ORIGINAL ARTICLE

Veterinary Research Forum. 2023; 14 (5) 249 - 257
doi: 10.30466/vrf.2022.549989.3410
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

Effects of dietary thyme and rosemary essential oils on biochemical parameters, anti-oxidant metabolism, small intestinal morphology and myofiber structure of superficial pectoral and biceps femoris muscles in broilers

Recep Gümüş[1]*, Adem Kara[2], Seçkin Özkanlar[3], Halit İmik[4], Nevra Aydemir Celep[5]

[1] Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Türkiye; [2] Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum, Türkiye; [3] Department of Biochemistry, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Türkiye; [4] Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ataturk University, Erzurum, Türkiye; [5] Department of Histology and Embryology, Faculty of Veterinary Medicine, Atatürk University, Erzurum, Türkiye.

Article Info

Article history:

Received: 08 March 2022
Accepted: 27 June 2022
Available online: 15 May 2023

Keywords:

Anti-oxidant
Broiler
Intestinal morphology
Rosemary
Thyme

Abstract

This study was aimed at determining the effects of dietary supplementation with thyme essential oil (TEO) and rosemary essential oil (REO) on blood parameters, the anti-oxidant metabolism in the liver, breast and drumstick muscle tissues, the morphology of the small intestine, and the myofibril structure of the superficial pectoral and biceps femoris muscles. For this purpose, 400 three-day-old male Ross 308 chicks were used. Five groups, each comprising 80 broilers, were established. The control group was fed on a basal diet alone and groups thyme-1, thyme-2, rosemary-1 and rosemary-2 received basal diets supplemented with 0.15 g kg^{-1} of TEO, 0.30 g kg^{-1} of TEO, 0.10 g kg^{-1} of REO and 0.20 g kg^{-1} of REO, respectively. The serum total cholesterol and low-density lipoprotein levels were decreased significantly in group thyme-1. Dietary TEO and REO significantly increased glutathione levels in all tissues. Drumstick catalase activity was significantly increased in groups thyme-1, thyme-2 and rosemary-2. Superoxide dismutase activity was significantly increased in the breast muscle of all groups that received dietary TEO and REO. Histomorphometrical analyses demonstrated that dietary supplementation with TEO and REO increased both crypt depth and villus height in the small intestine. In result, the tested doses of dietary TEO and REO were ascertained to improve the intestinal morphology and to increase the anti-oxidant metabolism mainly in the breast muscle, the drumstick muscle and liver.

Introduction

Since the prohibition of using of antibiotics as feed additives, multiple researches have been conducted on alternative non-residual and natural feed additives of plant origin that offer the potential of gaining recognition by consumers and being readily available at low cost. Some literature reports suggest that plant supplements prevent the establishment of pathogenic microorganisms in digestive tract, induce growth and function of intestinal villi, strengthen immunity, reduce stress and improve the antioxidant metabolism.[1,2] In this context, nowadays, plant essential oils and extracts have gained increased popularity as feed additives.[3] Two significant examples of these additives of plant origin are the aromatic plants thyme (*Thymus vulgaris* L.) and rosemary (*Rosmarinus officinalis* L.), both of which belong to the family Lamiaceae.[4]

Two major phenolic compounds found in thyme (*Thymus vulgaris* L.), namely, thymol and carvacrol, have various biological and protective effects related to their anti-oxidant, antibacterial, anti-inflammatory, immuno-stimulatory and intestinal morphology-improving activities.[4,5] Oxidative stressors, such as reactive oxygen species (ROS), cause major stress in all organisms. Animals have developed complicated intricate processes for protection against the toxic effects of ROS. Accordingly, endogenous enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) are involved in the anti-oxidant defense system.[3,6] In fact, previous studies have reported increased anti-oxidant enzyme activity in the serum of broiler chickens,[7] and the serum and liver of quails, and decreased levels of some lipid peroxidation end-products like malondialdehyde (MDA)[3] in response to the use of essential oils. Furthermore, it

***Correspondence:**

Recep Gümüş. DVM, PhD
Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Sivas Cumhuriyet University, Sivas, Türkiye
E-mail: rgumus@cumhuriyet.edu.tr

has been indicated that thyme essential oil significantly decreases blood parameters including triglyceride, total cholesterol and glucose levels.[8]

The main polyphenols found in rosemary (*Rosmarinus officinalis* L.) essential oil are diterpenes, carnosic acid, carnosol and rosmarinic acid, and these compounds have anti-oxidant, antimicrobial and anti-inflammatory activities.[9,10] Previous studies have demonstrated that rosemary essential oil, owing to its phenolic content, strongly affects the anti-oxidant defense system, scavenges free radicals and provides protection against oxidative stress.[9]

This study investigated the effects of different doses of thyme essential oil (TEO) and rosemary essential oil (REO), used as feed additives, on the blood profile, anti-oxidant metabolism in the liver, breast and drumstick muscle tissues, small intestinal morphology and myofiber structure of the superficial pectoral and biceps femoris muscles in broilers.

Materials and Methods

Animal material, experimental groups and feed. All experimental procedures were approved by the Local Ethics Board for Animal Experiments of Sivas Cumhuriyet University (Decision Number: 2020/342). Four hundred 3-day-old male broiler chicks (Ross 308) were randomly divided into five groups of 80 animals per treatment. Each group had 4 replications with 20 animals each. The control group was fed on a basal diet alone, and groups thyme-1, thyme-2, rosemary-1 and rosemary-2 received basal diets supplemented with 0.15 g kg^{-1} of TEO, 0.30 g kg^{-1} of TEO, 0.10 g kg^{-1} of REO and 0.20 g kg^{-1} of REO, respectively. The chicks were housed in floor cages measuring 150 × 150 × 85 cm until 42 days of age in a temperature-controlled room with a 23 hr constant light schedule. The ambient temperature was gradually decreased from 33.00 ˚C in the first week to 22.00 ˚C on day 14 and was kept constant afterwards. All animals were fed the same starter diet (from day 3 to day 21: crude protein 22.00%, metabolic energy 12.06 MJ kg^{-1}) and finisher diet (from day 22 to day 42: Crude protein 19.82%, metabolic energy 13.23 MJ kg^{-1}). The diets were formulated to meet the requirements of the NRC.[11] The nutritional composition of the diets was determined according to the AOAC[12].

