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Effects of essential oils and their combinations added to broiler diets on the mineral contents of some tissues and bone breaking strength*

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SUMMARY

The current research was conducted to determine the effect of thyme (*Thymus vulgaris L.*), rosemary (*Rosmarinus officinalis L.*), and French lavender (*Lavandula stoechas L.*) essential oils and their combinations on serum, bone and excreta mineral content and bone breaking strength in broilers. A total of 640 day-old broiler chicks (Ross 308) were randomly allocated to eight treatment groups with five replicates each. The experimental diets were prepared by adding essential oils to the control diet as follows: control (0 mg/kg), thyme essential oil (50 mg/kg), rosemary essential oil (50 mg/kg), French lavender essential oil (50 mg/kg), thyme + rosemary (25+25 mg/kg), thyme + French lavender (25+25 mg/kg), rosemary + French lavender (25+25 mg/kg) and thyme + rosemary + French lavender (16,7+16,7+16,7 mg/kg).

No difference was shown between the treatment groups in serum content of Ca, Mg, P or Zn (P > 0,05). Tibia Ca, Mg and P contents were statistically significant (P < 0,01), while tibia breaking strength was not significant (P > 0,05). Excreta Ca, Fe, Mg, Mn, P and Zn contents were significantly affected by the dietary treatments (P < 0,01), but excreta Cu content was not statistically significant (P > 0,05). The addition of thyme essential oil to the diet was effective in increasing tibia Ca, P and Mg content. The essential oils used, especially their binary mixtures, can be effective in reducing the amount of minerals excreted in the faeces.

KEY WORDS: Essential oil, broiler, bone, mineral

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INTRODUCTION

The importance of bioactive substances found naturally in medicinal and aromatic plants in terms of poultry health is a research topic that merits attention. These compounds of vegetable origin are called essential oils. Thyme, rosemary and lavender are plants rich in active ingredients, whose content may vary depending on the region where it is grown. In a previous study on the active substances of thyme in Turkey, Özcan and Chalchat (2004) reported that thyme (*Thymus vulgaris*) oil contained thymol (46,21%), γ-terpinene (14,08%), p-cymene (9,91%), myrcene (3,45%), α-pinene (2,97%), and carvacrol (2,44%). The major active substances of rosemary essential oil were cineol (20%), α-pinene (20%), camphor (18%), camphene (7%), borneol (5%), myrcene (5%), bornyl acetate (3%) and α-terpineol (2%), while linalool, limonene and caryophyllene were minor components (Saricaoğlu and Turhan, 2018). According to Białoń et al. (2019), the active substances of French lavender are camphor (18,18%), eucalyptol (8,03%), camphene (1,40%), α-pinene (1,31%), and bornyl acetate (1,32%).

Essential oils stimulate the appetite and digestive system, exhibiting antioxidant and antimicrobial activities (Sevim and Cufadar, 2017). They also positively affect poultry performance and health by stabilizing the normal intestinal microbiota and preventing pathogen colonization. They are also reported to stimulate the release of digestive enzymes, have positive effects on immunity, and promote the absorption and synergies of vitamins and minerals in the body (Stef and Gergen, 2012). Various studies conducted on these positive effects have shown that essential oils have antimicrobial (Zhang et al., 2019; Ayari et al., 2020; Zhai et al., 2020) and antioxidant Adaszynska and Szczerbinska, 2017; Zhang et al., 2019) effects and stimulate digestion (Zhai et al., 2020). Although essential oils have been reported to beneficially affect performance parameters in animals, their effects on the bioavailability of minerals are a complex issue (Cengiz et al., 2016). Several studies have showed that serum and small intestine Ca and P concentrations are not significantly affected in pigs (Lee et al., 2003) and broilers (Cao et al., 2010) fed diets containing vegetable essential oils. However, Amad et al. (2011) noted a linear increase in the ileal digestibility of Ca and P in broilers fed a diet containing thyme and anise oil. It has been reported that thyme essential oil, which contains the polyphenolic compounds thymol and carvacrol, may affect Ca and P metabolism and thus have positive effects on bone resorption (Cooper et al., 2015). Cengiz et al. (2016) found that rosemary essential oil stimulates oestrogen activity, which has a direct relationship with calcium, and therefore essential oils may contribute to protection of the skeletal structure. Research conducted on rats demonstrated that thyme and rosemary essential oils and monoterpenes are inhibitors of bone resorption and have a positive effect on bone formation, but thyme was more effective than rosemary in preventing bone resorption (Elbahnasawy et al., 2019). The effects of vegetable essential oils on mineral absorption, availability and accumulation in tissues may differ depending on their active substances, and an understanding of interactions between these active substances and minerals requires further research (Stef and Gergen, 2012).

