Evaluation of essential oils and a prebiotic for newborn dairy calves[1]

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ABSTRACT: A blend of essential oils (EO) and a prebiotic were combined (EOC) to formulate a colostrum-based liquid birth supplement and a separate feeding supplement (Start Strong and Stay Strong, Ralco Inc., Marshall, MN). These products were designed to promote immunity and stimulate appetite to diminish health challenges and stresses experienced by newborn calves. The hypothesis was that calves supplemented with an oral dose of liquid EOC at birth (10-mL aliquot at birth and 10 mL at 12 h of age) when fed the EOC feeding supplement would result in improved growth performance, health, and immunity. The objective was to determine if an additional feeding of liquid EOC at birth in combination with EOC in the milk replacer (MR) would allow calves to demonstrate improved growth, health, and immunity compare with calves only offered EO in MR. Sixty-one Holstein calves (18 males and 43 females) from a commercial dairy operation were blocked by birth date and randomly assigned to 1 of 3 treatments. Treatments were 1) Control (CON): a 24% crude protein (CP):20% fat (as-fed basis) MR; 2) EP: a 24:20 MR with EOC mixed at 1.25 g/d; or 3) EPC: a 24:20 MR with EOC mixed at 1.25 g/d in addition to calves receiving one 10-mL oral dose of liquid EOC at birth and 10 mL again at 12 h. The 24:20 MR was fed via bucket 2 times per day at a rate of 0.57 kg/calf daily for 14 d, increased to 0.85 kg/calf at 2 times per day until 35 d and was reduced to 0.43 kg at 1 time per day at 36 d to facilitate weaning after 42 d. Decoquinate was added to the MR at 41.6 mg/kg for coccidiosis control. Calves were housed in individual hutches bedded with straw with ad libitum access to a 20% CP-pelleted calf starter and water. All data were analyzed using PROC MIXED as a randomized complete block design. Calves in this study had similar ($P > 0.10$) average daily gains, body weight, and growth measurements. Calves fed EPC had significantly ($P < 0.05$) higher IgA titers on day 0 of the trial compared with calves fed EP or CON, which was expected as calves were supplemented with liquid EOC at birth and 12 h later demonstrating an increase in immune response. The use of a liquid EOC product being administrated after birth can improve IgA titers to improve the immune status of the new born calf to fight off potential diseases and pathogens. A formulation error resulted in the EOC being fed at half the rate of the previous experiment of 2.5 g/d, which appears to be below an efficacious dosage.

Key words: calf, essential oils, prebiotic

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INTRODUCTION

Pathogenic bacteria are the main cause of diarrhea in dairy calves resulting in dehydration, which is a leading cause of death (Urie et al., 2018). Traditionally, antibiotics have been used as an approach to control pathogens and shown to aid in rumen development, enhance calf performance, and reduce calf scours and mortality (Morrill et al., 1977; Santos et al., 2015). Concerns of antibiotic resistance and ramification of disease treatment for the general human population have caused the Food and Drug Administration (FDA) to restrict antibiotic use with a policy goal of phasing out antibiotic use in food animals. Thus, there is a need to find suitable antibiotic alternatives and one potential solution may be the use of essential oils (EO) and arabinogalactans in combination (EOC).

Essential oils have become an active area of research due to their ability to modify metabolism and growth of bacteria (Benchaar et al., 2007; Calsamiglia et al., 2007; R. Zhou et al., submitted for publication). One such example is the inhibition of *Escherichia coli* growth, a commonly found bacteria in the digestive system of ruminants (Marino et al., 2001). Furthermore, it has been reported that an oregano solution may be as effective as neomycin in preventing disease (Bampidis et al., 2006) and that EO have limited the opportunity for bacterial populations to develop spontaneous resistance, making them an ideal candidate for further study (Yap et al., 2014). Additional EO benefits have been reported, such as increased calf starter (CS) intake, feed efficiencies, and body weight (BW) gains (Hill et al., 2007) and increased beneficial bacteria in the gut flora (Santos et al., 2015).

The neonatal calf is born with no immunity, which is why colostrum consumption within the first hour of life is so important. Colostrum is a rich source of immunoglobulins, which include IgG, IgA, and IgM, that provides immunity and protection again inhaled and ingested pathogens. Immunoglobulin A represents a key first line of defense against pathogens at the mucosal surfaces (Woof and Kerr, 2004). Immunoglobulin A is also found as a second line of defense mediating elimination of pathogens that have breached the mucosal surface. Thus, the calf's development of immunity is crucial to the prevention and/or elimination of pathogens to maintain calf health.

