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Title: Effects of dietary dried Greek Oregano (*Origanum vulgare ssp. hirtum*) supplementation on blood and milk enzymatic antioxidant indices, on milk total antioxidant capacity and on productivity in goats

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## Highlights

- I studied the effects of oregano supplementation on antioxidant system's performance.
- Glutathione peroxidase activity was increased in both blood plasma and milk.
- Glutathione reductase activity was increased in both blood plasma and milk.
- FRAP values of milk were improved.
- Oregano supplementation can improve the antioxidant system performance in goats.

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Effects of dietary dried Greek Oregano (*Origanum vulgare ssp. hirtum*)  
supplementation on blood and milk enzymatic antioxidant indices, on milk total  
antioxidant capacity and on productivity in goats

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20 **Abstract**

21       The aim of the present study was to evaluate the effects of the dietary  
22 supplementation of dried Greek oregano (*Origanum vulgare ssp.hirtum*) as a whole  
23 plant on various blood and milk oxidative stress enzymatic indices, on milk total  
24 antioxidant capacity and on productivity in dairy goats. Twelve Alpine goats were  
25 used in a 4 week experiment and were allocated to 1 of 2 groups (CON, OR). The  
26 animals were fed 1.2 kg of alfalfa hay and 1.2 kg of concentrate mixtures (50 % basal  
27 and 50 % lactation ration) daily. The concentrate mixtures for the CON group were  
28 not supplemented with oregano, while oregano plants were incorporated into the  
29 lactation ration of the OR group, at a level of 30 g equivalent to a daily dosage of 1 ml  
30 of essential oil per animal. The goats were milked twice per day and the milk yield  
31 was recorded. At the end of each week of the experimental period, individual milk  
32 samples were obtained and analyzed for fat, milk and protein contents. Additional  
33 blood and milk samples were taken during the 3<sup>rd</sup> and the 4<sup>th</sup> week of the experimental  
34 period. The activities of the following antioxidant enzymatic indices were measured:  
35 superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase in blood  
36 and milk, glutathione transferase in blood and lactoperoxidase in milk. The OR group  
37 showed a significant increase in glutathione peroxidase and glutathione reductase both  
38 in blood ( $P<0.01$  and  $P<0.001$  respectively) and milk ( $P<0.001$  and  $P<0.001$   
39 respectively). In addition, the dietary oregano supplementation effectively enhanced  
40 FRAP values ( $P<0.001$ ) of the milk. It can be concluded that the dietary intake of  
41 dried oregano plants positively affected at least partially, some enzymatic and non  
42 enzymatic antioxidant defenses of blood and milk and thus, contributed to enhanced  
43 antioxidant capacity of milk.

44 *Key words: antioxidant enzymes; antioxidant capacity; essential oil; goat milk;*  
45 *oregano; oxidative stress.*

46 *Abbreviations: ADF, acid detergent fiber; BW, body weight; CLA, conjugated*  
47 *linoleic acid; CON, control; DM, dry matter; EO, essential oil; FRAP, ferric reducing*  
48 *antioxidant power; GC/MS, Gas chromatography–mass spectrometry;; NADPH,*  
49 *Nicotinamide adenine dinucleotide phosphate reduced form; NDF, neutral detergent*  
50 *fiber; NE<sub>L</sub>, net energy for lactation; N, nitrogen; OR, oregano; ROS, reactive oxygen*  
51 *species;*

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## 1. Introduction

The production of reactive oxygen species (ROS) during aerobic metabolism is a natural and inevitable phenomenon (Jóźwik et al., 2012). Although ROS are involved in various physiological functions (Nordeberg and Arner, 2001), when they are not scavenged efficiently, oxidative stress develops (Lykkesfeldt and Svendsen, 2007). Oxidative stress is influenced by physiological status (gestation, lactation), animal performance and nutrition as well as environmental factors (Festila et al., 2012; Jóźwik et al., 2012). This type of stress, while it does not demonstrate clinical symptoms, it has severe consequences on the immune system function as it contributes to an increased incidence of post partum diseases including mastitis and also to diminished milk quality (Castillo et al., 2013). The latter, can be affected through the oxidation of proteins, vitamins and lipids leading to a reduced nutritive value and organoleptic characteristics (Smet et al., 2008).

