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Effects of Ajwain (*Trachyspermum ammi*) and Thyme (*Thymus vulgaris*) Oils on Nutrients Digestibility, Blood Parameters and Growth Performance of Brown Swiss Neonatal Calves

Research Article

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ABSTRACT

The aim of this study was to compare the effects of dietary supplementation of ajwain (*Trachyspermum ammi*) and thyme (*Thymus vulgaris*) essential oils (EO) on nutrients digestibility, blood parameters and growth performance of neonatal dairy calves during eight weeks. Sixty suckling dairy calves with an initial body weight (43.2 ± 3.8 kg) were randomly allocated to five treatments including CO, control without essential oils (EO) supplementation; AJW-L, ajwain (AJW) oil mixed in milk at 1 mL/d per calf; AJW-H, AJW oil mixed in milk at 2 mL/d; THY-L, thyme (THY) oil mixed in milk at 1 mL/d and THY-H, THY oil mixed in milk at 2 mL/d. The experiment consisted of 7 d adaptation and 60 d sampling and data recording period. Experimental diets had no significant effects ($P>0.05$) on feed intake, average daily gain and feed conversion ratio. Dietary supplementation of both AJW and THY linearly improved ($P<0.01$) the apparent total tract digestibility of dry matter, organic matter, neutral detergent fiber, and acid detergent fiber while AJW improved ($P<0.01$) the digestibility of organic matter and neutral detergent fiber in a quadratic manner. AJW-L and THY-L decreased while their high level increased ($P<0.05$ for quadratic effect) white blood cell count. Also, increasing level of both EO increased ($P<0.05$) blood serum glucose linearly. Increasing level of AJW increased ($P<0.05$) total triglyceride but decreased ($P<0.05$) blood urea nitrogen. Overall, the results of this study suggest that THY and AJW oils improved digestion, biochemical parameters, and some blood hematological parameters without influencing the performance of neonatal dairy calves.

KEY WORDS ajwain oil, blood metabolites, dairy calf performance, nutrients digestibility, thyme oil.

INTRODUCTION

Antibiotics have been used in animal diets successfully to improve the efficiency of nutrient utilization as well as to prevent disease (Benchaar *et al.* 2008). However, the use of antibiotics as feed additives in livestock production systems is banned in the European Union due to the emergence of antibiotic resistant bacteria that may represent a risk to hu man health (European Commission, 2003). Consequently, researchers have been directed toward developing alternatives to antibiotics in animal feeds. It has been estimated that the elimination of antibiotics from livestock diets has demonstrated an increase in production costs of 3.5-5.0% (Carro and Ranilla, 2002). Therefore, it is necessary to determine alternative additives and strategies that will allow producers to maintain the current level of production with-

out increasing the cost or the prevalence of metabolic, digestive or respiratory upsets in young animals including neonatal dairy calves.

In the last years, essential oils (EO) of plants have been considered as a unique feed additive alternative to growth promoters in animal feeds. The EO, known as volatile or ethereal oils, are mixtures that give plants and spices their color and scent. Chemically, EO are mixures of secondary metabolites commonly composed of terpenoids and phenylpropanoids (Calsamiglia *et al.* 2007).

Many of the antimicrobial compounds are constitutively expressed by the plants, and others can be synthesized as a mechanism of self-defense in response to pathogens (Rauha *et al.* 2000). Thyme (THY) and ajwain (AJW) oils possess noticeable antimicrobial effect (Davidson *et al.* 2003). Thyme oil extracted from *Thymus vulgaris*, a perennial shrub belonging to the Lamiaceae family, has various beneficial effects as antiseptic and carminative with antimicrobial and antioxidative properties (Baranauskiene *et al.* 2003). Previous studies showed that the main constituents of THY 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). However, thymol is also a major compound of AJW oil which is extracted from AJW (*Trachyspermum ammi*), an annual herbaceous EO bearing plant that belongs to the Apiaceae family which grows in India, Iran, and Egypt (Zargari, 1996). For example in poultry, although improvements in animal performance by adding thymol and carvacrol, have been reported (Alcicek *et al.* 2003; Pournazari *et al.* 2017), there is limited data and also conflicting findings of using EO in ruminants (Bampidis *et al.* 2005; Chaves *et al.* 2008).

