Increasing intake of essential fatty acids from milk replacer benefits performance, immune responses, and health of preweaned Holstein calves

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ABSTRACT

The objective was to evaluate the effect of feeding increasing amounts of essential fatty acids (FA) in milk replacer (MR) during the first 60 d of life on growth, health, and immunity of Holstein calves. Calves were born from dams fed low concentrations of total and essential FA during the last 2 mo of pregnancy. Newborn calves were blocked by sex and parity of the dam and assigned randomly to receive 1 of 4 MR treatments (T). Hydrogenated coconut oil and soybean oil were mixed with emulsifier and commercial MR powder to prepare the following 4 MR containing 0.119 and 0.007 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), and 0.593 and 0.076 (T4) g of intake per kg of metabolic body weight ($BW^{0.75}$) of linoleic acid and α -linolenic acid, respectively. At 30 d of life, concentrations of essential FA (linoleic acid and α -linolenic acid) in liver increased, whereas concentrations of C12:0, C14:0, C16:0, and C20:3n-9 decreased linearly with increasing intake of essential FA. Body weight gain and feed efficiency were optimized when male calves consumed T2, whereas gain by female calves tended to increase linearly with increasing intake of essential FA during the first 30 d of age. However, these responses to treatment were not maintained after initiation of concentrate feeding at 31 d of life. Over the 60-d preweaning period, wither and hip heights were improved in both sexes as intake of essential FA increased up to T3. Some measures of health and immunity were affected by replacing some coconut oil with soybean oil. Severity of diarrhea tended to decrease linearly; plasma concentrations of haptoglobin during diarrhea were lower in T2, T3, and T4; phagocytosis by blood neutrophils tended to peak for calves fed T2; in vitro proliferation of stimulated blood lymphocytes was greater for calves fed T2; in vitro stimulated blood cells produced more IFN- γ (up to T3 for males and T2 for females), concentrations of

serum IgG against ovalbumin injections were increased in males fed T2 or T3; and skin-fold thickness increased in response to an intradermal antigen injection of all calves fed up to T4. Across sex and under the conditions of the present study, mean daily intakes of linoleic acid between 3 to 5 g/d and intakes of α -linolenic acid between 0.3 and 0.6 g/d during the first 30 d of life promoted growth of preweaned Holstein calves, possibly by improving their immune status.

Key words: calf, essential fatty acids, growth, immunity

INTRODUCTION

Attainment of good growth and health during the preweaning period is the main goal of those rearing dairy calves, as excess morbidity and mortality can significantly affect profit. Compared with that of other species, the immune system of calves is immature in early life (Godden, 2008). Calves must rely on their innate immune system for protection against initial pathogens as their adaptive immunity system develops (Chase et al., 2008). Dietary provision of the correct amounts of essential nutrients is beneficial to the growth and health of newborn calves. Linoleic acid, first identified as an essential nutrient for rats fed fat-free diets (Burr and Burr, 1930), should be beneficial to newborn calves but research to document this is lacking. Those authors identified poor growth, dermatitis, poor reproduction, and death as symptoms of long-term linoleic acid deficiency in rats suffering from “low fat disease.” Much later, α -linolenic acid was identified as an essential FA by the role of its derivative, docosahexaenoic acid (DHA; C22:6n-3), in brain development using specific tests of retinal and brain function in monkeys and rats (Neuringer et al., 1988; Anderson and Connor, 1989).

Supplementing milk replacer (MR) with different sources of FA improved the growth of preweaned calves (Jenkins et al., 1985, 1986; Jenkins and Kramer, 1986). However, for those studies, classical symptoms of an essential FA deficiency (e.g., poor growth, dermatitis, death) were not detected as reported by others feeding fat-free diets to preweaned ruminants (Cunningham

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and Loosli, 1954; Lambert et al., 1954). Moreover, feeding lower amounts of linoleic acid caused an immediate decline in concentrations of linoleic acid in plasma and erythrocytes of rats but an increase in that of preruminant calves, indicating that calves may have a lower dietary requirement, a greater efficiency of conservation, or a greater ability to mobilize linoleic acid from adipose tissue (Sklan et al., 1972). These ruminant studies used only growth, incidence of diarrhea, and FA profile of plasma and tissue, including the ratio of C20:3n-9 to C20:4n-6, to assess calf responses to dietary fats. Efforts should be made to establish FA intakes that optimize animal health, rather than identifying minimal amounts that are sufficient only for growth (Cunnane and Guesnet, 2011). Therefore, metabolic profile, severity of diseases, and measures of immune function should be considered in assessment of essential FA deficiency.

Several studies, performed primarily in rodents, have verified that the FA profile of the phospholipid fraction of immune cells reflects that of the diet and also that changes in proportions of n-6 and n-3 FA can modify the function of immune cells (Calder, 2008). Consuming sufficient amounts of linoleic acid should help the acute inflammatory response of the calf when exposed to environmental pathogens because linoleic acid serves as a precursor of proinflammatory mediators such as cytokines and eicosanoids (Calder, 2006). These compounds contribute to the activity of the all-important innate immune cells, such as monocytes and neutrophils in the early weeks of life. However, an exacerbated proinflammatory condition could impair calf health. The antiinflammatory properties of n-3 FA such as α -linolenic acid may aid in the resolution of inflammation by reducing the activity of signaling molecules and the expression of proinflammatory genes (Schmitz and Ecker, 2008). Therefore, it is critical to identify the symptoms of impaired metabolic, immune, and inflammatory processes associated with reduced intakes of essential FA to help determine actual dietary requirements.

Recent studies (Ballou and DePeters, 2008; Ballou et al., 2008) have focused on the effect of dietary n-3 FA on growth and markers of immune responses of pre-weaned dairy calves, whereas similar research involving n-6 FA is lacking. Hence, the hypothesis of the current study was that increasing intakes of linoleic acid and α -linolenic acid would improve immune responses of unweaned Holstein calves and, therefore, positively affect overall calf performance. The objective was to evaluate the effect of feeding increasing amounts of linoleic and α -linolenic acids in MR during the first 60 d of life on growth, health, and immunity of Holstein calves.

MATERIALS AND METHODS

Enrollment and Management of Pregnant Cows

The experiment was approved by the University of Florida's Institutional Animal Care and Use Committee and conducted at the University of Florida's dairy farm. A weekly cohort of pregnant nulliparous ($n = 34$) and previously parous ($n = 54$) Holstein cattle were enrolled in the study starting 8 wk before calculated calving day.

Prepartum animals were housed in a common lot and fed once daily (0800 h) with a single TMR formulated to have low concentrations of total (1.9%) and essential FA (0.60% linoleic acid and 0.35% α -linolenic acid, DM basis, Table 1). Nonsilage feedstuffs in the TMR were combined to form a grain mix. Bermudagrass silage and corn silage samples were collected once a week and dried for 1 h using a Koster Moisture Tester (Koster Crop Tester Inc., Strongsville, OH) for DM determination. Proportions of silages and grain mix in the ration were adjusted weekly based on weekly DM values to maintain the formulated silage-to-concentrate ratio (55.3:44.7, DM basis). Offered feed was adjusted daily to achieve 5 to 10%orts. Orts were collected and weighed daily. Weekly samples of silage and grain mix were ground to pass through a 1-mm screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). Samples were composited monthly, pooled in a single sample, and analyzed using wet chemistry procedures (Dairyland Laboratories Inc., Arcadia, WI; Table 1).

Calving Management and Colostrum Feeding

Calves were born between January 4 and April 5, 2011. Pregnant animals were monitored for signs of initiating parturition at least every 2 h. Within 2 h of birth, calves were weighed and ear-tagged, and the umbilical cord was disinfected with 10% betadine solution (Purdue Frederick Co., Norwalk, CT). Calves were housed temporarily (2 to 14 h of age) in individual pens (1 m $\times$ 1 m) equipped with a heat lamp before being moved to individual wire enclosures (1 m $\times$ 1.5 m) bedded with sand.

