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Effect of Essential Oils Supplementation on Growth Performance, Nutrient Digestibility, Health Condition of Holstein Male Calves During Pre- and Post-Weaning Periods

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Abstract: One hundred Holstein male calves were assigned to investigate the effect of essential oil mixture (eucalyptus oil, menthol crystal, mint oil) supplementation in milk replacer (0, 94, 187 and 281 mg/calf/day) during the 8 weeks (pre-weaning period) and in drinking water (0, 15.6, 31.2 and 46.8 mg/liter) for the next 16 weeks (post-weaning period) on growth performance, nutrient digestibility, health condition as well as on some blood serum parameters. Calves receiving 94, 187 and 271 mg EOM/calf/day during pre-weaning period had lower ($p>0.05$) concentrate intake across the whole period by about 6.1, 6.1 and 1.2% respectively when compared with control but had no effect on roughage intake while reduced the total dry matter intake across the whole pre-weaning period by about 3.6, 3 and 1.2% respectively although, EOM had no effect on body gain and improved feed conversion ratio. EOM improve nutrient digestibility and increase total protein and albumin concentrations in the blood serum. Moreover, EOM treatment reduced the incidence of diarrhea, reduces the average number of diarrheic days and improves the general health score compared with the control. Although there were bad effects of the higher doses of EOM supplementation on health, it can be concluded that the supply of EOM reduced the necessity of antibiotics treatments against digestive and respiratory diseases. On the other hand, during post-weaning period it was clear from this study that calves administrated 15.6 mg of EOM/liter of drinking water may increase daily body gain, reduced feed intake and improved FCR by about 3.8, 3.2 and 8.15% respectively when compared with control while, the higher dose of EOM administration less effective and uneconomical to be used.

Key words: Claves, essential oil mixture, growth performance, nutrient digestibility, animal health

INTRODUCTION

Under current husbandry conditions, veal calves are often affected by diarrhea and respiratory disease. Diarrhea is the main cause of morbidity and mortality in the early life of veal calves and the first peak of respiratory diseases often emerges at 4 weeks of age, causing substantial economic losses due to medication and growth depression (Postema *et al*., 1987). Various factors could cause the high incidence of intestinal and respiratory disease in veal calves. After birth, calves are separated from their mothers, preventing the calf from picking up the protective gut flora from its mother (Fuller, 1989). Furthermore, at a very young age, the animals are faced with major stress events like transportation, marketing, dietary changes and exposure to a variety of infectious agents. Consequently, animals consume less milk, (Loerch and Fluharty, 1999), are predisposed to loss of barrier function of the gut (Nabuurs *et al*., 2001; Soderholm and Perdue, 2001) and may suffer from impaired immune function (Blecha *et al*., 1984; Sheridan *et al*., 1994). Moreover, the protective potential of the microbial gut flora tends to decrease (Cray *et al*., 1998). For example, during stress events, the trend is for the productive lactobacilli to decrease and for coliforms to increase (Fuller, 1989). To prevent the opportunistic pathogenic flora from flourishing, current practice is to treat calves with antibiotics during the first 5-10 days after arrival. However, the antibiotics diminish not only the activity of pathogenic flora, but also that of the productive flora.

However, intensive beef production systems rely on feeding large quantities of cereal grains, however highly fermentable diets may result in ruminal acidosis, bloat and digestive and metabolic upsets (Nocek, 1997). Ionophores have been used successfully to improve the efficiency of beef production and to prevent or decrease the incidence of digestive upsets (Chalupa *et al*., 1980) but the use of ionophores in animal feeds will be restricted in the European Union in 2006 (OJEU, 2003). It has been estimated that the elimination of antibiotics from ruminant feeds will result in an increase in production costs of 3.5-5.0% (Carro and Ranilla, 2002). Therefore, it is necessary to identify potential feeding strategies and/or additives that will allow producers to maintain the current level of production without increasing the cost or the incidence of metabolic, digestive or respiratory upsets in both suckling and post weaning calves.

Natural plant extracts contain secondary metabolites that have shown antimicrobial activity (Cowanc, 1999). Some of them have already been tested for their effects on ruminal microbial fermentation, including sarsaponns

(Ryan *et al*., 1997), phenolic compounds (Evans and Martin, 2002) and essential oils (McIntosh *et al*., 2003; Cardozo *et al*., 2004; Busquet *et al*., 2005; Cardozo *et al*., 2005; Beauchemin and McGinn, 2006; Cardozo *et al*., 2006 and Yang *et al*., 2007). Most of these studies have been conducted using ruminal fluid from dairy cows fed high forage diets and little studies using ruminal fluid from steer calves *in vitro* and very rare studies explained the effect of EOM on growth performance for short period. Therefore, the search for additives that will help to replace ionophores in beef calves during both pre and post weaning periods needs to be tested.

The objective of this study was to through light on the effect of EOM (based on Eucalyptus oil, menthol and Mint oil) supplementation at different doses in milk replacer during pre-weaning period and in water during post-weaning period of the calves on health condition, feed intake, growth performance, nutrient digestibility, some blood serum parameters.

MATERIALS AND METHODS

The experiment took place at the Safwat Alalam trading Establishment Farm in the Kingdom of Saudi Arabia (KSA) during summer season and lasted for 24 weeks. The first 8 weeks of the calves age after arrival (pre-weaning period) and the next 16 weeks (post-weaning period).

