Colostrum supplementation with n-3 fatty acids does not alter calf outcome on a healthy commercial farm

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ABSTRACT

Our objective was to supplement colostrum with n-3 fatty acids (FA) to provide anti-inflammatory mediators that may improve the immune response of neonatal calves. Elevated markers of inflammation have been associated with increased occurrence of calf disease in early life, thus decreasing animal productivity. We hypothesized that a colostrum supplement containing 60-mL of a 1:1 ratio fish:flaxseed oil blend with or without 200 mg of α -tocopherol might provide an advantageous start to early life by decreasing oxidative stress and regulating the inflammatory response. Calves were blocked by birth order and randomly assigned to 1 of 3 treatments: no supplement added to colostrum (control), 60 mL of 1:1 fish:flaxseed oil blend, and 60 mL of 1:1 fish:flaxseed oil blend with 200 mg of α -tocopherol. In total, 180 heifer calves ($n = 60$ per treatment) were enrolled on a commercial farm. After colostrum feeding, all calves were housed in individual hutches and fed milk replacer 3 times per day. Health was scored 3 times per week until weaning at 8 wk of age. Weight, wither height, and heart girth were measured after birth, 3 wk, and 8 wk of age to assess preweaning growth. A subgroup of 54 calves (18 blocks or 18 calves per treatment) were sampled 2 d (± 8 h) after birth to evaluate oxidant status, serum total protein, and inflammatory gene and cytokine protein expression in blood after an *in vitro* lipopolysaccharide (LPS) challenge as indicators of health and immunity. At 9 wk, calves were transported 18 h to another farm, and medical records were kept as an indicator of disease incidence up to 13 wk of age. Calf mortality was 1.8%, which is below industry average, and exceptional health scores were observed throughout the study. Health scores and growth were similar throughout the preweaning period regardless of treatment. Serum total protein indicated successful passive transfer in

all calves, and oxidant status index was not affected by treatments on d 2 of age. Concentrations of tumor necrosis factor α increased with LPS stimulation, but the increase was not altered by treatment. Likewise, leukocyte gene expression of tumor necrosis factor α , IL-8 and IL-10, and cyclooxygenase-2 increased upon LPS stimulation, but the fold change did not differ with treatment. In conclusion, 60 mL of 1:1 ratio fish:flaxseed oil colostrum supplement did not enhance preweaning calf performance. Supplementing n-3 FA in a one-time meal may not provide the anti-inflammatory benefits observed with continuous feeding.

Key words: colostrum supplementation, calf health

INTRODUCTION

Calves may experience oxidative stress at birth because production of reactive oxygen and nitrogen species (**RONs**) is increased following onset of independent metabolic and respiratory processes (Gaál et al., 2006; Abuelo et al., 2019). Free radical damage to cellular components may cause inflammation and increase disease susceptibility. One potential way to reduce oxidative stress is to supplement calves with n-3 fatty acids (**FA**), which produce oxylipids during metabolism that can decrease oxidative stress and inflammation (Gabbs et al., 2015; Mavangira and Sordillo, 2018). Proinflammatory prostaglandins are produced via cyclooxygenase-2 (**COX-2**) enzymes. Human research indicates certain n-3 FA can decrease COX-2 expression (Massaro et al., 2006; Jaudszus et al., 2013) and other proinflammatory mediators such as tumor necrosis factor- α (**TNF- α**) and IL-8 (Li et al., 2014). Additionally, n-3 FA can increase anti-inflammatory mediators such as IL-10 (Jaudszus et al., 2013). In calves, the immune response is modulated with n-3 FA-rich fish or flaxseed oil supplemented to milk replacer (Ballou and DePeters, 2008; Karcher et al., 2014), and flaxseed oil in calf starter enhances growth (Hill et al., 2009). We speculated similar benefits may occur from a substantial one-time dose of fish and flaxseed oils in colostrum. The fat content of colostrum is greater than

Received December 11, 2019.

Accepted July 5, 2020.

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milk at 5 mo postpartum (5.3 vs. 3.7%), and the ratio of n-6 FA to n-3 FA is less than milk (3 vs. 7; Contarini et al., 2014). Therefore, we aimed to further enhance n-3 FA in colostrum.