In the study by Cardozo *et al.* (2006) adding some active compounds of EO (thymol, eugenol, vanillin, and limonene) mixed in milk replacer of calves had no effect on feed intake. Chaves *et al.* (2008) showed that supplementation of carvacrol into the diet of lambs did not result in any change in live weight. However, Williams and Losa (2001) reported that EO of THY has a stimulating effect on digestion in poultry. On the other hand, other researchers reported that 300 mg/kg as fed of plant extracts (gentian root, juniper oil, THY oil, tannins, and silicic acid) supplementation in the diet of piglets significantly increased total leukocyte counts, total neutrophils, and lymphocytes (Savoini *et al.* 2002).

To the best of our knowledge, no research has synchronously compared the effects of AJW and THY as feed additives in neonatal dairy calves nutrition. Despite the large number of studies on supplementation of EO in diets of poultry and dairy cows (Benchaar *et al.* 2008; Kung *et al.* 2008), very limited data is available related to the effects of AJW and THY essences on calves performance. In the present study, it was hypothesized that EO from AJW and THY mixed in milk as alternatives to antibiotics would improve the growth performance, nutrients digestibility, blood hematological and biochemical parameters of neonatal dairy calves.

Therefore, this experiment was conducted to compare the effects of supplementation of milk with two levels of EO extracted from AJW and THY on growth performance, hematological parameters and some blood metabolites of Brown Swiss suckling calves.

MATERIALS AND METHODS

Preparation and analysis of essential oils
Both THY and AJW grow in the semi-arid climate of Iran. The EO of these plants were obtained from Golghatrehtoos Co. (Mashhad, Iran). To analyse the chemical composition of both EO, gas chromatographic analysis was performed using a Gas Chromatograph (PU 4500, 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.

Animals and experimental design
Sixty newborn and milk-fed Brown Swiss calves with an average initial body weight of 43.2 ± 3.8 kg and age of 3 d were used in a completely randomised design. Calves were fed colostrum for 3 d then switched to milk until the end of the trial.

Moreover, the calves were adapted over a period of 7 d and the study lasted for 60 d after adaptation period. Animals were randomly assigned to one of the five treatments: control without EO supplementation (CO), supplement of 1 mL/d per calf AJW oil in milk (AJW-L), supplement of 2 mL/d AJW oil in milk (AJW-H), supplement of 1 mL/d THY oil in milk (THY-L), and supplement of 2 mL/d THY oil in milk (THY-H).

Diet and animal management
The EO additives of THY and AJW were mixed with milk just before feeding at 7:00 and 18:00. Whole pasteurized milk was fed at 10% of body weight per day with *ad libitum* access to a calf starter formulated to meet daily nutrient requirements of a neonatal calf (NRC, 2001; Table 1).

Table 1 Ingredients and nutrient composition of the starter and milk fed to calves

Ingredient composition of starter	% Dry matter (DM)
Barley grain	30.0
Yellow corn grain	30.0
Soybean meal	30.0
Wheat bran	7.0
Salt	1.0
Mineral-vitamin premix[1]	2.0
Nutrient composition of starter	% DM, otherwise stated
Dry matter (DM) (% as fed)	92.1
Organic matter	91.9
Net energy for maintenance (MJ/kg DM)	9.37
Net energy for growth (MJ/kg DM)	7.45
Neutral detergent fiber	16.4
Acid detergent fiber	6.55
Crude protein	22.6
Ether extract	3.20
Starch	31.4
Sugar	7.65
Ash	8.00
Calcium	1.60
Phosphorus	0.50
Magnesium	0.21
Chloride	0.72
Potassium	1.05
Sodium	0.42
Sulphur	0.22
Composition of milk (%)	
Total solid	11.8
Solid non-fat	8.34
Fat	3.94
Protein	3.31
Lactose	4.55

[1] Premix composition per kg: vitamin A: 500000 IU; vitamin D_3: 100000 IU; vitamin E: 100 mg; Ca: 196 g; P: 96 g; Mg: 19 g; Na: 71 g; Cu: 300 mg; Fe: 3000 mg; Mn: 200 mg; Zn: 300 mg; I: 100 mg; Co: 100 mg and Se: 100 mg.

