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Research Article

The Effects of the Supplementation of Lamb Rations with Oregano Essential Oil on the Performance, Some Blood Parameters and Antioxidant Metabolism in Meat and Liver Tissues [1]

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Abstract

In this study, the effects of oregano essential oil, on performance, some blood parameters, the antioxidant metabolism such as lipid oxidation (LPO) and glutathione (GSH) levels with superoxide dismutase (SOD) and catalase (CAT) enzyme activities in the liver and the *Musculus longissimus dorsi* (meat) tissue were investigated. The study consisted of three groups; the control group fed a basal ration, the OEO1 group fed a basal ration added 200 mg/kg Orego-Stim and the OEO2 group fed a basal lamb ration added 400 mg/kg Orego-Stim. The groups did not differ for performance parameters. In groups control and OEO2, serum calcium and potassium levels were significantly higher than the group of OEO1 ($P<0.05$). However, the serum magnesium level were significantly higher in OEO1 group ($P<0.05$). While there are no meaningful changes on superoxide dismutase activity, LPO and GSH rates with CAT activity had significant changes in liver tissue ($P<0.05$). LPO and GSH levels with SOD and CAT enzyme activities were significantly affected in *M. longissimus dorsi* tissue ($P<0.05$). As a result, while oregano essential oil did not affect the performance parameters and lipid profile, it significantly affected the antioxidant metabolism in meat and liver tissues.

Keywords: *Oregano essential oil, Lamb, Performance, Antioxidant enzyme, Liver, Meat*

Kuzu Rasyonuna İlave Edilen Kekik Yağının Performans, Bazı Kan Parametreleri İle Et ve Karaciğer Dokularında Antioksidan Metabolizma Üzerine Etkisi

Özet

Bu çalışmada kekik yağının performans ve bazı kan parametreleri ile karaciğer ve *Musculus longissimus dorsi* dokularında lipid oksidasyon (LPO) ve glutatyon (GSH) oranları ile süperoksit dismutaz (SOD) ve katalaz (CAT) enzim aktivitelerinin antioksidan metabolizma üzerine etkileri araştırılmıştır. Çalışma üç grup halinde yürütülmüştür. Gruplandırma ise bazal rasyon verilen kontrol grubu, bazal rasyona ilave olarak 200 mg/kg Orego-Stim verilen OEO1 grubu ve bazal rasyona ilave olarak 400 mg/kg Orego-Stim verilen OEO2 grubu şeklinde yapılmıştır. Grupların performans parametreleri arasında fark bulunmamıştır. Kontrol ve OEO2 gruplarının serum kalsiyum ve potasyum seviyeleri OEO1 grubuna göre yüksek bulunmuştur ($P<0.05$). Ancak serum magnezyum seviyesi OEO1 grubunda daha yüksek olduğu tespit edilmiştir ($P<0.05$). Karaciğer dokusunda süperoksit dismutaz aktivitesine önemli bir etkisi olmazken, LPO ve GSH oranları ile CAT aktivitesini önemli olarak değiştirmiştir ($P<0.05$). *M. longissimus dorsi* dokusunda LPO ve GSH oranları ile SOD ve CAT enzim aktivitelerinin önemli oranda etkilendiği belirlenmiştir ($P<0.05$). Sonuç olarak kekik yağının performans parametreleri ve lipid profilini etkilemediği, karaciğer ve kas dokularında antioksidan metabolizma üzerine önemli etkilerinin olduğu tespit edilmiştir.