We supplemented n-3 FA in calves' first colostrum via fish and flaxseed oils and found altered oxylipid and isoprostane concentrations indicative of decreased oxidative stress and increased n-3 FA profiles (Opgenorth et al., 2020a). Our results indicated n-3 FA decreases the n-6:n-3 FA ratio in plasma resulting in altered production of oxylipids derived from both n-6 and n-3 FA, potentially decreasing oxidative stress during the first week of life. The inclusion of α -tocopherol may also be important in enhancing the beneficial effects of n-3 FA (Opgenorth et al., 2020a). This may be due to α -tocopherol's antioxidant functions and roles in protecting n-3 FA from peroxidation (Tappel, 1972). However, general health and growth rates were unaffected. Tao and Dahl (2013) cautioned against results of health and growth of studies with lower sample sizes such as treatment $n = 10$. Seeing that the n-3 FA supplements were safe to feed calves with no observed negative effects on passive transfer or general health and growth, we sought to increase sample size to 180 calves at a commercial dairy farm.

A short-term decrease in oxidative stress that attenuates inflammation during the first few days of life may benefit calves during the preweaning period by advancing health and, thus, growth. The n-3 FA may alleviate oxidative stress; thus, we hypothesized that supplementing n-3 FA in colostrum would improve preweaning health and growth. Our primary objective was to determine if fish and flaxseed oil or fish and flaxseed oil with an additional 200 mg of α -tocopherol supplemented to colostrum would decrease health scores and disease incidence and increase growth. Our secondary objective was to examine the in vitro response of an immune challenge on inflammatory markers.

MATERIALS AND METHODS

The study was conducted during the months of March to May of 2019 at a commercial Michigan dairy farm and was approved by the Michigan State University Animal Care and Use Committee (approval no. 03/18-035-00). One hundred eighty Holstein-Friesian heifer calves were assigned to blocks as they were born and to 1 of 3 treatments within block in a randomized block design. Based on the variation in health scores of 0.5, we used the power procedure of SAS version 9.4 (SAS Institute Inc., Cary, NC) to determine that 60 calves per treatment would provide a 92% chance of detecting a difference in health scores greater than

0.3 at $P < 0.05$. Treatments were (1) no supplement added to colostrum (**CON**); (2) supplement of 60 mL of a 1:1 fish:flaxseed oil blend with 20% polysorbate 80 for emulsification (**FF**); and (3) supplement of FF with 200 mg of α -tocopherol. Sixty calves were enrolled each week within a 4-d window for 3 consecutive weeks in March.

Colostrum was acquired from freshening cows at the farm. If Brix $> 22\%$, the colostrum was pooled and stored in a bulk tank at 4°C . The bulk tank was emptied twice daily and pooled colostrum was distributed into 2.8-L bottles with 45 mL of 50% potassium sorbate solution in water to prevent bacterial growth. Bottles were stored at -20°C and then warmed before feeding. Supplements were added directly to the colostrum bottle and inverted to mix. All calves were fed by esophageal tube within 2 h after birth. Calves were ear notched to test for bovine leukemia virus and given 4 mL of an intramuscular VetOne Vitamin E-AD (MWI Animal Health, Boise, ID) injection that includes 300 IU of vitamin E, 100,000 IU of vitamin A, and 10,000 IU of vitamin D₃ per mL. Calves received a second feeding of 1.9 L of colostrum with no supplements by nipple bottle 12 to 18 h after birth. Calves that refused to drink the full 1.9 L were fed with esophageal tube to complete the meal. Only the first colostrum feeding contained treatment supplements.

Calves were placed in a heated pen within 1 h after birth. Within 24 h, they were placed in individual outdoor hutches bedded with straw and sawdust and had no contact with other calves for the rest of the study. At 2 d of age, calves were vaccinated against bovine respiratory virus (Inforce 3, Zoetis, Parsippany, NJ). Dehorning paste was applied within the first week of life.