All calves had free access to water and were housed in individual tie stalls on mattresses bedded with straw in pens (2.0×1.5 m).

All pens were located in a covered barn. The calf house was equipped with controlled ventilation where temperature (17 ˚C) and relative air humidity (65%) were controlled and monitored.

Feed intake, average daily gain and feed conversion rate

Amounts of starter offered and refused were recorded daily for each animal to calculate the starter intake throughout the trial. The animals were weighed at the beginning of the experiment and with two weeks interval thereafter until the end of the experiment. Body weight gain was calculated as weight gain= (final body weight-initial body weight), and the average daily gain (ADG) was determined by regression. Feed conversion ratio (FCR) was calculated by dividing total feed intake per calf by the total body weight gained per the same animal.

Apparent nutrients digestibility

Fecal samples were collected at 4 h after marker dosing, followed by every 6 h for 72 h. The samples were analyzed for the Cr content. Fecal samples for determining digestibility were collected every day during the last 5 d of the collection period. All Faeces during the last 5 d of the experiment were collected, mixed, and weighed. A sub-sample was collected for each calf and stored (-20 ˚C) for further analysis. Before chemical analysis, the samples were thawed at room temperature, dried for 48 h at 60 ˚C, and ground in a Retsch GM 200 mill with 1-mm screen (Retsch Technology GmbH, Haan, Germany). Dry matter (DM), organic matter (OM), ether extract (EE), calcium and phosphorous contents of starter and milk were analysed according to the methods of Association of Official Analytical Chemists (AOAC, 2005). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were analysed as proposed by Van Soest *et al*. (1991). Both NDF and ADF were not corrected for ash.

Starch content of starter was analysed using an enzymatic method (Karkalas, 1985). Milk samples were analysed for fat, protein, lactose and total solid by milko-tester (MilkoScan, FOSS Electric A/S, Denmark). Milk ash was calculated by difference. Total tract apparent digestibility coefficients of DM, OM, NDF and ADF were calculated by the equation (total nutrient consumed minus the nutrient excreted) divided for total nutrient consumed and expressed as percentages.

Blood sampling and analysis

Two hours after morning feeding, blood samples were collected by jugular vein puncture from all calves at days 20, 40, and 60 of the experiment. Two milliliters of blood were anticoagulated with ethylenediaminetetraacetic acid (EDTA) for hematological measurement and plain tubes supplied serum for measurement of some biochemical parameters. Blood samples were centrifuged at 3000 rpm for 10 min at 4 °C and collected serum was immediately transported to the laboratory and stored at -20 °C until required for analysis. Anticoagulated blood was analysed shortly after collection for number of red blood cell (RBC), hemoglobin (Hb), packed cell volume (PCV), number of white blood cell (WBC), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) by an automatic hematology cell counter (Nihon kohden, Celltaca, Tokyo, Japan). Manual WBC differential counting was performed by microscopic examination of Wright-Giemsa-Stained smears. Stored serum samples were analysed for concentration of glucose (GLU), total cholesterol (TC), albumin (ALB), total protein (TP), blood urea nitrogen (BUN), total triglyceride (TG) and creatinine (CR) by Selectra E Auto Analyzer (Vital Scientific NV, DIERN, Netherland), using commercial kits (Pars Azmoon, Tehran, Iran).

Statistical analysis

The data of blood parameters including whole-blood hematological and serum biochemical values were analysed using the MIXED procedure of SAS (2004) with considering time of sampling as a repeated measure. Feed intake, performance and diet digestibility coefficients were analysed by one way ANOVA. Significant differences between treatment LSMEANS were assessed by the linear and quadratic pre-planned contrasts and significance was adopted for values of P < 0.05 for all parameters.