The present study investigated the effects of diets containing thyme, rosemary and French lavender essential oils, individually or in combination, on tissue mineral contents, bone breaking strength, and mineral content in the faeces of broilers.

MATERIAL AND METHODS

All experimental procedures were carried out according to Directive 2010/63/EU of the European Parliament and of the Council of September 22, 2010 on the protection of animals used for scientific purposes. The animal material consisted of 640 day-old Ross 308 broiler chicks of both sexes. Chicks were randomly allocated to 8 treatment groups with 5 replicates, each with 16 chicks. The temperature was 33°C in the first week and stabilized at 21°C in the 4th week by decreasing it 3°C/week. A lighting programme was applied 23 hours/day. Feed and water were available to the chicks ad libitum throughout the experiment.

Experimental diets were formulated to meet the nutritional recommendations of the National Research Council (NRC, 1994) and the Ross broiler management manual (Table 1). Thyme (*Thymus vulgaris L.*; TEO), rosemary (*Rosmarinus officinalis L.*; REO) and French lavender (*Lavandula stoechas L.*; FLO) essential oils obtained from a commercial company (Ecodab) were added to the diets in the amount of 50 mg/kg of essential oil. In groups receiving combinations of essential oils, each essential oil was used in equal amounts. The ingredients and calculated nutrient compositions of the experimental diets are presented in Table 1. All chicks were fed a starter diet at 0-3 weeks and a finisher diet at 4-6 weeks of the experiment.

Table 1

Ingredients and calculated nutrient composition of starter (0-3 weeks) and finisher (0-6 weeks) diets

Ingredients (%)	Starter diet (0-3 weeks)	Finisher diet (4-6 weeks)
Maize	51,30	56,00
Soybean meal (40,8% CP)	38,80	33,60
Vegetable oil (8800 kcal/kg ME)	6,10	6,70
Limestone	1,00	1,20
Dicalcium phosphate	2,10	1,83
Salt	0,30	0,30
Premix[1]	0,25	0,25
L-Lysine	0,02	0,02
DL-Methionine	0,13	0,10
Total	100	100
Calculated nutrient composition (%)		
Crude protein	22,08	19,99
Metabolizable energy, kcal/kg	3104	3194
Calcium	1,00	0,99
Available phosphorus	0,50	0,44
Methionine	0,48	0,42
Methionine + cystine	0,85	0,76
Lysine	1,31	1,16

[1]Premix provided per kg of diet: vitamin A 15,000 IU, vitamin D_3 1500 IU, vitamin K 5,0 mg, vitamin B_1 3 mg, vitamin B_2 6 mg, vitamin B_6 5 mg, vitamin B_{12} 0,03 mg, niacin 30 mg, biotin 0,1 mg, calcium D-pantothenate 12,0 mg, folic acid 1,0 mg, choline chloride 400 mg, manganese 80 mg, Iron 35 mg, zinc 50 mg, copper 5,0 mg, iodine 2 mg, cobalt 0,04 mg

Determination of blood serum and bone mineral content

At the end of the experiment, approximately 5 cc of blood was taken from broilers, and the serum was separated by centrifugation at 3000 rpm for 5 min. Serum mineral concentrations were determined directly in an atomic emission spectrometer (AX-ICP, Varian Vista). Subsequently, the tibia was removed from the slaughtered broilers, and the meat and bone marrow were cleaned and dried at 105°C for 24 h. Samples were taken from similar parts of each tibia (middle axis). The mineral content of the tibias was determined with an atomic emission spectrometer (ICP, VISTA AX CCD Simultaneous ICP-AES) after wet decomposition of the tibia in a microwave (Mars Xpress Technology Inside).

