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Ruminant Nutrition

RUMINANT NUTRITION

Feeding a calf starter containing monensin alone or in combination with an oregano, and cobalt blend to Holstein calves

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Abstract

Gut health is critically important for growing neonatal calves, and nutritional technologies are needed to prevent disease and stress challenges. Previous work feeding monensin (**MON**) in combination with an oregano, prebiotic, and cobalt-lactate (**EOC**) blend had demonstrated improved calf gut health and growth performance. The objective of this study was to evaluate the growth performance of calves fed MON and EOC alone or in combination. Eighty (80) newborn Holstein (37) female and (43) male calves were randomly assigned to one of four treatments arranged in a 2 × 2 factorial (MON and EOC). Treatments were: 1) **Control**: without MON or EOC added to the calf starter (**CS**); 2) MON: 50.8 mg/kg CS (Elanco, Greenfield, IN); 3) EOC: 44.1 mg/kg CS (Rum-A-Fresh, Ralco Inc. Marshall, MN); 4) **MON + EOC**: MON and EOC added to CS. Calves were fed colostrum followed by whole milk through weaning at 42 d, while CS was fed ad libitum through the 70-d experimental period. The MON by EOC interaction was found to be nonsignificant ($P > 0.41$) for growth performance. Calves fed without or with MON demonstrated similar ($P > 0.70$) body weight (**BW**; 68.7 and 68.9 kg without and with MON, respectively), while calves fed EOC demonstrated greater ($P < 0.01$) BW (67.3 and 70.4 kg without and with EOC, respectively) compared with calves fed without EOC. Calves fed a CS containing MON were similar ($P > 0.47$) in average daily gain (**ADG**; 0.88 and 0.91 kg/d) compared with calves fed without MON; however, feeding calves a CS with EOC increased ($P < 0.01$) ADG (0.84 and 0.95 kg/d) by 13% through the 70-d experimental period compared with calves not fed EOC. Frame measurements indicated that the greater ADG was due to increased ($P < 0.10$) frame growth for calves fed essential oils (**EO**) compared with calves fed without EO. A MON by EOC interaction ($P < 0.01$) for serum propionate concentration demonstrated calves fed MON + EOC and EOC were greater ($P < 0.05$) compared with calves fed Control, while calves fed MON were intermediate and different ($P < 0.05$). Feeding calves a CS with EOC increased ($P < 0.04$) immunoglobulin A, immunoglobulin G, and immunoglobulin M concentrations compared with calves fed without EOC. A MON by EOC interaction was detected ($P < 0.01$) for total tract starch digestibility for calves fed EOC or MON + EOC demonstrating greater ($P < 0.05$) starch digestibilities than Control-fed calves. These data demonstrate that EOC and MON fed in combination was not beneficial for enhancing the growth performance, but that calf growth performance can be improved with EOC compared with MON.

Key words: calf, essential oils, monensin, oregano, starter

Abbreviations

ADF	acid detergent fiber
ADG	average daily gain
AIA	acid insoluble ash
BW	body weight
CP	crude protein
CS	calf starter
DM	dry matter
DMI	dry matter intake
IgA	immunoglobulin A
IgG	immunoglobulin G
IgM	immunoglobulin M
NDF	neutral detergent fiber
TSP	total serum proteins
VFA	volatile fatty acids

Introduction

Antibiotics have existed for more than 60 yr and substantially benefited public health and livestock production (Guardabassi, 2013). However, many countries have banned the feeding of antibiotics at subtherapeutic amounts for improving animal performance by preventing health and disease challenges (Kuehn, 2014; Qiao et al., 2018). The neonatal calf has a critical need for antibiotic alternatives, due to their susceptibility to numerous calfhood stresses and disease challenges that can potentially lead to high mortality (NAHMS, 2016). To provide an antibiotics alternative, our group has conducted several trials evaluating oregano as a viable antibiotic alternative for calves (Froehlich et al., 2017; Swedzinski et al., 2019; Liu et al., 2020). Albeit, historically, there have been some challenges when feeding EO, that is, oregano.

One challenging issue has been determining the appropriate feeding rate that does not result in overfeeding EO that results in palatability and/or consumption problems in contrast to the other extreme of feeding insufficient amounts to elucidate growth responses and prevention of health challenges. For example, Froehlich et al. (2017) conducted a titration study reporting that the most efficacious dosage was one-half of the manufacturer's recommendation of 5 g/d. However, recent data published by Swedzinski et al. (2020) reported that feeding one-half (1.25 g/d being one-quarter of the manufacturer's recommendations) of the low dose (2.5 g/d) used in the Froehlich et al.'s (2017) study resulted in no performance or health improvements, that is, dosage too low. However, these trials are being used in a current modeling effort to derive an efficacious dosage.

EO (specifically, oregano) has been reported to have broad antibacterial, antiviral, antifungal, insecticidal, and herbicidal properties, causing conformational changes in the cell membrane to become less impermeable (Calsamiglia et al., 2007). EO are known to inhibit Gram-positive more than Gram-negative bacteria but can inhibit Gram-negative *Escherichia coli* growth (Marino et al., 2001). In addition, recent work by our group has demonstrated that oregano can shift the rumen microbial community (Zhou et al., 2020), which enhances propionate concentrations. In addition, feeding a combination of oregano and a prebiotic has resulted in the discovery of a new Prevotellaceae microbe in the rumen of Holstein calves fed oregano and a prebiotic (Poudel et al., 2019).

The ruminal fermentation shift when feeding a blend of oregano, prebiotics, and cobalt (**EOC**) led to an increase in ruminal propionate concentrations (Zhou et al., 2020), which may be similar to the observed monensin (**MON**) ruminal fermentation shifts, that is, ionophore-like effect (Duffield et al., 2012). Cobalt (**Co**) lactate has been shown to improve fiber digestion, both in vitro (Allen, 1986) and in vivo studies (Lopez-Guisa and Satter, 1992; Kuester and Casper, 2015; Pretz, 2016), but its impact on calf fiber digestion would be minimal due to low fiber concentrations in calf starter (**CS**). MON, as an ionophore, is effective against Gram-positive bacteria (Duffield et al., 2012). Therefore, both technologies could be achieving similar ruminal fermentation shifts, through different mechanisms to enhance growth performance and intestinal health. What is not scientifically known is if these technologies would be synergistic, neutral, or agonistic to neonatal calf growth. MON is typically added to CS for growth promotion and cocci control (Bagg et al., 2000), while oregano has typically been added for disease challenges and outbreaks (Liu et al., 2020).