Calves and housing

Pre-weaning period: On hundred male Holstein calves were selected from dairy farms of 49.2 kg averaged body weight and aged 2-3 weeks to determine the effect of milk replacer supplemented with different levels of Essential Oil Mixture (EOM) on growth performance, nutrient digestibility and health condition. Calves were housed individually in metal stalls (70 X 170 cm). On arrival and considered as day 0, the calves were randomly allotted into 4 groups (25 calves per each) based on body weight distributions.

Post-weaning period: The same calves of each group were assigned to four separate pens (25 calves per pen).

Feeding and experimental design

Pre-weaning period: The animal fed basically on milk replacer contains 22.0% crude protein and 18.0% fat (Table 1). The milk replacer was reconstituted in hot water (45°C according to the instruction from the producer) and fed at a temperature of approximately 41°C.

On the day of arrival, the animals were fed twice with one liter of an electrolyte solution (Electrolyte mixes contain phosphorus 90 g, calcium 60 g, sodium 30 g, magnesium 12 g, iron 2 g, cobalt 7 mg, iodine 10 mg, copper 45 mg, manganese 300 mg and zinc 500 mg for each liter "produced by Pharma Care, KSA"). One day after arrival, the animals received 2.0 L containing 250 g of air-dry milk replacer per meal, the volume being gradually increased to 3.0 L containing 750 g of air dry milk replacer per meal after two days. The calves were fed twice a day at 7:0 am and 15:0 pm, with one of the reconstituted milk replacers presented in plastic buckets. EOM added to the milk replacer during its preparation at different concentration as presented in Table 2.

Water was available ad libitum daily and dry pellet calf starter feed was available ad libitum. to each calf shortly after the arrival while berseem hay was offered ad libitum also after 10 days from the arrival of the calves to the farm. Physical and chemical analysis of calf starter and berseem hay are presented in Table 3.

Table 1: Physical and chemical composition of used Milk Replacer (MR)*

Physical composition		Chemical composition (reconstituted MR)	
Ingredients	%	Item	%
Whey Powder	30.5	Moisture	88.5
Vegetable fat (Refined)	18.0	Dry matter	11.5
Whey powder (reduced in sugar)	10.0	Organic matter	10.4
Soy protein Conc.	14.0	Crude protein	2.80
Dextrose	7.0	Ether extract	2.3
Pea marc (extruded)	7.0	Crude ash	1.1
Wheat swell powder	5.0	Calcium	0.13
Premix (Additives)**	4.5	Phosphorus	0.09
Wheat protein (Hydrolyzed)	2.0		
Yeast	2.0		

*Milk replacer (Supramil T) produced by Josera Co. "Germany". ** Premix = contain the following per kg (48000 IU vitamin A, 4000 IU vitamin D_3, 100 mg vitamin E, 16 mg vitamin B_1, 8 mg vitamin. B_2, 6 mg vitamin B_6, 50 mcg vitamin B_{12}, 50 mg niacin; 2 mg vitamin K_3, 250 mg vitamin C, 25 mg Calcium pantothenate, 1 mg folic acid; 300 mg choline chloride; 200 mcg biotin; 90 mg iron; 4 mg copper; 0.4 mg selenium; 48 mg manganese and 40 mg zinc).

Table 2: The experimental design during experimental periods

Group No.	Diet	Essential Oil (EO) Supplementation (Aeroforte Product)*	EO (mg/L. MR)	EO (mg/ Calf/day)
1	MR (Control)	-----	-----	----
2	MR	1 ml/10 L MR	15.6 mg/L	94 mg
3	MR	2 ml/10 L MR	31.2 mg/L	187 mg
4	MR	3 ml/10 L MR	46.8 mg/L	281 mg

*"Aeroforte" produced by Kanters Special products Co. (Netherlands) and composed from (Ethanol, 22.5%; Emulsifiers, 15.3%; Eucalyptus oil 7%; Menthol crystal 6.6%; Mint oil 2.0% and demineralized water up to 100%).

Post-weaning period: EOM treatments were offered daily through mixing in the drinking water at levels (0.0, 10 ml, 20 or 30 ml of areoforte/100 liter water) to supply 0.0, 15.6, 31.2 and 46.8 mg of EOM/1 liter water respectively. Animals had ad libitum access to concentrate and berseem hay offered once daily at 8:0 am.

Table 3: Physical and chemical composition of the used feed

Concentrate						
Ingredients composition			Chemical analysis			
	starter	finisher		starter	finisher	Berseem hay chemical analysis
Ingredients	%	%	Item	%	%	Item %
Yellow corn	39.0	43.4	Moisture	12.1	11.6	Moisture 8.2
Sorghum	9.5	19.0	Dry matter	87.9	85.4	Dry matter 91.8
Barley	27.35	20.0	Organic M.	83.5	83.0	Organic M. 79.9
Soybean meal	18.5	13.0	Crude protein	18.2	16.2	Crude P. 24.9
Vegetable oil	0.5	0	Ether extract	3.1	2.8	Ether E. 3.6
DCP*	0.25	0	Ash	4.4	5.4	Ash 11.9
Lime stone	1.4	1.1	Calcium	0.9	0.5	Ca 1.3
Salt	0.4	0.4	Phosphorus	0.4	0.3	Phosphorus 0.3
Premix **	0.1	0.1				
Molasses	3.0	3.0				

*DCP = Dicalcium phosphate (20% phosphorus and 25% calcium). **Cattle premix produced by Centraly's Co. (France) contains the following elements per Kg. (10000000 IU vitamin A, 1000000, IU vitamin D_3, 10000 mg vitamin E, 100000 mg magnesium, 50000 mg manganese, 45000 mg zinc, 50000 mg iron, 6000 mg copper, 800 mg iodine, 100 mg selenium.