Our previous work demonstrated that supplementing 2.5 g/d of an EOC blend resulted in greater average daily gains (ADG) and BW, and increased immunity for calves compared with calves fed the control and higher EOC inclusion rates (Froehlich et al., 2017). Further investigations on synergistic combinations were proposed and hypothesized that feeding a EOC (1.25 g/d) in combination with a liquid EO blend (liquid EOC; a 10-mL aliquot at birth and again at 12 h of age) will demonstrate promise to replace antibiotics to reduce neonatal stress while improving growth performance, health, and immunity. The study objective was to determine whether an additional feeding of liquid EOC at birth in combination with EOC in the milk replacer (MR) would allow calves to demonstrate improved growth, health, and immunity compared with calves only offered EO in MR.

MATERIALS AND METHODS

Calf Feeding and Management

This research project was conducted at the South Dakota State University (SDSU) Department of Veterinary Science's Animal Research Wing (ARW, Brookings, SD) from 20 June to 26 August 2016. All procedures were approved by SDSU Institutional Animal Care and Use Committee before the start of the study. Sixty-one Holstein calves (18 males and 43 females) were sourced from a commercial dairy farm (Mr. Edward Kavaungh, KC Dairies, LLC, Elkton, SD) and housed in Calf-Tel Deluxe II hutches (220 by 122 by 138 cm; Hampel, Germantown, WI) with approximately 2.7 m^2 with wheat straw bedding. Calves were blocked by birth date and randomly assigned to 1 of 3 treatments. All calves were sourced within a 11-d period, and the study was conducted during

June, July, and August 2016. Treatments were as follows: 1) Control (CON), a 24:20% crude protein (CP):fat (as-fed basis) MR (24:20 MR); 2) EOC mixed into the 24:20 MR at a rate of 1.25 g/d (EP); or 3) EOC mixed into the 24:20 MR at a rate of 1.25 g/d in addition to calves receiving two 10-mL oral doses of liquid EOC at birth and again at 12 h (EPC). The EP is a proprietary blend of oregano and thyme EO, arabinogalactan prebiotics, vitamins, probiotics, and a microbial catalyst, whereas liquid EOC is a colostrum-based supplement containing a proprietary blend of oregano and thyme EO, arabinogalactan prebiotics, bioactives, and antioxidants being manufactured by Ralco, Inc. (Start Strong and Stay Strong for Dairy Calves; Marshall, MN, respectively).

Prior to study enrollment, calves were tested for successful passive transfer of immunoglobulins from colostrum. Successful passive transfer was determined by blood serum samples that were collected by jugular puncture approximately 3 d after birth and read for blood total serum protein (TSP). Samples were read using a Brix refractometer (Industrial Electronics, Inc., Knoxville, TN) and collected at the dairy operation prior to transporting to the SDSU-ARW. Calves with TSP greater than 5.0 to 5.2 g/dL were considered to have successful transfer of passive immunity in healthy calves that are not dehydrated (Tyler et al., 1996). All study calves had greater than 5.0 g/dL TSP. On arrival at the ARW, calves were intranasally vaccinated with Inforce 3 (Zoetis Inc., Florham Park, NJ).

All 61 calves were sourced from the dairy operation within 11 d and fed pooled colostrum before their arrival at SDSU for the first 2 d of life. Calves on the EPC treatment were administered liquid EOC in 2 separate 10-mL doses. The first dose was at birth and the second dose 12 h later, and all doses were completed prior to arriving at SDSU. A 24:20 MR was fed in a bucket at a rate of 0.28 kg DM/calf at each feeding (0630 and 1730 h) daily for 14 d. On day 15, feeding rate was increased to 0.43 kg per calf at 2 times per day through day 35. On day 36 feedings were reduced to 1 time per day to facilitate weaning after day 42. Decoquinate (Zoetis, Inc., Parsippany, NJ) was added to MR at 41.6 mg/kg (as-is basis) for coccidiosis control. A 22% (as-fed) CP-pelleted CS (Table 1) and fresh municipal water were offered ad libitum throughout the study. All MR and CS were sourced from Hubbard Feeds Inc. (Mankato, MN). Intakes and refusals of MR (if any) and CS were recorded daily in the morning.

Feed Analysis

During the study, MR and CS samples were collected biweekly and stored frozen (−20°C). At the end of the study, 3 MR and 3 CS samples were shipped to Analab Analytical Laboratory (Agri-King, Inc., Fulton, IL) for nutrient analyses. Samples were analyzed using the following AOAC International (2016) methods: DM (930.15), CP (990.03), fat (2003.05), neutral detergent fiber (2002.04), ADF (973.18), lignin (973.18), ash (942.05), Ca (985.01), P (985.01), Mg (985.01), potassium (985.01), sulfur (923.01). Soluble protein was analyzed using the procedures of Krishnamoorthy et al. (1982). Starch was analyzed using the Glucose Reagent Set (AMRESCO Inc., Solon, OH and ALPKEM Corporation, Portland, OR 1990), and ME was calculated using an National Research Council (2001) equation.