Herbal additives. Thyme essential oil (Talya Herbal Products, Antalya, Türkiye) was composed of thymol 82.05%, carvacrol 5.48%, para-cymene 4.78%, beta-bisabolene 2.98%, trans-caryophyllene 2.54%, alpha-pinene 1.02%, alpha-terpinene 0.85%, beta-myrcene 0.30%. Rosemary essential oil (Talya Herbal Products Co. Ltd.) was composed of limonene 58.75%, terpinolene 9.53%, para-cymene 3.90%, 1.40 cineol 3.78%, eucalyptol 2.94%, geraniol 2.22%, citral 2.08%, Alpha-pinene 1.96%, isopropyl myristate 1.68%, gamma-terpinene 1.54%, beta- myrcene 0.96%, menthonomethene 0.64%, linalool 0.44%, geranyl nitrile 0.39%, camphene 0.36%, alpha-terpineol 0.34%, dihydromycenol 0.33%, menthone 0.30%, nerli nitrile 0.29%, alpha-phellandrene 0.27%, para-cymen-8-ol 0.24%, isoterpinolene 0.23%, epoxyterpinolene 0.16%, sabinene 0.13%, other 6.96%.

Collection of blood, liver, breast and drumstick tissue samples. The animals included in this study were slaughtered at a slaughterhouse located at a distance of 100 m to the pens in which they were raised. Thus, transport stress was eliminated. It was ensured that both the slaughterhouse and the materials used for slaughtering the animals were aseptic. Prior to slaughter, the knives used for the slaughter of each animal were washed in alcohol and passed through a flame for the sterilization of the outer surface. At the end of the fattening period, 60 broilers, including 12 animals from each group (three broilers per repetition) were slaughtered. Prior to slaughter, the broilers were fasted for 10 hr. The slaughtered animals were bled for 120 sec. Then, 5.00 mL blood samples were collected into tubes (Becton Dickinson, Franklin Lakes, USA) without an anti-coagulant and were centrifuged at 4.00 ˚C and 1,792 *g* for 10 min in a cooled centrifuge (Hettich 38R; Hettich, Tuttlingen, Germany). The harvested serum samples were stored at – 80.00 ˚C until use. Liver and breast (superficial pectoral muscle) and drumstick (biceps femoris) muscle samples were taken from the slaughtered animals. After being homogenized, the tissue specimens were frozen in liquid nitrogen at – 80.00 ˚C and stored until being used for biochemical analyses.

Determination of serum biochemical parameters. Serum glucose, total cholesterol (TC), triglyceride, high-density lipoprotein (HDL), low-density lipoprotein (LDL), uric acid, creatinine, total protein (TP), albumin, calcium (Ca^{+}), phosphorus (P) and magnesium (Mg) levels were determined with an automatic biochemistry analyzer (BS-200; Mindray, Shenzhen, China) using commercial test kits (Mindray Bio Medical Co. Ltd., Shenzhen, China).

Determination of anti-oxidant markers. The activities of the enzymes SOD and CAT and the levels of glutathione (GSH) and MDA in the liver, breast and drumstick muscle tissues were determined. To prepare the tissue homogenates, the liver samples and breast and drumstick muscle tissues were ground in liquid nitrogen using a tissuelyser device (Qiagen, Hilden, Germany) at a frequency of 1 sec^{-1} for 30 sec. These tissue samples were later used for biochemical analyses. All biochemical measurements were performed using commercial test kits (Cayman Chemical Co., Ann Arbor, USA) in an ELISA reader (μQuant; BioTek, Vermont, USA). The SOD, CAT, GSH and MDA measurements were made spectrophotometrically at absorbances of 440 - 460, 540, 405 - 414 and 530 - 550 nm, respectively. Total anti-oxidant status (TAS) and total oxidative status (TOS) measurements were performed using commercial test kits (Rel Assay Diagnostics, Gaziantep, Türkiye) in the

ELISA reader (BioTek). In tissue samples homogenized with appropriate homogenate buffers the following indices were measured: SOD (Cayman Chemical Co.), CAT (Cayman Chemical Co.), GSH (Cayman Chemical Co.), MDA (Cayman Chemical Co.), TAS (Rel Assay Diagnostics), and TOS (Rel Assay Diagnostics).

Histological analysis and tissue preparation. Samples of the superficial pectoral and biceps femoris muscles were taken from the slaughtered animals and fixed in 10.00% formaldehyde solution for 3 days. After fixation, the tissue samples were dehydrated in an increasing gradient of alcohol concentrations, cleared with xylene and embedded in paraffin blocks. The paraffin blocks were cut into 5.00-µm-thick transverse sections. For histological measurements, the sections taken from each block were stained with Crossman's modified triple stain and evaluated under a trinocular light microscope (Zeiss, Oberkochen, Germany). For histomorphometrical analyses, the height of the villus epithelium, crypt depth and villus height were estimated in sections of the duodenum, jejunum and ileum (Fig. 1).

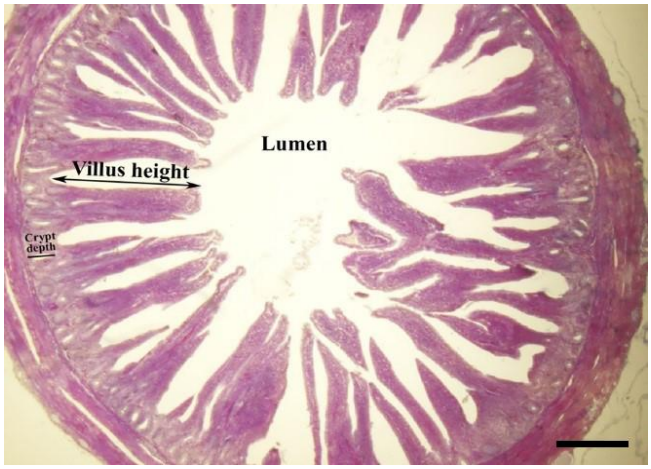

Fig. 1. Histomorphometrical analysis of the duodenum, jejunum and ileum sections of the small intestine for all groups (Crossman's modified triple staining; Bar = 200 µm).

Tissue stereological estimation of muscle transverse area using the nucleator method. The muscle sections of each animal were placed on a motor-driven stage attached to a microscope and projected onto the monitor via a camera at 200× magnification. The transverse areas of the muscle fascicules were estimated for each histological tissue section using the Stereo Investigator Software (version 9.0; Microbrightfield, Williston, USA) based on a previously described method[13] as shown in Figure 2.

Statistical analyses. The data obtained was assessed using the SPSS Software (version 20.0; IBM Corp., Armonk, USA). Differences between the groups were determined with the one-way analysis of variance test and Duncan's post-test. The biochemical data were expressed as mean ± standard error of mean (SEM). Histomorpho-metrical and stereological data were expressed as mean value ± SD. The threshold for establishing significance among the groups was set at $p < 0.05$ and $p < 0.01$.