RESULTS AND DISCUSSION

Chemical composition of essential oils

Analysis of EO extracted from THY indicated that thymol (45.2%) was the major component followed by gamma ter-

pinene (28.1%), p-cymene (17.3%), carvacrol (2.64%), β-pinene (1.70%), and terpinene-4-ol (0.80%). Thymol (46.2%) was the major component of EO extracted from AJW followed by gamma-terpinene (14.1%), P-cymene (9.90%), myrcene (5.93%), linalool (4.00%), α-thujene (2.80%), carvacrol (2.46%), and α-Pinene (0.30%).

Feed intake, average daily gain and feed conversion ratio

The feed intake, ADG, and FCR were not affected (P>0.05) by treatments with the exception for a tendency (P=0.07) for an quadratic increase in feed intake during day 15 and an quadratic increase (P=0.05) in ADG during day 30 of study due to THY (Table 2). As might be expected, the time effect was significant (P<0.01) for the mentioned parameters, since the requirements increase with advancing the age of growing animals.

Apparent nutrient digestibility

Dietary supplementation of both AJW and THY linearly improved (P<0.01) the apparent total tract digestibility of DM, OM, NDF and ADF while AJW improved (P<0.01) the digestibility of OM and NDF in a quadratic manner with more impact being detected with AJW-L and AJW-H, respectively (Table 3). Furthermore THY improved (P<0.01) digestibility of DM and OM, and tended (P=0.06) to improve digestibility of ADF in a quadratic manner with the greatest effect from THY-L.

Haematological blood serum

As shown in Table 4, low level of both AJW and THY decreased whereas their high level increased the WBC count (P<0.05 for quadratic effect). The MCH, MCHC, and count of RBC were not affected (P>0.05) by EO supplementation. Hb and PCV were reduced (P<0.05) by AJW in a quadratic manner.

Moreover, low level of both EO increased Hb and PCV (P<0.05 for quadratic effect). Low level of THY decreased MCV (P<0.05 for quadratic effect). Percentage of monocytes and eosinophils were not affected (P>0.05) by EO supplementation whereas increasing level of THY reduced (P<0.05) percentage of lymphocytes linearly but high level of THY increased (P<0.01 for quadratic effect) percentage of neutrophiles.

Blood metabolites

Increasing level of both AJW and THY increased and decreased (P<0.05) blood GLU and total TG, respectively (Table 5). Blood TG increased linearly (P<0.01) by increasing the level of AJW but was unaffected (P>0.05) by THY. Supplementation with EO did not influence (P>0.05) blood TP while increasing THY decreased (P<0.05) blood TC in a quadratic manner.

Table 2 Effects of two doses of ajwain and thyme essential oils on feed intake and performance in neonatal dairy calves

Parameters / collection day	Treatment[1]					SEM	Contrast			
	CO	AJW-L	AJW-H	THY-L	THY-H		Linear of AJW	Quadratic of AJW	Linear of THY	Quadratic of THY
Feed intake (g DM/d)										
1-15 day	185	193	212	211	197	8.5	0.291	0.602	0.293	0.069
16-30 day	690	727	727	738	695	33.3	0.445	0.688	0.925	0.281
31-45 day	1206	1235	1217	1270	1208	38.7	0.875	0.699	0.979	0.279
46-60 day	1944	1832	2097	2079	1962	57.5	0.318	0.161	0.905	0.342
Average daily gain (g/d)										
1-15 day	240	253	240	213	207	35.4	1.00	0.748	0.489	0.809
16-30 day	380	400	380	547	347	53.2	0.844	0.312	0.743	0.047
31-45 day	607	653	547	707	520	81.0	0.619	0.464	0.474	0.178
46-60 day	800	820	720	840	707	83.5	0.318	0.161	0.905	0.342
Feed conversion ratio										
1-15 day	0.77	0.76	0.88	0.99	0.95	0.221	0.794	0.235	0.460	0.666
16-30 day	1.81	1.81	1.91	1.34	2.00	0.382	0.709	0.220	0.746	0.109
31-45 day	1.98	1.89	2.22	1.79	2.32	0.442	0.912	0.115	0.799	0.288
46-60 day	2.43	2.23	2.91	2.47	2.77	0.685	0.390	0.238	0.875	0.692