Nasal Secretion Collection and Analysis

Nasal secretion samples were collected from calves twice throughout the study. Secretion samples were collected when the calves arrived (day 0) at the SDSU-ARW before an intranasal vaccination of Inforce 3 and again on week 3 (day 21). A 50 × 55 mm foam plug (VWR, Radnor, PA) was cut into quarters, and 1 quarter was inserted into the nasal cavity of the calf and was allowed to sit for 5 to 7 min or until the foam was saturated. The foam was then pulled out and inserted into a 10-mL plastic syringe that was used to squeeze the nasal secretions into a 1-mL microcentrifuge tube that was

Table 1. Ingredient composition of the pelleted calf starter

Ingredient	Formula %, as-is basis
Wheat midds	34.1
Soybean meal, 48% CP	20.2
Soyhulls	15.7
Corn, ground fine	15.0
Corn distillers grains with solubles	5.0
Molasses, liquid	3.8
Alfalfa meal	2.5
Fat, animal	0.6
Minerals[1]	2.8
Vitamins[2]	0.2
Decoquinate, 60,000 mg/kg	0.1

[1]Provided 0.9% Ca, 0.96% P, 0.3% Mg, 0.5% Na, 0.5% Cl, 0.2% S, 0.7 ppm Co, 23 ppm Cu, 95 ppm Fe, 1.1 ppm I, 125 ppm Mn, 0.3 ppm added Se, and 125 ppm Zn.

[2]Provided 25,176 IU/kg of vitamin A, 5,533 IU/kg of vitamin D, 165 IU of vitamin E, 0.1 ppm of biotin, 25 ppm of niacin, 3 ppm of choline, and 0.5 ppm of thiamine.

then stored frozen (−20°C) until analyzed. Nasal secretions were analyzed for specific IgA titers to Inforce 3 vaccination against bovine respiratory syncytial virus using an ELISA procedure published previously by Froehlich et al. (2017).

Body, Fecal, and Health Measurements

Body weights were taken weekly using a Wrangler Jr. digital scale (Digi-Star, Fort Atkinson, WI) mounted on an all-terrain vehicle running gear after the morning feeding, during the time frame of 0900 to 1200 h, on either Monday, Wednesday, or Friday to coincide, as closely as possible, with 7-d increments after the birth date. Body measurements including wither height (WH) and hip height (HH) were measured using a Ketchum Teletape with a level (Ketchum Manufacturing Inc., Brockville, ON, Canada), hip width (HW) was measured using a Hip-O-Meter (Elanco, Greenfield, IN), and heart girth (HG) and body length (BL) measured using a Nasco Dairy Calf Weigh Tape (Nasco, Fort Atkinson, WI). Body measurements were taken every 2 wk at the same time as BW were taken. Body surface temperature of each calf was recorded once weekly using a thermal imaging camera (Fluke Ti25 Infrared Camera, Fluke Inc., Everett, WA) targeted at the chest directly posterior to the elbow joint.

Fecal grab samples were collected on receiving at SDSU-ARW and again during week 5 of the study from each calf. Fecal grab samples were analyzed for *Bifidobacter*, *Clostridium perfringens*, *E. coli*, *Lactobacillus*, and *Salmonella* via real-time PCR analysis (Ahmed et al., 2008). Total genomic DNA was purified from feces using DNeasy Powersoil kit (Qiagen, Germantown, MD) according to manufacturer's instructions. The DNA was diluted 10-fold to minimize inhibition of real-time PCR. Standard DNA was purified from a known concentration of control bacteria and 10-fold serial dilutions were prepared from 10^9 to 10^3 colony forming units (CFU) for the standard curve. Genus- and species-specific forward and reverse primers, respectively are as follows: 1) *Bifidobacter* spp: GGTGTTCTTCCCGATATCTACA and CTCCTGGAAACGGGTGG; 2) *Clostridium perfringens:* ATGATTGGGATTATGCAGCAA and TCCATCCTTTGTTTTGATTCCA; 3) *E. coli*: CATGCCGCGTGTATGAAGAA and CGGGT AACGTCAATGAGCAAA; 4) *Lactobacillus* spp: AGCAGTAGGGAATCTTCCA and CACCGC TACACATGGAG: and 5) *Salmonella* spp: CGTT TCCTGCGGTACTGTTAAT and AGACGGCT GGTACTGATCGATAA. All qPCR were performed in duplicate on a CFX96 real-time system (BioRad, Hercules, CA) using 2-step amplification and melting protocol consisting of 1 cycle 95°C for 3 min followed by 40 cycles of 95°C for 10 s, 30 s at the annealing temperatures of 65, 65, 57 60, and 57°C for *Bifidobacter*, *Clostridium*, *Escherichia*, *Lactobacillus*, and *Salmonella*, respectively. Followed by melt curve analysis from 65 to 95°C in increments of 0.5°C to ensure amplicons from target samples matched those of the standards. Individual reactions consisted of 1× iTaq universal SYBR green supermix (BioRad), 500 nm each of forward and reverse primer, and 5 µl of diluted sample DNA in a total volume of 20 µl. The concentration of CFU/g was calculated by multiplying the quantitative mean times the dilution volume of the extracted DNA.