Parturient cows were milked within 6 h of calving and colostrum was harvested. The concentration of total IgG in colostrum was measured initially using a colostrometer (Biogenics, Mapleton, OR), and only colostrum of good quality (>50 g/L of IgG) was frozen at -20°C in 4-L amounts. Immediately after being weighed, calves were given 4 L of thawed and warmed colostrum from dams participating in this study using an esophageal feeding tube.

Table 1. Ingredient and chemical composition of diet fed to nonlactating, pregnant Holstein animals

Item	
Ingredient, % of DM	
Bermuda silage	46.5
Corn silage	8.8
Citrus pulp	31.7
Soybean meal	9.2
Mineral mix ¹	3.8
Nutrient composition, DM basis	
CP, %	13.80
NE _L , ² Mcal/kg	1.46
NDF, %	39.85
FA, %	1.87
C18:0, %	0.06
C18:2n-6, %	0.60
C18:3n-3, %	0.35
Ash, %	8.12
Ca, %	1.26
P, %	0.34
Mg, %	0.40
K, %	1.52
Na, %	0.18
Cl, %	0.87
DCAD, mEq/100 g	0.40

¹Contained (DM basis) 34.5% corn meal, 5.0% dicalcium phosphate, 16.0% calcium carbonate, 10% calcium sulfate, 5% magnesium oxide, 10% magnesium sulfate, 4% sodium chloride, 1.7% Zinpro 4-plex (Zinpro, Minneapolis, MN), 0.4% Rumensin 80 (Elanco Animal Health, Greenfield, IN), 0.35% Sel-Plex 2000 (Alltech Biotechnology, Nicholasville, KY), 0.002% Ca iodate, and a vitamin premix. Each kilogram contained 24.5% CP, 9.8% Ca, 1.5% P, 4.2% Mg, 3.2% S, 1.7% Na, 10.7% Cl, 475 mg of Zn, 160 mg of Cu, 456 mg of Mn, 7.4 mg of Se, 37.4 mg of Co, 13.2 mg of I, 118,000 IU of vitamin A, 27,500 IU of vitamin D, 2,600 IU of vitamin E, and 770 mg of monensin.

²Calculated from the estimation of energetic values of individual ingredients using NRC (2001) and considering intake at 3× maintenance.

Measure of Adequate Passive Immune Transfer

Blood and colostrum sampling and measure of adequate passive transfer (APT) were performed according to Garcia et al. (2014a) with some modifications. Briefly, colostrum fed and serum samples of newborn calves were measured for concentrations of bovine total IgG using a single radial immunodiffusion method (Triple J Farms, Bellingham, WA). Colostrum and serum samples were diluted with sterile saline (0.9% NaCl) at final concentrations of 15 and 70%, respectively. Diluted samples (5 µL) were applied to the wells of serial radial immunodiffusion plates containing agarose gel with anti-bovine IgG. Plates were left undisturbed for 27 h at room temperature. Resulting ring diameters were measured with a monocular comparator (VMRD Inc., Pullman, WA). A standard curve was plotted with reference sera (1.96, 14.02, and 27.48 g/L of IgG) supplied by the manufacturer. Interassay CV was 3.6%. Calves were considered to have APT if serum concentration of total IgG was ≥10 g/L 24 h after feeding of colostrum (Tyler et al., 1996).

Dietary Treatments, Feeding Management, Growth Measures, and Feed Analyses

Calves were blocked by sex (54 females and 34 males) and by parity of the dam and assigned randomly to receive 1 of 4 MR from 1 to 60 d of life. A low-fat, powdered MR was prepared commercially (9.5% fat, DM basis; Land O'Lakes Animal Milk Products Co., Shoreview, MN) to serve as the base liquid feed. Milk replacers were prepared by partially replacing hydrogenated coconut oil (CCO; Welch, Holme and Clark Co. Inc., Newark, NJ) with increasing amounts of soybean oil (SBO; Winn Dixie Co., Jacksonville, FL) which was the source of linoleic and linolenic acids in 4 milk replacers. The treatment (T) ratios of CCO and SBO were as follows: 100:0 (T1), 96.0:4.0 (T2), 87.9:12.1 (T3), and 71.8:28.2 (T4). Individual calves were offered amounts of MR to achieve intakes of 0.119 and 0.007 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of metabolic BW (BW^{0.75}) of linoleic acid and α-linolenic acid, respectively. These feeding rates for linoleic acid were selected to be below and above the recommended intake of linoleic acid by rats (NRC, 1995) and from results of our previous study with preweaned Holstein calves (Garcia et al., 2014b). The FA profiles of CCO, SBO, basal MR, and emulsifier are described in Table 2.

The amounts of SBO and CCO (stored at ~4°C) required at each feeding were heated to between 70 and 80°C using a microwave oven followed by the addition of an emulsifier (Grindsted Mono-Di HV 52 K-A, Danisco USA Inc., Madison, WI) added at 3% of the oils. Immediately, the blend of oils and emulsifier was transferred into insulated containers. Powdered MR and warm water (40 to 43°C) were mixed using an electric drill with a wire whisk attachment to prepare an 11% DM solution. The blend of oils and emulsifier was added to the MR base solution and whisked again. Oil droplets were not observed on the surface after mixing. The temperature of the liquid MR fed to the calves ranged between 35 and 38°C. Each calf was monitored to ensure that the MR offered in plastic pails was consumed within 10 min of offer. Those rare calves not willing to drink within 10 min were fed using a nipple bottle (preferably) or an esophageal feeder. The temperature of MR was verified to be within the desired temperature range and the MR was warmed in a hot water bath if needed for these calves.

Milk replacer was fed at 33 g of DM per kg of BW^{0.75} divided in 2 daily feedings at 0600 and 1230 h. This intake would support approximately 300 g of BW of ADG (NRC, 2001). Amounts of MR DM offered were increased weekly based upon BW measured at birth and once weekly throughout the 60-d study. However,

Table 2. Fatty acid profile of sources of oil, emulsifier, basal milk replacer, and concentrate

FA	Coconut oil ¹	Soybean oil ²	Emulsifier ³	Milk replacer ⁴	Concentrate
C6:0	0.6	ND ⁵	ND	0	ND
C8:0	7.8	ND	ND	4.1	2.0
C10:0	6.2	ND	ND	3.1	0.0
C12:0	50.0	ND	ND	29.4	0.0
C14:0	18.1	0.1	0.1	14.4	0.3
C16:0	8.4	11.4	11.1	17.7	21.9
C16:1	ND	0.1	ND	0.6	0.2
C18:0	8.7	4.0	87.9	7.0	1.8
C18:1	0.1	20.5	0.1	15.8	13.3
C18:2n-6	0.0	55.3	0.1	5.3	53.8
C18:3n-3	0.0	8.1	0.0	0.3	4.7
C20:0	0.1	0.3	0.5	0.1	0.2
Other	0.0	0.2	0.2	2.2	1.8

¹Welch, Holme and Clark Co. Inc. (Newark, NJ).

²Winn Dixie Co. (Jacksonville, FL).

³Grindsted Mono-Di HV 52 K-A (Danisco USA Inc., Madison, WI).

⁴Prepared by Land O'Lakes (Shoreview, MN).

⁵Not detected.

calves that lost BW in a 7-d period were offered the same amount of MR as that offered in the previous week. The afternoon MR feeding was replaced with electrolytes if diarrheic calves were not willing to drink voluntarily. Body weight and wither and hip heights were recorded as measures of growth at 0, 30, and 60 d of age. Body weight at 0 d of age was measured before feeding of colostrum, and height was measured within 12 h of birth, whereas BW and height at 30 and 60 d of age were measured before the morning feeding of MR. Calves were fed MR exclusively during the first 30 d of life. A single concentrate mix of a low concentration of linoleic acid was offered in ad libitum amounts starting at 31 d of age (Table 3). Clean water was available in ad libitum amounts at all times. Powdered MR and grain mix were sampled weekly, composited monthly, and then composited in a single sample for chemical analyses (Dairyland Laboratories Inc., Arcadia, WI).