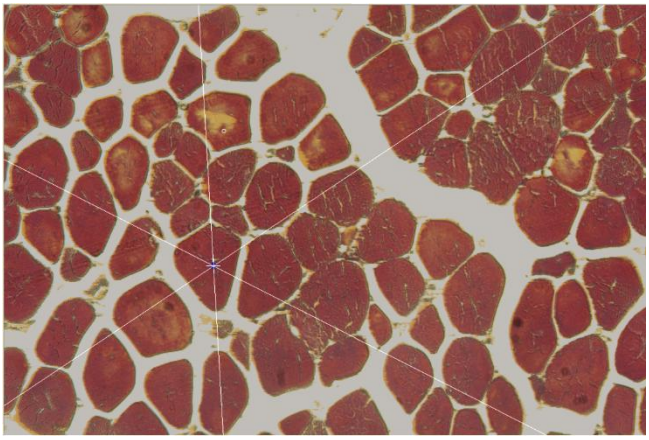

Fig. 2. Illustration of the myofiber transversal areas of the superficial pectoral and biceps femoris muscles of broiler chickens using the stereological nucleator method (Crossman's modified triple staining; 200×).

Results

Biochemical findings. The evaluation of the serum parameters demonstrated that TC, LDL, creatinine, TP and Ca^{+} levels had significantly decreased in only group thyme-1 ($p < 0.05$), and showed statistically insignificant alterations in the other groups ($p > 0.05$; Table 1). No significant difference was detected between the study groups for serum triglyceride, HDL, glucose, uric acid, albumin, P and Mg levels ($p > 0.05$; Table 1). When compared to the control group, all groups that received TEO and REO displayed mathematically higher CAT activity in the liver, yet this increase was statistically significant in only group rosemary-1 ($p < 0.05$; Table 2). Comparison to the control group revealed that liver SOD activity was significantly decreased in groups thyme-2 and rosemary-2 ($p < 0.01$), and GSH levels was significantly increased in groups thyme-1, thyme-2, rosemary-1 and rosemary-2 ($p < 0.05$; Table 2). MDA was measured at statistically similar levels in all of the groups ($p > 0.05$; Table 2). The study groups, which received dietary TEO and REO, displayed mathematically lower total anti-oxidant status (TAS) and total oxidant status (TOS) levels ($p > 0.05$). All groups that received dietary TEO and REO, presented significantly increased SOD activity and GSH levels ($p < 0.05$), and decreased MDA levels ($p < 0.01$) in the breast muscle (Table 2). On the other hand, breast muscle CAT activity was found to be statistically similar in all of the groups ($p > 0.05$). Moreover, it was ascertained that dietary supplementation with TEO and REO increased the oxidative stress index (OSI) and TOS levels, and decreased the TAS levels ($p < 0.05$; Table 2).

All tested doses of dietary TEO and REO increased the level of GSH and decreased the level of MDA in the drumstick muscle ($p < 0.01$; Table 2). Drumstick CAT activity was statistically similar in the control group and group rosemary-1, and was significantly increased in groups thyme-1, thyme-2 and rosemary-2 ($p < 0.01$; Table 2). Drumstick SOD activity was significantly decreased in group thyme-2 ($p < 0.05$), however, was similar in the other groups ($p > 0.05$; Table 2). The statistically similar TAS and OSI levels were observed in the control group and the

Table 1. Effects of basal diets supplemented with thyme and and rosemary essential oils on some serum biochemical parameters in broilers (n = 12). Data are presented as mean ± SEM.

Parameters	Groups				
	Control	Thyme-1	Thyme-2	Rosemary-1	Rosemary-2
Glucose (mg dL^{-1})	256.88 ± 4.87^{ab}	237.89 ± 12.09^{b}	244.44 ± 3.04^{b}	241.40 ± 2.83^{b}	269.33 ± 11.44^{a}
Cholesterol (mg dL^{-1})	124.75 ± 6.08^{a}	106.89 ± 7.37^{b}	121.11 ± 2.06^{ab}	115.40 ± 4.55^{ab}	126.22 ± 5.14^{a}
Triglyceride (mg dL^{-1})	38.00 ± 5.32	41.44 ± 6.27	37.00 ± 3.09	42.90 ± 2.83	48.33 ± 4.32
HDL (mg dL^{-1})	88.00 ± 3.85	76.00 ± 5.03	85.78 ± 1.27	80.70 ± 2.87	86.67 ± 3.37
LDL (mg dL^{-1})	54.63 ± 3.43^{a}	43.11 ± 3.60^{b}	50.89 ± 1.36^{ab}	48.20 ± 2.28^{ab}	54.22 ± 2.71^{a}
Uric acid (mg dL^{-1})	4.30 ± 0.29	3.80 ± 0.36	4.19 ± 0.32	3.82 ± 0.33	5.53 ± 1.18
Creatinine (mg dL^{-1})	0.09 ± 0.03^{a}	0.01 ± 0.01^{b}	0.04 ± 0.02^{ab}	0.05 ± 0.02^{ab}	0.09 ± 0.02^{a}
Total protein (g L^{-1})	3.16 ± 0.12^{a}	2.72 ± 0.16^{b}	2.98 ± 0.07^{ab}	3.04 ± 0.09^{ab}	3.24 ± 0.18^{a}
Albumin g L^{-1})	1.11 ± 0.05	1.00 ± 0.07	1.03 ± 0.02	1.05 ± 0.03	1.10 ± 0.06
Calcium (mg dL^{-1})	10.31 ± 0.36^{a}	9.17 ± 0.37^{b}	9.61 ± 0.18^{ab}	9.86 ± 0.36^{ab}	9.68 ± 0.31^{ab}
Phosphorus (mg dL^{-1})	6.11 ± 0.32	5.64 ± 0.11	5.61 ± 0.10	5.85 ± 0.12	5.77 ± 0.09
Magnesium (mg dL^{-1})	2.22 ± 0.18	2.11 ± 0.16	2.02 ± 0.05	2.06 ± 0.05	2.23 ± 0.07

Control: Basal diet alone, Thyme-1: Basal diet+0.15 g kg^{-1} of thyme essential oil (TEO), Thyme-2: Basal diet+0.30 g kg^{-1} of TEO, Rosemary-1: Basal diet+0.10 g kg^{-1} of rosemary essential oil (REO), and Rosemary-2: Basal diet+0.20 g kg^{-1} of REO. HDL: High density lipoprotein, LDL: Low density lipoprotein

^{ab} Superscript letters in each row indicate statistical difference at $p < 0.05$.