Anahtar sözcükler: *Kekik yağı, Kuzu, Performans, Antioksidan enzim, Karaciğer, Et*

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INTRODUCTION

Feed additives are used to ensure healthy growth and increase the yield and quality of animals. Therefore, for many years antibiotics have been used as feed additives. However, the use of these substances as feed additives bearing a risk to human health due to the development of resistance in microorganisms against antibiotics. That is why, the practice of using vitamins and plant extracts as feed additives has started to become commonplace [1-3]. Plant extracts are shown to stimulate the secretion of enzymes in the digestive system and regulate the microbial eco-system the effect on the performance of animals [4]. Already, plant extracts are used commonly in the pharmaceutical, cosmetics, perfumery, and food sectors, owing to their various biological effects. Both thyme itself and its extracts (thyme essential oil) contain substances that induce more than 60 effects, such as antiseptic, antioxidant, antimicrobial and aroma-regulating effects. It has been reported that thyme contains phenols, thymol (68.1%), carvacrol (3.5%), monoterpene hydrocarbons, p-cymene (11.2%) and γ-terpinene (4.8%) [5].

The free radicals occur as a result of metabolic activities and they generate oxidative stress and create damage to cells. Conversely, cells have several mechanisms that are capable of repairing harmful effects or are capable of preventing the effects harmful effects of reactive oxygen species (ROS). By the enzymes as primarily [SOD, CAT, myeloperoxidase (MPx), glutathione S-transferase (GST), glutathione reductase (GR) and glutathione peroxidase (GPx)] and by secondary antioxidant vitamins, GSH, plant extracts and many macro and micro molecules have reduced the formed damage by free radicals and is kept low and the specific concentrations of free radicals in cells [6].

The antioxidant substances in the composition of oregano were determined in the laboratory analysis [7]. These substances explain the effect on the living organisms given to animals and full disclosure of the antioxidant metabolism in cells. Unal and Kocabagli [8] reported that, dietary supplementation of oregano to lambs' ration had no effects on performance parameters. Information related to oregano growth promoting effects when added to sheep diets is scarce [9].

In this study, the effect of Orego-Stim was determined on the performance and some blood parameters in the ruminants to the determined details of the effect on the antioxidant metabolism through LPO and GSH rates with SOD and CAT activities in the meat and liver tissue.

MATERIAL and METHODS

Animals, Experimental Design and Feed

The study was conducted at the Animal Husbandry Research and Application Unit of Atatürk University, Faculty of Veterinary Medicine. 24 male Akkaraman lambs (which were weaned when they were 3 months old on average) were divided to 3 groups as control and two treatment (OEO1 and OEO2) groups, each consisting of four replicates of eight lambs. The lambs two animals were allocated to each compartment measuring 280×200×120 cm. This study was approved by the ethic committee of Faculty of Veterinary Medicine in Ataturk University [Decision No: 2010/700 (Decision No: 2006/5g)].

In this study the control group was fed a basal lamb diet, the OEO1 group was fed a basal lamb diet + 200 mg/kg Orego-Stim and the OEO2 group was fed a basal lamb diet + 400 mg/kg Orego-Stim *(Table 1)*. The Orego-Stim was added in place of bran (Orego-Stim was obtained from Ecopharm Hellas S.A., Kilkis, Greece. Orego-Stim was contains 5% essential oil of *Origanum vulgare subsp. Hirtum* plants and 95% natural feed grade inert carrier). Concentrate feed was specially produced on a monthly basis in a factory, in order to prevent the spoiling of oregano essential oil while it was stored.

The animals were provided with pre-weighed feed, twice a day at 08:00 am and 05:00 pm. The study lasted for 70 days (14 days were used as adaptation followed by

Table 1. *Composition of lamb diets used in the study, %*

Ingredient	Groups		
	Control	OEO1	OEO2
Barley	30	30	30
Corn	20	20	20
Sunflower seed meal	13.33	13.33	13.33
Cotton seed meal	13.2	13.2	13.2
Bran	9.70	9.68	9.66
Corn gluten	5	5	5
DDGS[1]	5	5	5
Marble powder	2.05	2.05	2.05
Molasses	1.12	1.12	1.12
Salt	0.5	0.5	0.5
Vitamin mineral premix[2]	0.1	0.1	0.1
Orego-Stim[3]	-	0.02	0.04
Rates of nutrient, %			
Crude protein	18.53	18.52	18.51
Crude fibre	12.74	12.74	12.73
Crude ash	7.2	7.2	7.2
Acid detergent fibre	13.67	13.67	13.67
Neutral detergent fibre	27.3	27.3	27.3