Health scores included fecal, nasal, eye, and ear scores and were recorded daily before the evening feeding. Health scores were visually assessed according to the University of Wisconsin calf health scoring chart (McGuirk, 2013), and were based on a scale of 0 to 3. Fecal scores were established as 0) normal, 1) semi-formed and/or pasty, 2) loose but stays on top of bedding, and 3) watery and/or sifts through bedding. Ear scores were 0) normal, 1) ear flick or head shake, 2) slight unilateral droop, and 3) head tilt or bilateral droop. Eye scores were 0) normal, 1) small amount of ocular discharge, 2) moderate amount of bilateral discharge, and 3) heavy ocular discharge. Nasal scores were 0) normal serous discharge, 1) small amount of unilateral cloudy discharge, 2) bilateral, cloudy or excessive mucus discharge, and 3) copious bilateral mucopurulent discharge. All health incidents and treatments were recorded for the length of the study.

Statistical Analysis

All data were checked for normality and outliers using the UNIVARIATE procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) before any statistical analyses were conducted. All data were then subjected to least squares ANOVA for a randomized complete block design (Steele and Torrie, 1980) having 3 treatments using the PROC MIXED procedure of SAS (version 9.4, SAS Institute Inc.). The statistical model used was: $Y_{ijk} = \mu + B_i + T_j + WK_k + (T_j \times WK_k) + Cov + e_{ijk}$, where Y_{ijk} = dependent variable, μ = overall mean, B_i = block effect, T_j = treatment effect, WK_k = week of study, and $(T_i \times WK_k)$ = treatment by week interactions, Cov = covariate (if taken), and e_{ijk} = random error.

Calves were blocked by birth date. Experimental wk was considered a repeated measurement in time having an autoregressive covariance structure. Treatment, wk, and treatment × wk were considered to be fixed effects with calf within treatment as a random effect. Least squares means were separated by PDIFF. Body weights and measurements were adjusted by the covariate (0 d) to account for differences in initial measurements. Each daily fecal, nasal, or eye/ear score was summarized by tallying by week as the number of day having a specific score, i.e. # d of score 0, and analyzed as weekly averages and overall experiment. The data were checked for normality and found to be normally distributed ($P > 0.15$). Fecal bacterial counts were converted to $\log_{10}$ base and analyzed as separate time points and the change over time was calculated as week 5 minus initial. The IgA titers were determined by subtracting control wells (noncoated) from corresponding optical density reading for the value of the sample well (coated) to account for nonspecific binding and converted to $\log_2$ values (Rivera et al., 2002). Orthogonal contrasts were designed to compare Control versus EO supplements and contrast for EP vs. EPC. Whenever significant differences attributed to treatment were detected, the Fisher's least significant difference test (Steele and Torrie, 1980) was used to separate least squares treatment means. Significance was declared at $P < 0.05$ and trends at $0.05 < P < 0.10$.

RESULTS AND DISCUSSION

Feed Analysis

The nutrient composition of the 24:20 MR fed to calves indicated the formulation agreed with CP specification (Table 2); however, the fat concentrations were below formulated values and are acceptable and would still be considered adequate for neonatal calves (National Research Council, 2001), especially during the summer. The CS met or exceeded formulation specifications to provide nutrients for meeting or exceeding the National Research Council (2001) nutrient requirements for growing dairy calves.

Body Growth and Measurements

There were 5 mortalities in the EP treatment that appeared unrelated to treatment. These deaths included, one due to a congenital heart defect, three clostridial infections, and one to internal umbilical infection. However, after the death of the second calf to clostridium, it was discovered that a mathematical error had resulted in the EO being fed at 1/2 of the desired rate to both the EP and EPC treatments. The targeted feeding rate was 2.5 g/d (Froehlich et al., 2017), but the actual feeding rate was 1.25 g per calf per day. Considering the healthy status of calves with adequate transfer of passive immunity (TSP > 5.2 mg/dL), it is imaginable misfeeding an appropriate EO dosage may have allowed for the opportunity of a clostridium infection to proliferate leading to the death of 3 calves. Any calf that died was necropsied by the SDSU Veterinary Science Department and calves that died were not replaced.