Table 2. Effects of diets supplemented with thyme and rosemary essential oils on antioxidant enzymes in liver, breast and drumstick muscles tissues in broiler (n = 12). Data are presented as mean ± SEM.

Parameters	Groups				
	Control	Thyme-1	Thyme-2	Rosemary-1	Rosemary-2
Liver tissue					
CAT (nmol g^{-1} protein)	4,905.24 ± 477.81^{b}	5,581.13 ± 269.66^{ab}	6,145.97 ± 650.05^{ab}	6,309.84 ± 387.57^{a}	5,353.46 ± 97.27^{ab}
SOD (U mg^{-1} protein)	42.29 ± 1.42^{A}	34.98 ± 2.96^{AB}	26.37 ± 3.41^{C}	35.11 ± 1.69^{AB}	29.04 ± 3.18^{BC}
MDA (nmol mg^{-1} protein)	5.23 ± 0.72	5.91 ± 0.68	5.60 ± 0.92	4.83 ± 0.48	5.34 ± 0.49
GSH (nmol mg^{-1} protein)	28.44 ± 1.16^{b}	36.41 ± 5.86^{a}	47.26 ± 8.06^{a}	36.53 ± 6.65^{a}	37.01 ± 6.59^{a}
TAS (mmol g^{-1} protein)	0.67 ± 0.03	0.63 ± 0.04	0.61 ± 0.06	0.55 ± 0.03	0.55 ± 0.03
TOS (µmol g^{-1} protein)	0.64 ± 0.07	0.59 ± 0.04	0.60 ± 0.08	0.65 ± 0.04	0.49 ± 0.03
OSI	0.09 ± 0.01^{b}	0.10 ± 0.01^{ab}	0.12 ± 0.02^{ab}	0.13 ± 0.01^{a}	0.09 ± 0.01^{b}
Breast muscle tissue					
CAT (nmol g^{-1} protein)	1,071.05 ± 128.54	1,187.74 ± 152.24	1,240.46 ± 242.41	1,160.22 ± 367.27	1,254.54 ± 95.66
SOD (U mg^{-1} protein)	22.32 ± 3.73^{b}	30.30 ± 0.97^{a}	27.39 ± 1.96^{a}	32.85 ± 1.66^{a}	32.17 ± 2.69^{a}
MDA (nmol mg^{-1} protein)	3.27 ± 0.62^{A}	1.03 ± 0.08^{B}	1.10 ± 0.10^{B}	1.45 ± 0.11^{B}	1.38 ± 0.20^{B}
GSH (nmol mg^{-1} protein)	6.02 ± 0.47^{b}	9.40 ± 1.01^{a}	13.08 ± 1.32^{a}	11.29 ± 3.60^{a}	9.09 ± 2.95^{a}
TAS (mmol g^{-1} protein)	0.10 ± 0.01^{c}	0.12 ± 0.01^{bc}	0.17 ± 0.00^{a}	0.15 ± 0.01^{ab}	0.17 ± 0.03^{a}
TOS (µmol g^{-1} protein)	0.34 ± 0.02^{a}	0.26 ± 0.01^{bc}	0.27 ± 0.01^{bc}	0.24 ± 0.02^{c}	0.30 ± 0.02^{ab}
OSI	0.30 ± 0.02^{a}	0.24 ± 0.01^{b}	0.18 ± 0.01^{c}	0.20 ± 0.01^{bc}	0.17 ± 0.03^{c}
Drumstick muscle tissue					
CAT (nmol g^{-1} protein)	1,268.72 ± 88.60^{b}	1,828.17 ± 122.39^{a}	1,833.20 ± 186.43^{a}	1,341.83 ± 65.74^{b}	2,397.08 ± 701.24^{a}
SOD (U mg^{-1} protein)	36.49 ± 3.09^{a}	31.42 ± 0.86^{ab}	29.59 ± 0.78^{b}	34.82 ± 1.80^{ab}	36.06 ± 2.55^{a}
MDA (nmol mg^{-1} protein)	3.46 ± 0.86^{A}	1.75 ± 0.11^{B}	2.24 ± 0.31^{B}	1.89 ± 0.24^{B}	1.46 ± 0.24^{B}
GSH (nmol mg^{-1} protein)	19.87 ± 4.53^{b}	32.21 ± 1.02^{a}	35.38 ± 3.43^{a}	30.08 ± 1.89^{a}	42.44 ± 8.96^{a}
TAS (mmol g^{-1} protein)	0.20 ± 0.02^{ab}	0.22 ± 0.02^{a}	0.15 ± 0.02^{ab}	0.13 ± 0.03^{b}	0.15 ± 0.02^{b}
TOS (µmol g^{-1} protein)	0.55 ± 0.04^{a}	0.50 ± 0.02^{ab}	0.43 ± 0.05^{b}	0.53 ± 0.03^{ab}	0.47 ± 0.04^{ab}
OSI	0.27 ± 0.03	0.24 ± 0.03	0.34 ± 0.05	0.36 ± 0.04	0.36 ± 0.06

Control: Basal diet alone, Thyme-1: Basal diet+0.15 g kg^{-1} of thyme essential oil (TEO), Thyme-2: Basal diet+0.30 g kg^{-1} of TEO, Rosemary-1: Basal diet+0.10 g kg^{-1} of rosemary essential oil (REO), and Rosemary-2: Basal diet+0.20 g kg^{-1} of REO. SOD: Superoxide dismutase, CAT: Catalase, GSH: Glutathione, MDA: Malondialdehyde, OSI: Oxidative stress index =TOS/(TAS × 10), TAS: Total antioxidant status, TOS: Total oxidative status.

$^{abc, ABC}$ Superscript lower and uppercase letters in the each row indicate a statistical difference at $p < 0.05$, and $p < 0.01$, respectively.

groups that received dietary TEO and REO. The TOS levels were significantly decreased in group thyme-2 ($p < 0.05$) and was mathematically decreased in the other groups ($p > 0.05$; Table 2).

Histomorphometrical findings. The histomorphometrical results for the epithelium height, crypt depth and villus height of all groups are presented in Table 3 and Figure 3. Histomorphometrical analyses demonstrated that the epithelium height, crypt depth and villus height of the duodenum were not different among the study groups ($p > 0.05$). The jejunal epithelium height were not different among the groups either ($p > 0.05$). However, jejunal crypt depths were significantly lower in the groups treated with both doses of thyme and rosemary oils compared to the control group ($p < 0.05$). Furthermore, the jejunal villus heights of the groups treated with both doses of thyme and rosemary oils were significantly higher than those of the control group ($p < 0.05$). The epithelium height and crypt depth of the ileum were similar in all groups ($p > 0.05$). However, the ileal villus height was significantly higher in the groups treated with both doses of thyme and rosemary oils, when compared to the control group ($p < 0.05$).