On d 2, 3, and 4, calves received 2.8 L of milk per feeding at 0500 and 1900 h. Milk on these days was a blend of unsaleable milk from the farm and milk replacer (HerdFirst, Cargill, Minneapolis, MN). Milk replacer was blended in varying amounts per feeding depending on the quantity of unsaleable milk available each day. At 3 to 4 d of age, calves received water and calf starter grain ad libitum. Starting on d 5, calves were fed 2.8 L of milk 3 times per day at 0500, 1300, and 1900 h. At 6 to 7 wk, the milk per feeding was decreased to 1.9 L. At 7 to 8 wk of age, milk per feeding was decreased to 0.94 L. At 8 to 9 wk of age, calves were fed 0.94 L at the 0500 h feeding only. At 9 to 10 wk of age, calves were transported for 18 h to another farm after receiving their final 0.94 L feeding. Calves were weighed within 1 h after birth. Initial heart girth and wither height were measured at 2 to 3 d of age. Calves were weighed and measured similarly at 3 wk of age and just before wean-

ing at 8 wk of age. Eye, nasal, fecal, and ear scores were assigned for each calf 3 d per wk by trained researchers blind to treatment using the health scoring system of University of Wisconsin–Madison (2020). Scores were assigned from 0 to 3 as follows: fecal: 0 = normal, 1 = semi-formed, 2 = loose, 3 = watery/blood; eye: 0 = no discharge, 1 = minor discharge, 2 = bilateral moderate discharge, 3 = severe discharge; nasal: 0 = normal or clear discharge, 1 = minor cloudy discharge, 2 = bilateral moderate discharge, 3 = severe mucopurulent discharge; ear: 0 = normal alertness, 1 = flick of ears or shaking head, 2 = minor ear droop, 3 = bilateral ear droop or tilted head. Health scores were averaged by week of age. Calves received appropriate medical attention by farm employees (health scores approximately ≥ 2). Severe diarrhea was treated with Polyflex (Boehringer Ingelheim Animal Health USA, Duluth, GA) and pneumonia with Draxxin (Zoetis, Parsippany, NJ) if necessary. Records of any medications given to calves were recorded as a measure of disease incidence from birth to 1 mo posttransport (until 13 wk of age).

Blood Collection and Analysis

In each weekly cohort, 6 blocks of calves born within a 16-h time period were selected for blood sampling so that 18 blocks (54 calves) were sampled for the experiment. Blood was sampled at 48 ± 8 h after birth via jugular venipuncture for 1 serum separator tube and 2 EDTA plasma tubes using the BD Vacutainer system (Becton, Dickinson and Company, Franklin Lakes, NJ). Blood was placed on ice immediately after collection before processing. Serum separator tubes were spun at $1,700 \times g$ at 4°C for 15 min, and 2 mL of serum was immediately aliquoted, flash frozen in liquid nitrogen, placed in dry ice, and stored at -80°C upon returning to campus laboratories. Remaining serum was tested for serum total protein with a digital Brix refractometer to assess passive transfer. Calves with total protein greater than or equal to 52 g/L were considered to have successful passive transfer (Calloway et al., 2002).

Within 2 mo of storage, serum was assessed for antioxidant potential (Re et al., 1999) and RONS (OxiSelect In Vitro ROS/RNS Assay Kit, Green Fluorescence, Cell Biolabs Inc., San Diego, CA) to calculate the oxidant status index (OSi), which quantifies the balance of RONS and antioxidants and indicates the potential for oxidative stress. Both assays assessing oxidant status are detailed by Putman et al. (2018). Free radicals in samples convert an added reagent to a fluorescing product that can be measured to relate to total free radical concentration as described previously (Putman et al., 2018).

Values of antioxidant potential (AOP) were quantified with a photometric plate reader. 2,2'-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid was used as a radical cation to be reduced by antioxidants to measure reduction potential of each sample. This assay measuring Trolox equivalents, a synthetic analog of vitamin E, is further described in Re et al. (1999). Whole blood from the 2 EDTA plasma tubes was transferred to 2 tubes of 5 mL each for LPS stimulation of immune cells. Methods were adapted from Karcher et al. (2014). One tube was inoculated with LPS (from *Escherichia coli* O111:B4; InvivoGen, San Diego, CA) at $5 \mu\text{g/mL}$ blood, and the other tube was inoculated with control medium. Blood was incubated for 3.5 h in a 37°C water bath. After incubation, blood was centrifuged at $1,300 \times g$ at 4°C for 10 min. Plasma was flash frozen in liquid nitrogen, placed on dry ice for transportation, and stored at -80°C until analyzed for TNF- α proteins (Bovine TNF- α ELISA Kit, Thermo Scientific, Frederick, MD). The remaining leukocyte layers were processed with QIAamp RNA Blood Mini Kit (Qiagen, Germantown, MD) to remove erythrocytes. The remaining white blood cells were suspended in $600 \mu\text{L}$ of buffer RLT for RNA isolation, flash frozen in liquid nitrogen, placed on dry ice during transportation, and stored at -80°C until analyzed for gene expression.