Table 2. Milk replacer and calf starter nutrient analysis

Item[1]	Milk replacer	Calf starter
DM, %	96.2	88.2
CP, %	24.4	25.0
Soluble protein, % of CP	—	24.6
NDF, %	—	32.0
ADF, %	—	16.6
Lignin, %	—	2.69
Fat, %	19.1	3.77
Starch, %	—	21.7
Ash, %	9.58	5.0
Calcium, %	0.78	1.02
Phosphorus, %	0.68	0.60
Magnesium, %	0.14	0.39
Potassium, %	2.20	1.36
Sulfur, %	0.37	0.33
ME[2], Mcal/kg	4.66	3.44

[1]Nutrient analysis conducted by Analab (Fulton, IL). Values are reported as % DM. CON = control [a 24:20% CP:fat (as-fed basis) MR without EOC]; EP = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d; EPC = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d in addition of two 10-mL oral doses of liquid EOC at birth and 12 h. All MR were fed at 0.28 kg 2 times per day for 14 d, then 0.43 kg 2 times per day from days 15 to 35, and then MR were fed 1 time per day from day 36 to weaning at day 42. DM = dry matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; ME = metabolizable energy.

[2]Metabolizeable energy, calculated according to the equations by National Research Council (2001).

The initial mean BW of all the calves was 37.3 ± 1.2 kg (Table 3) and was similar ($P > 0.10$) among treatments. Body weight data were covariate adjusted using initial BW as a covariate minimizing pretreatment differences due to the random assignment of calves coming from the dairy operation. The orthogonal contrasts of Control vs. EO supplements and EP vs. EPC were found to be nonsignificant ($P > 0.10$) for all growth parameters. Total BW gain and final BW were similar ($P > 0.10$) among treatments. Average daily gains through 56

Table 3. Body weight (BW), average daily gain (ADG), and frame measurements of body length (BL), heart girth (HG), wither height (WH), hip height (HH), and hip width (HW) for calves fed milk replacer (MR) containing essential oils (EO)

	Treatments[1]			
Item	CON	EP	EPC	SEM
No. calves	20	16	20	—
BW, kg				
Initial	35.4	38.6	37.8	1.22
Final	64.5	69.5	67.1	2.31
Gain	29.1	30.9	29.3	2.10
ADG, kg	0.52	0.55	0.52	0.03
BL, cm				
Initial	60.3	62.1	61.1	0.98
Final	73.5	75.0	74.7	1.03
Gain	13.2	12.9	13.7	1.08
HG, cm				
Initial	76.7	79.8	78.3	0.85
Final	92.3	94.8	93.2	1.00
Gain	15.6	15.1	14.9	0.79
WH, cm				
Initial	73.5	76.6	75.3	0.98
Final	83.6	86.5	84.9	0.95
Gain	10.1	9.9	9.6	1.50
HH, cm				
Initial	78.4	81.6	79.9	1.03
Final	87.5	89.5	89.0	0.91
Gain	9.1	7.9	9.2	0.85
HW, cm				
Initial	42.4	44.9	44.7	1.41
Final	63.1	67.3	65.5	2.34
Gain	20.7	22.4	20.9	2.22

[1]CON = control [a 24:20% CP:fat (as-fed basis) MR without EOC]; EP = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d; EPC = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d in addition of two 10 mL oral doses of liquid EOC at birth and 12 h. All MR were fed at 0.28 kg 2 times per day for 14 d, then 0.43 kg 2 times per day from days 15 to 35, and then MR were fed 1 time per day from day 36 to weaning at day 42.

d averaged 0.53 kg/d and were similar ($P > 0.10$) among treatments. These growth measurements are higher compared with a similar study evaluating different EO by Santos et al. (2015) who fed lower amounts of EO, but similar amounts of MR. In that study, Santos et al. (2015) reported an ADG of 0.38 kg for calves supplemented with a blend of EO (0.4 g/kg of carvacrol, cineole, cinnamaldehyde, pepper oil resin mix with mannooligosaccharide as a vehicle). However, the results in this study are lower than expected, most likely attributed to the product being fed at 1/2 the recommended rate due to a formulation error. The recommended feeding rate is 2.5 g per calf per day based on the results of Froehlich et al. (2017), which evaluated the same EOC product, supplemented similarly as the EP treatment. Feeding at 2.5 g er calf per day has an ADG through 56 d of 0.71 kg/d, which tended $P < 0.10$) to be greater than control calves fed no EO (Froehlich et al., 2017). Starter supplemented with EP had beneficial effects on preweaned calf performance with reported increases of ADG (Jeshari et al., 2016; Kazemi-Bonchenari et al., 2018). Had the supplementation met the recommended feeding rate increased ADG would have been expected.