[1] DDGS: Dried Distillers Grains with Solubles; [2] The vitamin & mineral premix provided the following (per kg): 4.000.000 IU vitamin A, 800.000 IU vitamin D_3, 5.000 IU vitamin E, 400 mg vitamin B_2, 2 mg vitamin B_{12}, 5.000 mg vitamin PP, 1.000 mg D-pantothenic acid, 20.000 mg choline, 50 mg Co, 5.400 mg Fe, 185 mg I, 6.900 mg Mn, 800 mg Cu, 6.400 mg Zn, 14 mg Se; [3] Orego-Stim was contains 5% essential oil of Origanum vulgare subsp. Hirtum plants and 95% natural feed grade inert carrier

56 days for data collection). The daily amount of roughage provided to the lambs was 125 g of wheat straw per animal (the chemical composition of wheat straw on the basis of dry matter content was as follows: crude protein: 3.1, crude ash: 6.69, Neutral Detergent Fiber (NDF): 77.45, Acid Detergent Fiber (ADF): 50.32) Concentrate feed and water was supplied *ad libitum* during the trial.

Feed Analysis

The rations fed to the animals were formulated in accordance with the recommendations of the NRC [10] *(Table 1)*. The raw feed materials used in the study were crude ash, crude protein, crude oil analysis according to the Weende Analysis System of AOAC [11] and if the crude cellulose according to Crampton and Maynard [12]. NDF and ADF analysis were according to Soest and Robertson [13] with Goering and Van Soest [14].

Determination of Performance Parameters

The body weight of the animals was measured at the beginning of the trial, and on days 14, 28, 42 and 56 (final) in the morning, before they were given feed. Daily feed intake was determined by weighing the concentrate remaining in the feeders prior to morning feeding. As the subgroups included two animals, individual daily feed intake was calculated by dividing the daily feed intake values by two. Feed efficiency was calculated as the proportion of daily feed intake to daily weight gain (kg/kg).

Collection of Blood, Liver and Muscle Tissues Samples

Blood samples were collected from jugular vein of the twenty-four animals on the last day of the trial, prior to morning feeding, for use in biochemical analyses. The blood samples, collected in volumes of 5 cc into dry tubes (Becton Dickinson Co. USA),were centrifuged at +4°C and 4.000 rpm for 10 min in a cooled centrifuge (Hettich 38R, Hettich Zentrifugen, Tuttlingen, Germany). The harvested serum samples were stored at -80°C until use.

At the end of the study 6 animals were slaughtered from each group. Longissimus muscle and liver tissues were homogenised using liquid nitrogen and then stored at -80°C until the biochemical investigations.

Biochemical Analyses

- Determination of Serum Biochemical Parameters

Serum concentrations of glucose, urea, uric acid, triglyceride, calcium, phosphorus, magnesium, sodium, potassium and chlorine and were measured with an automatic analyzer using commercial test kits (Cobas 8000 Analyzer, Roche).

- *Determination of the Lipid Profile (Thin Layer Chromatography)*

Thin layer chromatography was performed using a 20 x 10 cm Silica Gel 60 F254 High Performance Thin Layer Chromatography (HPTLC) Plate. 1 ml of serum homogenate or serum was added 1 ml of n-hexane/iso-propanol (2:1 (v/v)) mixture in a tube. After being mixed thoroughly, the tube content was maintained for 10 min and mixed once again. This procedure was repeated for a further two times [15]. Subsequently, the tubes were centrifuged at 8.000 rpm for 10 min and the upper phases were loaded onto the HPTLC plate. The plates were developed in a hexane: diethyl ether: formic acid (80:20:2 (v/v/v) mixture for 15 cm and then dried. The spots on the dry plates were made visible by means of treatment with 3% $CuSO_4$ in 8% phosphoric acid followed by burning on hot plates [16]. The hydrocarbon, triacylglycerol, steroid and polar lipid parameters were measured for lipid profile.