Since MON and oregano had been shown independently to improve calf gut health and growth rates, the hypothesis of this experiment was that MON and oregano may be synergistic for further improvements in calf gut health and growth performance. The study objective was to evaluate the gut health and growth performance of calf fed MON and EOC alone or in combination during the first 70 d of life.

Materials and Methods

Animals and experimental design

This experiment was conducted at the Lintao Huajia Animal Husbandry Co., Ltd., approximately 100 km south of Lanzhou, Gansu, P. R. China. The experiment was conducted according to the Chinese Standards for the Use and Care of Research Animals (He et al., 2016) through the Gansu Agricultural University, Lanzhou, Gansu, P. R. China. Eighty (80) Holstein calves (37 females and 43 males split across treatments) from a paternal half-sib family were sourced from the Huajia Dairy Farm, which is a commercial dairy and research farm. Calves were housed in individualize calf pens bedded with wheat straw under an open-sided naturally well-ventilated barn. Supplemental heat via a coal furnace could be supplied if needed during colder weather. Calves were blocked by birth date and randomly assigned to one of four treatments with treatments arranged in a 2 × 2 factorial design (Steel and Torrie, 1980). The factorial main effects were without or with MON or without or with EOC. The individual treatments were: 1) **Control**: CS without MON or EOC; 2): MON: CS with MON added at the rate of 50.8 mg/kg; 3) EOC: a blend of oregano, prebiotic, and Co added to CS at 44.1 mg/kg; and 4) **MON + EOC:** MON and EOC fed in combination at 50.8 mg and 44.1 mg/kg of CS, respectively. The ingredient formulations of the experimental CS are given in Table 1. The EOC contained 1.3% a proprietary blend of oregano EO (carvacrol, caryophyllene, *p*-cymete cineole, terpinene, and thymol), Co lactate (CoMax; Co = 1,425 ppm), and 98.7% natural feed grade carrier, which is manufactured by Ralco, Inc. (Rum-A-Fresh; Marshall, MN). The ingredient formulations of the experimental CS were designed to meet or exceed the NRC (2001) nutrient requirements for calves during the first 2 mo of life. The experiment was conducted from February through September 2017. For the study duration, temperature varied widely from a low of −8.4 °C in February to a high of 29.1 °C in July (Table 2). The experimental period was 70 d from the date of birth, and the calves were fed CS for ad libitum consumption starting on day 3 after birth through the end of the study (70 d) with weaning occurring after day 42 of age.

Table 1. Ingredient composition of CS fed to calves

Ingredient	Treatment[1] CS	MON	EOC	MON + EOC
	------------------- (% of mix) -------------------			
Corn, ground	40.3	40.3	40.3	39.6
Soybean meal, 48%	35.0	35.0	35.0	35.0
Corn distillers	11.0	11.0	11.0	11.0
Cottonseed meal	6.80	6.80	6.80	6.80
Molasses, beet	4.00	4.00	4.00	4.00
Calcium carbonate	1.63	1.63	1.63	1.63
$CaHPO_4$	0.10	0.10	0.10	0.10
Salt, white	0.10	0.10	0.10	0.10
Magnesium oxide	0.07	0.07	0.07	0.07
Selenium yeast	0.02	0.02	0.02	0.02
Soybean oil	0.80	0.80	0.80	0.80
MON, 19.8%	—	0.36	—	0.36
Essential oil/Co mix[2]	—	—	0.40	0.40
Premix	0.14	0.14	0.14	0.14

[1]CS, calf starter; MON, CS containing monensin; EOC, CS containing essential oil and cobalt; MON+EOC, CS containing monensin, essential oils and cobalt.
[2]Premix contains 9,000 IU vitamin A, 2,400 IU Vitamin D, 47.22 mg, 206.74 mg Fe, 33.49 mg Cu, 108.8 mg Zn, 79.99 mg Mn, 0.44 mg Se, 0.60 mg I, and 0.36 mg Co per kg of premix (Rum-A-Fresh, Ralco, Inc., Marshall, MN).

Table 2. Monthly temperature (°C) data during the CS study at Lintao, China[1]

Month	Minimum	Average	Maximum
February	−8.4	−0.4	7.6
March	−3.2	2.8	8.8
April	2.6	9.8	10.9
May	5.8	13.6	21.3
June	10.3	17.5	24.6
July	13.9	21.5	29.1
August	12.8	18.0	23.1
September	9.3	14.8	20.2

[1]Source: China National Historical Weather Data.

At birth (day 0), a blood sample was collected from the calf for total serum protein (**TSP**) measurements. Calves were fed 2 liters of colostrum at the morning and evening feedings for 2 d. However, if a calf was born in the afternoon or evening, this was considered day 1, and it may have received only one feeding of colostrum before being blood sampled for the day 2 TSP measurements; however, all calves received the required colostrum feedings. Calves were fed 2 liters of fresh whole milk after colostrum feeding to 10 d of age per feeding at 8:00 a.m. and 5:00 p.m. (i.e., 2×/d). From day 11 to 35, calves were fed 3 liters of fresh whole milk per feeding (i.e., 2×/d). The weaning protocol was started on day 35 through day 42 by feeding calves 3 liters of fresh whole milk once daily at 8:00 a.m. (i.e., 1×/d). Weaning occurred by discontinuing fresh whole milk feeding after day 42. The fresh whole milk was produced by the lactating dairy cows at the Huajia Dairy operation and milk nutrient composition was measured weekly via an emulsion analyzer (Model GT017830, Hangzhou Zhejiang University U-Micron Technology Co., LTD). This trial overlapped with the study by Liu et al. (2020), and the fresh whole milk nutrient composition and variation are presented in that paper.

Feed intake and analysis

Starting on day 3, the CS amounts offered and refused were recorded daily using a digital scale with 1-g display. Samples of base CS (without additives) were collected on the same day weekly and composited at the end of the study into monthly lots (eight samples) for nutrient analyses. Fresh amounts of each experimental CS were mixed daily. At 8 wk of age, a single CS sample and individual fecal samples were collected from each calf. Fecal samples were collected at various times of the day when sufficient feces was available to be sampled without bedding contamination. All feed and 8 wk fecal samples were dried at 60 °C and ground to pass through a 1-mm screen. Samples were analyzed using the following AOAC International (2019) methods for dry matter (**DM**; 930.15), crude protein (**CP**; 990.03), neutral detergent fiber (**NDF**; 2,002.04), acid detergent fiber (**ADF**; 973.18), starch (Hall, 2009), Ca (985.01), P (985.01), and acid insoluble ash (**AIA**; 955.03). Dry matter intake (**DMI**) was calculated as a 7-d weekly average using daily fed amounts times DM content of milk or CS, while apparent total tract nutrient digestibilities were calculated using AIA as an internal digestibility marker.