In order to counteract these negative effects, the organism is equipped with an antioxidant defense system which can scavenge ROS sufficiently under normal physiological conditions (Jóźwik et al., 2012). The antioxidant defense system consists of non-enzymatic scavengers such as vitamin A, vitamin C and vitamin E and proteins including enzymatic scavengers of ROS (Lindmark-Mansson et al. 2001). The latter include primarily superoxide dismutase and catalase which decompose  $O_2^-$  and  $H_2O_2$  (Lindmark-Mansson and Akesson, 2000) respectively. Glutathione peroxidase removes various organic peroxides including  $H_2O_2$  (Rezapour and Taghinejad-Roudbaneh, 2011). Other antioxidant enzymes include glutathione transferase which catalyzes the conjugation of the reduced form of glutathione to various oxidative substrates (Hayes et al., 2005), glutathione reductase which reduces the oxidized glutathione using NADPH (Azambuja et al., 2011) and lactoperoxidase

which catalyzes the oxidation of various organic and inorganic substrates by hydrogen peroxide (Kohler and Jenzer, 1989). All the aforementioned factors can be used as antioxidative indices.

Several *in vivo* studies showed that aromatic plants can improve the antioxidant system performance by means of increased activity of antioxidant enzymes activity in broiler chicken (Akbarian et al., 2014), rats (Lv et al., 2012) and fish (Azambuja et al., 2011). Furthermore, some *in vivo* studies showed that the intake of aromatic plants made a positive impact on the performance of dairy cows (Kraszewski et al., 2002; Tekippe et al., 2011). However there has been little or no research carried out on small ruminants, and more specifically goats (Heidarian Miri et al., 2013) in spite of the fact that goat milk consumption and derived dairy products are increasing worldwide due to the recognition of the beneficial effects of these products on human health (Garcia et al., 2014). In addition, the farming of dairy goats plays an important role in the economy of many Mediterranean countries (Park et al., 2007).

Greek oregano (*Origanum vulgare ssp. hirtum*) is an aromatic plant that belongs to the Labiatae family and is commonly found in Mediterranean-type ecosystems (Vokou et al., 1993). Greek oregano contains high amounts of carvacrol or/and thymol which are phenolic compounds that demonstrate potent antioxidant properties (Milos and Makota, 2012). Essential oils (EOs) as well as other natural antioxidants, after absorption in the digestive tract can be transferred into the blood stream and consequently a minor part is incorporated into milk (Pizzoferato et al., 2007). Thus, the main objective of this study was to assess the effects of the consumption of dietary dried Greek oregano on blood and milk antioxidant enzymatic indices (superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase,

lactoperoxidase, glutathione transferase) and on susceptibility of milk to oxidation assessed by FRAP assay in lactating goats. The second objective was to evaluate the yield and chemical composition of the milk.

## 2. Materials and methods

### 2.1. Plant material

The oregano plants were collected from the Greek island of Ikaria which is located in the Aegean Sea, in July of 2013 during the midflowering stage. The collected plants (including stems, leaves, and inflorescences but excluding the roots) were naturally dried in a partly closed space for 30 days with an average ambient temperature of 30°C.

### 2.2. Animals and diets

Twelve healthy and lactating (mid lactation;  $120 \pm 7$  days in milk) multiparous Alpine goats balanced for age (4-5 years) were used in one 4 week experimental trial and animals were allocated to two groups, with an average body weight of  $50.25 \pm 3.20$  kg (control group; CON) and  $48.83 \pm 2.95$  kg (treatment group; OR). The experiment was conducted in agreement with the guidelines of the Agricultural University of Athens concerning the care and welfare of agricultural animals in order to avoid any unnecessary discomfort to the animals.