Calf Scoring for Health Assessment and Incidence of Health Disorders

Attitude, fecal consistency, and nasal discharge were scored daily after the first daily feeding of MR by a single observer using a scale from 0 to 3 (http://www.vetmed.wisc.edu/dms/fapm/fapmtools/8calf/calf_health_scoring_chart.pdf). Calves given a fecal score >1 were considered to have diarrhea and those with a score of 3 were considered to have severe diarrhea. Calves given a score >0 for other criteria were considered as having the disorder. Weekly averages of all scores were generated per calf for statistical analysis.

Incidences of health disorders were recorded daily for individual calves. Rectal temperature was measured daily during the first 14 d of age, as well as on days when the calf displayed clinical signs of disease such as diarrhea, bloat, coughing, increased respiratory frequency, depression, or lack of appetite. Calves with a rectal temperature $\geq 39.5^{\circ}\text{C}$ were categorized as febrile. Duration in days and number of episodes of each illness event was recorded. To distinguish between different episodes, intervals of 4, 4, and 10 d between diagnoses of fever, diarrhea, and pneumonia, respectively, had to elapse to establish a new disease event. A calf was considered pneumonic if it had fever with at least one clinical sign of respiratory disease (mucopurulent nasal discharge, cough, increased respiratory rate, increased respiratory effort, or abnormal lung sounds auscultated by the herd veterinarian). Calves without fever but being depressed and showing at least 2 clinical signs of respiratory disease also were considered pneumonic. A navel was considered infected if it was enlarged, firm, and painful on palpation, regardless of purulent discharge. Calves were vaccinated according to the herd vaccination protocol. Calves with digestive and respiratory problems were treated by farm personnel according to protocols established by the herd veterinarian. Use of antiinflammatory drugs were avoided, except for 4 calves that were treated once. Three calves were treated with flunixin meglumine (Banamine; Merck Animal Health, Summit, NJ) at 2 (T1), 57 (T1), or 59 (T4) d of age, and 1 calf (T4) was treated with dexamethasone (Zoetis, Madison, NJ) at d 2 of age. Calves given these antiinflammatory drugs were not evaluated for measures of immune responses during the periods of drug clearance.

Hormone and Metabolite Analyses

Calves were bled (16 mL) at 24 to 30 h after colostrum feeding and at 8, 15, 22, 29, 36, 43, 50, and 57 ± 1 d of age from the jugular vein. Blood was collected into tubes without anticoagulant and with K₂EDTA and then centrifuged at 4°C for 15 min at $2,095 \times g$ for harvesting of serum and plasma, respectively. Hematocrit concentration was determined in blood collected into K₂EDTA tubes using micro-hematocrit capillary tubes (Fisherbrand, Thermo Fisher Scientific Inc., Waltham, MA), centrifuged for 3 min (Microspin 24 tube micro hematocrit centrifuge; Vulcon Technologies, Grandview, MO) and read in a micro hematocrit tube reader (model CR; Damon/IEC, Needham Heights, MA). Before freezing, serum total protein (**STP**) was measured using an automatic temperature-compensated refractometer (Reichert Jung; Cambridge Instruments Inc., Buffalo, NY).

Concentrations of glucose, plasma urea N (**PUN**), BHBA, and cholesterol were determined in all 9 plasma samples. A Technicon Autoanalyzer (Technicon Instruments Corp., Chauncey, NY) was used to measure concentrations of plasma glucose (Bran and Luebbe Industrial Method 339-19; Gochman and Schmitz, 1972) and PUN (Bran and Luebbe Industrial Method 339-01; Marsh et al., 1965). Each run included a common control sample. Intra- and interassay CV, respectively, were 1.0 and 1.3% for glucose and 3.0 and 4.2% for PUN. Plasma concentrations of BHBA were determined using a commercial kit (Autokit 3-HB; Wako Diagnostics Inc., Richmond, VA). Intra- and interassay CV were 5.6 and 7.8%, respectively. Concentrations of total cholesterol in plasma were determined using a commercial kit (Cholesterol E kit; Wako Diagnostics Inc., Richmond, VA). Intra- and interassay CV were 3.2 and 6.8%, respectively.

Insulin and IGF-I were measured in serum taken before feeding of colostrum and in plasma samples taken at 24 to 30 h after colostrum feeding and at 15, 29, 43, and 57 ± 1 d of age. Concentrations of IGF-I were analyzed following the manufacturer's protocol (Quantine ELISA; Human IGF-I Immunoassay, R&D Systems Inc., Minneapolis, MN). The intra- and interassay CV were 3.6 and 8.1%, respectively. Concentrations of insulin were analyzed by a double-antibody RIA (Badinga et al., 1991). Intra- and interassay CV were 7.3 and 14.6%, respectively.

A liver sample was collected via intercostal needle biopsy at 30 d of age, frozen in liquid N₂, and freeze-dried. A freeze-dried liver sample (~0.50 g) was placed into a screw-cap tube and 1 mL of C19:0 (internal standard), 0.7 mL of 10 N KOH, and 5.3 mL of MeOH were added to produce methyl esters of FA (O'Fallon et

Table 3. Ingredient and chemical composition of milk replacer and concentrate fed to preweaned Holstein calves

Item	Milk replacer	Concentrate
Ingredient, % of DM		
31:7 milk replacer ¹	89.80	—
Oil combination ²	9.68	—
Emulsifier ³	0.48	—
Steam rolled barley	—	51.7
Soybean meal	—	16.5
Beet pulp shreds	—	24.5
Sugarcane molasses	—	5.3
Mineral mix ⁴	—	2.0
Nutrient composition, DM basis		
Lactose, %	39.70	—
CP, %	29.70	18.30
FA, %	15.52	1.86
Ash, %	6.08	5.42
Ca, %	0.77	0.57
P, %	0.72	0.45
Mg, %	0.13	0.35
K, %	2.12	0.92
S, %	0.39	0.26
Na, %	0.76	0.16
Cl, %	1.27	0.32
Mn, mg/kg	49.5	55.0
Zn, mg/kg	53.0	57.0
Cu, mg/kg	11.7	16.0
Fe, mg/kg	132.0	362.0
Mo, mg/kg	0.6	1.4

¹Prepared by Land O'Lakes (Shoreview, MN). Contained whey products, dried skim milk, dried milk protein, coconut oil, vitamins, and minerals. Each kilogram contained 1.03% Ca, 0.80% P, 0.11 mg of Co, 10.9 mg of Cu, 1.1 mg of I, 110 mg of Fe, 49,560 IU of vitamin A, 12,400 IU of vitamin D, and 241 IU of vitamin E.

²Contained proportions of coconut oil:soybean oil according to following treatments (T): T1 = 100:0, T2 = 96.0:4.0, T3 = 87.9:12.1, and T4 = 71.8:28.2.

³Grindsted Mono-Di HV 52 K-A (Danisco USA Inc., Madison, WI).

⁴Each kilogram of DM contained 8.8% Ca, 4.2% P, 11.4% Mg, 12.4% Cl, 0.49% K, 8.1% Na, 0.36% S, 58 mg of Co, 263 mg of Cu, 26 mg of I, 1,933 mg of Fe, 923 mg of Mn, 8.46 mg of Se, 1,109 mg of Zn, 259,000 IU of vitamin A, 70,000 IU of vitamin D, and 2,400 IU of vitamin E.

al., 2007). Tubes were incubated for 1.5 h at 55°C with vigorous shaking for 5 s every 20 min. After cooling to below room temperature, 0.58 mL of 24 N H₂SO₄ was added. Again, tubes were incubated for 1.5 h at 55°C with vigorous shaking for 5 s every 20 min. After cooling, 3 mL of hexane was added and vigorously vortexed, and the layer of hexane was placed into GLC vials. The FA were determined using a GLC (Hewlett-Packard 5890A) fitted with a 100 m × 0.25 mm × 0.20 μm SP-256 capillary column as described by O'Fallon et al. (2007).