- Antioxidant Enzymes

Superoxide dismutase and CAT enzyme activities and the amounts of GSH and LPO in the tissues were determined. To prepare the tissue homogenates, the muscle tissues were ground with liquid nitrogen in a mortar. 0.5 g of tissue was then treated with 4.5 ml of appropriate buffer (SOD: pH 7.4/0.2mMTris-HCl buffer, CAT: pH 7/50 mM phosphate buffer, GSH: pH 7.4/50mMTris-HCl buffer, LPO: 10% KCl solution). The mixtures were homogenised on ice using an ultra-turrax homogeniser for 15 min. Homogenates were filtered and centrifuged at 4°C. These supernatants were then used for biochemical measurements. All biochemical measurements were carried out using a UV-Vis spectrophotometer.

- SOD Activity

SOD activity was measured according to Sun et al.[17]. SOD estimation was based on the generation of superoxide radicals produced by xanthine and xanthine oxidase, which reacts with Nitro Blue Tetrazolium (NBT) to form formazan. SOD activity was then measured at 560 nm by the degree of inhibition of this reaction, and was expressed as mmol/min/mg of tissue.

- CAT Activity

Decomposition of H_2O_2 in the presence of CAT was followed at 240 nm [18]. The CAT activity was defined as the amount of enzyme required to decompose 1mmol of H_2O_2 per minute at 25°C at pH 7.8. Results were expressed as mmol/min/mg of tissue.

- Total GSH

The amount of GSH in the tissues was measured according to the method described by Sedlak and Lindsay [19]. The muscles tissues were homogenised in 2 ml of 50mMTris-HCl buffer containing 20mM EDTA, at pH 7.5. After adding 2 ml ethanol (to precipitate the proteins), the homogenate was centrifuged at 3.000 g for 40 min at 4°C. The supernatant was used to determine GSH level using 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB). The absorbance was measured at 412 nm. Following the GSH level of the muscles was expressed as nmol/g tissue.

- Determination of LPO

The level of LPO in the tissues was determined by estimating malondialdehyde (MDA) using the thiobarbituric acid test [20]. The muscles were scraped, weighed and homogenised in 10 mL of 100 g/L KCl. The homogenate (0.5 mL) was added with a solution containing 0.2 mL of 80 g/L sodium lauryl sulphate, 1.5 mL of 200 g/L acetic acid, 1.5 mL of 8 g/L 2-thiobarbiturate and 0.3 mL of distilled water. The mixture was incubated at 98°C for 1 h Upon cooling, 5 mL of n-butanol:pyridine (15:l) was added. The mixture was vortexed for 1 min and centrifuged for 30 min at 4.000 rpm. The absorbance of supernatant was measured at 532 nm. The standard curve was obtained using 1,1,3,3-tetramethoxypropane. The recovery rate was over 99%. The results were expressed as nmol MDA/g tissue.

Statistical Analysis

Data were analysed using SPSS version 20.00 package software [21]. All values measured were tested with One-way ANOVA (total). Differences between the groups were determined by the Duncan Multiple Comparison Test with the P<0.05 value for significance.

RESULTS

Oregano essential oil had no significant effect on the performance parameters of the lambs *(Table 2)*. In *Table 3*, the serum levels of glucose, urea, uric acid and triglyceride were similar in all three groups. Furthermore, while serum phosphorus, sodium and chlorine levels were found to be similar in all three groups, in group OEO1 serum calcium and potassium levels were determined to have significantly decreased, when compared to with groups control and OEO2 (P<0.05) *(Table 3)*. The serum magnesium levels were ascertained to have significantly increased in group OEO1 (P<0.05) and to have slightly increased in group OEO2. The investigation of the lipid profile that, the hydrocarbon, triacylglycerol, steroid and polar lipid levels of all three groups were found to be similar *(Table 3)*.