The animals were kept in tie stalls equipped for individual feeding and were fed 1.056 kg of DM/d of alfalfa hay and 1.068 kg of DM/d of concentrate mixtures which consisted of 50 % basal and 50 % lactation ration (Table 1). The OR group



received the same rations as the CON group the only difference being the addition of 30 g of ground oregano plants in the lactation ration, in order to provide a daily dosage of 1 mL of oregano EO per animal. This dosage was chosen because some studies showed it has a positive effect on milk quality. Simitzis et al. (2007) reported a positive effect on the milk protein of lactating ewes when a dosage of 1 ml of oregano EO/kg of concentrate was applied. Similar results have been demonstrated by Offer et al. (2005) in dairy cows.

The mixing of the feedstuff and the ground oregano herbs took place every 15 days based on the results of a previous study (Paraskevakis et al., 2015) in order to minimize essential oil losses from the concentrate mixture during storage. Goats were weighted every week. All animals had *ad libitum* access to fresh water throughout the experimental period.

### 2.3. Measurements and analytical methods

Concentrates and alfalfa hay were analyzed for DM, crude protein, ether extract and ash according to methods 930.04, 978.04, 930.09 and 930.05 respectively of the Association of Official Analytical Chemists (1997). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to Van Soest et al. (1991). NDF was analyzed without  $\alpha$ -amylase or sodium sulfite and NDFom and ADFom values are expressed without residual ash (Table 1). Feed was offered twice daily at 08:00 and 16:00 h and feed intake was recorded by weight the feed offered to the animals and subtracting what remained uneaten.

In order to quantify the EO content of the dried oregano plants, a ground sample of 50 g of the whole plant (excluding roots) was submitted to hydrodistillation

in a Clevenger apparatus (Model Teknik 303.01, Ambala, India) for 3 h. The extracted EOs were dried with anhydrous magnesium sulphate and stored at  $-18^{\circ}\text{C}$  until analysis by gas chromatography–mass spectrometry (GC–MS). EO analysis was performed using a Hewlett Packard 5890 II GC coupled with a Hewlett Packard 5972 MSD (Agilent technologies inc., Santa Clara, USA) operating in the EI mode at 70 eV. A non-polar Rtx-5MS capillary column (30 m X 0.25 mm, film thickness 0.25  $\mu\text{m}$ ) used with a programmed temperature gradually increased from  $60^{\circ}\text{C}$  to  $250^{\circ}\text{C}$  at a rate of  $3^{\circ}\text{C}/\text{min}$ . Injector and MS transfer line temperatures were set at  $220^{\circ}\text{C}$  and  $290^{\circ}\text{C}$ , respectively. One micro liter of sample was injected in the splitless mode. Helium, the carrier gas, was set at 2.5 psi. Identification of the compounds was based on the comparison of their relative retention time and mass spectrum with those of NIST 98, Wiley 275 library data of the GC/MS system and literature data (Adams, 2007). All analyses were performed in triplicate.

Individual blood samples were obtained by jugular venipuncture before the morning feeding at the end of the third and fourth week of the experimental period. Blood samples were centrifuged (2,000 g for 20 min) and the upper layer (blood serum) was stored at  $-80^{\circ}\text{C}$  until analysis. The enzymatic antioxidant indices in the blood included Superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione transferase. Superoxide dismutase activity was measured according to the method described by McCord and Fridovich (1969). Catalase activity was measured according to the method described by Beers and Sizer (1952). Glutathione peroxidase activity was measured using the method described by Wendel (1980). Glutathione reductase activity was measured according to the method described by Mavis and Stellwagen (1968). Glutathione transferase activity was measured using to the method described by Habig et al., (1974). In all enzyme assays

absorption was determined photometrically using a Shimadzu UV-1800/Vis Spectrophotometer (USA). All analyses were performed in triplicate.