Determination of bone breaking strength

The left tibias of 4 broilers (2 females and 2 males) randomly selected from each replicate were taken to determine mechanical properties. After cleaning they were stored in a freezer at -20ºC until analysis. Bone mechanical properties were determined by a load-deformation curve from a three-point bending test performed using an Instron Universal Testing Instrument (Model 1122; Instron, Canton, MA) and the Test Works 4 software package (version 402; MTS System Corporation, Eden Prairie, MN), as described by Wilson and Ruszler (1996). To determine the shear force, an apparatus produced according to ANSI/ASAE standard S459 DEC 01 was used, with a constant crosshead speed of 5 mm/min. The shear force was measured on a 15 mm section in the middle of the bone.

Determination of excreta mineral content

To determine the excreta mineral content, all birds in each subgroup were placed in 60x60x40 cm cages for one day between 35 and 40 days of the experiment. All birds were placed in an individual cage for collection of excreta samples, and when sufficient droppings had accumulated, the animals were returned to their original cages. The samples were weighed after collection and stored in a freezer (-20°C) until analysis. Faeces taken from the freezer were dried at 105°C for 24 h, and approximately 0,15 g of sample was weighed out. Following the addition of 5 cc nitric acid, 2 cc hydrogen peroxide and 3 cc perchloric acid, the samples were digested in a microwave (Cem Mars 5) at 175 PSI and 190°C for 40 min. Then the mineral content of the samples, completed to 50 cc with distilled water, were determined by ICP-AES (VISTA AX CCD Simultaneous ICP-AES).

Statistical analysis

Data were subjected to one-way analysis of variance (SPSS, 2016). Where the P-value was less than 0,05, Duncan's multiple comparison test was used to determine the differences between the treatment groups (Duncan, 1955).

RESULTS

Blood serum mineral concentrations

The effect of the addition of essential oils to broiler diets on serum Ca, Fe, Mg, P and Zn concentrations are shown in Table 2. Differences between serum Ca, Mg, P and Zn concentrations in broiler chickens fed diets with added thyme, rosemary and French lavender essential oils and their combinations were shown to be insignificant ($P > 0,05$).

Table 2

Effect of thyme, rosemary, and French lavender essential oils and their combinations added to broiler diets on serum mineral concentrations

Treatments	Ca, mg/L	P, mg/L	Mg, mg/L	Zn, mg/L
C	118,4	132,9	23,9	1,60
TEO	113,9	130,0	23,3	1,50
REO	111,5	131,5	23,5	1,60
FLO	110,9	126,4	23,2	1,50
TEO+REO	114,9	136,9	22,9	1,70
TEO+FLO	115,1	131,4	23,2	1,50
REO+FLO	114,3	132,5	22,9	1,40
TEO+REO+FLO	112,1	138,3	23,9	1,50
SEM[a]	2,45	3,23	0,42	0,060

C: control, TEO: thyme essential oil, REO: rosemary essential oil, FLO: French lavender essential oil, TEO+REO: thyme essential oil + rosemary essential oil, TEO+FLO: thyme essential oil + French lavender essential oil, REO+FLO: rosemary essential oil + French lavender essential oil, TEO+REO+FLO: thyme essential oil + rosemary essential oil + French lavender essential oil. [a]Pooled standard error of mean

Tibia mineral concentrations and bone breaking strength

The tibia Ca, P and Mg concentrations in broilers fed a diet supplemented with essential oils and their combinations showed significant ($P < 0,01$) differences between groups, while the effects of the treatments on bone breaking strength were not statistically significant ($P > 0,05$). Tibia Ca concentration was significantly higher in the group receiving thyme essential oil than in the other groups, except for the group containing rosemary essential oil. The tibia Ca concentration in the group containing a mixture of all essential oils (TEO+REO+FLO) was significantly lower than in the other groups, except those whose diets contained French lavender and rosemary + French lavender essential oil. Tibia P and Mg concentrations were significantly higher in the groups receiving thyme essential oil than in the other groups. Tibia P concentration was significantly lower in the group receiving a mixture of all essential oils (TEO+REO+FLO) than in the control, thyme, and rosemary groups.