Stereological findings. The stereological estimations of the myofiber transverse areas of the superficial pectoral and biceps femoris muscles of all groups are shown in Table 4. The stereological estimations of the myofiber transverse areas of the superficial pectoral muscle were significantly higher in groups thyme-2 and rosemary-2, compared to the controls and groups rosemary-1 and thyme-1 ($p < 0.05$). The largest myofiber transverse area of the superficial pectoral muscle was determined in group thyme-2 ($p < 0.05$). The myofiber transverse area of the biceps femoris muscle was not significantly different between groups thyme-1 and rosemary-1 and the control group ($p > 0.05$). However, the transverse areas of the biceps femoris muscle were significantly larger in groups thyme-2 and rosemary-2 compared to the other groups ($p < 0.05$).

Table 4. Effect of diets supplemented with thyme and rosemary essential oils on estimation of the transverse areas (μm^2) of the superficial pectoral and biceps femoris muscles by the stereological nucleator method in broiler (n = 12). Data are presented as mean ± SEM.

Groups	Superficial pectoral muscle	Biceps femoris muscles
Control	1,445.11 ± 232.56[a]	2,462.85 ± 391.36[a]
Thyme-1	1,541.93 ± 393.68[a]	2,372.57 ± 302.78[a]
Thyme-2	1,805.93 ± 475.53[c]	2,747.98 ± 365.86[b]
Rosemary-1	1,421.89 ± 266.84[a]	2,395.38 ± 316.09[a]
Rosemary-2	1,655.71 ± 245.82[b]	2,645.45 ± 356.81[b]

Control: Basal diet alone, Thyme-1: Basal diet+0.15 g kg^{-1} of TEO, Thyme-2: Basal diet+0.30 g kg^{-1} of TEO, Rosemary-1: Basal diet+0.10 g kg^{-1} of REO and Rosemary-2: Basal diet+0.20 g kg^{-1} of REO.

[abc] Superscript letters in each column indicate statistical difference at $p < 0.05$.

Table 3. Effects of diets supplemented with thyme and rosemary essential oils on histomorphometrical measurements (µm) of intestinal sections in broiler. Data are presented as mean ± SEM (n = 12).

Groups	Duodenum			Jejunum			Ileum		
	Epithelium height	Crypt depth	Villus height	Epithelium height	Crypt depth	Villus height	Epithelium height	Crypt depth	Villus height
Control	30.43 ± 5.26	67.43 ± 7.16[b]	1,427.85 ± 112.34	31.71 ± 5.82	88.14 ± 12.33[a]	1,063.71 ± 159.22[b]	25.71 ± 5.47	70.14 ± 7.69	677.43 ± 99.05[b]
Thyme-1	32.01 ± 4.97	71.11 ± 8.03[a]	1,450.86 ± 103.72	28.14 ± 5.01	71.57 ± 12.75[b]	1,381.85 ± 169.82[a]	29.35 ± 6.34	73.71 ± 11.32	869.85 ± 119.41[a]
Thyme-2	31.28 ± 5.12	74.71 ± 9.35[a]	1,398.57 ± 124.23	31.29 ± 6.90	68.43 ± 10.67[b]	1,387.14 ± 88.53[a]	28.41 ± 7.24	70.52 ± 9.36	774.71 ± 97.34[a]
Rosemary-1	32.71 ± 7.23	70.71 ± 8.93[a]	1,466.29 ± 98.18	28.57 ± 5.08	64.14 ± 7.43[b]	1,559.71 ± 190.45[a]	26.65 ± 5.24	69.46 ± 9.24	756.45 ± 104.56[a]
Rosemary-2	30.28 ± 6.29	77.71 ± 9.71[a]	1,390.14 ± 134.32	28.25 ± 2.54	69.29 ± 19.88[b]	1,312.11 ± 179.84[a]	28.73 ± 6.12	73.36 ± 10.13	824.12 ± 116.42[a]

Control: Basal diet alone, Thyme-1: Basal diet+0.15 g kg^{-1} of thyme essential oil (TEO), Thyme-2: Basal diet+0.30 g kg^{-1} of TEO, Rosemary-1: Basal diet+0.10 g kg^{-1} of rosemary essential oil (REO), and Rosemary-2: Basal diet+0.20 g kg^{-1} of REO.

[ab] Superscript letters in each row indicate statistical difference at $p < 0.05$.

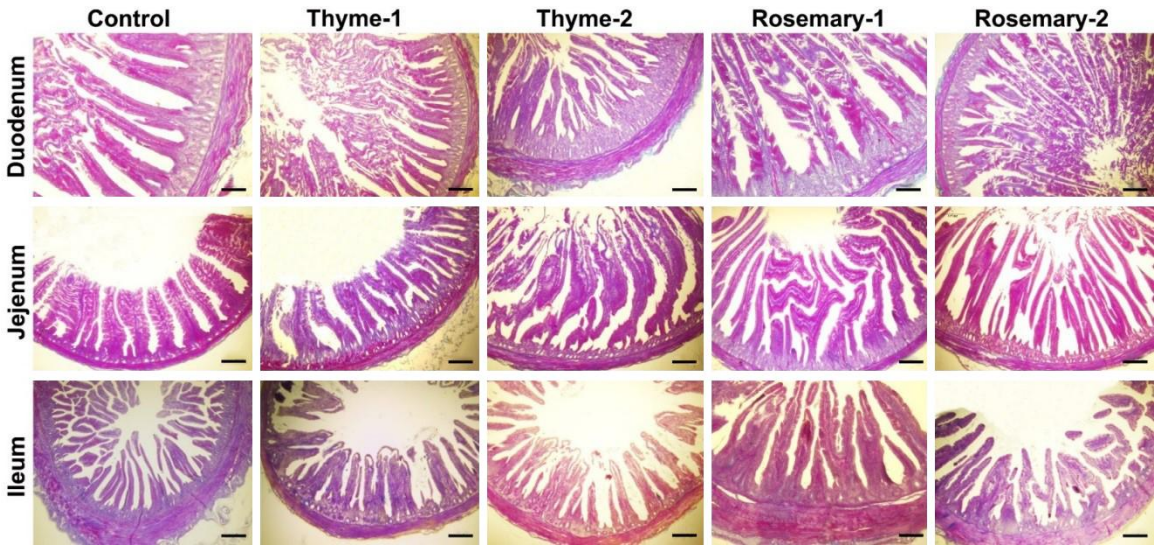

Fig. 3. Histologic illustration for basal diets supplemented with thyme and rosemary essential oils on histometric measurements of intestinal sections (Crosman's modified triple staining; Bars = 100 µm).