CO: control; AJW-L: 1 mL/d per calf ajwainoil; AJW-H: 2 mL/d ajwain oil; THY-L: 1 mL/d thyme oil and THY-H: 2 mL/d thyme oil.
SEM: standard error of the means.

Table 3 Effects of two doses of ajwain and thyme essential oils on apparent digestibility of nutrients measured during the last 5 days of the experiment in neonatal dairy calves

Digestibility (%)	Treatment					SEM	Contrast			
	CO	AJW-L	AJW-H	THY-L	THY-H		Linear of AJW	Quadratic of AJW	Linear of THY	Quadratic of THY
DM	75.8	82.5	86.3	87.4	83.7	0.68	< 0.001	0.090	< 0.001	< 0.001
OM	80.2	92.9	88.7	89.8	86.6	0.50	< 0.001	< 0.001	< 0.001	< 0.001
NDF	70.6	72.9	76.8	79.7	78.9	1.57	0.001	< 0.001	< 0.001	0.500
ADF	53.8	51.7	58.9	64.1	62.5	3.72	0.011	0.723	0.004	0.055

CO: control; AJW-L: 1 mL/d per calf ajwainoil; AJW-H: 2 mL/d ajwain oil; THY-L: 1 mL/d thyme oil and THY-H: 2 mL/d thyme oil.
DM: dry matter; OM: organic matter; ADF: acid detergent fiber and NDF: neutral detergent fiber.
SEM: standard error of the means.

Table 4 Effects of different two doses of ajwain and thyme essential oils on blood haematological parameters in neonatal dairy calves

Parameters / collection day	Treatment					SEM	Contrast			
	CO	AJW-L	AJW-H	THY-L	THY-H		Linear of AJW	Quadratic of AJW	Linear of THY	Quadratic of THY
WBC ($10^3/\mu L$)	8.44	7.49	9.34	7.94	9.28	0.349	0.059	0.001	0.107	0.037
RBC ($10^6/\mu L$)	7.98	7.99	8.04	8.70	8.02	0.321	0.877	0.964	0.928	0.091
Hb (g/dL)[2]	9.45	10.5	9.48	10.6	8.42	0.283	0.930	0.007	0.020	< 0.001
PCV (%)	30.1	31.9	29.4	32.3	26.9	0.75	0.484	0.024	0.006	< 0.001
MCV (fL)	37.5	37.6	36.5	36.7	38.2	0.58	0.180	0.370	0.420	0.012
MCH (pg)	11.8	12.0	11.5	11.4	11.8	0.19	0.259	0.156	0.955	0.118
MCHC (g/dL)	31.6	31.6	32.0	31.4	31.5	0.25	0.317	0.613	0.652	0.567
Neutrophil (%)	35.1	34.8	35.0	36.2	48.3	1.27	0.667	0.834	< 0.001	0.002
Lymphocyte (%)	60.8	61.3	60.7	58.3	47.5	1.67	0.644	0.418	< 0.001	0.521
Monocyte (%)	2.62	2.60	2.96	3.91	2.90	2.088	0.895	0.850	0.322	0.068
Eosinophil (%)	1.30	1.30	1.34	1.59	1.32	0.135	0.814	0.896	0.917	0.132

CO: control; AJW-L: 1 mL/d per calf ajwainoil; AJW-H: 2 mL/d ajwain oil; THY-L: 1 mL/d thyme oil and THY-H: 2 mL/d thyme oil.
Hb: haemoglobin concentration; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MCV: mean corpuscular volume; PCV: packed cell volume; RBC: red blood cell and WBC: white blood cell.
SEM: standard error of the means.

