Thyme (Thymus vulgaris) extract consumption darkens liver, lowers blood cholesterol, proportional liver and abdominal fat weights in broiler chickens

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Abstract

A study with 160-day-old broiler chickens was conducted to investigate the effects of Thymus vulgaris (Thyme) extract in drinking water on plasma cholesterol, triglyceride, high density lipoprotein (HDL-c), low density lipoprotein (LDL-c) and very low density lipoprotein (VLDL-c), proportional abdominal fat, liver weights and liver color index. The chickens were divided into four groups and received 0 (ZT), 0.2 (LT), 0.4 (MT) or 0.6% (HT) thyme extract from day one to day 42 of age. All the birds receiving the Thyme extract had lower plasma total cholesterol, LDL-c concentrations and proportional liver weights compared with the control birds (P<0.05). Liver absolute weight of MT birds and proportional fat weight of HT birds were lower than those of ZT birds (P<0.05). Liver color index of HT and MT birds was higher than that of ZT birds. In orthogonal comparisons, thyme extract supplementation diminished the plasma triglyceride, total cholesterol, LDL-c and VLDL-c, decreased the proportional and absolute liver weight and proportional abdominal fat weight and increased the (P<0.05) liver color index as compared to control. Significant negative correlation (P<0.01) was found between the thyme extract supplementation and plasma cholesterol, proportional abdominal fat weight and liver proportional weight, and a positive correlation (P<0.01) between the thyme extract supplementation and liver colour index. In conclusion, Thyme consumption in broiler chickens improved the carcass quality to the consumers and net returns of the producers.

Introduction

Heart disease is an important occurring case in modern human societies and is directly related with increased levels of plasma cholesterol. Hypercholesterolemia and low high density lipoprotein (HDL-c) levels are often associated with endothelium dysfunction and inflammation, which are often followed by atherosclerosis (Barbalho et al., 2009). Moreover, cellular cholesterol concentration is as well related to other disease such as Alzheimer (Michikawa, 2003). Animal products with high fat content are one of the risk factors for cardiovascular diseases and are not desirable for consumers (Dong et al., 2007). Although chicken meat is a healthier product, compared with other animal protein sources for human nutrition, having low cholesterol and fat content, several different dietary treatments have been used to attempt a further decrease of these ingredients in poultry meat (Ponte et al., 2004). Nowadays, medicinal plants are receiving high attention as feed additives, due to their depressing effects on fat contents of animal products. In this regard, beneficial effects of dietary alfalfa, rosemary, thyme and garlic have been revealed in human and animals (Adler and Holub, 1997; Konjufca et al., 1997; Mottaghibitalab and Taraz, 2002; Ponte et al., 2004; Radwan et al., 2008). Thymus vulgaris is a perennial medicinal herb in the Lamiaceae family, cultivated worldwide for culinary, cosmetic and medical purposes. This species has special activities such as antispasmodic, expectorant, antiseptic, antimicrobial and antioxidant (Hertrampf, 2001; Abu-Darwish et al., 2003) and thymol (5-methyl-1-2-isopropyl phenol) and carvacrol (5-isopropyl-2-methyl phenol) are the main phenolic components in Thymus vulgaris that have hypocholesterolemic effects (Massada, 1976). Hypcholesterolemic and performance improvement effects of thyme herb (El-Ghoussein and Al-Beitawi, 2009) or its essential oil (Bolukbasi et al., 2006) have been revealed previously. Nevertheless there is no information about thyme extract effects on abdominal fat (as a negative carcass characteristic), liver color index and weight (as the liver lipid content indices). Fat accumulation in carcasses of broilers, particularly in abdominal and visceral areas, represents a waste product to consumers from the nutritional and health points of view. Such obese broilers are unpleasant to the consumers, lead to decreased saleability and reduce the net returns of the producers (Rahie and Szilagyi, 1998).

Moreover, colour intensity indicates the cholesterol or lipid contents of the liver and indirectly meat quality. The liver colour associates to liver lipid contents in broiler chickens, and high dietary lipids (Trampel et al., 2005) or cholesterol (Lee et al., 2003) have resulted in lighter colour of the liver. So far, few studies are available regarding the potential effects of medicinal plants on these important indices in broiler chickens. In an experiment, dietary supplementation of 1.5% dried thyme herb alleviated the abdominal fat weight and redness of the skin colour (Schleicher et al., 1998).

Therefore, this experiment was conducted to evaluate the effects of thyme extract supplementation in drinking water on abdominal fat weight, liver colour and weight, rather than plasma lipoproteins.