Initial, final, and gains through 56 d for BL, HG, WH, HH, and HW were similar ($P > 0.10$) among treatments (Table 3). Previous research has demonstrated preweaned calves supplemented with an EO dose of 2.5 g/d to a 24:20 MR and EO dose of 1 g/kg of starter DM had enhanced body frame measurements (Froehlich et al., 2017; Kazemi-Bonchenari et al., 2018). Feeding the same EO product as this study, significant gains were found in WH, HH, and HW ($P < 0.05$) and numerically had the highest gains in BL and HG compared with control fed calves (Froehlich et al., 2017). Wither height in calves supplemented EO (thymol, eugenol, vanillin, limonene, and guaiacol) in starter were increased ($P < 0.05$); however gains were not reported (Kazemi-Bonchenari et al., 2018). Had the authors reported on gains, perhaps more of the body measurements would have indicated significance as EO-supplemented calves numerically had increased WH, HG, and body barrel gains compared with non-EO supplemented calves (Kazemi-Bonchenari et al., 2018).

Growth and frame measurements were expected to be greater in EO treatments compared with non-EO supplemented calves. Similarity between treatments in growth and frame rates were attributed to the formulation error, resulting in a low EO supplementation rate of 0.63 g per feeding (1.25 g/d). The recommended feeding rate is 1.25 g per feeding (2.5 g/d), which is twice the rate fed in this study (Froehlich et al., 2017). These data demonstrate that a supplementation rate of 0.63 g per feeding (1.25 g/d) is too low to have any advantageous growth effects. Overall, growth rates were lower than anticipated based on the feeding program, but could be the result of conducting the trial during a warm summer and having clostridial issues compared with Froehlich et al. (2017), which was conducted in the fall months.

Dry Matter Intake and Feed Efficiency

Calf starter intake on a DM basis, total dry matter intake (MR plus CS), and gain to feed

through 56 d were similar ($P > 0.10$) among treatments (Table 4). The orthogonal contrasts of Control vs. EO supplements and EP vs. EPC were found to be nonsignificant ($P > 0.10$). Similar studies supplementing EO have reported varying results. Froehlich et al. (2017) found no significant difference in gain per feed, or total dry matter intake (MR plus CS) through 56 d. This is further supported by Santos et al. (2015), who observed similar CS intake in calves supplemented with a blend of EO (0.4 g/kg MR) and nonsupplemented calves. Total dry matter intake was decreased in calves supplemented 0.09, 0.187, or 0.281 g per calf per day of EO mix (eucalyptus oil, menthol crystal, and mint oil) in a 22:18 MR (Soltan, 2009). However, feed conversion ratio (total feed intake divided by BW) was not affected and dry matter digestibility was increased in EO-supplemented calves compared with the control (Soltan, 2009). In contrast, data reported by Hill et al. (2007) demonstrated a commercial EO blend increased CS intake and efficiency. Furthermore, preweaned calves supplemented with EO have been reported to increase gain to feed (Kazemi-Bonchenari et al., 2018), and feed intake Jeshari et al. (2016) compared with control fed calves.

Health Performances and Fecal Pathogens

Blood TSP averaged 5.6 ± 0.15 mg/dL (Table 5). The orthogonal contrasts of Control vs. EO supplements and EP vs. EPC were found to be nonsignificant ($P > 0.10$). The mean TSP concentrations were similar ($P > 0.10$) between treatments

Table 4. Dry matter intake (DMI) of calf starter (CS), total DMI (milk replacer plus CS), and feed efficiency (gain:feed) of calves fed milk replacer (MR) containing essential oils (EO)

	Treatments[1]			
Item	CON	EP	EPC	SEM
No. calves	20	16	20	—
CS DMI, kg/d				
1 to 56 d	0.56	0.65	0.57	—
Total DMI, kg/d				
1 to 56 d	1.06	1.14	1.06	0.05
Gain per feed				
1 to 56 d	0.49	0.48	0.50	0.02

[1]CON = control [a 24:20% CP:fat (as-fed basis) MR without EOC]; EP = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d; EPC = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d in addition of two 10-mL oral doses of liquid EOC at birth and 12 h. All MR were fed at 0.28 kg 2 times per day for 14 d, then 0.43 kg 2 times per day from days 15 to 35, and then MR were fed 1 time per day from day 36 to weaning at day 42.