Feed intake and body weight: Dry calf starter, B. hay and milk replacer intakes for each calf were recorded daily throughout the pre-weaning period and average daily feed was calculated. During post-weaning period the diet consisted of berseem hay and concentrate. The concentrate (calf starter) during the first month post-weaning and changed after that to the calf finishing concentrate (Table 3) offered in pellet form. The diet designed to meet or exceed nutrient recommendations of the calves (NRC, 2001). Water and feed intake for each group were recorded daily throughout post-weaning period.

Calves were weighed individually on arrival and then every 4 weeks. Body weight gains calculated as: weight gain = (Final Body weight-Initial body weight) and the Average Daily Gain (ADG) was calculated for pre- and post-weaning periods. Feed Conversion Ratio (FCR) was calculated by dividing total feed intake per calf by the total body weight gain per the same animal for the pre-weaning period while feed intake and gain for each groups was calculated during post-weaning time.

Analytical procedure: Collected feed and feces samples were analyzed for Dry Matter (DM), moisture and ash contents according to (AOAC, 1985), crude protein using Kjeldahl method according to Randhir and Pradhan (1981) and ether extract was determined according to Bligh and Dyer (1959) technique as modified by Hanson and Olly (1963). Phosphorus was determined by using spectrophotometer according to (Cockerell and Holliday, 1975) while, calcium was estimated by titration with EDTA according to David (1976). Feed and fecal samples were analyzed for Acid Insoluble Ash (AIA) content by dissolving the obtained ash in hydrochloric acid following the ISO 5985 (1998).

Apparent total-tract digestibility

Fecal Collection: On day 43-49 of the pre-weaning period of the experiment, 4 samples were collected from each treatment daily. Fecal samples were collected fresh from calves upon rectal stimulation. Feces were weighed and stored at -20°C and subsequently thawed, dried at 55°C for 48 h and ground through a 1 mm screen for moisture, crude protein, ether extract, ash, acid insoluble ash, calcium and phosphorus analysis. Apparent Digestibility Coefficients (ADC) of nutrients were determined using an internal marker Acid Insoluble Ash (AIA) in the diets. ADCs for the different nutrients are expressed as the fractional absorption of these nutrients from diets in calves: ADC nutrient = 1-(AIA_{diet}/AIA_{feces}X$nutrient_{feces}$/$nutrient_{diet}$) X100, where AIA_{diet} is the AIA in the diet (%), AIA_{feces} is the AIA in the faeces (%), $nutrient_{feces}$ is the nutrient in the feces (%) and $nutrient_{diet}$ is the nutrient in the diet (%).

Blood sampling and analysis: Blood sample were collected by jugular vein puncture and placed in non-additives blood collection tubes to produce serum from a sub-sample of 5 randomly selected calves from each treatment groups at the end of the pre-weaning period. Serum was separated by centrifugation at 3000 rpm for 10 min and analyzed for concentration of blood serum glucose, total protein, albumin, globulin and total lipid according to (Doumas *et al*., 1981; Reinhold, 1953; Coles, 1974; David, 1976) and Giorgio, 1974) respectively.

Health monitoring: Visual health of the calves were recorded twice daily by a veterinarian and by the manager of the calf facility who was responsible for treatment on the ranch. The occurrence of diarrhea was recorded daily during the whole pre-weaning period of

Table 4: Effect of essential oil mixture supplementation on average feed intake (kg/calf) during pre-weaning period of veal calves

	EOM supplementation mg/calf/day			
	0	94	187	281
Calves age (week)	(N = 14)			
Concentrate intake				
0-2 weeks	0.29±0.03[a]	0.25±0.03[a]	0.29±0.03[a]	0.29±0.03[a]
3-4 weeks	0.65±0.04[a]	0.57±0.04[a]	0.62±0.04[a]	0.68±0.04[a]
5-6 weeks	1.03±0.06[a]	0.94±0.06[a]	0.98±0.06[a]	0.97±0.05[a]
7-8 weeks	1.31±0.10[a]	1.30±0.10[a]	1.19±0.07[a]	1.33±0.10[a]
Average daily intake				
(0-8 weeks)	0.82±0.06[a]	0.77±0.06[a]	0.77±0.05[a]	0.81±0.06[a]
Roughage intake				
0-2 weeks	0.07±0.03[a]	0.07±0.03[a]	0.07±0.03[a]	0.07±0.03[a]
3-4 weeks	0.36±0.03[a]	0.37±0.03[a]	0.37±0.03[a]	0.37±0.03[a]
5-6 weeks	0.28±0.02[a]	0.27±0.02[a]	0.27±0.02[a]	0.27±0.02[a]
7-8 weeks	0.32±0.02[a]	0.31±0.02[a]	0.31±0.02[a]	0.31±0.02[a]
Average daily intake				
(0-8 weeks)	0.26±0.02[a]	0.26±0.02[a]	0.26±0.02[a]	0.26±0.02[a]
Average dry matter intake(ADMI) (kg/calf/day)				
ADMI (kg/calf/day	1.69	1.63	1.64	1.67
ADMI (%Relative to control)	100	-3.6	-3.0	-1.2

Values are means ± standard error. Mean values with the same letters at the same row not differ significantly at (P>0.05). N = No. of observations.

the experiment. Recording was done per animal and the feces of each calf were inspected daily and stool appearance was described as following:

Mild diarrhea: Most probably from nutritional origin, would be present if calves were vivid and had yellow and mostly liquid feces.