Blood ALB was decreased by low level of AJW and high level of THY but it was increased by low level of THY (P<0.05 for quadratic effect). Blood CR was also increased (P<0.05) by the low level of both AJW and THY.

Feed intake, average daily gain and feed conversion rate
The lack of EO supplements effects on feed intake, ADG, and FCR are supported by Cardozo *et al*. (2006) who reported that 2 or 4 g/d of an EO mixture (consisting of thym-

ol, eugenol, vanillin, and limonene) mixed with milk replacer of calves had no effect on feed intake. No differences infeed intake of sheep supplemented with 250 mg/d of oregano oil (Wang *et al*. 2009) or in feed intake, ADG, and FCR of feedlot cattle with dietary supplementation of THY and cinnamon (Vakili *et al*. 2013) have been reported. Furthermore, ADG of the piglets fed EO blend (0, 150 and 300 mg/kg) were not affected (Manzanilla *et al*. 2004).

Santos *et al*. (2015) reported that EO mixture had no effects on acceptance of liquid or solid diet suggesting that there was no or minor change in palatability. However, Calsamiglia *et al*. (2007) observed a decrease in feed intake in cattle supplemented with EO that might be related to palatability problems. Generally, the differences in effects of EO supplementation on feed intake might vary with the EO source, type of diet (Yang *et al*. 2010a; Yang *et al*. 2010b) and factors such as body weight and animal growth stage (Yang *et al*. 2010b).

Apparent nutrients digestibility

Results of this study showed the improvement in digestibility of DM and OM in calves fed 2 mL/d AJW oil which was equal to those in calves fed 1 mL/d THY oil. The improvement in DM and OM digestibility in calves with EO supplementation compared with control is consistent with Soltan (2009) who supplemented a mixture of EO in milk replacer of calves and observed significantly increased DM digestibility. Moreover, the lower digestibility with the higher dose of THY in our study is in agreement with findings of Kamalak *et al*. (2011), who reported that high doses of orange oil significantly decreased DM and OM digestibility. Due to the increase in digestive enzyme secretion and improved nutrient utilization, herbal products have appetizing and stimulating effects on the animal digestive system (Pattnaik *et al*. 1997). Such products have traditionally been used to enhance the production of endogenous secretions in the small intestine, pancreas, and liver, thus aid digestion (Cross *et al*. 2007). In the young animals, the improvement in nutrients digestibility may be due to those phytogenic compounds can dictate digestive efficiency; improved secretion of endogenous enzymes and increased digestion of protein and starch in the upper ileum make more nutrients available for absorption (Soltan, 2009).

Haematological blood serum

The effects of high level of EO supplements on WBC count in the present study are consistent with Zhu and Zhou (1997) who reported an increase in WBC count when a blend of traditional Chinese medicinal herbs was added to piglets diet. On the other hand, the impact of EO on WBC count in our experiment disagrees with the findings of Maass *et al*. (2005) who reported dried herb of purple cone-

flower (*Echinacea purpurea*) had no impact on WBC count of the pigs regardless of dosage and dosage form. However, the effects of EO on most hematologic parameters in our experiment are in agreement with a previous research (Jurcic *et al*. 1989).

In this study, differential counting of WBC showed significant differences when EO of THY but not AJW was added to the diet in neutrophils and lymphocytes. Savoini *et al*. (2002) reported that total neutrophils and lymphocytes were increased by dietary supplements of 300 mg/kg as fed of plant extracts in piglets when compared with the control. On the other hand, Namkung *et al*. (2004) reported that feeding herbal extracts of cinnamon, THY, and oregano did not significantly affect WBC differentials in newly weaned pigs. In our experiment, the impact of treatments on WBC differentials is an indicator for the non-specific stimulation of cellular immunity. 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 (Craig, 1999).