Body, fecal, and health measurements

Body weights (**BWs**) were taken weekly using a digital platform scale (Model XK3190-A12±E, Shanghai Yaohua Co., LTD) before the morning feeding. Body frame measurements were taken at 0, 14, 28, 42, 56, and 70 d and included wither height, body length, heart girth, abdominal girth, and cannon bone were measured using a Biltmore stick and flexible ruler (Jiangsu Animal Husbandry Veterinary Equipment Manufacturing Co., Ltd., Jiangsu, China).

Fecal consistency scores were recorded every day for each calf throughout the trial based on a 1- to 5-point scale (1 = stiff, 2 = pasty, 3 = normal, 4 = loose, and 5 = watery; Stamey et al., 2012). Calves were monitored daily for body temperature and respiration rate. Any abnormality or health condition that arose was treated immediately with appropriate medicines and treatments recorded.

Blood samples were collected from the jugular vein of all calves 2 to 3 h after the morning feeding on 0, 2, 14, 28, 42, 56, and 70 d. An additional blood sample was collected at week 5 for the measurement of blood volatile fatty acid (**VFA**) concentrations as an index of rumen development. Blood samples were collected using a 10-mL Vacutainer serum separation tube with an 18-gauge needle (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Samples were allowed to clot and serum was harvested by centrifugation at 2,000 × *g* for 10 min at 20 °C and stored frozen in 5-mL polystyrene tubes until samples were thawed for the measurement of immunity indexes (TSP, immunoglobulin A [**IgA**], immunoglobulin M [**IgM**], and immunoglobulin G [**IgG**]) using an enzyme standard instrument (Beijing Liuyi Biotechnology Co., LTD) with Elisa Kit (Wuhan BeiYin Biotechnology Co., LTD).

The blood serum samples collected during week 5 were analyzed for VFA concentrations via gas chromatography (model 6890 N, Agilent Technologies, Wilmington, DE, USA). Samples were prepared according to the procedures of Oba and Allen (2003) with some modifications. The column was a nonbonded poly (biscyanopropyl siloxane) phase that was a 30 m × 0.32 mm × 0.25 μm film thickness, fused-silica column (SP-3560; Sigma-Aldrich, Co., St. Louis, MO) with a column flow of 2.0-mL min^{-1} nitrogen as the carrier gas used for chromatographic separations. The injector temperature was set at 220 °C and

1 uL of the sample was injected with a split ratio of 40:1, and the initial oven temperature was programmed at 120 °C and maintained for 3 min, then increased to 180 °C at 10 °C min^{-1} and held at 180 °C for 1 min. The flame ionization detector was maintained at 250 °C.

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. The box and whisker plots and Shapiro Wilk Test were used to verify that data were normally distributed ($P > 0.15$). All data were subjected to least squares ANOVA using a randomized complete block design (Steel and Torrie, 1980) with four individual treatments having a 2 × 2 factorial treatment arrangement via the MIXED procedure of SAS. The statistical model used was:

$$\begin{aligned} Y_{ijkl} = \mu + Block_i + MON_j + EOC_k + MON_j \times EOC_k) \\ + Day_l + (MON_j \times Day_l) + (EOC_k \times Day_l) \\ + (MON_j \times EOC_k \times Day_l) + Cov + e_{jkl} \end{aligned}$$

Where Y_{ijkl} = dependent variable, μ = overall mean, Blocki = block effect, MON_j = main effect of MON, EOC_k = main effect of EOC, Day_l = day on study, and $MON_j \times Day_l$ = interaction of MON by study day, $EOC_k \times Day_l$ = interaction of EOC by study day, $MON_j \times EOC_k \times Day_l$ = interaction of MON by EOC by study day, COV = covariate (initial measurement), and e_{ijkl} is the random error. Study day (Day_l) was considered a repeated measurement in time having an autoregressive covariance structure. Main effects (MON or EOC), interaction main effects, day, and main effects × study day interactions were considered as fixed effects. Block was considered a random effect. Least squares means were separated by the PDIFF statement when the *F* test was significant. All other data were summarized utilizing the same model described above but excluded study day and/or covariate. Initial measurements when appropriate were used as a covariate, that is, BW. Significance was declared at $P < 0.05$ and trends at $0.05 < P \le 0.10$. Daily feed intake and orts were compiled as weekly averages (7 d) and reported as a study day average for that week, and DMI were calculated. Each daily fecal score was summarized across the study (number of incidences of score 1) and analyzed.

Results and Discussion

CS nutrient composition

The nutrient composition of the base experimental CS (without additives) met or exceeded the nutrient formulation specifications for CP, fat, and minerals (Table 3). Because the CS was blended as a mix and then the respective treatments were added to the mix daily, mixed and fed to the calves, only the base CS was sampled for nutrient concentrations. These nutrient concentrations would meet or exceed the nutrient requirements guidelines for neonatal calves (NRC, 2001), especially for the warmer study months.

Body growth and ADGs

The initial BW (birth weight) used as a covariate was significant ($P < 0.01$). The MON by EOC interaction was nonsignificant ($P > 0.41$; Table 4) for BW, BW gain, and average daily gain (**ADG**). The MON main effect ($P > 0.38$) was nonsignificant indicating that the addition of MON to the CS did not enhance the growth performance when expressed as BW, BW gain, and ADG. Improvements in BW gains and ADG are common with MON addition to a CS (Duffield et al., 2012) but not always, but the more important reason to add MON to a CS is for coccidiosis control (Bagg et al., 2000). Wu et al. (2020) reported that young growing Holstein bulls fed MON demonstrated greater BW gains compared with control-fed bulls in a 280-d experiment.

Table 3. Nutrient composition of base CS (Control) prior to adding MON, or EOC, or a combination of both MON and EOC (MON + EOC)

Nutrient	Control
Dry matter, %	87.8
CP, %	27.4
NDF	9.82
ADF	5.71
Fat	4.35
Ash	3.73
Ca	0.81
P	0.49
ME, Mcal/kg[1]	1.45

[1]Calculated from NRC (2001).