The goats were milked twice per day at 07:00 and 17:00 h and the milk yield was recorded at each milking. At the end of each week of the experimental period, individual milk samples were obtained and stored at 4 °C until analyzed for fat, lactose and protein contents with a Milkoscan (133/Foss Electric, Hillerød Denmark). The milk was not submitted for pasteurization. Additional individual milk samples were taken at the end of the third and fourth week of the experimental period and stored at -80 °C until all subsequent analysis was performed. The enzymatic antioxidant indices in the milk included superoxide dismutase, glutathione peroxidase, catalase, glutathione reductase and lactoperoxidase. Before analysis, the milk samples were centrifuged at 10,000 g for 30 min at 4 °C to remove fat. Superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activities were measured according to the methods used for blood plasma. Lactoperoxidase activity was determined according to the method described by Keeseey (1987). All results are expressed as Units/ml. Antioxidant capacity in milk was measured using the FRAP assay described by Smet et al. (2008) and the results were expressed as FRAP values ( $\mu\text{mol Fe}^{+2}/\text{mL}$  of milk). Before measuring the absorbance, a brief centrifuging step was applied (5 min x 1,300g) which resulted in the production of a clear medium. All analyses were performed in triplicate.

#### 2.4. Statistical analysis

Obtained data on dry matter intake (kg/day, g/kg<sup>0.75</sup> BW), organic matter intake (kg/day, g/kg<sup>0.75</sup> BW), nitrogen intake (g/day, g/kg<sup>0.75</sup> BW), body weight, milk production, milk composition, blood and milk enzymes and FRAP values were analyzed by repeated measures analysis of variance (RM-ANOVA) considering the sampling time (3<sup>rd</sup> and 4<sup>th</sup> week) as repeated measures. When the normality and sphericity assumptions for applying RM-ANOVA were violated, Friedman non-parametric test, followed by Dunns multiple range test were used to identify significance. The normality of data distribution was tested with a Kolmogorov-Smirnov test. Statistical analysis was performed using the SPSS statistical package, release 17.0.0 (SPSS, Chicago, IL, USA). For all tests, a P value of less than 0.05 was considered to be statistically significant.

### 3. Results

#### 3.1. *Oregano* EO yield and profile

The dried oregano plants yielded an average EO content of 3.2 % (v/w) by hydrodistillation. Carvacrol was the predominant compound of the oregano EO as 91.5 % of the EO was comprised of it, while thymol was the lowest (Table 2). The rest of the elements were lower than 2.6 %.

#### 3.2. *Feed intake, body weight and productivity*

Dry matter intake, organic matter intake, nitrogen intake, body weight, milk yield and milk composition (Table 3) were similar among treatments (P>0.05).

Sampling time had no effect on any of the researched parameters while there was no interaction between diet and sampling time.

### 3.3. *Enzymatic antioxidant indices in blood plasma*

Glutathione transferase, superoxide dismutase and catalase activities were similar among treatments (Table 4). However glutathione peroxidase and glutathione reductase activities were found to be higher ( $P=0.003$ ) in the OR group compared with the CON group. Sampling time had no effect on any of the studied enzymes while there was no interaction between diet and sampling time.

### 3.4. *Enzymatic antioxidant indices and FRAP assay*

Lactoperoxidase, superoxide dismutase and catalase activities were similar among treatments ( $P>0.05$ ). Glutathione peroxidase and glutathione reductase activities were found to be higher ( $P<0.001$ ) in the OR group compared with the CON group (Table 5). FRAP value was higher ( $P<0.001$ ) in the OR group compared with the CON group. Sampling time had no effect on any of the studied parameters while there was no interaction between diet and sampling time.

## 4. Discussion

### 4.1 *Feed intake and milk yield*

Overall, the lack of considerable effects on feed intake and performance can be attributed to the low daily dosage of EO. This concurs with previous *in vivo* studies where similar dosages failed to improve small ruminant performance. Simitzis et al.