Severe diarrhea: Most probably from infectious origin, was scored if calves were apathetic and feces had an unpleasant odor and was slimy, watery, green or yellow with blood present. Where necessary, body temperature was assessed to confirm the visual observations made. The incidence of various diseases was estimated from the number of antibiotics treatment assigned by farm veterinarian against digestive, respiratory or other diseases such as joint eye, skin or umbilicus infections. Antibiotics use per animal was recorded during the complete period and classified as therapeutic treatments needed for gastrointestinal, respiratory or others diseases. The sum of all therapeutic treatments was calculated.

To monitor overall health in each group, a General Health Score (GHS) was designed according to Timmerman *et al*., 2005. The incidence of diarrhea and therapeutic treatments for digestive, respiratory or other diseases were weighed differently in the following formula:

GHS = 15 - 1X total number of diarrheic days - 2X the number of individual therapeutic treatments for digestive diseases - 3X the number of individual therapeutic treatments for respiratory diseases - 2 X the number of individual therapeutic treatments for infections other than digestive or respiratory - 2X the number of antibiotic treatment on a herd basis.

Table 5: Effect of essential oil mixture supplementation on body weight development (kg) and variation coefficient percent during pre-weaning of calves

	EOM supplementation mg/calf/day			
	0	94	187	281
Calves age (week)	(N = 25)			
Body weight development (kg)				
W0	49±1.1[a]	49±1.3[a]	49±1.1[a]	49±1.0[a]
W4	70±1.4[a]	69±1.1[a]	70±1.6[a]	69±1.0[a]
W8	92±2.1[a]	91±1.4[a]	91±2.2[a]	91±1.2[a]
Body weight variation coefficient %				
W0	10.6	12.7	11.5	10.0
W4	9.8	8.3	11.6	7.2
W8	11.3	7.6	11.8	6.7

Values are means ± standard error. Mean values with the same letters at the same row not differ significantly at (p>0.05). N = No. of observations.

Statistical analysis: The analysis of variance for the obtained data was performed using Statistical Analysis System (SAS, 1996) to assess significant differences.

RESULTS AND DISCUSSION

Pre-weaning period (0-8 weeks)

Feed intake: Calves receiving 94, 187 and 281 mgEOM/day in milk replacer had lower (p>0.05) concentrate intake across the whole pre-weaning period by about 6.1, 6.1 and 1.2% when compared to the control (Table 4). However, EOM supplementation had no effect

on roughage intake and on the other hand EOM at different levels of calves milk replacer reduced average dry matter intake across the experimental period by about 3.6, 3.0 and 1.2% (groups 2-3 respectively) when compared with the control. The present results indicated that higher inclusion level of EOM in calves milk replacer slightly improved the calves appetite compared with low inclusion rate of EOM and that may be due the treated milk replacer with high EOM addition irritating the calf's mouth. There are no previous reports on the effects of a mixture of Eucalyptus oil, Menthol crystal and Mint oil on feed intake in calves during pre-weaning period. In contrast the decreased DMI observed for EOM in the present trial is contrary to previous studies with botanical supplementation in calves' milk replacer (BFI Innovations, UK 2001) who reported that botanical supplementation in milk replacer for younger calves showed a 27% increase in milk replacer intake when compared with untreated calves. Those difference may be related to the authors depend on milk replacer feeding system as the main ingredients without dry feed supplementation.

Growth performance: EOM supplementation at different levels in milk replacer for calves had no effect ($p>0.05$) on body weight development (Table 5) when compared with control while, a slight lower body weight was observed in calves fed on treated milk replacer by about 1.1% for all groups when compared with the control. However, EOM improve the final body weight uniformity (Table 5) by about 33%, 41% in calves fed on 94 or 281 mg EOM/calf/day respectively when compared with the control, while EOM supplementation at 187 mg/calf/day had a slight negative effect with a lack of explanation and unclear reason for that difference. Generally uniformity of body weight considers as important parameter to the production economics.

Average Daily Gain (ADG) was not statistically different (Table 6) between treatment groups ($p>0.05$) but was numerically highest for the control group. There was a trend for the EOM treated groups (2-4) to have an improved FCR across the pre-weaning period by about 7.2, 4.6 and 4.6% respectively when compared with the control. The trend of EOM supplementation in the present study to lower ADG is in contrast with BFI Innovations, UK (2001) stated that calves received milk replacer and weaner diets containing either no treatment (control) or 250g/ton of a commercial botanical product. Overall the botanical product improved weight gain by 9.7% and FCR by 14%.

Apparent nutrient digestibility: An improvement ($p>0.05$) in nutrient digestibility due to treatment was observed (Table 7). EOM supplementation at different levels (groups 2-4) non significantly improved organic matter, crude protein and ether extract digestibility when compared with the control while, significantly ($p<0.05$) increased dry matter digestibility by about 5.8, 6.8 and 7% respectively when compared with control. Moreover, EOM supplementation at 94, 187 or 281 mg/calf/day improved ($p<0.05$) ash and phosphorus absorption by about (15.2, 30 and 38%) and (14, 13 and 12%) respectively when compared with control while, EOM supplementation had no effect on calcium absorption. Improvement of nutrient digestibility may be due to that EOM increase the gastric secretion, such as enzymes production from the pancreas which is very important in young animals, where gut maturation can dictate digestive efficiency, improved secretion and increased digestion of protein and starch in the upper ileum, making more nutrients available for absorption. This reduces the amounts of undigested feed particles passing down the gut and being utilized by bacteria within the colon. These changes in nutrient fermentation are associated with many digestive disorders, especially in calves. The trend of nutrient digestibility improvement is in agreement with (Williams and Losa, 2001) reported that phytogenics may assist in improving digestive process in piglet. Also, Meyer *et al.* (2007a) reported that a non significant improvement of total tract DM and OM digestibilities in steer fed on EOM treated diet when compared with control.