Materials and methods

A total of 160 mixed one-day-old broiler chicks (Ross 308) were purchased from a local hatchery, weighed on arrival and randomly allocated to 16 pens (1x1 m) of 10 birds each, with equal numbers of male and females (four replicates per each treatment). Water and feed were available ad libitum. All chickens were fed the similar starter (day 1-21 of age) and grower (day 22-42 of age) diets in pellet form (Table 1), but received 0.0%, 0.2%, 0.4% and 0.6% alcoholic extract of Thymus vulgaris (0.06% thymol and pH=5) in drinking water during the exper-
Results

Plasma lipoproteins, total cholesterol and triglyceride

The effect of thyme extract on these parameters is shown in Table 2. There was no significant difference between the treatments for plasma triglyceride, HDL-c and VLDL-c, but plasma total cholesterol and LDL-c concentration of thyme extract received birds were lower (P<0.05) compared with those of ZT birds. Moreover, compared with the control, thyme extract supplementation in drinking water decreased (P<0.05) the plasma triglyceride, total cholesterol, LDL-c and VLDL-c.

Liver colour index, liver and abdominal fat proportional and absolute weights

All the thyme extract received birds had a lower proportional liver weight (P<0.05) compared with control birds (Table 3). Liver absolute weight of MT received birds was lower than that of ZT birds, but no differences were observed between the liver absolute weights of other treatments. No differences were observed between the treatments for abdominal fat absolute weight. Although all the thyme extract received birds had a lower proportional fat weights compared with control birds, but only HT birds had a significantly lower value (P<0.05). HT and MT birds had a higher liver colour index compared with ZT birds, while no difference was observed between the liver colour index of LT and ZT birds (P>0.05). Besides, lower (P<0.05) proportional and absolute liver weight, proportional abdominal fat weight and higher (P<0.05) liver colour index were observed with thyme extract supplementation compared with the control.

Table 1. Composition of experimental diets.

| Ingredients | Starter, 0-21 d | Grower, 22-42 d |
|-------------|----------------|-----------------|
| Corn, %     | 54.87          | 61.78           |
| Soybean meal (44% protein), % | 36.72 | 26.36 |
| Fish meal, % | 1.31 | 4.50 |
| Vegetable oil, % | 3.00 | 4.00 |
| Limestone, % | 1.15 | 1.05 |
| Dicalcium phosphate, % | 1.94 | 1.49 |
| Vitamin and mineral premix*, % | 0.50 | 0.50 |
| Salt, %     | 0.30           | 0.30            |
| DL-methionine, % | 0.21 | 0.02 |
| Total       | 100.00         | 100.00          |

Calculated analysis

Metabolizable energy, kcal/kg 2937 3100
Crude protein, % 21.44 19.37
Calcium, % 1.05 1.00
Available phosphorus, % 0.51 0.50
Sodium, % 0.16 0.14
Arginine, % 1.41 1.23
Methionine + Cystine, % 0.91 0.69
Lysine, % 1.20 1.10
Tryptophan, % 0.31 0.26

*Provide per kg of diet: retinol, 15,000 U; colecalciferol, 8000 U; menadione, 3 mg; cyanocobalamin 15 µg; niacin, 32 mg; choline, 84 mg; biotin, 40 µg; thiamine, 4 mg; riboflavin, 6.6 mg; pyridoxine, 5 mg; folic acid, 1 mg; zinc, 80 mg; manganese, 100 mg; selenium, 200 mg; iron, 80 mg; magnesium, 12 mg; copper, 10 mg; calcium, 13 mg; iodine, 1 mg.

Table 2. Blood parameters in broiler chickens* receiving 0, 0.2, 0.4 and 0.6% thyme extract in drinking water.

| Treatment | Triglyceride, mg/dL | Total cholesterol, mg/dL | HDL-c, mg/dL | LDL-c, mg/dL | VLDL-c, mg/dL |
|-----------|---------------------|-------------------------|--------------|--------------|---------------|
| ZT        | 47.2                | 231.3*                  | 94.4         | 127.5*       | 9.44          |
| LT        | 45.0                | 146.2                   | 90.6         | 46.5*        | 9.00          |
| MT        | 44.9                | 140.9                   | 92.3         | 39.5*        | 9.00          |
| HT        | 45.7                | 136.5                   | 97.9         | 30.9*        | 8.15          |
| P value   | 0.06                | <0.0001                 | 0.45         | <0.0001      | 0.06          |
| Pooled SEM | 0.34             | 7.23                    | 1.84         | 7.4          | 0.07          |

*Two birds per replicate (8 per treatment) were used for the determinations; orthogonral contrast: T vs C=contrasting birds supplemented with thyme vs control birds.