In *Table 4*, oregano essential oil has a noticeable effect on the LPO rate in liver and meat tissues. While the LPO rate of the OEO1 group was significantly lower than the control and the OEO2 groups (P<0.01), LPO rates were similar in the control and OEO2 groups in the liver. LPO rates of the OEO1 and OEO2 groups were significantly lower than the control group in the *Musculus longissimus dorsi* (P<0.01).

The effects of oregano essential oil has on the SOD activity in liver and meat tissues are presented *(Table 4)*. Oregano essential oil has no determined significant effect on SOD activity in the liver. However, SOD activity of the OEO1 group was significantly lower than the control and OEO2 groups in meat tissue (P<0.01).

In *Table 4*, the oregano essential oil has given effects on the CAT activity in the liver and longissimus muscle tissue. Oregano essential oil has a determined significantly increase the CAT activity in the liver and the meat tissues (P<0.01).

Oregano essential oil has a determined significant effect on the GSH rates in the liver and the meat tissues *(Table 4)* (P<0.01).

DISCUSSION

Oregano essential oil has positive effects on performance via the assessment of food in the digestive system. Approximately 15% of the digested nutrients, converted to methane and CO_2 gas in particularly at the end of microbial digestion in the rumen, can not be used by the animals. The antimicrobial properties of the oregano oils modify the rumen metabolism by regulating/developing microbial activity in the rumen [22,23]. Oregano leaves [9] or extracts [24] supplementation of lamb ration has no known effects on the performance of animals. It is also reported that dietary supplementation of oregano oil to lambs'

Table 2. *The effects of the oregano essential oil on body weight, feed intake and feed efficiency of the lambs*

Variable Parameters	Groups			*P*-value
	Control	OEO1	OEO2	
Initial body weight, kg	34.73±4.45	34.11±3.51	34.58±3.98	0.953
14th day body weight, kg	37.10±5.16	37.91±4.20	38.46±4.82	0.858
28th day body weight, kg	40.38±5.12	40.08±3.48	39.57±7.34	0.960
42nd day body weight, kg	43.20±5.00	43.34±4.30	44.94±4.30	0.728
Final body weight, kg	46.06±4.47	46.75±4.74	48.61±3.86	0.526
Body weight gain, g/day	203±46.83	226±66.08	250±38.75	0.226
Feed intake, g/day	1813	1773	1702	-
Feed efficiency, kg/kg	8.93	7.85	6.81	-

All values are given as mean ± standard error of mean (SEM), (n=8); ***Control:*** *basal ration alone, OEO1: basal ration+200 mg/kg of Orego-Stim, OEO2: basal ration+400 mg/kg of Orego-Stim*

Table 3. *The effects of the oregano essential oil on some serum biochemical parameters the lambs*

Parameters	Groups			*P*-value
	Control	OEO1	OEO2	
Glucose, mg/dL	90.00±7.91	81.63±9.27	84.88±10.60	0.213
Urea, mg/dL	42.00±5.95	44.38±5.21	40.75±4.10	0.376
Uric acid, mg/dL	0.15±0.05	0.16±0.09	0.13±0.12	0.707
Triglyceride, mg/dL	23.50±7.50	22.38±6.70	34.50±16.25	0.074
Calcium, mg/dL	11.27±0.35^{a}	10.40±0.98^{b}	11.02±0.50^{a}	0.043
Phosphorus, mg/dL	8.52±0.72	8.49±1.68	8.39±1.11	0.977
Magnesium, mg/dL	2.43±0.22^{b}	2.97±0.54^{a}	2.56±0.30^{b}	0.026
Sodium, mmol/L	147.88±2.03	147.88±1.36	148.13±1.25	0.936
Potassium, mmol/L	4.82±0.22^{a}	4.56±0.14^{b}	4.89±0.25^{a}	0.010
Chlorine, mmol/L	107.88±1.81	108.63±2.33	108.38±1.92	0.756
Lipid profile, %				
Hydrocarbon	66.06±2.65	66.15±1.50	65.03±3.15	0.611
Triacylglycerol	3.45±0.52	4.09±1.15	4.44±0.55	0.062
Steroid	14.10±1.16	14.49±2.05	13.59±1.62	0.707
Polar lipid	16.81±2.22	15.61±2.01	17.43±1.01	0.376