Markers of Immunity Analyses

Concentrations of haptoglobin (**Hp**) and acid-soluble protein (**ASP**) were measured in all 9 plasma samples collected as previously described. Haptoglobin was determined by measuring the differences of H₂O₂ with

Hp-hemoglobin as described previously (Makimura and Suzuki, 1982). Concentrations of Hp are reported as arbitrary units [optical density (OD) $\times$ 100] resulting from the absorption reading at 450 nm. Intra- and interassay CV were 6.0 and 10.9%, respectively. Concentrations of ASP were determined by extraction with 0.6 M perchloric acid using the bicinchoninic acid kit for protein determination (Sigma-Aldrich, St. Louis, MO). The intra- and interassay CV were 2.6 and 5.9%, respectively.

Blood was collected from puncture of the jugular vein into tubes containing heparin at 7, 14, 28, and 42 ± 2 d of age at least 30 min but not more than 2 h after the first daily feeding of MR. Tubes were transported to the laboratory at environmental temperature maintaining constant gentle inversion and analyzed within 2 h of collection. Quantification of individual cells and cell populations were performed using a ProCyt Dx hematology analyzer (Idexx Laboratories Inc., Westbrook, ME). Phagocytosis and oxidative burst of blood neutrophils were measured using a dual-color flow cytometry assay with the method modified from Smits et al. (1997) and according to the details provided by Martinez et al. (2012).

Proliferation of lymphocytes in whole blood cells was induced by a 72-h incubation without (control) or with concanavalin A (**ConA**, Sigma-Aldrich). Control and ConA samples were run in triplicate in 96-well flat-bottomed sterile plates (Sigma-Aldrich). Briefly, blood samples from experimental calves at 14, 28, and 42 ± 2 d of age were diluted 1:32 with RPMI-1640 medium without phenol red (Invitrogen, Life Technologies, Grand Island, NY) containing 1% antibiotics (Gibco Antibiotic-Antimycotic, Invitrogen) and containing or not 5 $\mu\text{g/mL}$ of ConA. Plates were incubated at 37°C with 5% CO_2 . After 48 h of incubation, cells in each well were pulsed with 0.2 μCi of ^3H -thymidine (MP Bio-medicals, Irvine, CA). Incorporation of ^3H -thymidine into DNA was assessed by liquid scintillation counting in a Beckman LS6000 counter (Pegasus Scientific Inc., Rockville, MD). Stimulation index was calculated as the ratio of the average value of counts per minute of the ConA wells to the average count per minute value of the control wells.

Production of cytokines by whole blood cells was induced by stimulation with 0.2 $\mu\text{g/mL}$ of phytohemagglutinin (**PHA**, L1668; Sigma-Aldrich Co.) and 1 $\mu\text{g/mL}$ of LPS (*Escherichia coli* 0111:B4; Sigma-Aldrich Co.). This dose was selected from the 3 following doses of PHA + LPS tested (0.2 + 1, 1 + 5, and 5 + 25 $\mu\text{g/mL}$) based upon maximal concentrations of tumor necrosis factor (**TNF**)- α and IFN- γ produced upon stimulation. Stimulation of whole blood cells

was performed following the protocol of Hulbert et al. (2011) with some modifications. Briefly, blood samples of experimental calves at 14, 28, and 42 ± 2 d of age were diluted 1:5 with RPMI-1640 medium without phenol red (Invitrogen) containing 1% antibiotics (Gibco Antibiotic-Antimycotic, Invitrogen) and containing or not the 1 $\mu\text{g/mL}$ of LPS and 0.2 $\mu\text{g/mL}$ of PHA. Stimulated (PHA + LPS) and nonstimulated samples were incubated in triplicate in sterile 24-well cell culture plates (Sigma-Aldrich Co.) for 48 h at 37°C and 5% CO_2 . After incubation, plates were centrifuged for 12 min at $1,455 \times g$ (Allegra X-15R centrifuge, Beckman Coulter Inc., Brea, CA). The supernatant was pooled and stored at -80°C until analyzed for bovine TNF- α and IFN- γ . Quantification of TNF- α and IFN- γ was performed only on stimulated supernatant samples because preliminary results from the validation test indicated that the concentrations of cytokines of non-stimulated cells were low (<12 pg/mL). Bovine TNF- α and IFN- γ assays (Vet Sets ELISA Development Kit; Kingfisher Biotech Inc., St. Paul, MN) were used according to manufacturer's procedures. Stimulated samples were analyzed in duplicate including a control. Standards were prepared with RPMI-1640 medium including 1% antibiotics; stimulated samples were not diluted. The intra- and interassay CV, respectively, were 2.0 and 11.4% for TNF- α , and 8.4 and 13.2% for IFN- γ . The sensitivity of the assay was 78 and 125 pg/mL for TNF- α and IFN- γ , respectively.

Cell-mediated hypersensitivity to epidermal injection of PHA (L1668; Sigma-Aldrich Co.) was performed in calves at 29 ± 1 and 58 or 59 d of age. The treated shoulder was shaved and cleaned with 78% alcohol. The area to be injected was identified by drawing a circle. The epidermal injection of PHA (200 μg dissolved in 100 μL of sterile isotonic saline solution) was made in the middle of the drawn circle using insulin syringes. Skin-fold thickness was measured at 0, 6, 24, and 48 h after injection using a digital caliper (Mitutoyo, Kawasaki, Kanagawa, Japan). The proportional increase in diameter of the skin-fold thickness was recorded to determine delayed-type hypersensitivity (**DTH**) response to PHA injection.

Concentrations of bovine IgG against ovalbumin (**OVA**) were measured in serum of calves injected s.c. with 0.5 mg of OVA (Sigma Aldrich) diluted in Quil A adjuvant (Accurate Chemical, Westbury, NY) solution (0.5 mg of Quil A in 1 mL of PBS) using sterile procedures at 1, 22, and 43 d of age. Bovine anti-OVA IgG were measured on the same days of injection and at 57 d of age by the method described by Mallard et al. (1997). Intra- and interassay CV were 3.6 and 3.8%, respectively.

Statistical Analyses

The experiment was a completely randomized design. Newborn calves were blocked by sex and parity of the dam and assigned randomly to receive 1 of 4 MR treatments on the day of birth. Nearly all dependent variables were measured repeatedly and analyzed using the PROC GLIMMIX procedure of SAS (release 9.2; SAS Institute Inc., Cary, NC) using the following model:

$$Y_{ijkl} = \mu + T_i + G_j + (TG)_{ij} + C_{(ij)k} + A_l \\ + (TA)_{il} + (GA)_{jl} + (TGA)_{ijl} + \varepsilon_{ijkl},$$

where Y_{ijkl} is the observation, μ is overall mean, T_i is the fixed effect of MR i (T1, T2, T3, and T4), G_j is the fixed effect of sex j (male and female), $(TG)_{ij}$ is the interaction of MR with sex, $C_{(ij)k}$ is the random effect of calf k nested within MR and sex (1, 2, 3, ..., n), A_l is the fixed effect of age l in weeks or days (0, 1, 2, ..., n), $(TA)_{il}$ is the interaction of MR with age, $(GA)_{jl}$ is the interaction of sex with age, $(TGA)_{ijl}$ is the interaction of MR with sex with age, and ε_{ijkl} is the residual error. For nonrepeated measures, the same model was used after removing the age effect and their interactions.

Data were tested for normality of residuals using the Shapiro-Wilk test of SAS (version 9.2; SAS Inst. Inc.), and transformation was performed to achieve normality as needed. Repeated measures were tested to determine the structure of best fit as indicated by the smallest Schwartz Bayesian information criteria. If repeated measures were taken on unequally spaced intervals, the spatial power [sp(pow)] structure was used.

Coefficients for testing of orthogonal contrasts when using unequally spaced quantitative treatments were generated using PROC IML of SAS (SAS Inst. Inc.). Orthogonal contrasts performed were as follows: (1) linear effect of treatment, (2) quadratic effect of treatment, (3) cubic effect of treatment, (4) sex effect, (5) interaction of contrasts 1 and 4, (6) interaction of contrasts 2 and 4, and (7) interaction of contrasts 3 and 4. If a 3-way interaction of age with the main effects of treatment and sex or interaction of age with sex had $P > 0.25$, the interactions were dropped from the model.