(2007) reported no effect on milk yield when the diet was supplemented with 1 ml of *Origanum vulgare* essential oil/kg in dairy ewes. In addition, Giannenas et al. (2011), which applied lower dosages (50-150 mg/kg of concentrated feed) in ewes and Heidarian Miri et al. (2013) which applied higher dosages (1.27 and 2.53 % of cumin seed extract of DM intake) in goats found no differences in milk composition.

#### 4.2 Enzymatic antioxidant indices in blood plasma

Lactation stage (especially early) is a very critical physiological state that can negatively affect the antioxidant defense system performance (Festila et al., 2012). High yielding animals are susceptible to oxidative stress (Jóźwik et al., 2012) and hence, it is suggested that the goats in the present study have experienced some degree of oxidative stress due to their high daily milk production ( $2116.2 \pm 498.25$ ). However, currently there is no reference panel for oxidative stress indices concerning ruminants and therefore the physiological ranges of antioxidant enzymatic activity during oxidative stress cannot be determined (Celi, 2010). On the other hand, antioxidant enzyme induction by EOs is a well known effect (Tisserland and Young, 2014). Thus, this study aimed at reducing oxidative stress by the supplementation of dairy goats' diets with oregano plants which are rich in phenolic bioactive natural compounds (Vokou et al., 1993; Tsimidou et al., 1995).

This is the first report in the literature concerning the impact of oregano on goat blood activities of glutathione peroxidase and glutathione reductase, *in vivo*. The findings of this study suggest that oregano EO at a daily dosage of 1 mL/animal has the potential to augment glutathione peroxidase and glutathione reductase activity.

These findings are consistent with those of Akbarian et al. (2014) which reported a significant increase in two antioxidant enzyme activities in the blood of broiler chickens fed with EO of *Curcuma xanthorrhiza*. At a level of 200 mg EO/kg only glutathione peroxidase activity was increased while at a level of 400 mg EO/kg both glutathione peroxidase and superoxide dismutase activities were increased. It appears that at low EO dosages glutathione peroxidase is the first antioxidant enzyme to be activated. These authors concluded that plant extracts rich in phenolics compounds were responsible for these effects. This probably explains why the rest of the rest of the studied antioxidant enzymes (superoxide dismutase, glutathione transferase, catalase) were unaffected by the treatment in this study. Similar results have been demonstrated by other researchers (Faix et al., 2009).

Increased glutathione peroxidase activity in blood plasma is associated with an increased protection from ROS during oxidative stress (Festila et al., 2012). Celi et al. (2008) suggested that when glutathione peroxidase activity is decreased in the blood, oxidative stress develops in dairy goats. The fact that glutathione reductase activity was also positively affected by oregano supplementation is very important as *in vitro* and *in vivo* studies showed that when there is a deficiency of glutathione reductase during oxidative stress, neutrophils demonstrate impaired performance (Yan et al., 2012). Glutathione peroxidase catalyzes the oxidation of glutathione by various peroxides including hydrogen peroxide (Lindmark-Mansson and Akesson, 2000). In the presence of glutathione reductase and NADPH, the oxidized form of glutathione is reduced. Thus, parallel enhancement in glutathione peroxidase and glutathione reductase activities can offer additional protection to the tissues and molecules (e.g. mobilized body fat reserves) from ROS during lactation. Oregano dietary supplementation can be proposed as a strategy to fortify the antioxidant system and

mitigate the oxidative stress in dairy goats. The results of the present study are not expected to differ when the situation changes (e.g. animals no longer under oxidative stress), however it would be pointless to adopt this strategy as ROS are scavenged efficiently under normal physiological conditions (Nordeberg and Arner, 2001).

