

## Effects of Oregano (*Origanum vulgare*) and Thyme (*Thymus vulgaris*) Oils on Growth Performance and Blood Parameters in Holstein Suckling Calves

### Research Article

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### ABSTRACT

This experiment was conducted to compare the effect of supplementation of milk with thyme (THY) and oregano (ORE) essences separately or a mixture of THY and ORE essences on growth performance, haematological parameters and some blood metabolites of Holstein suckling calves. Forty-eight Holstein calves (48.31±5.82 kg) were randomly allocated to four treatments including (1) control without essential oil supplement; (2) THY oil mixed in milk at 5 mL/d/calf; (3) ORE oil mixed in milk at 5 mL/d/calf; (4) 2.5 mL THY oil + 2.5 mL/d/calf ORE oil mixed in milk (TOM). According to the results, the experimental diets had no significant effect on body weight, average daily gain, and feed conversion rate in the entire experiment ( $P>0.05$ ), although the parameters of starter and total feed intake were increased ( $P<0.0001$ ) in the ORE and TOM groups. Values of packed cell volume, haemoglobin, mean corpuscular volume, and lymphocyte percentage were increased ( $P<0.05$ ) in the ORE group compared with the control. Also, our results demonstrate the supplementation of either oregano or thyme essential oils increased ( $P<0.05$ ) serum iron concentration, alanine aminotransferase, and aspartate transaminase activities. Concentrations of total cholesterol and total triglyceride were decreased ( $P<0.05$ ) in calves given both oils compared to the control group. Further confirmation of these results is warranted, and more research is needed to identify and clearly explain in the contribution of oregano or thyme oils (individual or mixtures); however our results suggested that oregano oil might be more useful as the promising feed additive for suckling calves nutrition.

**KEY WORDS** blood metabolites, calf, growth performance, oregano oil, thyme oil.

### INTRODUCTION

The use of antibiotics as feed additives in animal feeds due to the emergence of antibiotic resistant bacteria that may represent a risk to human health is banned in the European Union (European Commission, 2003). Consequently, it is necessary to determine alternative additives and strategies that will allow producers to maintain the current level of production without increasing the cost or the prevalence of metabolic, digestive or respiratory upsets in suckling calves. Essential oils (EO) known as volatile or ethereal oils

are compounds that give plants and spices their colour and scent. In the last few years, EO of plants have been considered potential alternatives to antibiotics in animal feeds. Among EO compounds, thyme (THY) and oregano (ORE) oils possess noticeable antimicrobial activity (Davidson, 2001). Thyme oil which extracted from *Thymus vulgaris*- a pleasant smelling perennial shrub belongs to the Lamiaceae family- has various beneficial effects as antiseptic, carminative, antimicrobial and antioxidative properties (Baranauskiene *et al.* 2003). Previous studies showed that the main constituents of thyme oil include thymol (5-

methyl-2- isopropyl phenol), carvacrol (5- isopropyl-2-methyl- phenol) and flavonoids (Exarchou *et al.* 2002). It has been reported that thymol has antibacterial and antiviral activity (Calsamiglia *et al.* 2007; Benchaar *et al.* 2008); the antimicrobial impact of carvacrol was also proven (Nostro and Papalia, 2012). Oregano (*Origanum Vulgare*) is an herbaceous essential oil bearing plant belonging to the Lamiaceae family and is also known as European, wild, common or grove marjoram as well as the joy of the mountain. It has been also reported that thymol and carvacrol are major compounds of oregano (Calsamiglia *et al.* 2007). Furthermore, it is also known that the effects of essential oils may be subjected to change based on the variations in the chemical composition of medicinal plants that may be observed due to the origin, the locality, the climate conditions, and the harvest time of the collected plant material (Özguven and Tans, 1998; Marino *et al.* 2001).

The beneficial effects of essential oil-bearing plants in animal nutrition may include the stimulation of appetite and feed intake, the improvement of endogenous digestive enzyme secretion, activation of immune response and antibacterial actions, have been confirmed in many studies (Benchaar *et al.* 2008). A study reported that adding some active compounds of EO (thymol, eugenol, vanillin, and limonene) in high concentrate diets of Holstein heifers had no effect on feed intake (Cardozo *et al.* 2006). Chaves *et al.* (2008) also showed supplement of carvacrol into the diet of lambs did not result in any change in live weight of the lambs. On the other hand, other researchers reported that 300 mg/kg as fed of plant extracts (gentian root, oil of juniper, oil of thyme, tannins and silicic acid) supplementation in the diet of piglets significantly increased ( $P<0.05$ ) total leucocyte counts, total neutrophils, and lymphocytes.

There is little research that has attempted to compare the synchronously effects of THY and ORE as feed additives in suckling calves nutrition. Although improvements in animal performance by adding thymol and carvacrol has been reported (Alcicek *et al.* 2003), there is limited data and also conflicting findings of using of EO in ruminants (Bampidis *et al.* 2005; Chaves *et al.* 2008). In the present study, it was hypothesized that THY and ORE essences separately or a mixture of THY and ORE essences mixed in milk fed to calves as an alternative to antibiotic would improve the growth performance, blood haematological and biochemical parameters of suckling calves. Despite the large number of studies on supplementation of essential oils in diets of dairy cows (Benchaar *et al.* 2008), very limited data are available related to the effects of thyme and oregano essences on calves' performance. Therefore, in the present work we have investigated the effect of essential oils and constituents obtained from oregano and thyme on growth

performance, haematological parameters and some blood metabolites of Holstein suckling calves.