Table 5. Total serum proteins (TSP) and health scores for calves fed milk replacer (MR) containing essential oils (EO)

	Treatments[1]			
Item	CON	EP	EPC	SEM
No. calves	20	16	20	—
TSP, g/dL	5.6	5.5	5.7	0.15
Fecal scores[2], d/wk				
Score = 0	4.4	4.3	4.5	0.22
Score = 1	2.1	2.1	2.0	0.19
Score = 2	0.5	0.6	0.5	0.10
Score = 3	<0.1	<0.1	<0.1	0.01
Total days of fecal scores[2]				
Score = 0	26.5	25.9	26.8	1.09
Score = 1	12.6	12.4	12.1	0.92
Score = 2	2.9	3.7	3.1	0.57
Score = 3	<0.1	<0.1	<0.1	0.03
Nasal scores[3], d/wk				
Score = 0	6.9	6.9	6.9	0.03
Score = 1	0.1	0.1	0.1	0.03
Score = 2	<0.1	<0.1	<0.1	0.01
Score = 3	—	—	—	—
Total days of nasal scores[3]				
Score = 0	41.3	41.6	41.6	0.19
Score = 1	0.7	0.4	0.4	0.18
Score = 2	0.1	0.1	0.0	0.05
Score = 3	—	—	—	—
Eye scores[4], d/wk				
Score = 0	6.9	6.9	6.9	0.05
Score = 1	0.1	0.1	0.1	0.05
Score = 2	<0.1	<0.1	<0.1	0.01
Score = 3	—	—	—	—
Total days of eye scores[4]				
Score = 0	41.2	41.5	41.6	0.32
Score = 1	0.8	0.4	0.4	0.31
Score = 2	0.1	0.1	0.1	0.08
Score = 3	—	—	—	—
Ear scores[5], d/wk				
Score = 0	6.9	6.8	7.0	0.05*
Score = 1	0.1	0.2	<0.1	0.04*
Score = 2	0.0	0.0	<0.1	0.01
Score = 3	—	—	—	—
Total days of ear scores[5]				
Score = 0	41.6	40.9	41.8	0.36
Score = 1	0.4	1.0	0.2	0.31
Score = 2	<0.1	<0.1	<0.1	0.07
Score = 3	—	—	—	—

[1]CON = control [a 24:20% CP:fat (as-fed basis) MR without EOC]; EP = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d; EPC = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d in addition of two 10-mL oral doses of liquid EOC at birth and 12 h. All MR were fed at 0.28 kg 2 times per day for 14 d, then 0.43 kg 2 times per day from days 15 to 35, and then MR were fed 1 time per day from day 36 to weaning at day 42.

[2]Fecal score of 0 to 3; 0 = normal and ≥2 = scours.

[3]Nasal score of 0 to 3; 0 = normal and 3 = excessive mucopurulent discharge.

[4]Eye score of 0 to 3; 0 = normal and 3 = heavy ocular discharge.

[5]Ear score of 0 to 3; 0 = normal and 3 = droopy.

*Contrast of EP vs. EPC, $P < 0.05$.

and were above 5.2 mg/dL, indicating successful passive transfer of immunity. Tyler et al. (1996) reported that TSP concentrations greater than 5.0 to 5.2 mg/d is correlated with successful passage of immunity in hydrated healthy calves.

Fecal, nasal, eye, and ear scores, where expressed as number of days per week or days of a specific score, were similar ($P > 0.10$) for calves fed all treatments (Table 5). The orthogonal contrast of EP vs. EPC was significant ($P < 0.05$) for number of days during a week for a score of 0 and 1; however, the contrast of Control vs. the 2 EO products was nonsignificant ($P > 0.10$). Reduced average number of diarrheic days and improved general health scores have been reported in preweaned calves supplemented EO in MR (Soltan, 2009). There might be some benefit to using an EO product immediately after birth and 12 h later but did not improve health performance compared with Control fed calves and could be a result of feeding 1/2 the recommended rate of EP in the MR.

The fecal bacteria counts of both pathogens (*Clostridia*, *Escherichia*, and *Salmonella*) and good bacteria (*Bifidobacter* and *Lactobacillus*) were similar ($P > 0.10$) among calves fed all treatments at day 0 (receiving), as well as, during week 5 (Table 6). The change in fecal bacteria counts were also similar ($P > 0.10$) among calves fed all treatments. The calculation of change was based on the formula, day 0 minus week 5, so that a negative change is an increase in the CFU of those bacteria with time. The increase in *Lactobacillus* would be expected, but the increase in *E. coli* was not. However, not all *E. coli* are pathogenic (Pupo et al., 1997), but no further pathogenic differentiation was attempted (Table 6).