Table 3

Effect of thyme, rosemary, and French lavender essential oils and their combinations added to broiler diets on tibia mineral concentrations and bone breaking strength

Treatments	Ca, %	P, %	Mg, %	Bone breaking strength, N
C	24,00^{BC}	9,30^{B}	0,30^{B}	1579,2
TEO	24,97^{A}	10,30^{A}	0,32^{A}	1458,2
REO	24,49^{AB}	9,08^{B}	0,29^{B}	1544,8
FLO	23,82^{BCD}	8,78^{BC}	0,29^{B}	1457,5
TEO+REO	23,99^{BC}	8,79^{BC}	0,29^{B}	1479,7
TEO+FLO	23,88^{BC}	8,74^{BC}	0,29^{B}	1517,8
REO+FLO	23,14^{CD}	8,42^{C}	0,28^{B}	1580,5
TEO+REO+FLO	22,93^{D}	8,35^{C}	0,28^{B}	1545,3
SEM[a]	0,303	0,423	0,002	41,01

C: control, TEO: thyme essential oil, REO: rosemary essential oil, FLO: French lavender essential oil, TEO+REO: thyme essential oil + rosemary essential oil, TEO+FLO: thyme essential oil + French lavender essential oil, REO+FLO: rosemary essential oil + French lavender essential oil**,** TEO+REO+FLO: thyme essential oil + rosemary essential oil + French lavender essential oil

[A,B]Values bearing different superscript letters in columns are statistically different, $P < 0,01$. [a]Pooled standard error of mean

Excreta mineral concentrations

The results of the Ca, P, Mg, Cu, Fe, Mn and Cu contents of the faeces of chicks in the experimental groups are presented in Table 4. Analysis of the faeces mineral contents of broiler chickens fed diets supplemented with essential oils and their combinations revealed significant differences between groups for Ca, P, Mg, Fe and Mn concentration, but not for Cu. The excreta Ca concentration was significantly higher in the control group than in the other groups, except for those whose diet contained thyme, rosemary and French lavender essential oil individually. Excreta P, Mg, Fe, Mn and Zn concentrations were highest in the thyme essential oil groups, but these differences were not significant with the following groups: control, rosemary, French lavender and thyme + rosemary + French lavender (TEO+REO+FLO) groups for P; control and rosemary groups for Mg and Mn; control, rosemary, French lavender and thyme+rosemary (TEO+REO) groups for Fe and Zn.

Table 4

Effect of thyme, rosemary, and French lavender essential oils and their combinations added to broiler diets on excreta mineral concentrations

Treatments	Ca, mg/L	P, mg/L	Mg, mg/L	Cu, mg/L	Fe, mg/L	Mn, mg/L	Zn, mg/L
C	$3,09^{A}$	$1,32^{AB}$	$0,32^{AB}$	31,8	$514,9^{AB}$	$280,9^{AB}$	$329,7^{AB}$
TEO	$3,07^{AB}$	$1,40^{A}$	$0,32^{A}$	34,5	$560,5^{A}$	$307,0^{A}$	$333,8^{A}$
REO	$2,98^{ABC}$	$1,33^{AB}$	$0,32^{AB}$	33,9	$527,9^{AB}$	$287,5^{AB}$	$323,9^{AB}$
FLO	$2,82^{ABCD}$	$1,28^{AB}$	$0,31^{BC}$	31,6	$492,2^{AB}$	$271,8^{BC}$	$301,9^{ABC}$
TEO+REO	$2,59^{CD}$	$1,21^{BC}$	$0,30^{CD}$	29,9	$507,9^{AB}$	$266,9^{BC}$	$303,8^{ABC}$
TEO+FLO	$2,61^{CD}$	$1,19^{BC}$	$0,30^{CD}$	29,9	$487,9^{B}$	$268,3^{BC}$	$289,5^{C}$
REO+FLO	$2,43^{D}$	$1,11^{C}$	$0,29^{D}$	35,5	$458,5^{B}$	$245,9^{C}$	$285,8^{C}$
TEO+REO+FLO	$2,70^{BCD}$	$1,24^{ABC}$	$0,30^{CD}$	29,6	$482,2^{B}$	$275,3^{ABC}$	$298,9^{BC}$
SEM[a]	0,66	0,003	0,006	1,82	14,65	7,44	7,14