Blood serum units: There was no significant effect of EOM supplementation at 94 or 187 mg/calf/day on serum glucose concentration (Table 8) during pre-weaning period of the calves while, higher dose of EOM (281 mg/calf/day) significantly ($p<0.05$) increased serum glucose concentration by about 26.4% when compared with the control. EOM supplementation (94 mg/calf/day) significantly ($p<0.05$) increased serum total protein concentration by about 20% when compared with the control, however, higher dose of EOM supplementation (187 or 281 mg/calf/day) non significantly ($p>0.05$) increased serum total protein concentration by about 4.9% for both levels when compared with the control. Moreover EOM supplementation at different levels increased serum albumin concentration by about 14, 0.5 and 23% respectively, while serum globulin concentration decreased within the higher addition levels of EOM. EOM supplementation at 94 or 187 mg/calf/day (group 2 and 3 respectively) recorded the highest values of globulin concentration and that may be attributed to enhance the resistance of calves against different stress factors. Higher serum total protein with EOM supplementation may be indicated that EOM had positive immune stimulatory effect of the calves during pre-weaning period. A higher serum concentration of total lipids in calves serum fed on treated milk replacer (15.6, 31.2 and 46.6 mgEOM/liter) by about 13, 65 and 37% respectively was observed when compared with control. There are no previous reports on the effects of a mixture of Eucalyptus oil, Menthol crystal and Mint oil on

Table 6: Effect of essential oil supplementation on average body weight gain (kg) and Feed Conversion Ratio (FCR) during pre-weaning period of calves

	EOM supplementation mg/calf/day			
	0	94	187	281
Calves age (week)	(N = 25)			
Body weight gain (kg)				
W0- W4	20.3±10.7[a]	19.5±0.6[a]	20.6±0.75[a]	20.1±0.5[a]
W5-W8	22.5±0.9[a]	22.2±0.6[a]	21.7±1.21[a]	21.7±0.6[a]
Total body gain	42.9±1.6[a]	41.7±0.8[a]	42.2±1.62[a]	41.8±0.9[a]
Average Daily gain	0.77±0.03[a]	0.75±0.01[a]	0.75±0.03[a]	0.75±0.02[a]
Feed Conversion ratio (FCR)				
W0-W4	2.0±10.2[a]	1.9±0.1[a]	1.9±0.1[a]	1.9±0.1[a]
W5-W8	2.9±0.3[a]	2.5±0.1[a]	2.8±0.2[a]	2.6±0.1[a]
FCR (W0-W8)	2.4±0.2[a]	2.2±0.1[a]	2.3±0.1[a]	2.3±0.1[a]
FCR (Relative to control)	100	- 7.2	- 4.6	- 4.6

Values are means ± standard error. Mean values with the same letters at the same row not differ significantly at (p>0.05). N = No. of observations.

Table 7: Effect of essential oil supplementation on the apparent nutrient digestibility and some mineral absorption during pre-weaning of calves

	EOM supplementation mg/calf/day			
	0	94	187	281
Items	(N = 28)			
Apparent nutrient digestibility (%)				
Dry matter	87.05±3.71[c]	92.11±0.24[bc]	92.99±0.44[ab]	93.13±0.80[ab]
Organic matter	93.24±1.03[a]	94.47±0.60[a]	93.89±0.35[a]	93.47±0.64[a]
Crude protein	84.70±0.55[a]	84.92±0.43[a]	85.74±0.56[a]	85.03±0.44[a]
Ether extract	89.77±2.42[a]	90.76±1.62[a]	89.72±1.35[a]	92.29±0.91[a]
Mineral absorption (%)				
Total ash	57.15±11.47[c]	65.85±3.05[bc]	74.28±1.62[a]	78.74±1.60[ab]
Calcium	78.56±9.04[a]	80.90±3.82[a]	81.72±5.93[a]	82.58±3.81[a]
Phosphorus	81.85±4.44[b]	93.23±1.02[a]	92.53±0.88[a]	91.49±1.46[a]

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$). N = No. of observations.

Table 8: Effect of essential oil mixture supplementation on the some blood serum parameters during pre-weaning period of calves

	EOM supplementation mg/calf/day			
	0	94	187	281
Items	(N = 5)			
Glucose (g/dl)	45.3±5.5[b]	49.5±4.2[b]	50.75±1.8[ab]	57.25±6.7[a]
Total protein (g/dl)	59.8±1.3[b]	72.0±2.0[a]	62.75±2.1[b]	62.75±0.5[b]
Albumin (g/dl)	38.3±0.6[b]	43.8±2.6[a]	38.50±2.0[b]	47.00±0.7[a]
Globulin	21.5±1.7[b]	28.3±1.3[ac]	24.25±3.9[bc]	15.75±0.9[b]
A/G ratio	1.8±0.2[b]	1.6±0.2[b]	1.74±0.3[b]	3.02±0.2[a]
Total lipid (mg/dl)	120.3±7.5[b]	136.3±9.0[b]	175.0±11.5[a]	165.25±14.7[a]

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$). N = No. of observations.

blood parameters in fattening calves. However, Soltan *et al*. (2008) observed an increase of serum protein, albumin and triglycerides serum concentrations in broiler chicks fed on diets containing 0.25 g anise seed/kg diet when compared with the control.