Discussion

Today, many countries have banned the use of antibiotics in feed, over increased concerns related to drug residues and antimicrobial resistance.[14] The prohibition of the use of antibiotics in poultry rations has obliged the search for favorable alternatives including phytogenic feed additives. In recent years, owing to their metabolic properties, essential oils have been suggested to be used for the protection of animal health and the increase of animal production.

Thymol and carvacrol, found in the composition of TEO, have a hypocholesterolemic effect which arises from reducing the activity of the enzyme 3-hydroxy 3-methyl-glutaryl coenzyme A (HMG-CoA) reductase involved in the synthesis of cholesterol.[3] The decreased total cholesterol levels detected in group thyme-1, in the present study, was in agreement with the literature reports referred to above as well as with the data of previous studies carried out in quails and broiler chickens.[8,15] Furthermore, the blood parameters determined in the groups other than group thyme-1 were observed to be similar and were also in agreement with previous results reported to have been obtained with dietary thyme and rosemary supplementation for cholesterol, triglyceride, HDL, LDL, glucose, total protein, uric acid, albumin, Ca^{+} and P levels.[15-17] There are also reports indicating increased serum triglyceride, cholesterol, LDL and HDL levels to be associated with the use of rosemary oil.[18] In a previous study carried out in quails, dietary supplementation with 150.00, 300 and 450 mg kg^{-1} of TEO was not affected serum cholesterol, triglyceride, HDL, glucose, total protein, Ca^{+}, P and Fe levels, however, decreased creatinine and LDL levels and increased Mg levels.[3] Along with the present study, researchers conducted to date showed certain differences which were attributed to environmental conditions, the amount of active substances found in plant oils and the usage of these oils.

The phenolic content of plants was indicated to be positively correlated with the anti-oxidant activity of plants. It is known that plants such as thyme and rosemary prevent free radical generation and induce the anti-oxidant system by means of the high phenolic compounds they contain (i.e., polyphenols).[3,19]

The GSH, as an integral component of the anti-oxidant defense system, plays an important role in preventing damage to several beneficial molecules and components and also constitutes the substrate of GSH-dependent enzymes including GPx and glutathione S-transferase (GST).[3] By means of the catalytic effect of GPx, glutathione detoxifies lipid peroxides and hydrogen peroxide (H_2O_2) and scavenges singlet oxygen ($^1O_2$) and the hydroxyl radical (OH).[20] Alterations that occur in GSH levels during oxidative stress may alter GPx activity, thus, may bring about alterations in the scavenging of free radicals and hydro-peroxides. In a previous study carried out in broiler chickens, it was determined that thymol and carvacrol prevented decrease and even caused an increase in GPx activity during oxidative stress.[15] Carvacrol has also been reported to be involved in the elevation of GSH levels.[21] In a study conducted by Hashemipour *et al.* the results obtained for serum and liver GSH-Px activity in broiler chickens were attributed to the active substance content of phytogenic products and the improvement achieved in the anti-oxidant status through increased anti-oxidant enzyme activity owing to the antioxidant effects of thymol and carvacrol.[22] In another study carried out in broiler chickens, animals supplemented with dietary oregano essential oil were determined to display increased serum, duodenal and jejunal GSH-Px activity.[23] Furthermore, increased anti-

oxidant activity was determined in the serum of broiler chickens given 7.00 g kg^{-1} of rosemary powder,[24] and the muscle tissue of broiler chickens given 50.00 - 100 mg kg^{-1} of oregano essential oil.[25] Another literature report indicated that TEO significantly increased liver GSH levels.[26] In agreement with the previous studies referred to above, the present study demonstrated that in the groups that received dietary TEO and REO, the GSH levels was significantly increased in the liver as well as in the breast and drumstick muscles. Based on the literature data referred to above, it was evident that free radical-scavenging enzymes such as GPx and GST, did not show any activity in the absence of GSH which would lead to the cellular accumulation of peroxides and various damages. Thus, the incorporation of TEO and REO into rations would provide benefits in terms of the prevention of such oxidative damages.

Anti-oxidant enzyme activity and oxidative product concentrations are important for the assessment of the oxidant status of animals. The SOD which constitutes the first line of defense against reactive oxygen species is an anti-oxidant enzyme that catalyzes the superoxide radical into hydrogen peroxide and molecular oxygen.[20] Subsequently, hydrogen peroxide is scavenged by CAT or GPx.[27] In a previous study carried out in broiler chickens, dietary supplementation with oregano essential oil was determined to increase serum, duodenal and jejunal SOD activity.[23] Furthermore, in another study in broiler chickens, Hashemipour *et al.* attributed the results they obtained for serum and liver SOD activity to the active substance content of phytogenic products and the improvement achieved in the anti-oxidant status of the animals through increased anti-oxidant enzyme activity induced by the anti-oxidant effects of thymol and carvacrol.[22] Dietary TEO has been reported to positively affect liver SOD activity in quails.[3] Positive effects have been reported on serum anti-oxidant activity with the use of 7.00 g kg^{-1} of rosemary powder[24] and on meat anti-oxidant activity with the use of 50.00 - 100 mg kg^{-1} of oregano essential oil.[25] Similarly, the present study demonstrated that breast muscle SOD activity was significantly increased with dietary TEO and REO, and this suggested that these supplements had positive effects on the anti-oxidant metabolism.

The enzyme catalase is the second line of anti-oxidant defense and prevents the accumulation of hydrogen peroxide by converting it into water and oxygen.[28] An increased level of catalase is an indicator of a stimulated anti-oxidant system.[19] It has been reported that, the dietary supplementation of broiler chickens with 100 and 200 mg kg^{-1} of rosemary extract increased serum CAT activity.[29] Furthermore, in a study in quails, doses of 150 and 300 mg kg^{-1} of dietary TEO were determined to significantly increase serum and liver CAT activity.[3] In agreement with these literature reports, the present study showed that dietary supplementation with TEO and REO had significantly increased CAT activity, in particular in drumstick muscles. However, in contrast to these reports, Drozd *et al.* reported that the incorporation of TEO and REO into quail rations did not have effect on liver CAT activity.[30]

During the initial stage of lipid peroxidation, due to the hydroxyl groups in the environment serving as hydrogen donors for intermediate radicals, the free radicals generated at excessive levels are scavenged by phenolic compounds such as thymol and carvacrol found in dietary TEO.[31] MDA is an end-product of the peroxidation of polyunsaturated fatty acids[26] and carvacrol has been reported to decrease MDA levels.[21] In their study in broiler chickens, Zidan *et al.* found that dietary supplementation with 200 and 400 mg kg^{-1} of thymol significantly decreased MDA levels in breast meat and drumsticks.[32] Another study carried out in broiler chickens showed that 100 and 200 mg kg^{-1} of dietary TEO significantly decreased MDA levels in breast meat and drumsticks.[33] The incorporation of 25.00 g kg^{-1} of rosemary leaves into broiler rations has been reported to decrease breast meat MDA levels.[34] Furthermore, the addition of TEO to drinking water has been reported to significantly decrease liver MDA levels in rabbits.[26] In agreement with the literature reports referred to above, the present study revealed significantly decreased MDA levels in breast and drumstick muscles in the groups that received dietary TEO and REO. This suggested that dietary TEO and REO protected tissues against oxidative damage.