## MATERIALS AND METHODS

### Preparation and analysis of essential oils

Thyme (*Thymus vulgaris*) and oregano (*Origanum vulgare*) oils were obtained from Golghatrehtos Co., Mashhad, Iran. To analyze the chemical composition of both EO, gas chromatographic analysis was performed using a Gas Chromatograph PU 4500 system (Shimadzu Corp., Kyoto, Japan) equipped with a capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven temperature was held at 50 °C for 5 min and then programmed to 250 °C at a rate of 3 °C/min. Injector and detector (FID) temperatures were 290 °C; helium was used as the carrier gas with a linear velocity of 32 cm/s. The chemical composition of AJW and THY oils were calculated by the peak areas without the use of response factors correction. The main compounds identified in these oils and their components are listed in Table 1.

### Calves, management and treatments

Forty-eight Holstein suckling calves with an average weight of  $48.31 \pm 5.82$  kg were used in a completely randomized design. The calves were housed in individual tie stalls on mattresses bedded with straw in pens ( $2 \times 1.5$  m<sup>2</sup>). All pens were located in a covered barn. The calf house was equipped with controlled ventilation. The study lasted for 9 weeks including one week adaptation period.

Calves were fed colostrum for 3 days and then received whole milk, using calf buckets, 4 kg of fresh milk/head day from day 4 to 15, 5 kg from day 16 to 45, and 4 kg from day 46 until the end of the trial (day of 63). The THY and ORE essential oils additives were mixed just prior to feeding in milk which offered twice (8:00 and 16:00 h) daily with a calf starter ad libitum. The starter diet was formulated based on NRC (2001) recommendations (Table 2). At the start of experiment, animals were randomly assigned to one of four treatments: CON: control without essential oil supplement, THY: supplement of 5 mL/d/calf THY oil in milk, ORE: supplement of 5 mL/d/calf ORE oil in milk, and TOM: supplement of 2.5 mL THY oil + 2.5 mL/d/calf ORE oil mixed in milk.

### Data collection

The amount of calf starter offered and refused were recorded daily for each animal to obtain the starter feed intake. The animals were weighed at the start of experiment and continued every two weeks until the end of the experiment.

**Table 1** Qualitative and quantitative composition of oregano and thyme essential oils (DM basis)

Plant species	Main Components	Composition (%)
<i>Origanum vulgare</i>	Thymol	37.30
	Carvacrol	29.06
	Gamma-terpinene	9.60
	P-cymene	4.50
	Carvacrol methyl ether	6.88
	Cis-alpha-bisabolene	6.80
	Eucalyptol	3.82
	Elemol	2.04
<i>Thymus vulgaris</i>	Thymol	45.20
	P-cymene	21.60
	Gamma-terpinene	28.10
	Carvacrol	2.60
	Beta-pinene	1.70
	Terpinene-4-ol	0.80

Body weight gain was calculated as weight gain= (Final body weight-initial body weight) and the average daily gain (ADG) calculated simply as the average amount of weight an animal has gained each day for two weeks. Feed conversion rate (FCR) was also calculated by dividing starter DM intake + milk DM (total DM) intake by ADG.

Blood samples were collected 2 hrs after morning feeding by jugular vein using vacutainer tubes containing sodium heparin from all calves at the end of the experiment (day of 63). Serum was separated by centrifugation at 3500 rpm for 10 min and stored at -20 °C until required for analysis. Anticoagulated blood was analyzed shortly after collection for the red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) by an automatic haematology cell counter (Nihon kohden, Celltaca, Tokyo, Japan). Manual white blood cell (WBC) differential counting was also performed by microscopic examination of Wright–Giemsa-stained smears (Jain, 1998). Stored serum samples were analyzed for blood urea nitrogen (BUN), total cholesterol (TC), total triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate transaminase (AST), calcium (Ca), phosphorus (P) and iron (Fe) by a Selectra E autoanalyzer (Vital Scientific NV, DIEREN, Netherland), using commercial kits (Pars azmoon, Tehran, Iran).

### Chemical analyses

For analysing the starter diet, all feed samples for each calf were mixed together. The samples were dried for 48 hrs at 60 °C for dry matter (DM) and grounded in a Retsch GM 200 mill (Retsch Technology GmbH, Haan, Germany). Ether extract (Tecator soxtec system HT 1043 extraction unit by Tecator, Foss North America, Eden prairie, MN), and crude protein (1030 micro- kjeldahl auto analyzer; Tecator, Foss North America) were analyzed according to the

methods of association of official analytical chemist (AOAC, 2005).

Milk samples were also analyzed for fat, protein, lactose and total solid by milko- tester (Micro scan; Foss Electric A/S, Denmark).

The composition of starter and milk was reported in Table 2.

### Statistical analysis

The data of feed intake and growth performance were analyzed as a completely randomized design with repeated measured by the PROC MIXED of SAS 9.1 (SAS, 2004) according to the model (1):

$$Y_{ijk} = \mu + T_i + C_{(ij)} + t_k + (T \times t)_{ik} + E_{ijk}$$

Where:

$Y_{ijk}$ : dependent variable.