Binary data were analyzed by logistic regression using the LOGISTIC procedure of SAS (SAS Inst. Inc.). The models included the effects of treatment and sex of calf. Adjusted odds ratio and 95% CI were calculated. Birth weight and height deviations within each sex were covariates for analysis of BW gain and growth, respectively. First-day measures of plasma metabolites and anti-OVA IgG were used as a covariate for the same compounds. Finally, serum total IgG concentration at 1 d of life was used as a covariate for health measures.

Differences discussed in the text were significant at $P \leq 0.05$ and tended to be significant at $0.05 < P \leq 0.10$.

RESULTS AND DISCUSSION

Adequate Passive Immunity

At 24 to 30 h after consumption of colostrum, mean concentrations of total IgG in serum and of STP were 21.5, 21.4, 19.8, and 22.9 g/L (SEM = 1.5 g/L) and 5.8, 5.9, 5.8, and 5.8 g/dL (SEM = 0.1 g/dL) for T1, T2, T3, and T4, respectively. Both IgG and STP values were above the minimum concentrations recommended to ensure APT (Tyler et al., 1996). Two calves assigned to T2 and 1 calf assigned to T3 failed to attain APT after colostrum feeding (serum total IgG <10 g/L), which was corroborated by their low concentration of STP (<5 g/dL) measured at 24 to 30 h after colostrum feeding; however, performance of these calves was not different from that of their treatment mates. Mean concentrations of STP exceeded 5.5 g/dL throughout the duration of the study and were not affected by increased intake of essential FA (data not shown).

Liver FA Concentration and Profile

Concentrations of total FA in liver of 30 d-old calves did not differ because of treatment and averaged 8.4 ± 0.5 g/100 g of DM. Contrary to the current finding, Jenkins and Kramer (1986) reported reductions of approximately 30 and 20% in proportions of FA in fresh liver of calves fed CCO-based MR compared with MR that replaced 5 or 7.5% of CCO with corn or canola oil, respectively (10.7, 7.2, and 8.2% of total liver DM, respectively, assuming liver tissue was 20% DM). However, those authors reported that calves fed CCO-MR had a better growth performance, which might indicate that liver function was not impaired by this increase in fat concentration.

Concentrations of C12:0 ($P = 0.01$) and C14:0 ($P = 0.01$) decreased linearly with increasing replacement of CCO with SBO in the MR (Figure 1A), as expected, based upon the medium-chain FA profile of the 2 FA sources (Table 2). Concentrations of the longer chain SFA were decreased moderately (C16:0, $P = 0.03$) or unchanged (C18:0, Figure 1B) by feeding more SBO because the 2 oil sources differed little in their concentrations of C16:0 and C18:0 (Table 2). The magnitude of linear increase ($P < 0.01$) in the essential FA concentrations with increasing intake of SBO was dramatic, being 63 and 145% for linoleic acid and α -linolenic acid, respectively (Figures 1C and D) and reflected dietary profiles of these 2 FA. Jenkins and Kramer (1986) also reported greater proportions of linoleic acid in calf liver

by replacing some or all of the CCO with a source of fat containing linoleic acid. Calves fed increasing amounts of SBO compared with CCO consumed more C18:1 *cis*-9 but liver concentrations of C18:1 *cis*-9 decreased with increasing intake of the 2 essential FA (Figure 1B). The decrease of C18:1 *cis*-9 likely reflected a substitution by the essential FA for C18:1 *cis*-9 in the triglyceride and phospholipid fractions of liver. This accompaniment of increasing linoleic acid and decreasing C18:1 *cis*-9 concentrations agrees with the FA patterns of the liver of male (Mohrhauer and Holman, 1963) and female growing rats (Pudelkewicz et al., 1968) fed increasing amounts of ethyl linoleate in otherwise fat-free diets. The concentration of C20:4n-6 (the main derivative of linoleic acid) was consistent and not affected by feeding more linoleic acid, averaging 11.4% of liver FA content (Figure 1C). Similarly, the concentration of C20:4n-6 did not change in liver of growing female rats fed increasing amounts of ethyl linoleate once the requirement for linoleic acid was thought to be met (Pudelkewicz et al., 1968). Concentration of C22:5n-3 appeared to increase linearly at the same rate as that of α -linolenic acid (Figure 1D), whereas other n-3 derivatives (C20:5 and C22:6) decreased or tended to decrease in liver of calves fed increasing amounts of α -linolenic acid (Figure 1E), suggesting a teleologic demand for C22:5n-3. Most of the studies addressing the essentiality of α -linolenic acid have focused on the role of its derivative, DHA, in brain development (Innis, 2007). A recent review by Kaur et al. (2011), primarily based on in vitro and in vivo studies in rodents, suggests unique and shared functions of docosapentaenoic acid (DPA) with DHA, such as enhanced endothelial migration, synthesis of DPA-related eicosanoids, and regulation of gene expression. In the current study, the FA profile of immune cells was not determined; however, several studies using rodents reported that the phospholipid fraction of immune cells was enriched in long-chain dietary FA (Calder, 2008). The phospholipid fraction normally accumulates more of the long-chain FA than does the triglyceride fraction. The FA profile of the phospholipid fractions in the liver were not measured in the current study. However, based on previous studies, the phospholipid fraction of liver as well as that of immune cells may have been enriched in essential FA derivatives.

Mead and Slaton (1956) reported that C20:3n-9, subsequently called mead acid, accumulated in organs and depot fat of male rats fed a fat-deficient diet. In the current study, in which diets were isolipidic, the mean concentration of C20:3n-9 in liver decreased linearly and dramatically ($P < 0.01$) with increasing intake of linoleic acid (Figure 1F). However, the ratio of C20:3n-9 to C20:4n-6 was <0.2 , which others have

used as a metabolic indicator of linoleic acid sufficiency in nonruminant animals (Holman, 1978). Lambs and calves have very low concentrations of linoleic acid at birth, but concentrations increase 12- to 14-fold by 30 d of age (Noble et al., 1975; Garcia et al., 2014a,b). Presumably, preruminant calves have developed a mechanism to optimize the efficiency of linoleic acid utilization under the conditions of a low supply by the dam, as also pointed out for the young lamb by Noble et al. (1972).

Measures of Growth and Feed Efficiency

Birth weight of calves did not differ across treatments and averaged 39.9 kg (Table 4), with males being heavier than females (42.0 vs. 37.7 kg; $P < 0.01$). Gain in BW during the first 30 d of life was below industry standards, with ADG of 111 g. Ballou and DePeters (2008) also reported ADG of <100 g when averaged over the first 4 wk of life for Jersey bull calves fed only MR formulated and fed for 300 g of ADG. Male calves assigned to T2 tended ($P = 0.09$) to consume more MR DM compared with males fed other MR treatments due to less refusal of MR, whereas female calves consumed similar amounts of MR across treatments (sex by cubic treatment interaction, Table 4). Body weight gain ($P = 0.02$) and feed efficiency (FE, $P = 0.04$), measured as kilograms of BW gain per kilogram of DMI, by male calves followed the same cubic pattern of MR intake (sex by cubic treatment interaction). This positive response of male calves fed T2 was not replicated in the second 30 d of life nor did it carry over for the entire 60-d study (Table 4). A slightly greater intake of MR by male calves fed T2 (17 to 20 g/d increase) compared with other male calves would not account nutritionally for the almost doubling of BW gain for this treatment group, suggesting that improved growth of T2-fed calves may have been due partly to meeting a metabolic demand for linoleic acid and (or) α -linolenic acid. However, an improved BW gain was not maintained by male calves fed T3 or T4 diets. Based upon dermal scores, concentration of C20:4n-6 in erythrocytes, BW gain, and FE, Pudelkewicz et al. (1968) reported that the optimum intake of linoleic acid for growing rats was narrow and that consumption of linoleic acid exceeding this optimum range resulted in reduced performance.