The morphological measurements of the small intestine of the broiler chickens included in the present study are presented in Table 3. Histomorphometrical analyses showed that, when compared to the control group, all of the tested doses of dietary TEO and REO had significantly increased crypt depth in the duodenum and the villus height in the jejunum and ileum. In agreement with our results, previous studies showed that dietary supplementation with essential oils increased duodenal villus height in laying hens,[1] and jejunal villus height in broiler chickens.[35] Both villus height and crypt depth are major indicators of digestive health in poultry and are directly related to the absorption capacity of the intestinal mucosa.[36] Higher villus-crypt ratios point out to higher digestion and absorption capacities.[37]

Muscle weight is described as the function of the total number of fibrils, fibril cross-section and fibril length.[13] It has been indicated that the high body weight of the genetically superior modern poultry breeds raised today are related to increased muscle yield, particularly of the major pectoral muscle which could be linked to the increased size of muscle fibrils.[38] In broiler chickens, the major pectoral muscle is almost completely composed of rapid glycolytic type IIB fibers.[39,40] In fast-growing broiler chickens with high production yields, type IIb fibers have a larger diameter thus, are hypertrophic in comparison with

the muscle fibers of slower-growing broiler chickens and laying hens.[39] A previous study in broiler chickens demonstrated that extending the slaughter age from 42 days to 56 days increased the number of myofibrils in the superficial pectoral and biceps femoris muscles.[13] In the present study, the number of myofibrils in the *superficial pectoral* and biceps femoris muscles were determined to be significantly higher in groups thyme-2 and rosemary-2. These results suggested that dietary supplementation with 300 mg kg^{-1} of TEO and 200 mg kg^{-1} of REO produced positive effects, however, further studies are needed to determine the quantitative alterations (volume and number of muscle fibers) and qualitative alterations (muscle fiber traits) that may occur in muscle tissue.

In conclusion, the incorporation of TEO and REO into broiler rations showed significant effects on the anti-oxidant metabolism of primarily breast muscle tissue and secondarily drumstick muscles and liver, and significantly decreased the MDA levels, an indicator of tissue lipid per-oxidation, in both breast and drumstick muscles. Another important finding related to the anti-oxidant defense system was the GSH levels, critical to the protection of tissues against the detrimental effects of free radicals that was increased in all tissues. The significant decrease observed in total cholesterol and LDL levels in association with dietary supplementation with 0.15 g kg^{-1} of TEO was interesting since a decrease in these parameters was critical to the prevention of cardiovascular diseases. It was also observed that dietary TEO and REO improved the morphology of the small intestine and the transverse sections of breast and drumstick muscle myofibrils. Based on these results, it was suggested that thyme essential oil (0.15 and 0.30 g kg^{-1}) and rosemary essential oil (0.10 and 0.20 g kg^{-1}) could be used as feed additives for broiler chickens.

Acknowledgments

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

References

1. Arslan C, Pirinç A, Eker N, et al. Dietary encapsulated essential oil mixture influence on apparent nutrient digestibility, serum metabolic profile, lymphocyte histo-chemistry and intestinal morphology of laying hens. Anim Biosci 2022; 35(5): 740-751.
2. Ghiasvand AR, Khatibjoo A, Mohammadi Y, et al. Effect of fennel essential oil on performance, serum biochemistry, immunity, ileum morphology and microbial population, and meat quality of broiler chickens fed corn or wheat-based diet. Br Poult Sci 2021; 62(4): 562-572.
3. Gumus R, Ercan N, Imik H. The effect of thyme essential oil (*Thymus vulgaris*) added to quail diets on performance, some blood parameters, and the antioxidative metabolism of the serum and liver tissues. Braz J Poult Sci 2017; 19(2): 297-304.
4. Emami Bistgani Z, Hashemi M, DaCosta M, et al. Effect of salinity stress on the physiological characteristics, phenolic compounds and antioxidant activity of *Thymus vulgaris L.* and *Thymus daenensis* Celak. Ind Crops Prod 2019; 135: 311-320.
5. Seifeldeen AR, Youssef HM, Arafa MM, et al. Effect of thyme and rosemary on chemical composition of some meat products. Alex J Vet Sci 2021; 69(2): 59-67.
6. Oblakova M, Nikolova G, Mincheva N, et al. Influence of some herbal essential oils on productivity, natural humoral immunity and oxidative status in broiler turkeys. J Hyg Eng Des 2021; 35: 183-193.
7. Talebi E, Rowghani Haghighi Fard E, Navabi M, et al. Evaluating the effect of two types of thyme essential oils (*Zataria multiflora & Ziziphora clinopodioides* Lam) on some productive traits and blood parameters in broilers. Poult Sci J 2021; 9(1): 107-119.
8. Khaksar V, van Krimpen M, Hashemipour H, et al. Effects of thyme essential oil on performance, some blood parameters and ileal microflora of Japanese quail. J Poult Sci 2012; 49(2): 106-110.
9. Ruff J, Tellez G Jr, Forga AJ, et al. Evaluation of three formulations of essential oils in broiler chickens under cyclic heat stress. Animals (Basel) 2021; 11(4): 1084. doi:10.3390/ani11041084.
10. Yang M, Yin Y, Wang F, et al. Effects of dietary rosemary extract supplementation on growth performance, nutrient digestibility, antioxidant capacity, intestinal morphology, and microbiota of weaning pigs. J Anim Sci 2021; 99(9): skab237. doi:10.1093/jas/skab237.
11. NRC. National Research Council, Nutrient requirements of poultry. 9th rev. ed. National Academy Press, Washington DC, 1994.
12. Cunniff P. Official methods of analysis of the AOAC international.16th ed. Arlington USA: Association of Official Analytical Chemists 1995; 2201-3301.
13. Çoban Ö, Laçin E, Aksu M, et al. The impact of slaughter age on performance, carcass traits, properties of cut-up pieces of carcasses, and muscle development in broiler chickens. European Poult Sci 2014; 78. doi:10.1399/eps.2014.60.
14. Diarra MS, Malouin F. Antibiotics in Canadian poultry productions and anticipated alternatives. Front Microbiol 2014; 5: 282. doi:10.3389/fmicb.2014.00282.
15. Saadat Shad H, Mazhari M, Esmaeilipour O, et al. Effects of thymol and carvacrol on productive performance, antioxidant enzyme activity and certain blood metabolites in heat stressed broilers. Iran J Appl Anim Sci 2016; 6(1): 195-202.
16. Abdel-Wareth AA. Effect of dietary supplementation of