We extracted white blood cell RNA using QIAcube automation (Qiagen) with QIAamp RNA Blood Mini Kit (Qiagen). The RNase-free DNase (Qiagen) digested DNA, and all RNA samples were suspended in $40\text{-}\mu\text{L}$ RNase-free water before undergoing cDNA synthesis with a High Capacity cDNA reverse transcriptase kit with RNase inhibitor (Applied Biosystems, Foster City, CA). Afterward, cDNA was amplified with Taqman PreAmp Kit (Applied Biosystems).

Samples were analyzed in triplicate for expression of *COX2*, *IL8*, *IL10*, and *TNFA* using quantitative real-time PCR (ABI QuantStudio 7 Flex PCR system, Applied Biosystems). The reaction mix for each well consisted of $5 \mu\text{L}$ of Taqman Gene Expression PCR Master Mix (Applied Biosystems), $2.5 \mu\text{L}$ of amplified cDNA sample, $0.5 \mu\text{L}$ of Taqman Gene Expression Assay Mix (Applied Biosystems), and $2 \mu\text{L}$ of RNase-DNase-free water. We included 3 endogenous controls of *B2M*, *PGK1*, and *GUSB* Taqman Assays for reference and 1 nontemplate control per PCR plate for contamination detection.

Standard method thermal cycling conditions were 48°C for 30 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s followed by 60°C for 1 min. The 3 reference genes of *B2M*, *PGK1*, and *GUSB* relative to the calibrator of CON, nonstimulated LPS samples normalized target gene abundance calculated by the relative quan-

tification method described by Livak and Schmittgen (2001).

Statistical Analysis

Data were analyzed using the mixed procedure of SAS 9.4. Fixed effects were treatment and cohort (starting week of study). Random effects were block within cohort and calf within block, cohort, and treatment. The linear model was as follows: $Y = \mu + C_i + B_j(C_i) + T_k + T_k \times C_i + e_{ijk}$, where μ = overall mean, C_i = fixed effect of cohort, $B_j(C_i)$ = random effect of block within cohort, T_k = fixed effect treatment, $T_k \times C_i$ = fixed effect of interaction between treatment and cohort, and e_{ijk} = residual error. Preplanned contrasts were (1) CON versus FF and FFE, and (2) FF versus FFE. Birth weights and initial heart girths and hip heights were used as covariates for growth rates. Health scores were analyzed based off a weekly average score per calf (Figure 1) and sum of abnormal days (days on which scores were greater than 0) per calf (Table 2). Changes between LPS and control gene expression were additionally analyzed with a paired *t*-test in SAS 9.4. Differences for main effects were considered significant if $P \leq 0.05$ and trends if $0.05 < P \leq 0.10$. Differences for interactions were considered significant if $P \leq 0.05$ and trends if $0.05 < P \leq 0.10$.

RESULTS

Calves were healthy throughout the study with an average of 0.43 medical intervention events per calf from birth to 13 wk of age where 119 of calves did not require any medical treatments. Two calves died during the preweaning trial period: one from failure to thrive less than 12 h postparturition and one from pneumonia. Most medications during the first 8 wk of life were to treat diarrhea. Pneumonia was the primary disease after transport. Treatment did not alter number of medications per calf ($P = 0.89$).

Because calves were healthy, health scores were generally low (Table 1; Figure 1). Average number of days for abnormal fecal scores (scores >0) were lower (13.7 vs. 14.6 d; $P = 0.05$) for FFE than FF, but these were not different than CON. Similarly, number of days with abnormal eye scores per calf was reduced in FFE compared with FF (6.4 vs. 6.6 d; $P = 0.02$). Number of days for abnormal nasal and ear scores did not differ across treatments. We estimated 92% chance of detecting a difference in health scores greater than 0.3 at $P < 0.05$. However, mean differences of health scores were lower than 0.3, indicating our chances of detecting differences among treatments were markedly reduced.