#### *4.3 Enzymatic antioxidant indices in milk*

The same antioxidant defenses in milk were also enhanced by oregano treatment. Glutathione peroxidase and glutathione reductase activities were significantly increased ( $P < 0.001$ ) in the OR group and that reflected an increased protection of milk lipids and various components from oxidation processes (Castillo et al., 2013). Undoubtedly, lipid oxidation is a process that affects milk quality negatively. Milk fat contains unsaturated fatty acids that are beneficial to human health including the group of conjugated linoleic acids (CLA's) and n-3 fatty acids (Butler et al., 2011). Polyunsaturated fatty acids are more susceptible to oxidation while the oxidation of lipids is one of the main causes of food off-flavors including milk and derived products (Rafalowski et al., 2014).

Yet, the responsible mechanisms by which enzymes are secreted into milk are poorly understood (Silanikove et al., 2006). Blood enzymes can be transferred to milk through leaky junctions between mammary cells (Fox and Kelly, 2006).

#### *4.4 Milk antioxidant capacity*

Nevertheless, increased total antioxidant capacity is associated positively with milk quality and the prolonged shelf-life of dairy products as it indicates a higher



level of antioxidants. Despite the fact that a combination of various assays is necessary in order to evaluate the total antioxidant capacity of milk (Chen et al., 2003), FRAP assay in this study showed a clear increase in the antioxidant capacity of the milk produced by the OR group. The observed increase in FRAP values can be attributed to the fact that EOs can be transferred into the blood stream and consequently into milk via absorption from the digestive system (De Feo et al., 2006) and exhibit antioxidant properties even in minor quantities in milk and cheese (Pizzoferrato et al., 2007).

Oregano EO demonstrates remarkable antioxidant activity and it is comparable with that of rosemary EO (Tsimidou et al., 1995). The antioxidant activity is attributed mainly to carvacrol and thymol, while in the present study carvacrol was the predominant compound. Furthermore, oregano contains other antioxidants such as caffeic acid, protocatechuic acid (Yanishlieva et al., 2006) and rosmarinic acid (Exarchou et al., 2002). Hence, both EOs and antioxidant compounds from oregano plants can contribute directly as ROS scavengers to the non enzymatic antioxidant system performance. Storage of milk at low temperatures (below 4 °C), pasteurization (72 °C, 15 s) and milk processing (e.g. cheese production) does not affect glutathione peroxidase activity significantly (Lindmark-Mansson et al., 2001). Thus, the increased activity of glutathione peroxidase in milk is anticipated to remain unchanged in the dairy products (pasteurized milk, cheese) and protect lipids and other components from oxidation processes contributing to an increased shelf-life for the product.

## Conclusions

The results of this study suggested that the dietary supplementation of dried oregano plants in lactating goats positively affected antioxidant system performance. Oregano plants exert antioxidant protection indirectly through antioxidant enzyme activation properties and directly through ROS scavenging by various antioxidant compounds contained in oregano. Thus, oregano plants as feed additives in ruminant diets may be a promising way of fortifying both the enzymatic and the non enzymatic antioxidant system and consequently minimize the oxidative damage during the lactation period and the deterioration of milk quality.

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Table 1. Ingredients of the concentrate mixtures and chemical composition of the concentrate mixtures and alfalfa hay offered to animals during the experimental period

	Basal ration	Lactation ration	Alfalfa hay
Ingredient composition			
DM (g/kg)			
Maize	520	545	
Barley	135	-	
Wheat bran	200	200	
Cottonseed hulls	100	-	
Soybean meal	-	220	
CaCO <sub>3</sub>	20	20	
Dicalcium phosphate	15	5	
NaCl	5	5	
Premix <sup>a</sup>	-	5	
Chemical composition			
Dry matter (g/kg)	890	890	880
DM (g/kg)			
Organic matter	924	930	910
Crude protein	115	172	172
Ether extract	35	35	11
NDFom <sup>c</sup>	390	500	450
ADFom <sup>d</sup>	170	100	300
Ca	10	12	12
P	7	9	2
NE <sub>L</sub> <sup>b</sup> (MJ/kg DM)	7.0	7.0	3.6

<sup>a</sup>Premix contained 10000 IU vit. A., 2000 IU vit., D<sub>3</sub>, 10 mg vit., E.