The main effect of EOC showed that calves fed a CS containing EOC demonstrated greater ($P < 0.01$) BW, BW gains, and ADG than calves fed without EOC. In addition, a significant EOC by day interaction was detected ($P < 0.01$) indicating that calves fed EOC were gaining more BW as the study progressed than calves fed the CS without EOC (Figure 1). Calves fed EOC were gaining approximately 6.2% more BW than calves not fed EOC. Froehlich et al. (2017) reported greater BW, BW gains, and ADG when a similar EOC product was fed through a milk replacer in a 56-d study, while Liu et al. (2020) reported greater BW, BW gains, and ADG when adding the same EOC product used in this study to a CS that also contained MON. In contrast, Swedzinski et al. (2020) reported similar BW, BW gains, and ADG when feeding the same EOC blend as Froehlich et al. (2017), but the inclusion rate was one-half of the optimal inclusion rate (1.25 g/d) that elicited a growth response. Santos et al. (2015) reported no growth performance improvements when feeding a commercial EO product, but the feeding rate may have been too low. These growth measurements approach or meet industry standards, in that calves double their birth BW by 60 d of age (Barringer et al., 2016). These results demonstrate that feeding EOC can improve the growth performance of neonatal calves through 70 d of age when the appropriate amounts are fed.

DMI and feed efficiency

The MON by EOC interaction was nonsignificant ($P > 0.41$) for CS DMI and feed efficiency (total BW gain/[milk DMI plus CS DMI]; Table 5). The MON and EOC main effects indicated no differences ($P > 0.19$) for CS intake when calves were fed MON or EOC. Calves fed all treatments averaged approximately 1 kg/d for the entire study. Duffield et al. (2012) in their meta-analysis reported that MON reduced DMI, but the responses in DMI are not always consistent. Liu et al. (2020) reported that calves fed EOC in a CS containing MON reported an increase in DMI, which substantiated that feeding EOC can enhance DMI. Froehlich et al. (2017) reported an increase in DMI for calves fed EOC after study week 4 compared with control-fed calves, whereas Swedzinski et al. (2020) reported no DMI response for calves fed EOC but the feeding rate was too low in that study. This study did not observe an increase in DMI when feeding EOC, which could be related

Table 4. BW, BW gain, and ADG for calves fed a CS without or with MON, or EOC, or a combination of both MON and EOC (MON × EOC)

	Treatments[1]					Main effects & interaction, *P*-value <[2]		
Measurement	CS	MON	EOC	MON + EOC	SEM	MON	EOC	MON × EOC
N	20	20	20	20	—	—	—	—
BW, kg								
0 d	44.7	44.7	44.8	44.7	0.84			
14 d	52.5	52.0	53.5	53.4	0.84			
28 d	60.5	60.3	63.3	63.4	0.84			
42 d	70.3	70.2	74.0	74.4	0.84			
56 d	81.3	81.7	85.9	86.8	0.84			
70 d	94.0	94.9	100.0	100.8	0.84			
Average, 0 to 70 d	67.2	77.3	70.2	70.6	0.76	0.70	0.01	0.81
BW gain, 0 to 70 d	49.4	50.7	55.2	58.0	1.22	0.36	0.01	0.41
ADG, kg/d								
0 to 14 d	0.55	0.52	0.63	0.62	0.02			
15 to 28 d	0.58	0.59	0.70	0.71	0.02			
29 to 42 d	0.70	0.71	0.76	0.79	0.02			
43 to 56 d	0.79	0.82	0.85	0.89	0.02			
57 to 70 d	0.91	0.94	1.01	1.00	0.02			
Average, 0 to 70 d	0.70	0.72	0.79	0.80	0.02	0.38	0.01	1.00
ADG, BW gain/70 d	0.83	0.85	0.93	0.97	0.02	0.47	0.01	0.78

[1]CS, calf starter; MON, CS containing monensin; EOC, CS containing essential oil and cobalt; MON+EOC, CS containing monensin, essential oils and cobalt.
[2]*P*-value for main effects of MON or EOC and interaction.

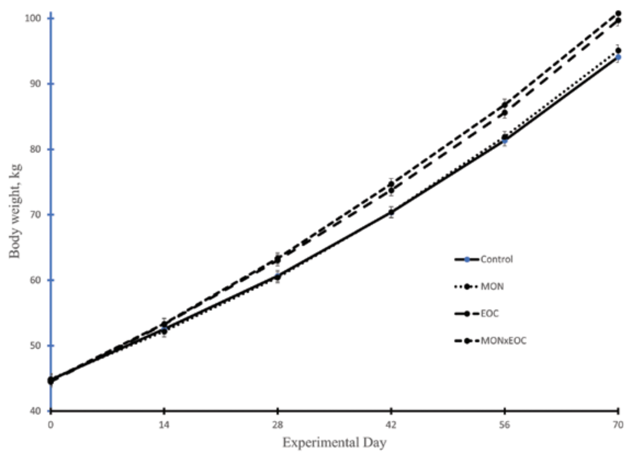

Figure 1. BW of calves fed a CS without (Control) or with MON, or EOC, or a combination of both MON and EOC (MON × EOC). Data are LSM and error bars represent SEM. Interaction of EOC × Experimental day, $P < 0.01$.

to the season during which the studies were conducted, that is, winter/spring vs. spring/summer/fall.

The MON main effect indicated similar ($P > 0.69$) feed conversions for calves fed a CS without or with MON (Table 6). However, when evaluating the feed efficiency by total BW gain/total DMI (milk plus CS), calves fed MON demonstrated greater feed conversions than calves fed without MON. Why the difference between the repeated measures evaluation of feed efficiency and the overall total experiment feed efficiency is likely due to the variation occurring during the 2-wk period measurements compared with final–initial BW/total DMI calculation, which would eliminate the week to week variation. In addition, BW would not have been covariate-adjusted when calculating the overall total study feed efficiency. Duffield et al. (2012) reported that several studies demonstrated improvements in feed conversions, but few other studies did not report significant improvements in feed efficiency, which leads to inconsistencies in responses to MON, which may be due to a number of factors, including management and/or disease challenges.