Body weight gain averaged 0.66 kg/d throughout the 8-wk preweaning period and was not altered by treatment ($P = 0.60$; Table 2). Heart girth gain during the preweaning period was not different for CON versus FF and FFE ($P = 0.12$) or FF versus FFE ($P = 0.13$). Treatments did not alter ($P = 0.13$) heart girth gain the first 3 wk of age. However, FFE tended ($P = 0.08$) to have more heart girth gain than FF. Treatments did not alter wither height gain during the first 3 wk ($P = 0.82$) or 8 wk ($P = 0.26$) of life.

Serum total protein concentrations at 2 d of age of the 54 calves out of 180 sampled were well above the threshold for successful passive transfer and were above the benchmark value of 52 g/L, indicating successful passive transfer of immunoglobulins. Treatment did not alter OSi, AOP, or ROS (Table 3). Treatment did not alter the LPS stimulation of TNF- α concentration (Table 3). Treatment did not alter expression of *IL8*, *TNFA*, *COX2*, or *IL10* in control blood. Inoculation with LPS markedly increased ($P < 0.001$) expression of these genes, indicating that LPS stimulation was successful. In LPS-stimulated blood, expression of *IL8* was not altered by treatment overall, but FF tended to decrease *IL8* expression compared with FFE ($P = 0.07$). Treatment did not alter expression of other genes in LPS-stimulated samples nor the LPS-induced changes in gene expression (Table 4).

DISCUSSION

Supplementing calves with a 60-mL blend of fish: flaxseed oil in colostrum with or without 200 mg of α -tocopherol did not improve markers of immune function, health, or growth during the first 8 wk of life on a well-managed commercial farm. Preweaned calves have an average mortality rate of 8% in the United States, excluding stillbirths (USDA NAHMS, 2007). We conducted our study at a commercial farm where mortality was 1% in preweaned calves during the study. The average number of medications given to calves displaying signs of disease was 0.43 per calf. Only one-third of research calves were given some form of medication from birth to 1 mo after weaning. In our previous studies (Opgenorth et al., 2020a,b), average medications per calf were 1.2 and 1.4. In those studies, supplementation seemed promising, but in this study with exceptionally healthy calves, we saw no benefit of n-3 FA supplementation. Fecal and eye scores decreased by FFE compared with FF, but the average of both was not different than CON. Overall, score differences were considered minimal regarding biological importance, so these tendencies may not provide confident conclusions regarding health status.

Figure 1. Changes in average fecal (panel A), eye (panel B), nasal (panel C), and ear (panel D) scores during the first 8 wk of life by CON, FF, and FFE. CON = control, no supplement added to colostrum; FF = 60 mL of 1:1 ratio fish:flaxseed oil blend added to colostrum; FFE = 60 mL of 1:1 ratio fish:flaxseed oil blend with 200 mg of α -tocopherol added to colostrum. Data shown are represented as LSM $\pm$ SE predicted from the model. Overall treatment $P = 0.09, 0.10, 0.45$, and 0.94 , respectively.

In the current study, *IL8* expression tended to decrease after endotoxin stimulation in FF compared with FFE, but the average of both treatments was not different than CON. Why supplementing n-3 FA, but not n-3 FA with 200 mg of α -tocopherol, tended to decrease *IL8* expression is not clear. Dietary supplementation of α -tocopherol was found to decrease leukocyte *IL8* in humans (Van Tits et al., 2000), so we expected *IL8* expression might have decreased in FFE relative to FF.

However, all calves received an adequate 300-IU injection of α -tocopherol following birth, and an additional 134 IU of α -tocopherol in 200 mg with the FF supplement may have been suboptimal in this case.

Additionally, no change in LPS-stimulated expression of *TNFA*, *IL10*, or *COX2* with n-3 FA colostrum supplementation was observed. In contrast, Karcher et al. (2014) found that n-3 FA supplementation in calves altered inflammatory gene expression where fish oil de-

Table 1. Effects of treatments on average number of abnormal health score days (scores >0) per calf

d >0 per calf	Treatment ¹			SEM	P-value ²		
	CON	FF	FFE		CvT	TvT	P
Fecal score (d)	14.0	14.6	13.7	0.34	0.62	0.05	0.13
Eye score (d)	6.6	6.6	6.4	0.07	0.45	0.02	0.04
Nasal score (d)	2.8	2.9	3.0	0.3	0.65	0.81	0.87
Ear score (d)	6.2	6.6	5.5	0.51	0.87	0.12	0.29

¹Treatment: CON = control, no supplement added to colostrum; FF = 60 mL of 1:1 ratio fish:flaxseed oil blend added to colostrum; FFE = 60 mL of 1:1 ratio fish:flaxseed oil blend with 200 mg of α -tocopherol added to colostrum.