$\mu$ : overall mean.

$T_i$ : treatment effect.

$t_k$ : time (of sampling) effect.

$(T \times t)_{ik}$ : treatment  $\times$  time interaction.

$C_{(ij)}$ : animal (within treatment) effect.

$E_{ijk}$ : residual effect.

Blood haematological and biochemical parameters were analysed as a completely randomized design by the PROC MIXED of SAS 9.1 (SAS, 2004) according to the model (2):

$$Y_{ij} = \mu + T_i + E_{ij}$$

Where:

$Y_{ij}$ : response variable.

$\mu$ : overall mean.

$T_i$ : treatment effect.

$E_{ij}$ : residual effect.

**Table 2** Ingredients and nutrient composition of the basal diet (DM basis)

Ingredient composition	% of DM
Alfalfa hay	10
Barely	25
Yellow corn	25
Soybean meal	30
Wheat bran	7
Salt	1
Mineral-vitamin premix <sup>1</sup>	2
Nutrient composition	% of DM
Dry matter	92.10
Net energy of maintenance (M cal/kg) <sup>2</sup>	2.24
Net energy of growth (M cal/kg) <sup>2</sup>	1.70
Crude Protein	22.40
Ether extract	3.20
Ash	8.00
Calcium <sup>2</sup>	1.60
Phosphorus <sup>2</sup>	0.50
Magnesium	0.21
Chloride	0.72
Potassium	1.05
Sodium	0.42
Sulfur	0.22

<sup>1</sup> Premix composition per kg: vitamin A: 500000 IU; vitamin D<sub>3</sub>: 100000 IU; vitamin E: 100mg; Ca: 196000 mg; P: 96000; Mg: 19000 mg; Na: 71000; Cu: 300 mg; Fe: 3000 mg; Mn: 200 mg; Zn: 300 mg; I: 100 mg; Co: 100 mg and Se: 100 mg.

<sup>2</sup> Calculated (NRC, 2001).

Significant differences between the averages of treatments were assessed by the Duncan's test with P-value of 0.05 for all parameters.

## RESULTS AND DISCUSSION

### Feed intake and calf performance

The daily intakes of starter diets (Figure 1) indicate that the starter feed intake was increased over the weeks. As shown in Figure 2, supplementation of ORE oil in milk improved ADG ( $P>0.05$ ) in the last phase of this experiment, even though from the 4th week the shape of the curve changed with a dramatic rise from the 6th week. As shown in Table 3, the starter and total feed intake was increased ( $P<0.0001$ ) in calves received ORE oil and mixture of THY and ORE oils, but supplementation with EO in milk did not affect ( $P>0.05$ ) final body weight, ADG and FCR. As might be expected, the time effect was significant ( $P<0.0001$ ) for all mentioned variables, since there is a higher need to consume more nutrients as calves age. There was an interaction between treatment and age for the starter and total feed intake ( $P<0.0001$ ), and total ADG ( $P=0.01$ ); this interaction was not observed for final BW and FCR ( $P>0.05$ ).