The EOC main effect indicated that calves fed EOC demonstrated greater ($P < 0.01$) feed efficiency compared with calves fed a CS without EOC. Feed efficiency improvement by feeding an EOC blend was also reported by Liu et al. (2020), but Froehlich et al. (2017) reported similar feed efficiencies. In this study, MON improved feed efficiency by approximately 4.9%, while feeding EOC improved feed efficiency by approximately 11.0%. Thus, EOC demonstrated an improved response in feed efficiency, but EOC would have more capabilities than MON for improving feed efficiency. In addition, these data would demonstrate that the addition of MON and EOC is not beneficial. Our work (Wu et al., 2020) with feeding MON and EOC in combination can actually be detrimental to the growth performance of growing Holstein bulls 70 through 310 d of age. The speculation is feeding the MON and EOC to a growing ruminant with a functioning rumen is resulting in excessive propionate production resulting in reduced growth performance (Wu et al., 2020). This situation would not be occurring for a neonatal growing calf without a functioning rumen but could become an issue as the rumen begins functioning.

Fecal scores

Daily fecal scores were tallied for total incidences by calf (Table 7). A fecal score of 1 was not recorded during the study, which indicates that gut health was consistently being challenged. The MON by EOC interaction was nonsignificant ($P > 0.15$) for diarrhea occurrences by calves, while both MON or EOC were reduced ($P < 0.02$) the incidences of mild and severe diarrhea. Similar results were reported by Froehlich et al. (2017) and Liu et al. (2020). MON is known to inhibit the growth of Gram-positive bacteria, while oregano EO contains phenolic compounds that can inhibit specific pathogenic microorganisms, such as Gram-negative bacteria

Table 5. CS DMI for calves fed a CS without or with MON, or EOC, or a combination of both MON and EOC (MON × EOC)

	Treatment[1]					Main effects & interaction, P-value <[2]		
Measurement	CS	MON	EOC	MON + EOC	SEM	MON	EOC	MON × EOC
N	20	20	20	20	—	—	—	—
DMI, kg/d								
0 to 7 d	0.02	0.02	0.02	0.02	0.04			
8 to 14 d	0.09	0.10	0.14	0.12	0.04			
15 to 21 d	0.10	0.12	0.15	0.17	0.04			
22 to 28 d	0.26	0.26	0.29	0.34	0.04			
29 to 35 d	0.59	0.59	0.49	0.65	0.04			
36 to 42 d	0.96	0.97	0.81	0.90	0.04			
43 to 48 d	1.37	1.39	1.26	1.24	0.04			
50 to 56 d	1.80	1.86	1.91	1.67	0.04			
57 to 63 d	2.13	2.01	2.19	2.14	0.04			
64 to 70 d	2.41	2.23	2.66	2.55	0.04			
0 to 70 d	0.97	0.96	0.99	0.98	0.02	0.49	0.19	0.94
Total DMI, kg	72.0	71.1	73.5	72.7	1.86	0.52	0.25	0.96

[1]CS, calf starter; MON, CS containing monensin; EOC, CS containing essential oil and cobalt; MON+EOC, CS containing monensin, essential oils and cobalt.
[2]P-value for main effects of MON or EOC and interaction.

Table 6. Feed efficiency of milk plus CS DMI into BW gain for calves fed a CS without or with MON, or EOC, or a combination of both MON and EOC (MON × EOC)

	Treatment[1]					Main effects & interaction, P-value <[2]		
Measurement	CS	MON	EOC	MON + EOC	SEM	MON	EOC	MON × EOC
N	20	20	20	20	—-	—	—	—
Feed conversion, kg BW/kg DMI								
0 to 14 d	0.77	0.72	0.84	0.82	0.03			
15 to 28 d	0.49	0.49	0.56	0.54	0.03			
29 to 42 d	0.33	0.33	0.40	0.36	0.03			
43 to 56 d	0.25	0.25	0.26	0.27	0.03			
57 to 70 d	0.20	0.23	0.21	0.21	0.03			
Average, 0 to 70 d	0.41	0.40	0.45	0.45	0.01	0.69	0.01	0.91
Total BW gain/ total feed intake, kg/kg	0.49	0.51	0.54	0.57	0.01	0.02	0.01	0.50

[1]CS, calf starter; MON, CS containing monensin; EOC, CS containing essential oil and cobalt; MON+EOC, CS containing monensin, essential oils and cobalt.
[2]P-value for main effects of MON or EOC and interaction.

(*E.* and *Salmonella typhimurium*; Benchaar et al., 2008). Gilling et al. (2019) reported the antimicrobial efficacy of plant EO (lemongrass, cinnamon, and oregano EO) against *E. coli*. The EO and their active components demonstrated significant ($P < 0.05$) >5.0 $-\log_{10}$ reductions for *E. coli* within 1 to 10 min in vitro. Moreover, Benchaar et al. (2008) suggested that certain EO may have the ability to inhibit parasites, such as cryptosporidium, coccidia, and nematodes. Thus, EOC could substitute for MON for coccidiosis control. The resulting improvement in the intestinal gut health environment may inhibit parasites, which may decrease diarrhea. However, Pempek et al. (2018) reported that the EO (cinnamaldehyde) had no effect on diarrhea incidence, but there was a risk of navel inflammation being lower ($P < 0.04$) for calves that received cinnamaldehyde compared with calves fed the control group, while Santos et al. (2015) reported no calf health benefits when feeding a commercial EOC. Different EO can and probably will have different characteristic responses that will require further research to elucidate specific benefits for feeding livestock.

Frame measurements

The MON by EOC interaction was nonsignificant ($P > 0.34$) for the frame measurements of body length, withers height, heart girth, abdominal girth, and cannon bone (Table 8). Calves fed without or with MON (main effect) were similar ($P > 0.27$) for body length, withers height, heart girth, and cannon bone, but tended ($P < 0.07$) to be greater in abdominal girth over the course of the experiment and greater ($P < 0.05$) calculated total gain (final-initial) for the entire 70-d experiment. The initial measurements as a covariate were significant ($P < 0.01$) for all frame measurements. The speculation is that the greater abdominal girth may be related to more fiber reaching the large intestine and being digested (to be discussed later) even though DMI was similar ($P > 0.49$) for calves feed without or with MON.