HDL-c, high density lipoprotein; LDL-c, low density lipoprotein; VLDL-c, very low density lipoprotein; ZT, 0% thyme extract treatment; LT, 0.2% thyme extract treatment; MT, 0.4% thyme extract treatment; HT, 0.6% thyme extract treatment; P<0.05 was considered as the non-parametric difference between the treatments for plasma triglyceride, HDL-c and VLDL-c, but plasma total cholesterol and LDL-c concentration of thyme extract received birds were lower (P<0.05) compared with those of ZT birds. Moreover, compared with the control, thyme extract supplementation in drinking water decreased (P<0.05) the plasma triglyceride, total cholesterol, LDL-c and VLDL-c.

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Correlation analysis

Significant negative correlations were found between thyme extract supplementation and plasma cholesterol \((r=-0.81, P=0.0001)\), proportional abdominal fat weight \((r=-0.49, P=0.004)\) and liver proportional weight \((r=-0.53, P=0.001)\) and a significant positive correlation between thyme extract supplementation and liver colour index \((r=0.51, P=0.002)\) (Table 4). Furthermore, plasma total cholesterol had significant correlation with proportional abdominal fat \((r=0.40, P=0.02)\) and liver weight \((r=0.63, P=0.0001)\) and negative correlation with liver colour index \((r=-0.50, P=0.003)\). Besides, significant negative correlation \((P=0.04)\) was observed between the proportional liver weight and liver colour index \((r=-0.36)\). However, no significant correlation \((P=0.72)\) was obtained between the proportional abdominal fat weight and proportional liver weight.

Discussion

The results of the present study indicate that thyme extract consumption in drinking water reduces the plasma total cholesterol, LDL-c, VLDL-c and triglyceride in broiler chickens, which is in agreement with the results of previous studies in laying hens by dietary supplementation of 0.25% thyme plant (Ali et al., 2007) and in broiler chickens by dietary levels of 0.5, 1.0, 1.5 and 2.0% crushed thyme (El-Ghousein and Al-Beitawi, 2009). In the same way, Case et al. (1995) reported 9% reduced serum cholesterol in 21-day-old leghorn chicks fed 15 ppm thyme essential oil. These results are also consistent with those of a previous study (Lee et al., 2003), where reduced plasma triglyceride was found in female broiler chickens at day 28 of age by dietary carvacrol supplementation. Nevertheless, higher plasma levels of triglyceride, HDL-cholesterol and LDL-cholesterol were reported in broilers by dietary supplementation of 100 and 200 ppm thyme essential oil (Bolukbasi et al., 2006). Undoubtedly different additives (thyme essential oil and thyme extract) and supplementation ways (dietary and drinking water) should be considered between the studies. Depressing effects of thyme extract supplementation on plasma total cholesterol, LDL-c, VLDL-c and possibly triglyceride of recent experiment may possibly be related to the reduced activity of HMG-CoA reductase by active components of thyme extract (thymol and carvacrol) or the formation of insoluble saponin-cholesterol complexes in gastrointestinal tract or both. Thyme extract has essential oil, tannins, glycosides, saponins and other components (ESCORP, 2003). Dietary thymol and carvacrol inhibit the hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, which is a key regulatory enzyme in cholesterol synthesis. The non-steroid products derived from mevalonate, which modulate HMG-CoA reductase activity (Goldstein and Brown, 1990), thymol and carvacrol might induce putative regulatory non-steroid products (Case et al., 1995; Elson, 1996). This action results in a reduction of the plasma cholesterol concentration (Case et al., 1995; Elson and Qureshi, 1995; Lee et al., 2003; El-Ghousein and Al-Beitawi, 2009). A positive correlation has been reported between the activity of HMG-CoA reductase and total cholesterol or LDL-cholesterol in broiler chickens (Qureshi et al., 1983). Besides the thymol and carvacrol, thyme extract possesses saponin components, which have beneficial effects on plasma lipids concentration. Saponins form insoluble complexes with cholesterol in the digesta and inhibit the intestinal absorption of endogenous and exogenous cholesterol (Oakenfull and Sidhu, 1990). Furthermore, saponin can affect the enterohepatic circulation of bile acids by forming mixed micelles, which directly changes the reabsorption of bile acids from the terminal ileum. In rat, feeding the Gypsophia saponin has decreased the plasma cholesterol (Souton et al., 1988).