Diarrhea incidence and duration: A slight reduction of mild diarrhea incidence and diarrheic days of treatment were observed (Table 9) in calves fed on the lower level of EOM supplementation (94 mg/calf) by about 4.0% and 20% respectively when compared with the control group. The positive effect which obtained in group 2 may be attributed to the antimicrobial effect of EOM (Gowan, 1999), such improvement indicate increased digestive efficiency, linked to several activities of the botanical components, for example increased endogenous enzyme secretion, improved gut environment and micro flora balance and increased liver function to better utilize fats and proteins This outcome agrees with that of other studies in calves (Abe *et al*., 1995; Donovan *et al*., 2002; Khuntia and Chaudhary, 2002; Timmerman *et al*., 2005). Incidence of sever diarrhea was observed only with high

inclusion rate of EOM (groups 3 and 4). The adverse effect of high EOM dose may be related to the highly reduction of gut microorganisms numbers and this reduction may be had bad effect on normal gut microbial activity. However, the higher doses of EOM (groups 3 and 4) which fed on the milk replacer supplemented by 31.2 and 46.8mg/1 liter respectively showed a higher incidence and treatment duration of diarrhea across the pre-weaning period by about (35% and 60%) and (73% and 176%) respectively when compared with the control. The present data confirmed that EOM supplementation is dose dependent and must be studied before advice to be used as feed additives for pre-weaning calves.

Digestive and respiratory diseases: An overall improvement in the therapeutic treatment against respiratory diseases were observed (Table 10) with EOM supplementation when compared with control and the improvement was approximately linearly related with the dose of EOM supplementation. A lower number of treatments per animal percent was observed by about 18.8 and 12.5% in calves fed on 94 or 187 mg EOM/calf/day respectively while, the higher dose had no effect when compared with the control. The obtained data indicated that higher doses of EOM had a negative effect on the healthy condition.

EOM (Eucalyptus oil, menthol and Mint oil) supplementation in calf's milk replacer may not only reduce losses due to direct and indirect costs of respiratory disease but they might contribute to reducing microbial resistance against veterinary and human antibiotics. There are no previous reports on the effects of a mixture of Eucalyptus oil, Menthol crystal and Mint oil on health condition calves during pre-weaning period. However, Phytogenics may assist in improving digestive processes, particularly during the early stages of life, finally resulting in higher performance (Williams and Losa, 2001).

General Health Score (GHS): EOM supplementation at 94 and 187mg/calf/day improved GHS of calves (Table 10) by about 27% and 19% respectively when compared with the control. On the other hand negative effect on GHS was observed in calves fed on the higher supplementation level of EOM (281 mg/calf/day) by about 3% when compared with control. The present results are supported by those obtained by (Leung and Foster, 1996) reported that botanical compound have been shown to be active against damaging compounds, such as mycotoxin, which they strongly bind and inhibit. Other plant extracts are associated with protecting cellular function directly or via antioxidant properties to stabilize metabolism, allowing the liver to function optimally (Fahim *et al*., 1999).

Post-weaning period (9-24 weeks)

Feed and water intake: Claves drinking water with EOM supplementation at 15.6, 31.2 and 46.8 mg/liter of drinking water had lower ($p<0.05$) concentrate intake by about 3.4, 3.9 and 3.6% respectively (Table 11) across the whole post-weaning period when compared with the control. Also EOM supplementation significantly ($p<0.05$) reduced roughage intake across the post-weaning period by about 1.7, 5.1 and 6.8% respectively when compared with the control. Higher level of EOM addition in the drinking water (46.8 mg/1 liter) more determinately affecting the roughage intake when compared with the other EOM doses. There are no previous reports on the effects of a mixture of Eucalyptus oil, Menthol crystal and Mint oil on feed intake in fattening calves. However, Busquet *et al*. (2003) observed a 12% reduction in concentrate Dry Matter Intake (DMI) in dairy cattle fed 0.6 g of cinnamaldehyde/kg of dry matter. Also Cardozo *et al*. (2006) reported that the cinnamaldehyde and eugenol mixture decreased ($p<0.05$) dry matter and concentrate intakes compared with control in growing heifers, however that mixture disagreement with these results as it had no effect on barley straw DMI. Moreover, Gurney *et al*. (1996) also observed that cinnamamide (the amide of cinnamaldehyde) decreased DMI by 17% in house mice. They noted that mice appeared to find the treated diet irritating to the mouth and paws. In contrast, the decreased feed intake observed for EOM in the present trial is contrary to previous studies with Cardozo *et al*. (2006) indicated that addition of capsicum and anise essential oil mixture to growing heifer diet increased ($p<0.05$) total DMI, concentrate intake compared with control.