^{a,b} Different letters in the same column represent a statistical significance between the groups; All values are given as mean ± standard error of mean (SEM), (n=8)

Table 4. *The effects of the oregano essential oil on LPO and GSH rates with SOD and CAT activities in the liver and M. longissimus dorsi muscle tissues*

Parameters	Groups			*P*-value
	Control	OEO1	OEO2	
Liver				
LPO, nmol/g tissue	2.669±0.112^{a}	2.089±0.09^{b}	2.646±0.113^{a}	0.003
SOD, mmol/min/mg tissue	0.223±0.005	0.218±0.003	0.222±0.004	0.095
CAT, mmol/min/mg tissue	0.721±0.045^{c}	1.337±0.056^{a}	1.083±0.068^{b}	0.005
GSH, nmol/g tissue	0.163±0.002^{b}	0.180±0.004^{a}	0.180±0.004^{a}	0.002
M. longissumus dorsi				
LPO, nmol/g tissue	0.263±0.018^{a}	0.197±0.025^{b}	0.220±0.025^{b}	0.004
SOD, mmol/min/mg tissue	0.366±0.018^{a}	0.312±0.025^{b}	0.348±0.025^{a}	0.003
CAT, mmol/min/mg tissue	1.006±0.207^{b}	1.571±0.050^{a}	1.466±0.050^{a}	0.005
GSH, nmol/g tissue	0.077±0.002^{b}	0.094±0.002^{a}	0.084±0.004^{a}	0.001

All values are given as mean ± SEM, (n=6); $^{a,b,c:}$ Different letters in the same column represent a statistical significance between the groups

ration did not affect the performance parameters [8,25]. In this study, no statistically significant effect was seen, although a certain improvement revealed daily live weight gain, feed consumption and feed efficiency *(Table 2)*. Our findings are in agreement with the previous literatures [9,24].

Unal and Kocabagli [8] has also reported that, thyme essential oil added to lamb rations did not affect the cholesterol, triglyceride, HDL and LDL ratios of the blood serum among the groups but changed within the groups depending on the time. Effects of oregano essential oil on blood metabolites in lambs have not been investigated widely. Vakili et al.[26] established that thyme essential oil in the diets of feedlot calves (5 g/day/calf) resulted in no changes in values of plasma triglyceride. Whereas in another study it has been reported that, concentrations of trigycerides can be influenced by oregano essential oil supplementation via changing of feed intake [27]. In the present study, while serum glucose, urea, uric acid and triglyceride levels were found to be similar in all three groups, in group OEO1 serum Mg levels were determined to have significantly increased, when compared to with groups control and OEO2. Also, in group OEO1 serum calcium and potassium levels were determined to have significantly decreased.

Today LPO is accepted as an important indicator of oxidative stress as well as tissue damages [28]. Many studies have determined the reduction at a significant level the lipid oxidation in the tissues, the antioxidants found in the structure of thyme essential oil [29-31]. In this study, either dose of oregano essential oil support by information the literature decrease the LPO rate in meat tissue. However, while it is similar with the literature information the 200 mg Orego-Stim is significant level reduced the rate of LPO in liver tissue, it is interesting that there was no effect on the rate of LPO of the group that were given 400 mg Orego-Stim. These results show different the effects antioxidative of oregano essential oil the rate of LPO in the liver and meat tissues. The study showed a high rate of LPO in the liver tissue of group OEO2 that probably can to come forward from fatty acids in the structure of the oregano essential oil. Also, more detailed studies are suggested to obtain more detailed information.