Calves fed EOC tended ($P < 0.06$) to demonstrate greater body length and wither height, while abdominal girth was greater ($P < 0.03$) than calves fed a CS without EOC, but the total body length and wither height gains for the experiment were nonsignificant ($P > 0.15$) due to having numerically more body length and wither height at the start of the experiment. These

Table 7. The occurrence of diarrhea by calves fed a CS without or with MON, or EOC, or a combination of both MON and EOC (MON × EOC)

	Treatment[1]					Main effects & interaction, *P*-value <[2]		
Measurement	CS	MON	EOC	MON + EOC	SEM	MON	EOC	MON × EOC
N	20	20	20	20	—	—	—	—
Score 1 (times)	—	—	—	—	—	—	—	—
Scores 2 & 3 (times)	58.1	57.0	62.4	60.4	0.30	0.01	0.01	0.15
Score 4 mild (times)	11.0	12.3	7.3	9.1	0.23	0.01	0.01	0.29
Score 5 severe (times)	0.9	0.8	0.3	0.5	0.16	0.70	0.02	0.25

[1]CS, calf starter; MON, CS containing monensin; EOC, CS containing essential oil and cobalt; MON+EOC, CS containing monensin, essential oils and cobalt.
[2]P-value for main effects of MON or EOC and interaction.

data were covariate-adjusted and the covariate was significant ($P < 0.01$). Calves fed without or with EOC were similar ($P > 0.12$) in heart girth and cannon bone. Calves fed EOC demonstrated greater ($P < 0.03$) abdominal girth compared with calves not fed EOC. Previous studies (Froehlich et al., 2017; Liu et al., 2020) feeding the same EO/EOC have reported greater frame growth, which may be related to the increase in BW, BW gains, and ADG. Santos et al. (2015) also reported increased frame growth when feeding an EOC blend. In contrast, Swedzinski et al. (2020) reported similar frame growth, but the feeding rate was too low. These data and literature suggest that feeding an EO or EOC blend can enhance frame growth. Future research may want to focus on changes in body composition and nutrients being supplied for metabolic processes.

Nutrient digestibility

A significant ($P < 0.01$) MON by EOC interaction was detected for apparent total tract starch digestibility and a tendency ($P < 0.07$) for a MON by EOC interaction for apparent total tract ADF digestibility, while the remaining MON by EOC interaction for nutrient digestibilities were similar ($P > 0.35$). Care must be taken when evaluating apparent total tract nutrient digestibilities because whole milk and CS can be highly digestible, such that small differences in nutrient intake and excretion can result in large changes in apparent total tract nutrient digestibility in combination with typical laboratory analytical errors for nutrients and internal markers. However, the results are similar to previous work evaluating apparent total tract nutrient digestibility by calves (Quigley, 2019; Liu et al., 2020). Calves fed MON + EOC were greatest ($P < 0.05$) in apparent total tract starch digestibility compared with calves fed EOC, which was greater ($P < 0.05$) than calves fed Control and lowest ($P < 0.05$) for calves fed MON. Why calves fed MON alone would decrease apparent total tract starch digestibility is not known; however, it could be argued that MON and EOC appear to be synergistic for improving apparent total tract starch digestion. The apparent total tract ADF digestibility followed the same trends as apparent total tract starch digestibility, except that calves fed MON were greater than calves fed Control.

The MON main effect demonstrated that calves fed MON demonstrated greater ($P < 0.01$) apparent total tract DM, CP, NDF, Ca, and P digestibilities than calves fed without MON. Improvements in gut health along with shifts in ruminal fermentation would be expected to increase apparent total tract nutrient digestibilities. Why improvements in apparent total tract nutrient digestibilities would lead to an increase in abdominal girth for calves fed MON compared with calves fed CS without MON is counterintuitive. To our knowledge, no literature data exist measuring abdominal girth when calves are fed MON.

The EOC main effect demonstrated that calves fed EOC demonstrated greater ($P < 0.01$) apparent total tract nutrient digestibilities for all nutrients measured compared with calves fed without EOC. The apparent total tract nutrient digestibility improvements are greater than the improvements observed with MON in this study. A shift in ruminal microflora via inhibiting pernicious bacteria has the potential to increase CS digestibility. Oregano oil contains a phenolic structure that can inhibit specific pathogenic microorganisms, such as the Gram-negative bacteria *Escherichia* and *Salmonella typhimurium* (Benchaar et al. 2008). Zhou et al. (2020) reported a shift in the ruminal microbial community when adding EOC to an in vitro system, while Poudel et al. (2019) identified a new Prevotellaceae bacteria along with an increase in *Prevotella* abundance when feeding EOC to calves. Liu et al. (2020) reported enhanced apparent total tract nutrient digestibility when feeding EOC in combination with MON in a CS. Kolling et al. (2018) reported that oregano EO tended to increase apparent total tract CP digestibility. Thus, feeding EOC can improve the nutrient availability to the calf through improvements in total tract nutrient digestibility.

Immunity

The MON by EOC interaction was nonsignificant ($P > 0.46$) for TSP, IgG, IgA, and IgM (Table 10). The MON main effect was similar ($P > 0.42$) for TSP, IgG, IgA, and IgM for calves fed without or with MON. To our knowledge, few studies have evaluated the effects of feeding MON on immune parameters, especially IgG, IgA, and IgM. Yasui et al. (2016) reported that feeding MON to transition dairy cows improved some aspects (polymorphonuclear neutrophils and monocytes) of immune function. So, the speculation was that MON would exert little effect on the immune response.

In contrast, the EOC main effect was significant ($P < 0.04$) for TSP, IgG, IgA, and IgM demonstrating that calves fed a CS with EOC had improved immunity compared with calves fed without EOC. Feeding an EOC blend may not only enhance the immune system response to a pathogen challenge but may also modulate the immunological response due to an inflammatory response that can be detrimental to the growing calf because of energy being directed to the immune system vs. growth (Froehlich et al., 2017). These calves were being immunologically challenged due to no fecal scores of 1, but diarrhea incidences were reduced when fed EOC. Thus, the immune system was enhanced by feeding EOC, but speculation is that the immune system was not overstimulated, which resulted in nutrients being directed to growth performance instead of the immune system. The study by Froehlich et al. (2017) demonstrated that feeding EOC demonstrated the greatest immunological response

Table 8. Body frame measurements by calves fed a CS without or with MON, or EOC, or a combination of both MON and EOC (MON ×EOC)