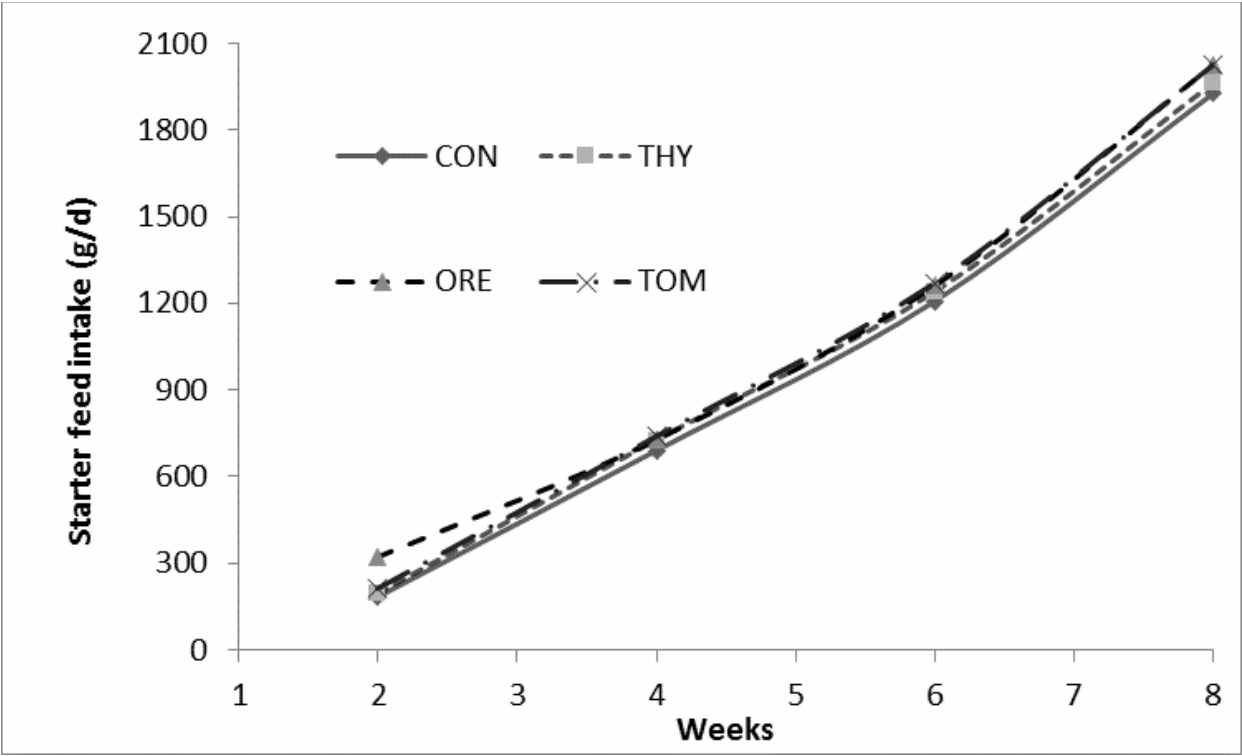
### Haematological blood serum

As shown in Table 4, statistical analysis indicated that ORE oil and mixture of THY and ORE oil groups increased ( $P<0.05$ ) RBC count compared with THY group, while there was no significant difference between the control group

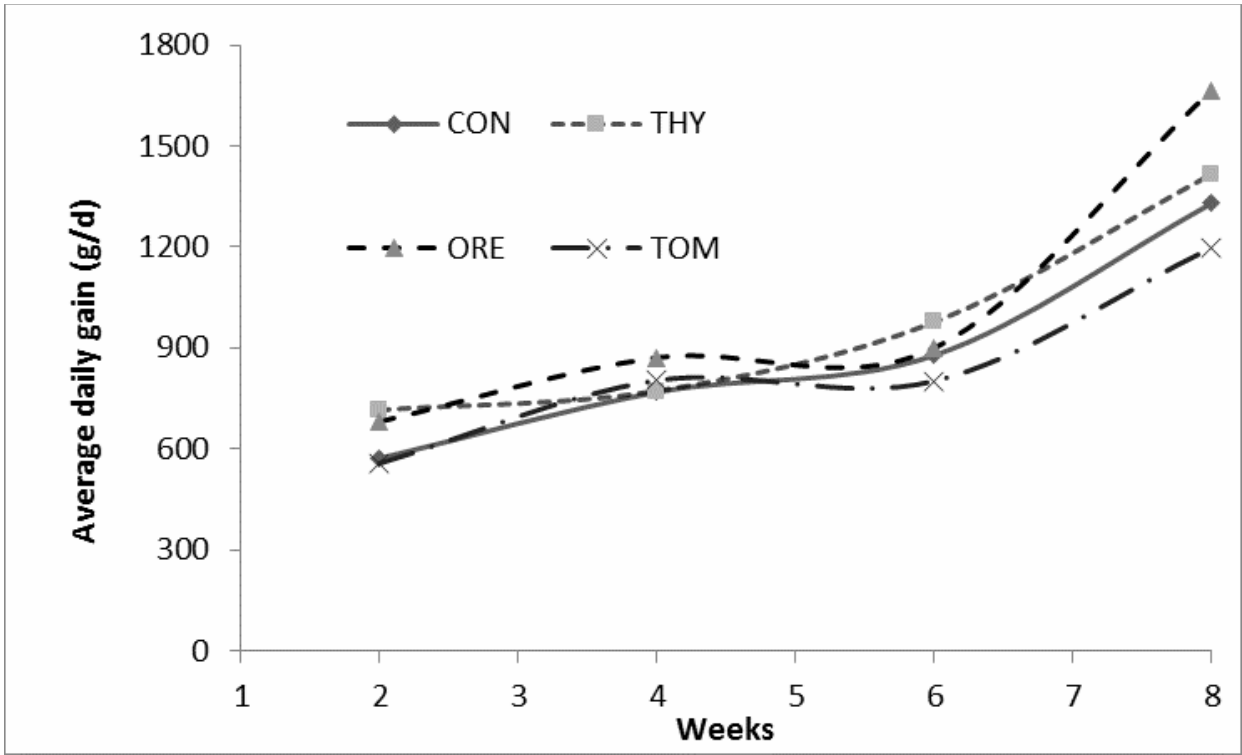
up with ORE and TOM groups. Values of PCV, Hb, and MCV were also higher ( $P<0.05$ ) in the ORE group compared with the control. In this experiment, differential counting of WBC showed no differences between treatments for monocyte and eosinophil counts. The proportion of lymphocyte was higher ( $P<0.05$ ) in calves given ORE oil compared with the control. Although in our study, the neutrophil percentage was slightly ( $P>0.05$ ) elevated in ORE group than in all the other groups. Band (young) neutrophil was also increased ( $P<0.05$ ) in calves given THY and ORE oils mixture group compared to the other groups.

### Blood metabolites

Table 5 represents the effects of the EO on some blood metabolites. Serum BUN concentration, ALT and ASP activities were significantly increased ( $P<0.05$ ) in calves given 5 mL/d/calf of ORE or THY essential oils when compared with other treatments. However, THY and ORE oils mixture lowered ( $P<0.05$ ) serum BUN concentration than other treatments except for the control group. Our results also indicated that ORE essential oil (5 mL/d/calf) increased ( $P<0.05$ ) phosphorus concentration in blood serum compared with the control (Table 4). Serum iron concentration was significantly increased ( $P<0.05$ ) in calves given THY or ORE groups and EO mixture supplementation when compared with the control (250.68, 251.85, 268.28 vs. 140.00 mg/dL, respectively). As shown in Table 5, HDL concentration was increased ( $P<0.05$ ) in calves fed on milk containing THY essential oil.