Total BW gain by female calves during the first 30 d tended ($P = 0.07$) to increase linearly with increasing intake of essential FA (2.6, 3.1, 3.3, and 3.4 kg for T1, T2, T3, and T4, respectively, Table 4). Feed efficiency of female calves followed a similar numeric increase (sex by linear diet interaction, $P = 0.11$). In a previous study from our laboratory, unweaned Holstein calves, regardless of sex, had lower ADG and FE when

Figure 1. Concentration of selected FA in liver of 30-d-old preweaned Holstein calves fed linoleic and α -linolenic acids at 0.119 and 0.007 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), and 0.593 and 0.076 (T4) g/kg of $BW^{0.75}$ in milk replacer.

fed reduced amounts of linoleic acid (0.14 g per kg of $BW^{0.75}$) compared with calves fed essential FA at 0.45 and 0.04 g of linoleic and α -linolenic acids per kg of $BW^{0.75}$, respectively (Garcia et al., 2014b). Jenkins and Kramer (1986) fed only MR formulated with hydrogenated CCO, hydrogenated CCO plus corn oil, hydrogenated CCO plus canola oil, or tallow to preweaned dairy calves for 6 wk. In that study, calculated mean intakes of linoleic and α -linolenic acids were 0.2 and 0, 5.0 and 0.0, 2.7 and 1.7, and 9.3 and 1.1 g/d; ADG was 600, 640, 671, and 731 g/d; and DMI was 935, 973, 990, and 1,018 g/d for calves fed the 4 MR, respectively.

Although performance appeared to be greater if some linoleic acid was fed, only tallow-fed calves had better ADG compared with those fed CCO alone.

Wither and hip heights of calves at birth were not different across treatments and averaged 75.3 ± 0.70 and 79.8 ± 0.73 cm, respectively. Over the 60-d study, growth at the withers ($7.6, 8.6, 9.1$, and 8.3 ± 0.47 cm for T1, T2, T3, and T4, respectively) and at the hip ($8.0, 9.1, 9.4$, and 8.5 ± 0.49 cm for T1, T2, T3, and T4, respectively) increased with increasing intake of essential FA up to T3 before decreasing for calves fed T4 across sexes ($P = 0.04$, quadratic effect).

Table 4. Dry matter intake, BW gain, and feed efficiency (FE, kg of BW gain/kg of DMI) of preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic and α -linolenic acids

Measure	Treatment ¹								Contrast, ² <i>P</i> -value							
	T1		T2		T3		T4		SEM	L	Q	Cb	S	L $\times$ S	Q $\times$ S	Cb $\times$ S
	M	F	M	F	M	F	M	F								
No. of calves	7	14	9	13	9	13	9	14								
Birth to 30 d																
Birth weight, ³ kg	41.1	36.8	43.4	38.8	40.3	38.2	43.2	37.2	1.57	0.76	1.00	0.13	<0.01	0.61	0.32	0.57
Milk replacer intake, kg of DM	16.6	15.2	17.1	15.5	16.5	15.6	16.6	15.5	0.15	0.95	0.54	0.03	<0.01	0.17	0.27	0.09
C18:2n-6 intake, g	62	57	96	87	154	153	303	279	3.4	<0.01	0.11	0.50	<0.01	0.01	0.06	0.24
C18:3n-3 intake, g	3.6	3.3	8.5	7.7	18.2	17.3	39.0	35.9	0.3	<0.01	0.42	0.85	<0.01	<0.01	0.36	0.55
BW gain, kg	2.75	2.62	5.26	3.09	2.80	3.30	2.70	3.44	0.54	0.66	0.44	0.01	0.49	0.07	0.98	0.02
FE	0.16	0.17	0.31	0.20	0.17	0.21	0.17	0.22	0.03	0.84	0.44	0.01	0.95	0.11	0.96	0.04
31 d to weaning																
Milk replacer intake, kg of DM	19.8	18.1	20.4	18.2	20.0	18.3	19.8	18.3	0.19	0.88	0.24	0.17	<0.01	0.17	0.67	0.13
GM ⁴ intake, kg of DM	16.3	13.7	13.4	13.3	16.1	13.5	15.6	13.3	1.66	0.92	0.88	0.30	0.11	0.82	0.95	0.40
BW gain, kg	12.5	19.2	21.3	18.9	23.7	19.1	24.1	19.3	1.39	0.51	0.84	0.34	<0.01	0.58	0.95	0.44
FE	0.63	0.60	0.63	0.60	0.65	0.60	0.68	0.60	0.02	0.12	0.90	0.67	<0.01	0.14	0.91	0.76
Birth to weaning																
Final BW, kg	68.3	59.5	68.7	59.7	68.6	60.1	68.9	60.4	1.35	0.61	0.91	0.92	<0.01	0.88	0.95	0.90
Total DMI, kg	52.8	47.0	52.6	46.9	52.6	47.3	52.1	47.1	1.7	0.94	0.97	0.52	<0.01	0.97	0.91	0.62
BW gain, kg	26.2	21.9	26.6	22.0	26.5	22.4	26.8	22.7	1.35	0.61	0.92	0.92	<0.01	0.88	0.95	0.90
FE	0.49	0.46	0.52	0.47	0.50	0.48	0.52	0.48	0.02	0.27	0.87	0.24	<0.01	0.99	0.86	0.38

¹Targeted intakes of linoleic and α -linolenic acids were 0.119 and 0.007 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of BW^{0.75}. Milk replacer was the only feed offered during the first 30 d of life.

²*P*-values for orthogonal contrasts of treatments, sex, and treatment by sex interactions: L = linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, S = sex.

³Birth weight deviations from the mean birth weight within each sex were covariates for all measures. Therefore, birth weight added to any later variable of gain will not give the expected BW.

⁴GM = grain mix. It was fed exclusively during the last 30 d of the preweaning period.

Metabolic and Hormonal Profiles

Increasing consumption of SBO (from T1 to T3) resulted in decreasing ($P = 0.02$) mean concentrations of glucose in plasma of female calves before concentrations rebounded in calves fed T4, whereas that of male calves did not differ due to treatment (Table 5). Mean plasma concentrations of the anabolic hormones insulin and IGF-I did not differ due to treatments. Mean plasma concentrations of insulin were greater (2.7 vs. 2.0 ng/mL, $P < 0.01$) and that of IGF-1 tended to be greater (42.0 vs. 39.0 ng/mL, $P = 0.06$) in male compared with female calves (Table 5), which coincides with greater DMI and BW gain for male compared with female calves (Table 4). Differences in plasma concentrations of anabolic metabolites and hormones are normally detected when calves experience different growth rates due to different intakes of nutrients (Smith et al., 2002).

Mean concentrations of plasma BHBA were lowest during the first 30 d of life (<0.70 mg/dL) before gradually increasing above 1.0 mg/dL after offering of dry feed was initiated (effect of age, $P < 0.01$). Synthesis of BHBA by ruminal epithelium during absorption of butyric acid from microbial fermentation of concentrates likely accounted for the increase in blood concentration of BHBA after 30 d of age. Mean concentrations of plasma BHBA across sexes tended ($P = 0.06$) to decrease linearly as intake of essential FA increased (0.88, 0.80, 0.76, and 0.76 mg/dL for T1, T2, T3, and T4, respectively, Table 5). Polyunsaturated FA are known to activate and bind to peroxisome proliferator-activated receptor- α (PPAR- α) in liver. Once PPAR- α is activated, it stimulates β -oxidation and glucose metabolism as well as regulates cholesterol and lipoprotein metabolism to reduce dyslipidemia (Forman et al., 1997; Li and Chiang, 2009). Calves fed MR enriched in medium-chain FA compared with MR enriched in linoleic acid had greater circulating concentrations of BHBA within the first 3 h of feeding (Sato, 1994; Garcia et al., 2014b). Production of BHBA by hepatocytes from unweaned Holstein bull calves was less when incubated for 48 h with linoleic or α -linolenic acid compared with C16:0 or C18:0 (Mashek and Grummer, 2003).