²P-value: CvT: P-value contrast of CON versus FF and FFE; TvT: P-value contrast of FF versus FFE; P: overall P-value of treatment effect.

creased LPS-stimulated *TNFA* expression and flaxseed oil reduced *IL8* expression (Karcher et al., 2014). However, they fed n-3 FA for several weeks before sample analysis, whereas we gave a one-time supplement and collected leukocytes 48 h (± 8 h) later. One supplemental dose in colostrum may not provide enough time for n-3 FA to provide a meaningful effect on inflammation, and daily supplementation of n-3 FA may be more beneficial in young calves.

We previously showed that n-3 FA decreased oxidative stress in calves supplemented with 60-mL fish:flaxseed oil. Supplementation with 200 mg of α -tocopherol decreased OSi (Opgenorth et al., 2020b), and fish:flaxseed oil without an additional 200 mg of α -tocopherol decreased 8-iso-prostaglandin-A2, a marker of oxidative stress (Opgenorth et al., 2020a). We speculate the additional 200 mg of α -tocopherol may have protected tissues from RONS damage, which decreased OSi. No difference in oxidative stress was reported in the current study, but sampling calves over the same period as we sampled in previous studies (d 1, 2, 4, 7, and

14), instead of only 2 d of age, may have allowed us to detect differences. Another difference between this study and our prior studies is that they were conducted at our university farm where calves were fed colostrum with bottles instead of an esophageal tube. However, Desjardins-Morrisette et al. (2018) determined there is no difference in the abomasal emptying rates between bottle- and tube-fed calves, so we speculate that feeding method did not alter n-3 FA absorption or effectiveness.

Previously, we found a fish:flaxseed oil supplement decreased the n-6:n-3 FA ratio for at least 2 wk in plasma phospholipid FA (Opgenorth et al., 2020a). However, increased plasma free fatty acid and oxylipid concentrations with fish and flaxseed oil supplementation returned to control levels by d 7 of age in that study, indicating that beneficial effects might be short term. We speculate that short-term benefits may not be able to provide the benefits seen from continuous n-3 FA supplementation. Long-term supplementation of n-3 FA provides greater benefits than short-term

Table 2. Average daily gain of weight, heart girth, and wither height wk 0 to 3 and wk 0 to 8 during the preweaning period

Item	Treatment ¹			SEM	P-value ²		
	CON	FF	FFE		CvT	TvT	P
Weight gain (kg/d)							
Week 0–3	0.58	0.59	0.60	0.01	0.67	0.80	0.88
Week 0–8	0.66	0.65	0.67	0.01	0.95	0.22	0.60
Heart girth gain (cm/d)							
Week 0–3	0.32	0.28	0.32	0.01	0.30	0.08	0.13
Week 0–8	0.38	0.36	0.37	0.01	0.12	0.14	0.10
Wither height gain (cm/d)							
Week 0–3	0.16	0.15	0.15	0.02	0.54	0.90	0.82
Week 0–8	0.21	0.20	0.21	0.01	0.27	0.23	0.26

¹Treatment: CON = control, no supplement added to colostrum; FF = 60 mL of 1:1 ratio fish:flaxseed oil blend added to colostrum; FFE = 60 mL of 1:1 ratio fish:flaxseed oil blend with 200 mg of α -tocopherol added to colostrum.

²P-value: CvT: P-value contrast of CON versus FF and FFE; TvT: P-value contrast of FF versus FFE; P: overall P-value of treatment effect.

Table 3. Effects of treatments on total protein, tumor necrosis factor- α , and oxidant status index 2 d after birth¹

Item ²	Treatment ³			SEM	P-value ⁴		
	CON	FF	FFE		CvT	TvT	P
STP (g/L)	64	65	65	0.12	0.60	0.85	0.85
TNF- α (Δ ng/mL)	1.65	1.61	1.11	0.26	0.36	0.17	0.26
OSi (AU)	68	55	65	6.4	0.30	0.28	0.32

¹Calves were sampled 40 to 56 h after birth, where calves within a block were all sampled at the same time. Thus, block within cohort would have accounted for any variation in means due to sampling time.