**Figure 1** Starter feed intake (g/day) of calves supplemented with essential oils in milk  
CON: control; THY: 5 mL/d/calf thyme oil; ORE: 5 mL/d/calf oregano oil and TOM: 2.5 mL thyme oil + 2.5 mL/d/calf oregano oil



**Figure 2** Average daily gain (g/d), according to week of age, of calves supplemented with essential oils in milk  
CON: control; THY: 5 mL/d/calf thyme oil; ORE: 5 mL/d/calf oregano oil and TOM: 2.5 mL thyme oil + 2.5 mL/d/calf oregano oil

**Table 3** Effects of oregano and thyme essential oils (EO) on feed intake and performance in suckling calves

Item	Treatments				SEM	P-value		
	CON	THY	ORE	TOM		Treatment	Time	Treatment × time
Final body weight (kg)	68.21	72.68	72.93	69.48	2.39	0.443	< 0.0001	0.370
Starter feed intake (g/d)	883.59 <sup>c</sup>	909.38 <sup>b</sup>	933.47 <sup>a</sup>	936.13 <sup>a</sup>	0.451	< 0.0001	< 0.0001	< 0.0001
Total feed intake (g/d)	1401.02 <sup>c</sup>	1425.33 <sup>b</sup>	1447.92 <sup>a</sup>	1450.76 <sup>a</sup>	0.609	< 0.0001	< 0.0001	< 0.0001
Average daily gain (g/d)	769.84	883.73	900.79	761.90	45.22	0.097	< 0.0001	0.010
Feed conversion rate (g/g)	1.82	1.75	1.98	2.13	0.171	0.455	< 0.0001	0.255

CON: control; THY: 5 mL/d/calf thyme oil; ORE: 5 mL/d/calf oregano oil and TOM: 2.5 mL thyme oil + 2.5 mL/d/calf oregano oil.

SEM: standard error of the means.

However, THY and ORE oils mixture decreased ( $P<0.05$ ) LDL concentration than other treatments. Concentrations of TG and TC was decreased ( $P<0.05$ ) in calves given essential oils compared to the control group.

### Feed intake and growth performance

In our experiment, the increase of the starter and total feed intake in the ORE and TOM groups, were in agreement with the previous findings (Morrill and Dayton, 1978; Thomas *et al.* 2007; Fathi *et al.* 2009) who reported using different flavours added to milk replacer or calf starters. Other findings were reported by Cardozo *et al.* (2006) who reported that 2 or 4 g/d of essential oil mixture (compounds consisting of thymol, eugenol, vanillin, and limonene) inclusion in milk replacer of calves had no effect on feed intake. Santos *et al.* (2015) also showed that EO blend had no effect on the acceptance of liquid or solid diet, suggesting that, the intake responses to oils have been variable. In a study with growing lambs, Chaves *et al.* (2008) found that addition of carvacrol or cinnamaldehyde (200 mg/kg of dietary DM) had no effect on feed intake. Other researchers reported that EO mixture had no effects on acceptance of liquid or solid diet suggesting that there was no or minor change in palatability (Santos *et al.* 2015). However, other researchers indicated the decrease in feed intake in cattle supplemented with EO might be related to palatability problems (Calsamiglia *et al.* 2007).

In our study, the effects of THY and ORE essential oils supplementation to milk on ADG and FCR were in agreement with the findings of Vakili *et al.* (2013) that supplementing feedlot cattle with thyme and cinnamon had no effect on ADG and FCR. Daily weight gain of the piglets fed EO blend (0, 150 and 300 mg/kg) were also not affected (Manzanilla *et al.* 2004). Generally in our study, in accord with the lack of EO effects on ADG and FCR may be related to EO source, type of diet (Geraci *et al.* 2012), and factors such as body weight and animal growth stage. Therefore in our study, the lack of EO effects on FI, ADG, and FCR may be related to EO source, type of diet, diet interactions or adaptation of rumen microbial population to EO (Geraci *et al.* 2012).

### Haematological blood serum

Since thyme and oregano extracts do have essential oil, tannins, glycosides, saponins and other components; other researchers reported low levels of saponins can increase intestinal villi diameter which is permeable to large molecules like ferritin. Therefore, ferritin concentration may be responsible for the increased Hb and RBC counts by the EO supplementation (Franchini *et al.* 2007).

In our experiment, the increase ( $P<0.05$ ) of RBC means that supplementation of ORE and TOM groups can stimulate to produce higher RBC to carry oxygen throughout the body.

An elevated percentage of lymphocytes in this experiment was in line with the results of Savoini *et al.* (2002), who reported lymphocytes were increased ( $P<0.05$ ) by 300 mg/kg as fed of plant extracts supplementation in diets of piglets when compared with the control. In our experiment, the increase of some WBC differential values are an indicator for the non-specific stimulation of cellular immunity and demonstrates that the influence of ORE and THY essential oils on the immune response can be determined in any case by the recorded parameters of the blood picture. It has also been known that the non-specific reactivity of the immune system of many animals can be enhanced by administration of certain plant extracts, possibly due to their antioxidant activity. Flavonoids, which are found in EO and extracts of many natural substances, have been shown to promote phagocytic activity (Ncube *et al.* 2008).