Almost all of the studies made to determine the antioxidant properties of oregano essential oil have been related to the LPO ratio [32,33]. In order to fully explain the mechanism the antioxidant effect of oregano essential oil must determine the rates of LPO and GSH with the SOD and CAT activities.

SOD enzyme, the first step of antioxidant defense, plays crucial role on the elimination of superoxide radical. The enzyme SOD catalyzes the dismutation of superoxide radicals to oxygen and hydrogen peroxide. Catalase enzyme constitutes the second step of the defense mechanism which prevents the accumulation of hydrogen peroxides and catalyzes the conversion of hydrogen peroxide to water and molecular oxygen. An increased activity of any enzyme could be linked with an enhanced substrate production during the metabolic processes. Therefore, both SOD and CAT activities inform us both about how the defense system works and the situation of oxidative stress [28].

In addition, it has been reported that the SOD activities are different according to the tissues [34]. But stress usually increases the SOD activity [28]. In the present study the SOD activity of group OEO1 is lower and it has been shown that the rate of superoxide radical is less than other groups and the most effective dose was 200 ppm of oregano oil in the *M. longissimus dorsi*. If it is not significant among the groups of activity SOD in liver tissue is shows different levels of superoxide radical formation in tissues and activity of SOD in the liver and *M. longissimus dorsi*. The free radicals rate has been known to cause an increase in the rate of free radicals in favour of the antioxidant balance between free radicals with antioxidants in the tissues. The rate of the SOD activity in diabetic rats was reported to be lower than in healthy animals [35].

Antioxidant strategies designed to either inhibit free radical formation or to scavenge free radicals may provide protection. An increased activity of any enzyme could be linked to enhanced substrate production during the metabolic processes. Indeed, the increased CAT activity would also suggest that the accumulation of H_2O_2 might be responsible for an increased LPO following heat stress. One may consider that the heat stress application increased the H_2O_2 formation; therefore, increased CAT activity could discharge these radicals from the medium [28]. Orageno essential oil increased significantly the CAT activity in the liver and meat tissues. These results of the oregano essential oil have been shown to increase in CAT activity for removed occurs intensive of hydrogen peroxide radicals in cells.

GSH forms an important part of the antioxidant defence system and plays an important role for the prevention of damage of many harmful molecules and compounds. Glutathione is the substrate of GSH-dependent enzymes such as GSH-peroxidase and GSH S-transferase. Organisms use GSH to eliminate hydrogen peroxide and other peroxides. These enzymes will not show any activity in the absence of GSH. Conversely, these enzymes use GSH against ROS, which leads to a decrease in the amounts of GSH. Briefly, GSH is a marker used to explain how the defence system acts directly, and the ROS amount indirectly. When GSH levels are insufficient, peroxides may accumulate, therein causing damage. Oregano essential oil has antioxidant components higher than GSH levels groups of OEO1 and OEO2 than the control group remove free radicals in liver and meat tissue. Glutathione (GSH) content was higher depending on the amount of essential oils obtained from plants [33].

As a result, Orego-Stim added as a feed additive in ration provides a significant effect on the rates of LPO and GSH with activities of CAT and SOD in *M. longissimus dorsi*, while it increased the activity of CAT with the rate of GSH in liver tissue. Also, the results show the different effect of different doses on SOD activities in the *M. longissimus dorsi* and on the rate of LPO in the liver. On the other hand, Orego-Stim was determined to have no significant effects on performance parameters. This study, after determining in the effect of antioxidants on the metabolism, will find more efficient and correct use areas, particularly in the food and pharmaceutical industry. There is a need for studies on different doses in order to obtain detailed information about oregano essential oil or derivatives added as a feed additive in ration.

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