Blood metabolites

The higher serum GLU in all calves receiving supplemental EO compared with control group is interesting. The higher serum GLU due to EO supplements is supported by Soltan (2009) who reported that high dose (281 mg/d) of EO mixture supplementation in milk replacer of calves enhanced serum GLU concentration by about 26.4%. Our results disagree with the previous researches (Tassoul and Shaver, 2009; Yang *et al*. 2010b) who reported EO supplements did not affect blood GLU concentration. Higher serum GLU of EO supplemented calves in the present study is consistent with higher digestibility of OM and suggests that the improved OM digestibility by EO supplies more GLU to the animal.

The lack of any effect due to dietary EO supplementation on serum TP concentration in early ages is contrary to the findings of Amad *et al*. (2011) who reported THY and anise EO increase serum TP concentration of male Cobb broilers. Soltan (2009) also reported increased serum TP concentration when 94 mg/d of supplemental EO was mixed with milk replacer of calves. However, in that study higher doses of EO supplementation (187 or 281 mg/d) only numerically increased serum TP concentration.

Decreasing BUN concentration due to increasing the level of both EO in our experiment is in accordance with previous research using different dosages of cinnamaldehyde supplementation in beef cattle (Yang *et al*. 2010a) and lambs (Chaves *et al*. 2011).

Table 5 Effects of two doses of ajwain and thyme essential oils on blood biochemical parameters in neonatal dairy calves

Parameters / collection day	Treatment					SEM	Contrast			
	CO	AJW-L	AJW-H	THY-L	THY-H		Linear of AJW	Quadratic of AJW	Linear of THY	Quadratic of THY
Glucose (mg/dL)	90.4	141	169	153	156	8.2	< 0.001	0.248	< 0.001	0.004
Total triglyceride (mg/dL)	58.5	63.9	73.9	56.6	61.8	3.36	0.001	0.572	0.485	0.394
Total cholesterol (mg/dL)	114	101	102	89.9	106	5.30	0.131	0.269	0.299	0.003
Total protein (g/L)	5.67	5.50	5.62	5.67	5.71	0.132	0.813	0.832	0.839	0.907
Albumin (mg/dL)	24.7	21.1	24.8	28.5	21.7	1.14	0.980	0.011	0.068	< 0.001
Blood urea nitrogen (mg/dL)	22.1	17.0	17.2	20.3	16.2	1.19	0.004	0.069	< 0.001	0.442
Creatinine (mg/dL)	1.30	1.49	1.30	1.43	1.34	0.03	0.980	< 0.001	0.461	0.023

CO: control; AJW-L: 1 mL/d per calf ajwainoil; AJW-H: 2 mL/d ajwain oil; THY-L: 1 mL/d thyme oil and THY-H: 2 mL/d thyme oil.
SEM: standard error of the means.

On the contrary, Vakili *et al*. (2013) found that BUN concentration was not affected by adding thymol or cinnamaldehyde separately. Similar results have been demonstrated by other researchers that used other EO (Tassoul and Shaver, 2009).

Moreover, the inconsistency between studies may be due to dosage rate and compounds of EO and experimental conditions (Benchaar *et al*. 2008). 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 which circulates in the blood. Consequently, 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 hyperammonia producing bacteria such as *Clostridium astyklandy* and *Peptostreptococcus anaerobius* resulting in reduced production of ammonia-N (Benchaar *et al*. 2008).

Affected concentrations of TG and TC in this experiment were in disagreement with the findings of Alsaht *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 THY or cinnamon EO. Nevertheless, our results contrast with the findings of Chaves *et al*. (2008) who reported that serum TG concentration was 18-fold higher in lambs supplemented with EO compared with the control.

Thus, in this experiment, the changes in blood metabolites propose that feeding EO from AJW and THY might have influenced 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).

CONCLUSION

Our results suggest that AJW and THY essential oils would improve nutrients digestibility and blood biochemical and hematological parameters of dairy calves. So, this study indicates that AJW and THY essences could be considered as suitable feed additives for use in dairy calves nutrition. However, contrary to our hypothesis, feed intake, ADG and FCR were not affected by AJW or THY supplementation in the present study. Moreover, different types and doses of EO or combination of different EO deserve further research in order to achieve better animal performance and growth promotion.

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