<sup>b</sup>Net energy value for lactation calculated using published values of feed ingredients. The following equation was used for calculating the efficiency of metabolisable energy for milk production:  $kl = 0.60 + 0.24 \times (Qm - 0.57)$  (INRA, 2007).

<sup>c</sup>NDFom; neutral detergent fiber not assayed with a heat stable amylase and expressed exclusive of residual ash.

<sup>d</sup>ADFom; acid detergent fiber expressed exclusive of residual ash.

Table 2. Constituents (%) of the composition of essential oil in oregano plants.

Serial number	Constituents	Percentage yield <sup>a</sup>	
		Mean	S.E
1	$\alpha$ -Thujene	Trace <sup>b</sup>	-
2	$\alpha$ -Pinene	0.10	0.002
3	$\beta$ -Pinene	0.10	0.004
4	$\alpha$ -Terpinene	0.30	0.005
5	p-Cymene	2.00	0.033
6	$\gamma$ -Terpinene	0.90	0.028
7	cis-Sabinene hydrate	0.10	0.004
8	Borneol	1.00	0.031
9	Terpinen-4-ol	0.70	0.025
10	Thymol	0.20	0.020
11	Carvacrol	91.50	0.027
12	$\beta$ -Caryophyllene	0.90	0.037
13	$\alpha$ -Farnesene	0.40	0.021

<sup>a</sup> Values are expressed as percentage of the total peak area of the chromatograms without correction factors and represent triplicate assays of three samples.

<sup>b</sup> Trace<0.08

Table 3. Impact of oregano dietary addition on feed intake, body weight and productivity.

	Treatment <sup>a,b</sup> (T)			Sampling time (S)					P		
	0	30	SEM	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	SEM	T	S	TXS
Dry matter intake (kg/day)	2.06	2.02	0.019	2.05	2.02	2.04	2.06	0.020	0.098	0.488	0.631
Organic matter intake (kg/day)	1.90	1.84	0.018	1.88	1.85	1.86	1.88	0.018	0.480	0.521	0.754
N <sup>c</sup> intake (g/day)	51.92	50.21	0.537	51.45	50.62	50.81	51.37	0.555	0.480	0.521	0.754
Body weight (kg)	50.10	49.57	2.859	49.54	49.10	50.08	50.63	2.046	0.898	0.083	0.469
Dry matter intake (g/kg <sup>0.75</sup> BW)	60.85	59.37	3.529	61.36	60.94	60.33	57.81	2.562	0.773	0.069	0.825
Organic matter intake (g/kg <sup>0.75</sup> BW)	31.88	31.38	1.841	31.18	30.91	31.31	33.12	1.346	0.300	0.854	0.838
N intake (g/kg <sup>0.75</sup> BW)	0.87	0.86	0.051	0.85	0.84	0.86	0.90	0.037	0.830	0.288	0.837
Production (g/day)											
Milk	2125.2	2507.1	189.71	2039.2	2375.4	2237.1	2012.9	138.62	0.947	0.077	0.278
Protein	63.57	58.58	4.358	57.74	69.47	65.08	52.00	3.481	0.437	0.064	0.281
Fat	62.96	66.24	4.958	69.08	73.94	63.26	53.12	3.665	0.650	0.065	0.064
Lactose	93.60	95.58	7.867	89.90	106.87	100.31	81.27	5.758	0.863	0.088	0.205

<sup>a</sup> Target grams of ground oregano plants per animal/day.

<sup>b</sup> Number of goats per treatment=6

<sup>c</sup>N-nitrogen

Table 3. (Continued)

	Treatment <sup>a,b</sup> (T)			Sampling time (S)					P		
	0	30	SEM	1 <sup>st</sup> week	2 <sup>nd</sup> week	3 <sup>rd</sup> week	4 <sup>th</sup> week	SEM	T	S	TXS
<b>Milk composition (g/100g)</b>											
Protein	3.02	2.80	0.113	2.87	2.95	2.95	2.89	0.093	0.195	0.630	0.128
Fat	3.01	3.17	0.159	3.39	3.16	2.86	2.97	0.122	0.497	0.060	0.077
Lactose	4.43	4.55	0.046	4.43	4.51	4.50	4.50	0.039	0.101	0.800	0.829

<sup>a</sup> Target grams of ground oregano plants per animal/day.