thymol, synbiotic and their combination on performance, egg quality and serum metabolic profile of Hy-Line Brown hens. Br Poult Sci 2016; 57(1): 114-122.
17. Abd El-Latif AS, Saleh NS, Allam TS, et al. The effects of rosemary (*Rosemarinus afficinalis*) and garlic (*Allium sativum*) essential oils on performance, hematological, biochemical and immunological parameters of broiler chickens. Br J Poult Sci 2013; 2(2): 16-24.
18. Rostami H, Seidavi A, Dadashbeiki M, et al. Effects of different dietary *Rosmarinus officinalis* powder and vitamin E levels on the performance and gut gross morphometry of broiler chickens. Braz J Poult Sci 2015; 17: 23-30.
19. Yousefi M, Hoseini SM, Vatnikov YA, et al. Rosemary leaf powder improved growth performance, immune and antioxidant parameters, and crowding stress responses in common carp (*Cyprinus carpio*) fingerlings. Aquaculture 2019; 505: 473-480.
20. Sen S, Chakraborty R, Sridhar C, et al. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. Int J Pharm Sci Rev Res 2010; 3(1): 91-100.
21. Luaibi NM, Mousa AH. A study effect of thyme on biochemical and histological changes in liver of male rats. Adv Environ Biol 2016; 10(10): 217-227.
22. Hashemipour H, Kermanshahi H, Golian A, et al. Effect of thymol and carvacrol feed supplementation on performance, antioxidant enzyme activities, fatty acid composition, digestive enzyme activities, and immune response in broiler chickens. Poult Sci 2013; 92(8): 2059-2069.
23. Zhang LY, Peng QY, Liu YR, et al. Effects of oregano essential oil as an antibiotic growth promoter alternative on growth performance, antioxidant status, and intestinal health of broilers. Poult Sci 2021; 100(7): 101163. doi:10.1016/j.psj.2021.101163.
24. Soltani M, Tabeidian SA, Ghalamkari G, et al. Effect of dietary extract and dried areal parts of *Rosmarinus officinalis* on performance, immune responses and total serum antioxidant activity in broiler chicks. Asian Pac J Trop Dis 2016; 6(3): 218-222.
25. Botsoglou NA, Florou-Paneri P, Christaki E, et al. Effect of dietary oregano essential oil on performance of chickens and on iron-induced lipid oxidation of breast, thigh and abdominal fat tissues. Br Poult Sci 2002; 43(2): 223-230.
26. El-Kaiaty AM, El-Moghazy GM, El-Manylawi MAF, et al. Impact of thyme oil and *Lactobacillus acidophilus* as natural growth promoters on performance, blood parameters and immune status in growing rabbits. World Vet J 2020; 10(1): 01-11. doi: 10.36380/scil.2020.wvj1.
27. Young IS, Woodside JV. Antioxidants in health and disease. J Clin Pathol 2001; 54(3): 176-186.
28. Kaushal J, Mehandia S, Singh G, et al. Catalase enzyme: Application in bioremediation and food industry. Biocatal Agric Biotechnol 2018; 16: 192-199.
29. Yildirim BA, Tunc MA, Gül M, et al. The effect of rosemary (*Rosmarinus officinalis* L.) extract supplemented into broiler diets, on performance and blood parameters. GSC Biol Pharm Sci 2018; 02(03): 001-009. doi: 10.30574/gscbps.2018.2.3.0057.
30. Drozd R, Rybarczyk A, Wasak A, et al. Influence of flaxseed combined with thyme, rosemary, and sage leaves as fodder additives on antioxidant status in the liver of Japanese quail. Turk J Vet Anim Sci 2016; 40(3): 359-364.
31. Yanishlieva NV, Marinova EM, Gordon MH, et al. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem 1999; 64(1): 59-66.
32. Zidan DE, Kahilo KA, El-Far AH, et al. Ginger (*Zingiber officinale*) and thymol dietary supplementation improve the growth performance, immunity and antioxidant status in broilers. Glob Vet 2016; 16(6): 530-538.
33. BölükbaşiSC, Erhan MK, Özkan A. Effect of dietary thyme oil and vitamin E on growth, lipid oxidation, meat fatty acid composition and serum lipoproteins of broilers. S Afr J Anim Sci 2006; 36(3): 189-196.
34. Loetscher Y, Kreuzer M, Messikommer RE. Oxidative stability of the meat of broilers supplemented with rosemary leaves, rosehip fruits, chokeberry pomace, and entire nettle, and effects on performance and meat quality. Poult Sci 2013; 92(11): 2938-2948.
35. Heydarian M, Ebrahimnezhad Y, Meimandipour A, et al. Effects of dietary inclusion of the encapsulated thyme and oregano essential oils mixture and probiotic on growth performance, immune response and intestinal morphology of broiler chickens. Poult Sci J 2020; 8(1): 17-25.
36. Buddle JR, Bolton JR. The pathophysiology of diarrhoea in pigs. Pig News Inf 1992; 13(1): 41N-45N.
37. da Silva MA, de Sousa Pessotti BM, Zanini SF, et al. Intestinal mucosa structure of broiler chickens infected experimentally with *Eimeria tenella* and treated with essential oil of oregano. Cienc Rural 2009; 39(5): 1471-1477.
38. MacRae VE, Mahon M, Gilpin S, et al. Skeletal muscle fibre growth and growth associated myopathy in the domestic chicken (*Gallus domesticus*). Br Poult Sci 2006; 47(3): 264-272.
39. Remignon H, Gardahaut MF, Marche G, et al. Selection for rapid growth increases the number and the size of muscle fibres without changing their typing in chickens. J Muscle Res Cell Motil 1995; 16(2): 95-102.
40. Sihvo HK, Airas N, Lindén J, et al. Pectoral vessel density and early ultrastructural changes in broiler chicken wooden breast myopathy. J Comp Pathol 2018; 161: 1-10. doi: 10.1016/j.jcpa.2018.04.002.