tissue. In conclusion, the data suggest that 200 mg/kg of the thyme oil could be successfully used as an antioxidant in broiler diets.

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