### Blood metabolites

The concentration of BUN in serum was lowered by EO with TOM group as an exception. The effect of AJW and THY oils on BUN concentration in our experiment is in accordance with previous research using different dosages of cinnamaldehyde supplementation in beef cattle (Yang *et al.* 2010) and lambs (Chaves *et al.* 2011). On the contrary, Vakili *et al.* (2013) found that BUN concentration was not affected by adding thymol or cinnamaldehyde separately. The inconsistency between studies may be due to dosage rate and compounds of EO and experimental conditions (Benchaar *et al.* 2008).



**Table 4** Effects of oregano and thyme essential oils (EO) on blood haematological parameters in suckling calves

Parameters	Treatments				SEM	P-value
	CON	THY	ORE	TOM		
RBC (106/ $\mu$ L)	5.40 <sup>ab</sup>	4.30 <sup>b</sup>	6.48 <sup>a</sup>	6.23 <sup>a</sup>	0.57	0.049
PCV (%)	26.75 <sup>c</sup>	34 <sup>ab</sup>	37.50 <sup>a</sup>	30.25 <sup>bc</sup>	2.24	0.003
Hb (g/dL)	8.50 <sup>c</sup>	11.30 <sup>ab</sup>	12.65 <sup>a</sup>	10.05 <sup>b</sup>	0.84	0.002
MCV (fL)	35.92 <sup>c</sup>	45.30 <sup>b</sup>	50.97 <sup>a</sup>	40.30 <sup>c</sup>	2.51	0.001
MCH (pg)	12.42 <sup>b</sup>	15.02 <sup>ab</sup>	16.77 <sup>ab</sup>	13.37 <sup>b</sup>	2.59	0.028
Lymphocyte (% of WBC)	60.25 <sup>b</sup>	65.25 <sup>ab</sup>	68.75 <sup>a</sup>	64.00 <sup>ab</sup>	2.26	0.032
Neutrophil (% of WBC)	22.00 <sup>b</sup>	25.25 <sup>b</sup>	28.75 <sup>ab</sup>	24.50 <sup>b</sup>	1.68	0.030
Monocyte (% of WBC)	1.20	1.60	2.00	1.40	0.31	0.074
Eosinophil (% of WBC)	1.20	1.20	1.20	1.00	0.28	0.095
Band neutrophil (% of WBC)	3.25 <sup>b</sup>	5.30 <sup>ab</sup>	3.50 <sup>b</sup>	6.82 <sup>a</sup>	1.14	0.043

CON: control; THY: 5 mL/d/calf thyme oil; ORE: 5 mL/d/calf oregano oil and TOM: 2.5 mL thyme oil + 2.5 mL/d/calf oregano oil.

RBC: red blood cell; PCV: packed cell volume; Hb: haemoglobin; MCV: mean corpuscular volume and MCH: mean corpuscular haemoglobin.

The means within the same row with at least one common letter, do not have significant difference ( $P>0.05$ ).

SEM: standard error of the means.

**Table 5** Effects of oregano and thyme essential oils (EO) on some blood biochemical parameters in suckling calves

Parameters	Treatments				SEM	P-value
	CON	THY	ORE	TOM		
BUN (mg/dL)	22.34 <sup>b</sup>	26.82 <sup>a</sup>	25.87 <sup>a</sup>	23.44 <sup>b</sup>	0.62	0.023
HDL (mg/dL)	102.33 <sup>b</sup>	130.93 <sup>a</sup>	117.36 <sup>ab</sup>	105.66 <sup>b</sup>	19.20	0.010
LDL (mg/dL)	44.60 <sup>a</sup>	41.54 <sup>a</sup>	31.56 <sup>ab</sup>	18.87 <sup>b</sup>	15.32	0.001
ASP (U/L)	39.37 <sup>b</sup>	59.82 <sup>a</sup>	62.50 <sup>a</sup>	40.57 <sup>b</sup>	6.89	0.013
ALT (U/L)	29.37 <sup>b</sup>	40.58 <sup>a</sup>	40.43 <sup>a</sup>	26.55 <sup>b</sup>	3.89	0.041
TG (mg/dL)	25.52 <sup>a</sup>	12.44 <sup>b</sup>	11.20 <sup>b</sup>	9.34 <sup>b</sup>	2.65	0.034
TC (mg/dL)	117.02 <sup>a</sup>	92.50 <sup>b</sup>	88.25 <sup>b</sup>	93.00 <sup>b</sup>	21.35	0.001
Ca (mg/dL)	6.47	6.09	7.34	7.04	1.04	0.076
P (mg/dL)	7.95 <sup>b</sup>	8.36 <sup>ab</sup>	9.34 <sup>a</sup>	8.43 <sup>ab</sup>	0.54	0.012
Fe (mg/dL)	140.00 <sup>b</sup>	250.68 <sup>a</sup>	251.85 <sup>a</sup>	268.28 <sup>a</sup>	43.67	0.033

CON: control; THY: 5 mL/d/calf thyme oil; ORE: 5 mL/d/calf oregano oil and TOM: 2.5 mL thyme oil + 2.5 mL/d/calf oregano oil.