The effect of treatment on plasma concentrations of cholesterol was opposite to that of BHBA. Mean concentrations of plasma cholesterol increased quadratically as intake of essential FA increased (77.3, 82.5, 89.2, and 86.9 mg/dL for T1, T2, T3, and T4, respectively; $P = 0.04$, Table 5). Similarly, feeding SBO compared with tallow or CCO in MR increased concentrations of plasma cholesterol in dairy calves (Stewart et al., 1978; Barrows et al., 1980). Although consumption of SFA usually results in increased circulating concentrations of cholesterol compared with consumption of unsaturated

Table 5. Plasma concentrations of glucose, insulin, IGF-I, BHBA, and total cholesterol of preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic and α -linolenic acids

Measure	Treatment ¹						Contrast, ² <i>P</i> -value											
	T1		T2		T3		T4				SEM	L	Q	Cb	S	L × S	Q × S	Cb × S
	M	F	M	F	M	F	M	F										
Glucose, mg/dL	90.1	89.2	87.8	87.6	90.2	84.9	86.6	90.3	1.46	0.70	0.18	0.46	0.53	0.12	0.02	0.23		
Insulin, ng/mL	2.66	2.20	2.66	1.72	2.48	2.03	2.92	2.04	0.29	0.63	0.40	0.44	<0.01	0.75	0.89	0.26		
IGF-I, ng/mL	39.5	38.4	45.1	38.6	41.1	39.5	42.3	39.4	2.22	0.68	0.66	0.25	0.06	0.93	0.94	0.21		
Cholesterol, mg/dL	78.6	76.0	83.8	81.2	92.3	86.0	94.4	79.4	4.08	0.03	0.04	0.98	0.03	0.09	0.81	0.90		
BHBA, mg/dL	0.92	0.84	0.81	0.78	0.81	0.71	0.82	0.69	0.06	0.06	0.17	0.58	0.04	0.35	0.96	0.64		

¹Targeted intakes of linoleic and α -linolenic acids were 0.119 and 0.007 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of BW^{0.75}. Milk replacer was the only feed offered during the first 30 d of life.

²*P*-values for orthogonal contrasts of treatments, sex, and treatment by sex interactions: L = linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, S = sex. Effect of age: $P < 0.01$ for all measures.

FA in humans, the intake of cholesterol is an important factor influencing this response (review by Goodnight et al., 1982). Because minimal, if any, cholesterol was consumed in the current study, this classical effect was not detected. The increased concentration of cholesterol in the current study due to increased intake of PUFA may simply reflect the preferential esterification of cholesterol esters with highly unsaturated FA.

Incidence of Diarrhea and Diseases

Treatment did not affect the incidence of pneumonia (18.1% incidence), navel infection (4.5% incidence), or fever (62.1% incidence). Based upon health scores and percentage of days with a health disorder, males were slightly healthier than females (Table 6). Mean scores for attitude were not affected by treatments. Severity of diarrhea increased at wk 2 of age ($P < 0.01$, Figure 2) and was accompanied by an increase in attitude score. Severity of diarrhea during the study tended ($P = 0.07$) to decrease linearly as intake of essential FA increased (Table 6). In addition, the age at first evidence of diarrhea tended ($P = 0.10$) to increase linearly from 7.0 to 7.6 d as the intake of essential FA increased (Table 6). Mean score and percentage of days with nasal discharge tended to increase ($P = 0.10$) or increased ($P = 0.04$), respectively, with increasing intake of essential FA before decreasing in calves fed T4.

Hematocrit, Select Blood Cell Populations, and Neutrophil Function

Mean values of hematocrit and blood cell populations (Table 7) were within the normal ranges for preweaned, healthy dairy calves (Brun-Hansen et al., 2006). Mean values for hematocrit and concentrations of platelets and lymphocytes were not affected by treatments. However, mean concentration of red blood cells tended ($P = 0.10$) to decrease linearly as intake of essential FA increased (8.2, 8.7, 8.3, and $7.9 \times 10^6/\mu\text{L}$ for T1, T2, T3, and T4, respectively, Table 7) which coincides with the tendency for increasing intake of essential FA to reduce diarrhea (Table 6). Increased concentrations of red blood cells as well as hematocrit are usually related to increased dehydration, often caused by increased incidence or severity of diarrhea.

Mean concentrations of blood neutrophils decreased ($P = 0.04$) between T1 and T3 before increasing at T4 (4.14, 3.89, 3.43, and $4.45 \times 10^3/\mu\text{L}$) for female calves, whereas that of male calves were not different across treatments (3.26, 3.35, 3.61, and $3.27 \times 10^3/\mu\text{L}$, Table 7). If the progressively decreasing concentration of neutrophils detected in female calves fed T1 to T3 were due to increased migration from the blood to sites

of inflammation, it would indicate that these calves were better able to mount an attack against infection; however, decreased production of neutrophils in bone marrow cannot be ruled out. Expression of receptors on neutrophil surfaces, which help regulate migration from the blood to injured tissues, was not evaluated. Male calves had 15% lower ($P \leq 0.01$) circulating concentrations of neutrophils compared with female calves (3.37 vs. $3.98 \times 10^3/\mu\text{L}$), which agrees with the better health indices reported in Table 6 for male calves. Perhaps the decreasing concentration of blood neutrophils in female calves fed T2 or T3 reflects improving health at the subclinical level.

Mean proportion of neutrophils undergoing phagocytosis tended ($P = 0.09$) to peak in calves fed T2 (62.1, 66.6, 64.2, and 62.8% for T1, T2, T3, and T4, respectively, Table 7). Ballou and DePeters (2008) also reported a curvilinear response of polymorphonuclear neutrophils from dairy calves fed increasing amounts of n-3 FA. In the current study, the proportion of neutrophils producing oxidative radicals did not differ with treatment (mean of 52.8%). Mean fluorescence intensities for phagocytic activity and production of oxidative radicals were not affected by treatment but were greatest ($P < 0.01$) at 7 d of age. Female calves fed T2 or T3, who had lower circulatory concentrations of blood neutrophils due to potential increased migration to the sites of inflammation along with improved phagocytotic activity of these neutrophils, might be more efficient to resist pathogenic invasions. However, regardless of sex, the only positive health effect was a tendency for reduced severity of diarrhea when feeding increased amounts of essential FA.

Circulating Concentration of Acute Phase Proteins

Plasma concentrations of Hp followed the same pattern as that for fecal and attitude scores. Plasma Hp reached the greatest concentrations at 8 d of life, with calves fed T1 having the greatest concentration, and falling to nadir values from 15 d until the experiment ended (treatment by age interaction; $P = 0.02$, Figure 3). Concentrations of Hp are absent in healthy calves but their concentrations increase dramatically even before the clinical signs of inflammatory disorders, such as diarrhea, appear (Gänheim et al., 2007; Cray et al., 2009). Calves fed T1 may have had a greater immune reaction to inflammation of the small intestine, suggesting that feeding more essential FA reduced the inflammatory response compared with feeding more medium-chain SFA, although feeding more PUFA also may have allowed a faster or greater resolution of diarrhea.

Mean plasma concentrations of ASP tended ($P = 0.08$, quadratic effect) to be lowest in calves fed T3

Table 6. Health scores¹ and percentage of days with poor attitude, fever, diarrhea, and nasal discharge of preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic and α -linolenic acids

Measure	Treatment ²								Contrast, ³ <i>P</i> -value							
	T1		T2		T3		T4		SEM	L	Q	Cb	S	L $\times$ S	Q $\times$ S	Cb $\times$ S
	M	F	M	F	M	F	M	F								
Attitude score	0.14	0.19	0.11	0.18	0.12	0.20	0.13	0.15	0.03	0.53	0.78	0.53	0.02	0.51	0.57	0.92
Fecal score	0.66	0.73	0.64	0.68	0.59	0.73	0.60	0.60	0.05	0.07	0.92	0.60	0.08	0.53	0.32	0.41
Nasal discharge ⁴	0.02	0.03	0.02	0.06	0.04	0.09	0.04	0.05	0.02	0.44	0.10	0.83	0.09	0.77	0.30	0.64
Rectal temp., °C	38.8	38.9	38.8	38.9	38.8	38.9	38.8	38.9	0.05	0.36	0.93	0.22	0.02	0.99	0.56	0.68
Days to diarrhea	7.2	6.8	7.2	7.5	7.7	7.4	7.7	7.5	0.35	0.10	0.39	0.72	0.54	0.85	0.86	0.30
Days with: ⁵ %																
Poor attitude	12.9	16.0	9.6	15.1	12.0	16.4	10.9	13.3	2.54	0.53	0.81	0.36	0.04	0.72	0.69	0.71
Diarrhea	14.7	18.6	15.7	15.8	14.2	18.3	15.1	13.7	2.00	0.28	0.81	0.61	0.25	0.30	0.59	0.24
Nasal discharge	2.05	3.02	1.54	5.37	4.99	8.25	3.19	4.34	1.85	0.51	0.04	0.54	0.08	0.79	0.46	0.60
Fever, first 14 d	4.86	8.54	5.69	5.12	3.93	6.61	4.06	7.05	2.30	0.74	0.61	0.75	0.18	0.81	0.78	0.36