EOM supplementation had variable effects on water intake of fattening calves during post-weaning periods (Table 12), but generally with higher addition levels of EOM (31.2 and 46.8 mg/1 liter of drinking water) increased water intake especially with the later period of the experiment. Calves drinking water supplemented with the higher EOM levels (groups 3 and 4) had higher ($p<0.05$) water intake by about 5 and 18.6% across the whole post-weaning period when compared with control. Lower EOM addition (15.6 mg/1 liter) had no effect ($p>0.05$) on water intake compared with control. There are no previous reports on the effects of a mixture of Eucalyptus oil, Menthol crystal and Mint oil on water intake in fattening calves. However, there is evidence that capsaicin, the active component of capsicum oil, increase water intake in rats (Zafra *et al*., 2003) and can stimulate appetite in humans (Calixto *et al*., 2000). Also, Cardozo *et al*. (2006) reported that capsicum oil addition increased water intake in growing heifers. The trend of EOM in the present study to increase water intake is in contrast to results of Cardozo *et al*. (2006) who tested the effect of anise and cinnamaldehyde plus eugenol mixture as additive in growing heifers diet and noticed lowering of water intake compared with control.

Growth performance: Addition of EOM in water for calves during post-weaning period significantly ($p<0.05$)

Table 9: Effect of essential oil mixture supplementation on the incidence and duration of diarrhea during pre-weaning of calves

	EOM supplementation mg/calf/day			
Variable	0	94	187	281
Mild diarrhea most probably of nutritional origin				
Percentage of animals with diarrhea	20.8	20.0	24.0	36.0
Average number of diarrheic days per animal percent	0.5	0.4	0.7	1.1
Severe diarrhea most probably of infectious origin				
Percentage of animals with diarrhea	0.0	0.0	4.0	8.0
Average number of diarrheic days per animal percent	0.0	0.0	0.1	0.1
Mild plus severe diarrhea				
Percentage of animals with diarrhea	20.8	20.0	28.0	36.0
Average number of diarrheic days per animal percent	0.5	0.4	0.8	1.38

Table 10: Effect of essential oil supplementation on the therapeutic treatments and General Health Score (GHS) during pre-weaning of calves

	EOM supplementation mg/calf/day			
Variable	0	94	187	281
Therapeutic treatments against digestive diseases				
Percentage of treated animals:	20.8	20.0	28.0	36.0
Average number of treatments per animal percent	0.5	0.4	0.8	1.3
Therapeutic treatments against respiratory diseases				
Percentage of treated animals:	12.5	8.0	4.0	4.0
Average number of treatments per animal percent	0.3	0.1	0.1	0.1
Total Number of therapeutic treatments[1]				
Percentage of treated animals:	44.0	44.0	48.0	52.0
Average number of treatments per animal percent	1.6	1.3	1.4	1.6
General Health Score (GHS)	7.32	9.29	8.73	7.09

1= this group includes therapeutic treatments for non digestive and non respiratory infections (e.g. joint, eye, skin or Umbilicus infections).

Table 11: Effect of essential oil mixture supplementation on feed intake (kg/calf/day) of calves during post-weaning period

	EOM supplementation mg/liter of drinking water			
	0	15.6	31.2	46.8
Calves age (week)	(N = 28)			
Concentrate intake (kg/calf/day)				
9-12 weeks	2.73±0.0^{a}	2.55±0.01^{b}	2.50±0.0^{c}	2.55±0.01^{b}
13-16 weeks	3.83±0.1^{a}	3.59±0.01^{b}	3.50±0.0^{c}	3.50±0.04^{c}
15-20 weeks	4.56±0.1^{a}	4.49±0.04^{a}	4.49±0.04^{a}	4.89±0.04^{a}
21-24 weeks	5.38±0.01^{a}	5.28±0.04^{b}	5.35±0.01^{a}	5.35±0.01^{a}
Average (9-24 weeks)	4.12±0.0^{a}	3.98±0.01^{b}	3.96±0.0^{c}	3.97±0.01^{b}
Roughage (kg/calf/day)				
9-12 weeks	0.55±0.0^{a}	0.51±0.01^{b}	0.50±0.0^{c}	0.51±0.01^{b}
13-16 weeks	0.66±0.0^{a}	0.63±0.0^{a}	0.63±0.0^{a}	0.60±0.0^{a}
15-20 weeks	0.55±0.0^{a}	0.52±0.0^{b}	0.52±0.0^{b}	0.52±0.0^{b}
21-24 weeks	0.60±0.0^{a}	0.57±0.0^{b}	0.57±0.0^{b}	0.57±0.0^{b}
Average (9-24 weeks)	0.59±0.0^{a}	0.58±0.0^{b}	0.56±0.0^{c}	0.55±0.0^{c}
Average total feed intake (kg/calf/day)	4.71	4.56	4.52	4.52

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$). N = No. of observations.

Table 12: Effect of essential oil mixture supplementation on water intake (liter/day) of calves during post-weaning period

	EOM supplementation mg/liter of drinking water			
	0	15.6	31.2	46.8
Calves age (week)	(N = 28)			
9-12 weeks	24.2±1.7^{a}	26.2±1.5^{a}	20.4±1.3^{a}	24.3±1.3^{a}
13-16 weeks	27.2±0.5^{c}	26.5±0.2^{c}	30.4±0.6^{b}	34.5±0.6^{a}
15-20 weeks	30.2±0.0^{c}	28.6±0.1^{d}	32.6±0.1^{b}	36.6±0.1^{a}
21-24 weeks	30.2±0.0^{c}	29.0±0.1^{d}	34.0±0.1^{b}	37.1±0.1^{a}
Average (9-24 weeks)	28.0±0.0^{b}	27.6±1.5^{b}	29.4±1.3^{a}	33.2±1.3^{a}

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$). N = No. of observations.