BUN: blood urea nitrogen; HDL: high density lipoprotein; LDL: low density lipoprotein; AST: aspartate transaminase; ALT: alanine aminotransferase; TG: total triglyceride; TC: total cholesterol; Ca: calcium; P: phosphorus and Fe: iron.

The means within the same row with at least one common letter, do not have significant difference ( $P>0.05$ ).

SEM: standard error of the means.

Ruminal ammonia-N in excess of the microbial requirement is absorbed across the rumen wall into portal blood, and most of it is converted to urea in the liver. Synthesis of urea in the liver is performed from ammonia absorbed from the rumen; as a result, urea N concentration in blood is highly correlated with the rumen ammonia-N concentration (Petit and Flipot, 1992; Davidson *et al.* 2003). Therefore, reduction of BUN concentration in our experiment was probably due to the impact of EO on hyper-ammonia producing bacteria resulting in reduced production of ammonia-N (Benchaar *et al.* 2008).

Reduced concentrations of TG and TC in this experiment were in disagreeing with the findings of Alsah *et al.* (2014) who observed that TC and TG concentrations were not affected by thymol and cinnamaldehyde mixture when compared with the control. Vakili *et al.* (2013) also reported no significant differences in the concentrations of TC and TG in calves fed on diets containing 5 g/d/calf thyme and cinnamon EO separately. Nevertheless, our result contrasts with the findings of Chaves *et al.* (2008) who reported that TG serum concentration was 18- fold higher in lambs supplemented by other EO compared to the control. Similar to our result, Elson and Qureshi (1995) found that isoprenoids

and end products of plant secondary metabolism suppresses cholesterol synthesis by inhibiting the production of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate controlling enzyme in the cholesterol synthetic pathway. Thus, in this experiment, the changes in blood metabolites propose that feeding EO from AJW and THY seems to have influences on the function of organs associated with the tested blood metabolites. In addition, differences in concentrations of serum TC and TG could be an indicator of the changes in fat mobilization (Chaves *et al.* 2008) and our study reveal lowering effect of either ORE or THY essential oils on TC and TG serum concentrations.

## CONCLUSION

Dietary supplementation of both oregano and mixed oregano and thyme essential oils improved ( $P<0.0001$ ) the parameters of starter and total feed intake. Furthermore, there was the significant increase in red blood cell count, haemoglobin concentration, haematocrit, mean corpuscular volume, lymphocyte percentage and serum phosphorous concentration by oregano group. Our results demonstrate the supplementation of either oregano or thyme essential oils

increased serum iron concentration, alanine aminotransferase, and aspartate transaminase activities, but serum triglyceride and total cholesterol concentrations were reduced by them. So, this study suggests that oregano oil could be considered as suitable feed additives for use in dairy calves nutrition. However, interpreting the results from this research must be with caution; and more research is needed to identify and clearly explain in the contribution of oregano or thyme essential oils (individual or mixtures) to improve nutrient utilization and growth performance in suckling calves.