<sup>b</sup> Number of goats per treatment=6

<sup>c</sup>N-nitrogen

Table 4. Impact of oregano dietary addition on antioxidant enzymes in blood plasma.

	Treatment <sup>a,b</sup> (T)			Sampling time (S)			P		
	0	30	SEM	3 <sup>rd</sup> week	4 <sup>th</sup> week	SEM	T	S	TXS
Superoxide dismutase <sup>c</sup>	40.00±17.88	46.84±17.88	5.164	36.07±17.87	50.77±17.87	5.160	0.461	0.180	0.527
Glutathione peroxidase <sup>d</sup>	0.88±0.19	1.19±0.19	0.055	0.90±0.18	1.17±0.18	0.054	0.003	0.500	0.104
Glutathione reductase <sup>e</sup>	0.03±0.01	0.05±0.01	0.003	0.03±0.01	0.04±0.01	0.003	0.003	0.089	0.380
Catalase <sup>f</sup>	20.96±10.57	23.22±10.57	3.053	20.53±11.19	23.65±11.19	3.233	0.686	0.589	0.628
Glutathione transferase <sup>g</sup>	0.12±0.02	0.14±0.02	0.008	0.12±0.02	0.14±0.02	0.008	0.215	0.320	0.109

<sup>a</sup>Target grams of ground oregano plants per animal/day.

<sup>b</sup> Number of goats per treatment=6

<sup>c,d,e,f,g</sup> Values are expressed as enzyme units per mL of blood plasma.



Table 5. Impact of oregano dietary addition on milk antioxidant enzymes and on antioxidant capacity.

	Treatment <sup>a,b</sup> (T)			Sampling time (S)			P		
	0	30	SEM	3 <sup>rd</sup> week	4 <sup>th</sup> week	SEM	T	S	TXS
Superoxide dismutase <sup>e</sup>	29.73 $\pm$ 13.82	30.03 $\pm$ 13.82	3.990	29.58 $\pm$ 13.84	30.18 $\pm$ 13.84	3.997	0.963	0.906	0.906
Lactoperoxidase <sup>f</sup>	0.58 $\pm$ 0.15	0.61 $\pm$ 0.15	0.046	0.59 $\pm$ 0.15	0.60 $\pm$ 0.15	0.046	0.661	0.882	0.579
Glutathione peroxidase <sup>g</sup>	1.00 $\pm$ 0.19	1.47 $\pm$ 0.19	0.055	1.184 $\pm$ 0.18	1.289 $\pm$ 0.18	0.054	<0.001	0.235	0.443
Glutathione reductase <sup>f</sup>	0.68 $\pm$ 0.50	1.82 $\pm$ 0.50	0.145	1.33 $\pm$ 0.47	1.17 $\pm$ 0.47	0.136	<0.001	0.489	0.578
Catalase <sup>g</sup>	25.47 $\pm$ 8.28	23.42 $\pm$ 8.28	2.392	22.22 $\pm$ 8.25	26.67 $\pm$ 8.25	2.382	0.577	0.195	0.265
FRAP value <sup>h</sup>	180.25 $\pm$ 11.68	240.83 $\pm$ 11.68	3.374	211.25 $\pm$ 11.50	209.83 $\pm$ 11.50	3.321	<0.001	0.691	0.726

<sup>a</sup> Target grams of ground oregano plants per animal/day.

<sup>b</sup> Number of goats per treatment=6

<sup>c,d,e,f,g</sup> Values are expressed as enzyme units per mL of milk.

FRAP value is expressed as  $\mu\text{mol Fe}^{+2}/\text{mL}$  of milk as assessed by FRAP method.