C: control, TEO: thyme essential oil, REO: rosemary essential oil, FLO: French lavender essential oil, TEO+REO: thyme essential oil + rosemary essential oil, TEO+FLO: thyme essential oil + French lavender essential oil, REO+FLO: rosemary essential oil + French lavender essential oil, TEO+REO+FLO: thyme essential oil + rosemary essential oil + French lavender essential oil

[A,B]Values bearing different superscript letters in columns are statistically different, $P < 0,01$. [a]Pooled standard of error mean

DISCUSSION

Blood serum mineral concentrations

The effect of adding different essential oils and their combinations to broiler diets at a level of 50 mg/kg on serum mineral concentrations was not significant.

Çetin and Göçmen (2013) reported that blood circulation is stimulated by essential oils in the diet. That study found that the addition of 4% garlic powder to the diet significantly decreased serum Ca and Mg levels. A study in laying quails investigated the effect of adding cinnamon, rosemary and their combinations to the diet (not exceeding 200 mg/kg) on serum Cu, Co, Cr, Zn, Fe, K, Na and Mg levels. At the end of the study, the serum K level was significantly higher in the cinnamon group ($P < 0,05$), while the serum Zn level was found to be non-significant in the cinnamon group ($P = 0,078$) and the serum Co level in the rosemary group ($P = 0,086$). Other findings indicate similar mineral contents between groups ($P > 0,05$) (Şimsek et al., 2015). In another study, Hajiazizi et al. (2016) found that the addition of 200 mg/kg of rosemary essential oil and 40 mg/kg of Zn to the diet of laying hens did not affect their performance parameters, and rosemary essential oil also did not affect the serum Zn level. Khaksar et al. (2012) reported that the addition of 1 g/kg of oregano essential oil to quail diets did not affect serum Ca and P levels. Cengiz et al. (2016) examined the effects of vitamin E and of thyme, rosemary, and fennel essential oils and their combinations added to the diet of broilers at varying levels on their performance and serum and tibia properties. Serum Ca levels were found to be significantly higher in the groups receiving 100 mg/kg of fennel essential oil and 400 mg/kg of a thyme + rosemary + fennel essential oil mixture than in the control and vitamin E groups. Ciftci et al. (2018) reported that a combination of the essential oils did not affect the serum Ca level in a study investigating the effects of a combination of thyme, bay leaf, and orange peel oil added to the diet of chukars at 200 mg/kg.

Many factors contribute to differences in results regarding the effects of essential oils and their combinations added to poultry diets. In particular, differences between treatment levels and the form in which the oils are added to the diets can be reflected in the research results.