	Treatment[1]					Main effects & interaction, P-value <[2]		
Measurement	CS	MON	EOC	MON + EOC	SEM	MON	EOC	MON × EOC
N	20	20	20	20	—	—	—	—
Body length, cm								
Day 0	78.3	79.0	79.6	80.0	0.63			
Day 14	80.7	80.8	81.6	82.2	0.63			
Day 28	82.9	82.8	83.8	84.5	0.63			
Day 42	85.4	85.2	86.3	87.1	0.63			
Day 56	88.2	87.7	89.3	90.2	0.63			
Day 70	92.1	90.6	92.6	93.7	0.63			
Average, 0 to 70 d	85.8	85.6	86.4	86.7	0.54	0.99	0.06	0.48
Gain 0 to 70 d	6.7	6.36	7.09	7.35	0.57	0.92	0.22	0.57
Wither height, cm								
Day 0	79.1	79.3	80.3	80.5	0.74			
Day 14	81.7	81.4	82.2	82.5	0.74			
Day 28	84.2	83.6	84.6	84.8	0.74			
Day 42	86.8	86.0	87.1	87.5	0.74			
Day 56	89.1	88.5	89.9	90.6	0.74			
Day 70	92.4	91.5	93.1	94.2	0.74			
Average, 0 to 70 d	86.4	86.2	86.7	87.3	0.66	0.68	0.10	0.34
Gain 0 to 70 d	6.73	6.48	6.91	7.48	0.58	0.78	0.28	0.44
Heart girth, cm								
Day 0	78.7	78.8	79.5	79.0	0.71			
Day 14	80.8	81.2	81.7	81.7	0.71			
Day 28	83.0	83.3	83.9	83.9	0.71			
Day 42	85.2	85.7	86.5	86.6	0.71			
Day 56	87.8	88.5	89.3	89.6	0.71			
Day 70	90.6	91.5	92.5	93.1	0.71			
Average, 0 to 70 d	85.1	85.7	85.9	86.4	0.60	0.27	0.12	0.96
Gain 0 to 70 d	6.13	6.21	6.93	7.47	0.59	0.30	0.15	0.98
Abdominal girth, cm								
Day 0	78.1	77.7	78.9	78.1	0.84			
Day 14	80.2	79.9	81.1	81.0	0.84			
Day 28	82.4	82.3	83.8	84.1	0.84			
Day 42	84.8	84.9	86.6	87.3	0.84			
Day 56	87.5	88.0	89.5	90.9	0.84			
Day 70	90.4	92.0	92.8	94.9	0.84			
Average, 0 to 70 d	84.6	85.3	85.6	87.1	0.74	0.07	0.02	0.49
Gain 0 to 70 d	6.43	7.15	7.33	8.91	0.55	0.05	0.03	0.47
Cannon bone, cm								
Day 0	9.40	9.55	9.72	9.95	0.16			
Day 14	9.85	10.1	10.2	10.5	0.16			
Day 28	10.6	10.8	10.9	11.1	0.16			
Day 42	11.4	11.5	11.8	11.9	0.16			
Day 56	12.2	12.3	12.6	12.7	0.16			
Day 70	13.0	13.1	13.5	13.6	0.16			
Average, 0 to 70 d	11.3	11.3	11.4	11.3	0.13	0.92	0.53	0.91
Gain 0 to 70 d	1.68	1.67	1.70	1.62	0.14	0.67	1.00	0.85

[1]CS, calf starter; MON, CS containing monensin; EOC, CS containing essential oil and cobalt; MON+EOC, CS containing monensin, essential oils and cobalt.
[2]P-value for main effects of MON or EOC and interaction.

and improved growth. Thus, feeding or administering an EOC blend can enhance the immune system (Froehlich et al., 2017; Liu et al., 2020; Swedzinski et al., 2020).

Volatile fatty acids

Serum blood samples collected during week 5 were analyzed for VFA concentrations as an indirect measurement of ascertaining ruminal development (Table 11). The MON by EOC interaction was nonsignificant ($P > 0.07$) for all blood VFA concentrations, except propionate ($P < 0.01$) and a trend ($P < 0.07$) for valerate. Calves fed MON + EOC demonstrated greater ($P < 0.05$) propionate concentrations than calves fed MON, while calves fed EOC were intermediate and similar ($P > 0.10$), but calves fed Control were lowest ($P < 0.05$) compared with calves fed the other treatments. These shifts in propionate concentrations appear to follow the changes observed in starch digestibilities for calves fed the different treatments (Table 9). While MON is known to shift ruminal fermentation to more propionate (Bagg et al., 2000; Duffield et al., 2012), EOC can also shift ruminal fermentation to increase propionate concentrations (Poudel et al., 2019;

Table 9. Nutrient digestibilities from calves fed a CS without or with MON, or EOC, or a combination of both MON and EOC (MON × EOC)

	Treatment[1]					Main effects & Interaction, P-value <[2]		
Nutrient	CS	MON	EOC	MON + EOC	SEM	MON	EOC	MON × EOC
N	20	20	20	20	—	—	—	—
DM, %	86.9	89.8	91.2	94.0	0.22	0.01	0.01	0.81
CP, %	85.3	88.7	90.1	92.8	0.33	0.01	0.01	0.35
NDF, %	50.5	55.4	58.4	62.4	1.02	0.01	0.01	0.64
ADF, %	49.4	53.7	57.1	58.4	0.80	0.01	0.01	0.07
Starch, %	93.7[c]	93.1[d]	97.5[b]	98.4[a]	0.04	0.01	0.01	0.01
P, %	83.5	86.8	88.2	90.9	0.04	0.01	0.01	0.51
Ca, %	71.1	75.3	76.4	78.9	1.01	0.01	0.01	0.40

[1]CS, calf starter; MON, CS containing monensin; EOC, CS containing essential oil and cobalt; MON+EOC, CS containing monensin, essential oils and cobalt.
[2]P-value for main effects of MON or EOC and interaction.
[a–d]Means in the same row with unlike superscripts differ, $P < 0.05$.

Table 10. Blood TSP and immunoglobulin concentrations of calves fed a CS without or with MON, or EOC, or combination of both MON and EOC (MON × EOC)