²STP = serum total protein; tumor necrosis factor- α (TNF- α) increase from nonstimulated plasma to LPS-stimulated plasma after 3.5 h incubation; oxidant status index (OSi) in serum. AU = arbitrary units.

³Treatment: CON = control, no supplement added to colostrum; FF = 60 mL of 1:1 ratio fish:flaxseed oil blend added to colostrum; FFE = 60 mL of 1:1 ratio fish:flaxseed oil blend with 200 mg of α -tocopherol added to colostrum.

⁴P-value: CvT: P-value contrast of CON versus FF and FFE; TvT: P-value contrast of FF versus FFE; P: overall P-value of treatment effect.

dietary inclusion, and n-3 FA effects are partially dependent on the duration consumed (Palozza et al., 1996). Therefore, the calf may benefit more from n-3 FA supplementation in colostrum if followed by further supplementation in milk replacer similar to what has previously been described (Ballou and DePeters, 2008; Karcher et al., 2014). When quality standards for calf feeding and care are achieved, we suggest that quality colostrum alone, without addition of n-3 FA, seems to promote excellent health for calves. Colostrum supple-

ments or replacers are only recommended when maternal colostrum is below caliber (Cabral et al., 2013).

CONCLUSIONS

Supplementing colostrum with 60 mL of a 1:1 blend of fish:flaxseed oil with or without 200 mg of α -tocopherol did not enhance measures of immune function on d 2 or health or growth of calves before weaning on a commercial farm where calves were exceptionally healthy.

Table 4. Effect of treatment means on inflammatory gene expression of leukocytes in nonstimulated and LPS-stimulated whole blood¹

Target gene (RQ)	Treatment			SEM	P-value ²		
	CON	FF	FFE		CvT	TvT	P
Δ^3							
<i>IL8</i>	62	47	71	8.7	0.37	0.07	0.13
<i>IL10</i>	11	19	12	1.8	0.64	0.98	0.90
<i>TNFA</i> ⁴	8.2	9.3	7.8	1.5	0.88	0.48	0.76
<i>COX2</i> ⁴	29	28	34	4.3	0.46	0.31	0.45
No LPS ⁵							
<i>IL8</i>	3.0	2.1	2.2	1.2	0.15	0.85	0.35
<i>IL10</i>	0.93	0.71	1.0	0.19	0.71	0.97	0.93
<i>TNFA</i>	1.7	1.6	1.4	0.32	0.69	0.59	0.80
<i>COX2</i>	1.8	1.5	1.7	0.39	0.77	0.72	0.90
LPS ⁶							
<i>IL8</i>	68	47	70	9.1	0.36	0.07	0.12
<i>IL10</i>	13	14	14	1.7	0.56	0.96	0.84
<i>TNFA</i>	9.8	11	9.0	1.6	0.92	0.39	0.68
<i>COX2</i>	30	30	35	3.6	0.62	0.30	0.52

¹Treatment: CON = control, no supplement added to colostrum; FF = 60 mL of 1:1 ratio fish:flaxseed oil blend added to colostrum; FFE = 60 mL of 1:1 ratio fish:flaxseed oil blend with 200 mg of α -tocopherol added to colostrum. RQ = relative quantification.

²P-value: CvT: P-value contrast of CON versus FF and FFE; TvT: P-value contrast of FF versus FFE; P: overall P-value of treatment effect.

³ Δ : average difference between nonstimulated and LPS-stimulated samples. Paired *t*-test between individual LPS- and LPS+ samples was *P* < 0.001 for all genes.

⁴*TNFA* = tumor necrosis factor- α ; *COX2* = cyclooxygenase-2.

⁵No LPS: average gene expression with no stimulation of LPS.

⁶LPS: average gene expression after LPS stimulation.

Perhaps this treatment might have been beneficial on a farm with health similar to industry averages, but daily supplementation of n-3 FA may provide greater benefits than a one-time dose.

ACKNOWLEDGMENTS

This project was supported by the Michigan Alliance for Animal Agriculture (#AA18-032; East Lansing), Michigan Milk Producers Association (Novi), and Michigan State University AgBioResearch (East Lansing). The authors express sincere appreciation for the De Saegher family and farm employees for their partnership with this project. The authors have not stated any conflicts of interest.

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