Table 13: Effect of essential oil mixture supplementation on body weight development (kg/calf) during post-weaning period of calves

	EOM supplementation mg/liter of drinking water			
	0	15.6	31.2	46.8
Calves age (week)	(N = 25)			
W8	92±2.1[a]	91±1.4[a]	91±2.2[a]	91±1.2[a]
W12	127±1.7[a]	120±1.9[b]	120±3.0[b]	121±1.9[b]
W16	163±3.4[a]	157±2.8[ab]	156±4.3[ab]	152±2.6[b]
W20	207±4.6[a]	208±3.6[a]	207±5.3[a]	207±3.4[a]
W24	239±5.6[a]	245±4.2[a]	235±6.0[a]	239±3.6[a]

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$). N = No. of observations.

Table 14: Effect of essential oil supplementation on body weight gain (kg) and Feed Conversion Ratio (FCR) during post-weaning period of calves

	EOM supplementation mg/liter water			
	0	15.6	31.2	46.8
Calves age (week)	(N = 25)			
Body weight gain (kg/calf)				
W9-W12	34±0.8[a]	29±0.9[a]	30±1.3[a]	31±1.1[a]
W13-W16	36±2.1[a]	37±1.5[a]	35±1.7[a]	31±1.7[a]
W17-W20	44±2.2[a]	50±1.7[a]	46±2.1[a]	54±3.2[a]
W21-W24	32±1.8[a]	37±1.6[a]	33±1.8[a]	34±1.9[a]
Total body gain	146±4.5[a]	153±4.1[a]	144±4.7[a]	149±3.8[a]
Average Daily gain	1.30±0.04[a]	1.35±0.04[a]	1.29±0.03[a]	1.33±0.03[a]
Feed Conversion ratio (FCR)				
W9-W12	2.7±0.2[a]	3.0±0.3[a]	2.8±0.1[a]	2.8±0.1[a]
W13-W16	3.0±0.3[b]	3.2±0.3[b]	3.3±0.2[b]	3.7±0.2[a]
W17-W20	3.3±0.1[a]	2.8±0.2[b]	3.0±0.2[a]	2.8±0.1[b]
W21-W24	5.2±0.3[a]	4.4±0.2[b]	5.0±0.2[a]	4.9±0.2[b]
Average FCR (W9 –W24)	3.7±0.1[a]	3.4±0.1[a]	3.6±0.1[a]	3.5±0.1[a]
FCR Improvement % (Relative to control)	100	- 8.1	- 2.7	- 5.4

Values are means ± standard error. Mean values with different letters at the same row differ significantly at ($p \leq 0.05$). N = No. of observations.

decreased live body weight at the end of the first month post-weaning by about 5.5%. 5.5 and 4.7% for the dose 15.6, 31.2 and 46.8 mg EOM/1 liter of drinking water respectively (Table 13) when compared with control.

However, body weight development highly improved in calves received EOM treated water within the 2nd and 3rd months post-weaning and at the end of the experimental period a higher ($p>0.05$) body weight in calves received 15.6 mg EOM/ 1 liter of drinking water by about 2.5% and was observed when compared with control while calves received water with higher levels of EOM showed a compatible body weight with the control.

Body gain not significantly ($p>0.05$) reduced (Table 14) during the 1st and 2nd months post-weaning with EOM supplementation in the drinking water while, non significantly improvement in body weight gain of the calves received EOM at different levels of the drinking water were observed when compared with the control. A higher ($p>0.05$) Average Daily Gain (ADG) was observed across the whole post-weaning period in calves received EOM treated water at 15.6 and 46.8 mg/liter by about 3.8 and 2.3% respectively when compared with control, while EOM supplementation at 31.2 mg/liter had lower DBG by about 0.8% when compared with control.

Feed Conversion Ratio (FCR) values followed the same trends of body weight and body gain as FCR was determinately affected during the 1st and 2nd months post-weaning in calves received EOM at different levels in the drinking water and at other periods EOM supplementation improved FCR when compared with control. EOM supplementation improved ($p>0.05$) FCR across the whole post-weaning period by about 8.1%, 2.7 and 5.4%) in groups 2-4 respectively when compared with control. The present results indicated that the effect of essential oil supplementation was dose and time dependent. There are no previous reports on the effect of a mixture of Eucalyptus oil, Menthol crystal and Mint oil on growth performance of fattening calves. However, Kamel (2000) observed improvement of overall ADG and FCR by 5% and 6% respectively in piglets fed on the diets containing a phytogenic blend when compared with control. Also Meyer *et al*. (2007b) concluded that steer fed on essential oil compound treated diet had non significantly higher ADG and improved FCR compared to the control group.

Conclusion: Dietary EOM supplementation (94 mg/calf/day) in the milk replacer of pre-weaning reduce

dry matter intake, improve FCR, nutrient digestibility and increase total protein and albumin concentrations in the blood serum. Moreover and EOM treatment reduced the incidence of diarrhea, reduces the average number of diarrheic days and improve the general health score compared with the control. Although there were bad effects of the higher doses of EOM supplementation, it can be concluded that the supply of EOM reduced the necessity of antibiotics treatments against digestive and respiratory diseases.

On the other hand, during post-weaning period it was clear from this study that calves administrated 15.6 mg of EOM/liter of drinking water may increase daily body gain, reduced feed intake and improved FCR and the higher dose of EOM administration less effective and uneconomical to be used.

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