	Treatment[1]					Main effects & Interaction, P-value <[2]		
Measurement	CS	MON	EOC	MON + EOC	SEM	MON	EOC	MON × EOC
N	20	20	20	20	—	—	—	—
TSP, g/L								
Day 0	47.9	46.9	48.2	48.7	0.53			
Day 2	50.4	50.1	50.8	51.3	0.53			
Day 14	51.8	50.9	51.7	52.7	0.53			
Day 28	53.8	53.8	55.1	55.6	0.53			
Day 42	56.1	56.2	58.1	59.4	0.53			
Day 56	59.7	61.6	63.1	64.1	0.53			
Day 70	62.1	65.4	68.0	69.4	0.53			
Average, 0 to 70 d	54.6	56.1	56.2	56.3	0.44	0.44	0.01	0.61
IgG, mg/dL								
Day 0	100.6	99.5	101.0	100.1	5.00			
Day 2	114.9	114.4	112.3	112.2	5.00			
Day 14	104.2	108.1	107.6	106.4	5.00			
Day 28	93.5	95.9	96.2	98.5	5.00			
Day 42	105.7	110.1	112.9	115.7	5.00			
Day 56	118.4	126.0	133.0	133.5	5.00			
Day 70	134.8	143.2	153.4	155.6	5.00			
Average 0 to 70 d	110.1	110.8	116.8	117.8	4.10	0.82	0.02	0.96
IgA, mg/dL								
Day 0	4.3	4.5	4.6	4.5	1.91			
Day 2	12.8	12.6	14.1	13.1	1.91			
Day 14	8.5	9.9	11.6	10.9	1.91			
Day 28	4.6	5.0	6.0	5.8	1.91			
Day 42	7.4	8.4	12.4	11.9	1.91			
Day 56	14.0	14.5	21.8	19.3	1.91			
Day 70	16.5	17.4	27.7	27.6	1.91			
Average, 0 to 70 d	9.7	10.3	14.1	13.2	1.51	0.93	0.01	0.50
IgM, mg/dL								
Day 0	6.2	5.8	6.6	6.2	1.25			
Day 2	15.2	15.4	16.5	15.7	1.25			
Day 14	8.3	7.8	8.8	8.8	1.25			
Day 28	5.9	5.2	8.3	8.5	1.25			
Day 42	8.8	9.2	13.0	12.3	1.25			
Day 56	12.0	10.4	15.3	15.1	1.25			
Day 70	15.9	16.9	27.6	22.2	1.25			
Average 0 to 70 d	10.4	11.5	12.5	12.5	0.85	0.46	0.04	0.46

[1]CS, calf starter; MON, CS containing monensin; EOC, CS containing essential oil and cobalt; MON+EOC, CS containing monensin, essential oils and cobalt.
[2]P-value for main effects of MON or EOC and interaction; A significant EOC * Day was found for TP, IgG, IgA, and IgM, $P < 0.01$.

Table 11. Blood VFA concentrations from calves fed a CS without or with MON, or EOC, or combination of both MON and EOC (MON × EOC)

	Treatment[1]					Main effects & Interaction, *P*-value <[2]		
Measurement	CS	MON	EOC	MON + EOC	SEM	MON	EOC	MON × EOC
N	20	20	20	20	—	—	—	—
Acetate, µmole/L	381.1	334.3	451.4	428.7	8.32	0.01	0.01	0.16
Propionate, µmole/L	41.5[c]	49.5[b]	55.2[ab]	59.5[a]	1.72	0.01	0.01	0.01
Isobutyrate, µmole/L	15.3	14.9	20.5	20.1	0.40	0.40	0.01	0.99
Butyrate, µmole/L	44.8	42.6	59.7	55.9	1.24	0.02	0.01	0.53
Isovalerate, µmole/L	14.4	14.6	23.4	23.5	0.44	0.81	0.01	0.93
Valerate, µmole/L	4.7	4.3	4.7	4.8	0.12	0.26	0.07	0.07
Total VFA, µmole/L	501.9	460.3	614.9	592.4	11.36	0.01	0.01	0.41

[1]CS, calf starter; MON, CS containing monensin; EOC, CS containing essential oil and cobalt; MON+EOC, CS containing monensin, essential oils and cobalt.
[2]P-value for main effects of MON or EOC and interaction.
[a–c]Means in the same row with unlike superscripts differ, $P < 0.05$.

Zhou et al., 2020). These data demonstrate that EOC can result in a larger increase in propionate concentration than MON (approximately 19% and 33% for MON and EOC, respectively). An enhanced shift to increased propionate concentrations could be the result of reduced methane production in the rumen. Zhou et al. (2020) reported approximately an 11% reduction in methane production in vitro when adding EOC to the fermenter.

The MON effect demonstrated that calves fed MON compared with calves fed without MON demonstrated reduced blood concentrations of acetate, butyrate, and total VFA, while isobutyrate, isovalerate, and valerate concentrations were similar ($P > 0.26$). The observed shift in ruminal fermentation would be expected to reduce blood acetate and butyrate concentrations (Duffield et al., 2012); however, the reduction in total blood VFA concentrations is unexpected. Several studies cited by Burrin and Britton (1986) reported that feeding MON reduced total VFA, acetate, and butyrate with no impact on isobutyrate, isovalerate, and valerate blood concentrations.

The EOC main effect demonstrated that calves fed EOC had greater ($P < 0.01$) blood concentrations of all individual VFA and total VFA, except valerate, which demonstrated a tendency ($P < 0.07$) for greater blood concentration compared with calves fed without EOC. The greater VFA concentrations would support the conclusion that feeding EOC is enhancing ruminal development through both shifting ruminal fermentation and papillae nutrient absorption. Several of these VFA are known to initiate epithelial and stimulate rumen papillae growth, and butyrate is considered the most effective followed by propionate and acetate (Sakata and Tamate, 1979). Thus, the greater VFA concentrations in this study are likely due to the enhanced nutrient digestibility (Table 9) leading to more nutrients being digested to enhance ruminal VFA concentrations, which, in turn, lead to enhanced blood VFA concentrations (Table 11). The combination of EO and Co lactate in the EOC blend appears to be synergistically enhancing ruminal development. Given the enhancement in nutrient digestibilities (Table 9) by feeding, the EOC blend would be expected to have more ruminal VFA production. Cobalt lactate is one component of the EOC blend. CO lactate has been shown to improve fiber digestion (Poudel, 2016), which could lead to more acetate and butyrate.

Conclusions

The lack of significant MON by EOC interaction for growth performance indicates that feeding MON and EOC as a combination is not beneficial for enhancing neonatal calf growth performance. Including MON in a CS enhanced feed conversion, while EOC incorporation in a CS resulted in larger improvements in BW, BW gains, and ADG. In addition, feeding a CS containing EOC is stimulating rumen and papillae development to enhance total tract nutrient digestion and VFA absorption to increase blood VFA concentrations. Calves fed MON or EOC in the CS demonstrated fewer diarrhea occurrences, but only feeding EOC improved TSP, IgG, IgA, and IgM immune parameters. The experimental database is growing to support the conclusion that feeding an EOC blend provides a natural viable alternative to antibiotics to minimize health challenges, while improving calf growth performance to improve the economic returns to the dairy calf raiser.

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Conflict of interest statement

The authors have declared no conflict of interest.

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