Immunological Performances

Immunoglobulin A titers were greater on day 0 for calves receiving EPC ($P < 0.05$) compared with calves receiving the other treatments (Table 7), which would be the result of calves receiving the liquid EOC dose at birth and again at 12 h of age compared with calves receiving CON and EP treatments. It could be postulated that the 2 liquid EOC applications provided extra protection against pathogens compared with the calves fed EP, however, that would not explain the lack of death loss for calves fed Control. However, the high health status of the calves (TSP > 5.2) in this study was influenced by feeding more than adequate amounts of colostrum at the commercial dairy farm may explain the lack of significant treatment responses combined with the lower than desired feeding rate. Titers from 21 d (Table 7) were similar ($P > 0.10$) among calves receiving all treatments. These results are in agreement with Froehlich et al. (2017) that

Table 6. Fecal pathogen colony forming units (CFU/g) reductions by calves fed milk replacer

	Treatments[1]			
Measurement	CON	EP	EPC	SE
Week 0, receiving				
Bifidobacter, $\log_{10}$	7.27	7.28	7.27	0.36
Clostridia, $\log_{10}$	1.13	1.24	1.91	0.66
Escherichia coli, $\log_{10}$	5.91	5.71	5.76	0.28
Lactobacillus, $\log_{10}$	8.25	8.37	8.46	0.28
Salmonella, $\log_{10}$	1.91	3.16	2.43	0.61
Week 5				
Bifidobacter, $\log_{10}$	6.93	6.81	6.95	0.49
Clostridia, $\log_{10}$	0.00	0.00	0.40	0.22
Escherichia coli, $\log_{10}$	6.53	6.69	6.63	0.25
Lactobacillus, $\log_{10}$	9.20	8.90	9.39	0.28
Salmonella, $\log_{10}$	0.86	0.50	0.49	0.40
Change				
Bifidobacter, $\log_{10}$	0.35	0.79	0.36	0.76
Clostridia, $\log_{10}$	1.13	1.24	1.50	0.68
Escherichia coli, $\log_{10}$	−0.63	−0.81	−1.16	0.40
Lactobacillus, $\log_{10}$	−0.96	−0.87	−0.56	0.41
Salmonella, $\log_{10}$	1.00	2.54	2.03	0.90

[1]CON = control [a 24:20% CP:fat (as-fed basis) MR without EOC]; EP = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d; EPC = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d in addition of two 10-mL oral doses of liquid EOC at birth and 12 h. All MR were fed at 0.28 kg 2 times per day for 14 d, then 0.43 kg 2 times per day from days 15 to 35, and then MR were fed 1 time per day from day 36 to weaning at day 42.

Table 7. Immunoglobulin A titer responses ($\log_2$ fold increase) to bovine respiratory disease (Inforce 3 vaccination; Zoetis Inc., Florham Park, NJ) and body temperature (BT) via thermal imaging cameras by calves fed milk replacer (MR) containing essential oils and prebiotic (EOC)

	Treatments[1]			
Item	CON	EP	EPC	SEM
No. calves	20	16	20	—
IgA				
0 d	0.02[b]	0.02[b]	0.03[a]	0.005
21 d	0.92	0.81	0.89	0.24
BT, °C	28.1	28.2	27.6	0.42

[a,b,c]Means with unlike superscripts differ, $P < 0.05$.

[1]CON = control [a 24:20% CP:fat (as-fed basis) MR without EOC]; EP = EOC mixed into the 24:20% CP:fat MR at a rate of 1.25 g/d; EPC = EOC mixed into the 24:20% CP:fat MR at a rate 1.25 g/d in addition of two 10-mL oral doses of liquid EOC at birth and 12 h. All MR were fed at 0.28 kg 2 times per d for 14 d, then 0.43 kg 2 times per day from days 15 to 35, and then MR were fed 1 time per day from days 36 to weaning at day 42.

reported feeding an EO product resulted in no difference in 21 d IgA and IgG titers.

CONCLUSIONS

The use of a liquid EOC product being administered after birth and again 12 h later can improve IgA titers to improve the immune status of the newborn calf to fight off potential diseases and pathogens. This study did not have the synergistic effect of improving growth or health of calves by combining the liquid EO and EO in the MR. Nor did it confirm our earlier results that supplementing an EO product to an MR improves calf growth performance. The targeted inclusion rate was to be 2.5 g per calf per day. However, due to the formulation error, and only feeding half of the recommended amount from our previous work (1.25 g/d), most likely resulted in an EO feeding rate that is below efficacious concentrations needed to enhance growth performance. Although this study was not designed to be a titration study, combining these data with the previous data would provide some information on what an efficacious EOC feeding rate could be for dairy calves.

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