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## REFERENCES

- Alcicek A., Bozkurt M. and Cabuk M. (2003). The effect of an essential oil combination derived from selected herbs growing wild in Turkey on broiler performance. *South Africa J. Anim. Sci.* **33**, 89-94.
- Alsah A.A., Bassiony S.M., Abdel Rahman G.A. and Shehata S.A. (2014). Effect of cinnamaldehyde thymol mixture on growth performance and some ruminal and blood constituents in growing lambs fed high concentrate diet. *Life Sci. J.* **11**, 240-248.
- AOAC. (2005). Official Methods of Analysis. Vol. I. 18<sup>th</sup> Ed. Association of Official Analytical Chemists, Arlington, VA, USA.
- Bampidis V.A., Christodoulou V., Florou-Paneri P., Christaki E., Spais A.B. and Chatzopoulou P.S. (2005). Effect of dietary dried oregano leaves supplementation on performance and carcass characteristics of growing lambs. *Anim. Feed Sci. Technol.* **121**, 285-295.
- Baranauskiene R., Venskutonis P.R., Viskelis P. and Dambraskiene E. (2003). Influence of nitrogen fertilizers on the yield and composition of thyme (*Thymus vulgaris*). *J. Agric. Food Chem.* **51**, 7751-7758.
- Benchaar C., Calsamiglia S., Chaves A.V., Fraser G.R., Colombatto D., McAllister T.A. and Beauchemin K.A. (2008). A review of plant-derived EO in ruminant nutrition and production. *Anim. Feed Sci. Technol.* **145**, 209-228.
- Calsamiglia S., Busquet M., Cardozo P., Castillejos L. and Ferret A. (2007). Essential oils as modifiers of rumen microbial fermentation: a review. *J. Dairy Sci.* **90**, 2580-2595.
- Cardozo P.W., Calsamiglia S., Ferret A. and Kamel C. (2006). Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef heifers fed a high-concentrate diet. *J. Anim. Sci.* **84**, 2801-2808.
- Chaves A.V., Dugan M.E.R., Stanford K., Gibson L.L., Brystom J.M., McAllister T.A., Van Herk F. and Benchaar C. (2011). A dose – response of cinnamaldehyde supplementation on intake, ruminal fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livest. Sci.* **141**, 213-220.
- Chaves A.V., Stanford K., Dugan M.E.R., Gibson L.L., McAllister T.A., Van Herk F. and Benchaar C. (2008). Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. *Livest. Sci.* **117**, 215-224.
- Davidson P.M. (2001). Chemical preservatives and naturally antimicrobial compounds. Pp. 593-627 in Food Microbiology: Fundamentals and Frontiers. M.P. Beuchat and L.R. Montville, Eds. ASM Press, Washington, DC, USA.
- Davidson S., Hopkins B.A., Diaz D.E., Bolt S.M., Brownie C., Fellner V. and Whitlow L.W. (2003). Effects of amounts and degradability of dietary protein on lactation, nitrogen utilization, and excretion in early lactation Holstein cows. *J. Dairy Sci.* **86**, 1681-1689.
- Elson C.E. and Qureshi A.A. (1995). Coupling the cholesterol- and tumor-suppressive actions of palm oil to the impact of its minor constituents on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. *Prostaglandins Leukot. Essent. Fatty Acids* **52**, 205-208.
- European Commission. (2003). Regulation (EC). No. 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. *Off. J. European Union*. **268**, 29-43.
- Exarchou V., Nenadis N., Tsimidou M., Gerothanassis I.P., Troganis A. and Boskou D. (2002). Antioxidant activities and phenolic composition of extracts from greek oregano, greek sage, and summer savory. *J. Agric. Food Chem.* **50**, 5294-5299.
- Fathi M.H., Riasi A. and Allahresani A. (2009). The effect of vanilla flavoured calf starter on performance of Holstein calves. *J. Anim. Feed Sci.* **18**, 412-419.
- Franchini M., Salvagno G.L., Montagnana M. and Lippi G. (2007). Serum ferritin levels correlate with haemoglobin concentration: a report on 589 outpatients from a single centre. *Blood Transfus.* **5(4)**, 244-245.
- Geraci J.I., Garcarena A.D., Gagliostro G.A., Beauchemin K.A. and Colombatto D. (2012). Plant extracts containing cinnamaldehyde, eugenol and capsicum oleoresin added to feedlot cattle diets: ruminal environment, short term intake pattern and animal performance. *Anim. Feed Sci. Technol.* **176**, 123-130.
- Jain N.C. (1998). Essentials of Veterinary Hematology. Lea and Febiger Publication, Philadelphia, USA.
- Manzanilla E.G., Perez J.F., Martin M., Kamel C., Baucells F. and Gasa J. (2004). Effect of plant extracts and formic acid on the intestinal equilibrium of early weaned pigs. *J. Anim. Sci.* **82**, 3210-3218.
- Marino M., Bersani C. and Comi G. (2001). Impedance measurements to study the antimicrobial activity of essential oils from



- Lamiaceae and Compositae. *Int. J. Food Microbiol.* **67**, 187-195.
- Morrill J.L. and Dayton A.D. (1978). Effect of feed flavor in milk and calf starter on feed consumption and growth. *J. Dairy Sci.* **61**, 229-232.
- Ncube N., Afolayan S.A.J. and Okoh A.I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African J. Biotechnol.* **7(12)**, 1797-1806.
- Nostro A. and Papalia T. (2012). Antimicrobial activity of carvacrol: current progress and future perspectives. *Recent Pat. Anti-infect. Drug Discov.* **7**, 28-35.
- NRC. (2001). Nutrient Requirements of Dairy Cattle. 7<sup>th</sup> Ed. National Academy Press, Washington, DC, USA.
- Özguven M. and Tans S. (1998). Drug yield and essential oil of *Thymus vulgaris* as influenced by ecological and on to genetic variation. *Turkish J. Agric. For.* **22**, 537-542.
- Petit H.V. and Flipot P.M. (1992). Feed utilization of beef steers fed grass as hay or silage with or without nitrogen supplementation. *J. Anim. Sci.* **70**, 876-883.
- Santos F.H.R., De Paula M.R. and Lezier D. (2015). Essential oils for dairy calves: effects on performance, scours, rumen fermentation and intestinal fauna. *Animal.* **9(6)**, 958-965.
- Savoini G., Bontempo V., Cheli F., Baldi A., Sala V., Mancin G., Agazzi A. and Dell'Orto V. (2002). Alternative antimicrobials in the nutrition of postweaning piglets. *Vet. Rec.* **151(19)**, 577-580.
- SAS Institute. (2004). SAS<sup>®</sup>/STAT Software, Release 9.1. SAS Institute, Inc., Cary, NC, USA.
- Thomas L.C., Wright T.C., Formusiak A., Cant J.P. and Osborne V.R. (2007). Use of flavored drinking water in calves and lactating dairy cattle. *J. Dairy Sci.* **90**, 3831-3837.
- Vakili A.R., Khorrami B., Danesh Mesgaran M. and Parand E. (2013). The effects of thyme and cinnamon essential oils on performance, rumen fermentation and blood metabolites in Holstein calves consuming high concentrate diet. *Asian Australasian J. Anim. Sci.* **26(7)**, 935-944.
- Yang W.Z., Benchaar C., Ametaj B.N. and Beauchemin K.A. (2010). Dose response to eugenol supplementation in growing beef cattle: ruminal fermentation and intestinal digestion. *Anim. Feed Sci. Technol.* **158**, 57-64.