Thyme leaves have indicated to increase the abdominal fat in broilers consumption (Ocak et al., 2008), which is connected to the faster grow rate of broilers due to thyme consumption; whereas thyme extract supplementation reduced the proportional abdominal fat weight in the recent study, which is in agreement with the results of Al-Kassei (2009), who found in broiler chickens fed 200 ppm thyme essential oil. This is related to the decreased cholesterol and triglycerides absorption from the gut or

| Table 3. Liver and abdominal fat absolute and proportional weights and liver colour index of broiler chickens* receiving 0, 0.2, 0.4 and 0.6% thyme extract in drinking water. |
| --- |
| Treatment | Liver weight | Abdominal fat | Liver colour index |
| | Proportional | Absolute | Proportional | Absolute |
| ZT | 3.05abc | 43.03a | 1.51b |
| LT | 2.56bc | 40.50ab | 1.84ab |
| MT | 2.55bc | 34.20a | 1.50b |
| HT | 2.46bc | 38.60a | 33.1 |
| P value | 0.003 | 0.03 | 0.14 |
| Pooled SEM | 14.10 | 16.08 | 23.7 |
| Orthogonal contrast | 0.0003 | 0.03 | 0.02 | 0.02 |

ZT, 0% thyme extract treatment; LT, 0.2% thyme extract treatment; MT, 0.4% thyme extract treatment; HT, 0.6% thyme extract treatment; *Lines with no common superscript letter in each column differ significantly \((P<0.05)\); \(\#\) liver and abdominal fat of two birds per replicate \((8\) per treatment) were taken for the analyses; *Orthogonal contrast. T vs C—contrasting birds supplemented with thyme vs control birds.

| Table 4. Pearson correlation coefficients between thyme extract supplementation (% in water) with plasma total cholesterol, proportional abdominal fat weight (% of live body weight), proportional liver weight (% of live body weight) and liver colour index of broiler chickens* receiving 0, 0.2, 0.4 and 0.6% thyme extract in drinking water. |
| --- |
| Thyme extract | Total cholesterol, mg/dL | Proportional abdominal fat weight | Proportional liver weight | Liver colour index |
| Thyme extract | -0.81 | -0.49 | -0.53 | 0.51 |
| P-value | 1.000 | <0.001 | 0.004 | 0.001 |
| P-value | 0.50 | 0.63 | 0.72 | 0.01 |
| Total cholesterol, mg/dL | -0.81 | 0.40 | 0.06 | -0.44 |
| P-value | <0.001 | 0.02 | 1.000 | 0.003 |
| Proportional abdominal fat weight | -0.49 | 0.06 | <0.001 | 0.01 |
| P-value | 0.004 | 0.02 | 1.000 | 0.04 |
| Proportional liver weight | 0.53 | 0.63 | 0.72 | 0.04 |
| P-value | 0.001 | <0.001 | 0.003 | 1.00 |
| Liver colour index | 0.51 | -0.50 | -0.50 | 1.00 |
| P-value | 0.002 | 0.003 | 0.01 | 0.04 |

*Two birds per replicate \((8\) per treatment) were used for the analyses.
synthesis in liver, because a significant correlation obtained between the plasma cholesterol and proportional abdominal fat weight. The other possible reason may be the abdominal lipids catabolism for gluconeogenesis process in thyme fed birds, since crushed thyme consumption in broiler chickens was reported to increase the serum glucose (El-Ghousein and Al-Beitawi, 2009).

No changes of liver weight were reported in broiler chickens fed 1 g/kg thyme powder (Demir et al., 2008). An increase in relative liver mass of broiler chickens was observed after the thyme essential oil consumption (Lee et al., 2003). In agreement with our results, the findings of another study demonstrated that feeding thyme essential oil to broiler chickens caused the lowering of liver weight (Bulubasri et al., 2006); although liver fat content was not determined in the present study, the reduced liver weight is likely related to its lower fat content, since significant correlation was shown between the proportional liver weight and plasma cholesterol, and proportional abdominal fat weight in present study. The higher liver colour index in thyme extract received birds further indicates the decreased liver fat content. A lower plasma cholesterol concentration (mg/dL) and liver proportional weight in broilers fed 100 ppm thyme essential oil have been reported already (Al-Kassei, 2009). Lee et al. (2003) observed higher plasma and liver cholesterol, and liver weight, in broiler chickens fed 1% cholesterol in diet. Moreover, they observed the yellowish livers in cholesterol fed 1% cholesterol in diet. Moreover, they observed the yellowish livers in cholesterol fed broilers and pinkish livers in birds fed the control diet (without cholesterol). In other consistent study, Trampel et al. (2005) indicated the higher liver weight and lighter colour in broiler chickens fed high dietary lipids and the redder colour in liver with low cholesterol content.

Conclusions

Based on the results of our experiment, we can conclude that thyme extract supplementation in drinking water decreases the plasma triglyceride, total cholesterol, LDL-c and VLDL-c, which in turn lower the liver and abdominal lipids, reduces the proportional liver and abdominal fat weights, and increases as well the liver colour intensity in broiler chickens. These effects of the thyme extract consumption are supposed to be exerted by the lower activity of HMG-CoA reductase enzyme, reduced fat absorption from the gastrointestinal tract or the lipid catabolism for gluconeogenesis process. Consequently, it can be concluded that thyme consumption in broiler chickens could improve the carcass quality, for a higher appreciation by the end users and profitable net returns for the producers.

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