Tibia mineral concentrations and bone breaking strength

Differences were observed between groups in bone Ca, Mg, and P levels in broilers fed diets supplemented with essential oils and their combinations. Bone Ca, Mg and P contents were higher in the group with thyme added to the diet than in the control group. The difference between treatment groups may be due to the fact that thyme essential oil stimulates osteoblast proliferation, suggesting that it may also affect bone mineral concentrations. Cengiz et al. (2016) investigated the effects of vitamin E and of thyme, rosemary, and fennel essential oils and their combinations on the performance and serum and tibia properties of broilers. At the end of the research, supplementation with fennel essential oil and the thyme + rosemary + fennel mixture at levels of 200 and 400 mg/kg significantly increased the bone Ca content compared with the other groups (control, vitamin E, thyme, rosemary, and essential oil mixture (thyme + rosemary + fennel) at 100 mg/kg), but bone P content in these groups (fennel, 200 and 400 mg/kg thyme + rosemary + fennel essential oil mixture) was only higher than in the control and vitamin E groups. Olgun (2016) investigated the effect of the addition of different levels of a phytogenic feed additive containing essential oil combinations (thyme, black cumin, fennel, anise, and rosemary essential oils) to compound feed on performance, eggshell quality, bone biomechanical properties, and bone mineralization in laying hens. The differences between groups in tibia P, Mg and Mn contents were not significant, while the essential oil combinations quadratically affected the tibia Ca, which reached the highest value at the level of 100 mg/kg. In the same study, essential oil combinations did not significantly affect tibia Zn content. The researchers also found no difference in Ca and P contents among treatment groups. Elkomy and Elsaid (2015) reported that the addition of a combination of sage, rosemary, and thyme at a level of 30 g/kg body weight to the diet of ovariectomized rats positively affected bone mineral density. Mühlbauer et al. (2003) reported that essential oils inhibit bone resorption in ovariectomized rats.

The effects of essential oils and their combinations added to poultry diets on bone Ca, Mg, and P concentrations can vary depending on the levels of essential oils used. In terms of bone mineral concentrations, studies that agree with our results as well as those that do not are mentioned above. Differences in the results may be due to the levels of essential oils used or to environmental conditions.

The differences between treatment groups in bone breaking strength depending on the addition of essential oils and their combinations to broiler diets were found to be insignificant. Bone breaking strength varied between 1457,5 N and 1580,0 N. Olgun (2016), in an experiment on laying hens, investigated the effect of the addition of different levels of a phytogenic feed additive containing essential oil combinations (thyme, black cumin, fennel, anise, and rosemary essential oils) on performance, eggshell quality, bone biomechanical properties and bone mineralization. Essential oil combinations at the level of 600 mg/kg were shown to significantly reduce tibia breaking strength, while tibia breaking strength was highest in the treatment group receiving 50 mg/kg of essential oil combinations. The results of the current research are not similar to those reported by Olgun (2016). Differences in the animal material and essential oil levels may explain the variability of results.

Excreta mineral concentrations

The effects of essential oils and their combinations added to broiler diets on excreta mineral concentrations were found to be significant, except for Cu. Ca and P excretions were lower in the treatments with binary and ternary combinations of essential oils compared to the control group. Excretions of Ca, Fe, Mg, Mn and P were lower in the treatment with the binary combination of rosemary and French lavender essential oils than in the control group. The lower mineral content excreted in the treatment groups compared to the control group can be explained by synergistic stimulation of digestive enzymes and better absorption in the small intestine. Very few studies have investigated the effect of essential oils and their combinations on excreta mineral concentrations. Olgun and Yıldız (2014) studied the effect of different levels of thyme, black cumin, fennel, anise, and rosemary essential oils added to the diet on performance and mineral excretion in breeder quails. The results indicated that 400 and 600 mg/kg of the essential oil combination (all 5 oils) increased Ca, P, Mg, Mn and Zn excretion compared to the control group, whereas the addition of the essential oil combination to the diet at 200 mg/kg and lower levels made no difference in terms of Ca, P, Mg, Mn and Zn excretion compared to the control group. In this study, the decrease in excreta mineral content in the treatment groups with binary and ternary combinations of essential oils can be explained by the increase in mineral absorption due to the reduction in the small intestine pH.

CONCLUSIONS

The effects of thyme, rosemary, and French lavender essential oils and their binary and ternary combinations added to the diets of broilers at a level of 50 mg/kg on bone breaking strength and serum mineral concentrations were found to be insignificant, but the addition of thyme essential oil to the diet had a positive effect on tibia Ca, P and Mg concentrations. Important effects were observed among the treatment groups in terms of excreted minerals. The groups receiving essential oils, especially thyme essential oil, were similar to the control group, but the addition of binary or ternary combinations caused a significant decrease in the minerals excreted in the excreta. The addition of combinations of essential oils to the diet can be said to increase the availability of minerals and, although not observed in this study, this may be positively reflected in performance in future studies.

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