¹Attitude score scale: 0 = responsive, 1 = nonactive, 2 = depressed, and 3 = moribund. Feces score scale: 0 = firm feces, no diarrhea; 1 = soft feces, no diarrhea, 2 = mild diarrhea, and 3 = watery, severe diarrhea. Nasal score scale: 0 = normal serous discharge, 1 = small amount of unilateral cloudy discharge, 2 = bilateral cloudy or excessive mucus discharge, and 3 = copious bilateral mucopurulent discharge.

²Targeted intakes of linoleic and α -linolenic acids were 0.119 and 0.007 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of BW^{0.75}. Milk replacer was the only feed offered during the first 30 d of life.

³*P*-values for orthogonal contrasts of treatments, sex, and treatment by sex interactions: L = linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, S = sex. Effect of age: *P* < 0.01 for all score measures.

⁴Treatment by age interaction: *P* = 0.08.

⁵Percentage of days with health issue over a 60-d period unless indicated. Poor attitude and nasal discharge if score >0, fever if temperature $\geq 39.5^\circ\text{C}$, and diarrhea if score >1.

Figure 2. Average weekly fecal scores of preweaned Holstein calves fed linoleic and α -linolenic acids at 0.119 and 0 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of $BW^{0.75}$ in milk replacer. Possible scores were 0 for firm feces, no diarrhea; 1 for soft feces, no diarrhea; 2 for mild diarrhea; and 3 for watery, severe diarrhea. Linear effect of treatment ($P = 0.07$) and effect of age ($P < 0.01$).

(90.1, 88.5, 82.5, and 89.4 mg/L, Table 8). Likewise, the mean concentration of ASP in plasma was less in Holstein calves fed more linoleic acid by partly replacing CCO with porcine lard in MR (García et al., 2014b). Acid-soluble protein has dual inflammatory and immunomodulatory properties (Hochepied et al., 2003). Interpretation of ASP values in the current study may be aided by examining the effects of sex on ASP. Female calves had greater mean concentrations of plasma ASP (91.3 vs. 83.8 mg/L, $P = 0.02$) and Hp (0.87 vs. 0.78 OD $\times$ 100, $P = 0.06$) compared with males, which agrees with the better health indices (Table 6) and lower concentrations of blood neutrophils

Figure 3. Concentrations of haptoglobin in plasma of preweaned Holstein calves fed linoleic and α -linolenic acids at 0.119 and 0 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of $BW^{0.75}$ in milk replacer. Effect of treatment by age interaction ($P = 0.02$); slice effect at d 8 and 43 ($P < 0.05$).

Table 7. Mean values of hematocrit and concentration of select blood cells, and phagocytosis, oxidative burst, and geometric mean fluorescence intensity (GMFI) of neutrophils in peripheral blood of preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic and α -linolenic acids

Measure	Treatment ¹								Contrast, ² <i>P</i> -value							
	T1		T2		T3		T4		SEM	L	Q	Cb	S	L $\times$ S	Q $\times$ S	Cb $\times$ S
	M	F	M	F	M	F	M	F								
Hematocrit, %	33.1	32.3	32.8	32.9	32.3	32.2	32.1	32.1	0.65	0.23	0.80	0.62	0.62	0.74	0.69	0.65
Red blood cells, $10^6/\mu\text{L}$	8.3	8.0	8.8	8.6	8.4	8.2	8.2	7.6	0.32	0.10	0.24	0.11	0.13	0.50	0.69	0.85
Neutrophils, $10^3/\mu\text{L}$	3.26	4.14	3.35	3.89	3.61	3.43	3.27	4.45	0.31	0.57	0.43	0.94	0.01	0.55	0.04	0.71
Lymphocytes, $10^3/\mu\text{L}$	4.11	4.74	4.67	4.71	4.63	4.73	4.60	4.47	0.28	0.95	0.36	0.55	0.41	0.28	0.60	0.45
Platelets, $10^3/\mu\text{L}$	488	521	527	515	561	415	518	447	49.8	0.48	0.84	0.56	0.15	0.27	0.13	0.66
Phagocytosis																
%	60.2	64.0	67.4	65.8	64.3	64.1	62.9	62.7	2.28	0.58	0.27	0.09	0.81	0.64	0.50	0.38
GMFI	21.2	22.8	23.2	24.6	21.2	24.5	23.0	21.4	2.10	0.78	0.62	0.42	0.45	0.41	0.45	0.71
Oxidative burst																
%	48.9	53.9	55.8	55.9	53.4	53.8	50.5	50.4	2.52	0.24	0.18	0.15	0.46	0.46	0.55	0.53
GMFI	32.2	33.1	35.5	35.5	31.5	36.8	36.9	34.6	2.90	0.45	0.88	0.36	0.63	0.62	0.29	0.52

¹Targeted intakes of linoleic and α -linolenic acids were 0.119 and 0.007 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of $BW^{0.75}$. Milk replacer was the only feed offered during the first 30 d of life.

²*P*-values for orthogonal contrasts of treatments, sex, and treatment by sex interactions: L = linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, S = sex. Effect of age: $P < 0.01$ in phagocytosis and oxidative burst GMFI.

(Table 7) for male calves. Therefore, lower ASP values with increasing intake of essential FA may reflect an improving immune status.

Humoral and Cell-Mediated Immune Responses

Neonates are born with a polarized and biased T helper (Th)-2 against Th-1 response, making them less able to mount a proper immune response to infectious agents. As reviewed by Adkins et al. (2004), a high number of naïve neonatal CD4⁺ T cells, fetal origin of these cells, and local environment can influence the changes in Th-1 versus Th-2 responses and therefore influence the responses of many other immune cells. Others have reported that neonates are born with high proportions of naïve T cells that can suppress immunoglobulin production (Clement et al., 1990) and also that neonates have a decreased capacity to produce Th1-type cytokines such as IFN- γ (Wilson, 1986). Therefore, an overall change of polarized Th-2 response to a Th-1 response should enhance the cell-mediated and humoral immune responses of preweaned calves.

Lymphocytes from calves fed T2 demonstrated greater ($P = 0.01$) mean proliferation across all 3 measuring days of age (14, 28, and 42 d) as reflected in stimulation index means of 26.9, 39.1, 29.0, and 28.5 for T1, T2, T3, and T4, respectively (Table 8). Lymphocytes from calves fed T3 proliferated better than those from calves fed T1 only at the last sampling time of 42 d of age (Figure 4). Gorjão et al. (2007) reported that 25 μ M linoleic acid stimulated IL-2-induced proliferation of human lymphocytes, preventing apoptosis and necrosis, but that greater concentrations of linoleic acid (75 and 100 μ M) reduced proliferation of lymphocytes compared with those in the control medium. Calder et al. (2002) reviewed studies that fed recommended amounts of α -linolenic acid (2 g/d) or amounts 6 times above recommendation and concluded that lymphocyte proliferation was reduced only when intake reached 13 g/d. Therefore, the amount of α -linolenic acid fed in the current study should not have had a marked antiinflammatory effect that could lead to immunosuppression.

Concentration of TNF- α in supernatant of stimulated whole blood cells increased in a nonsignificant quadratic pattern (337, 373, 424, and 367 pg/mL for T1, T2, T3, and T4, respectively, $P = 0.20$). Mean concentrations of IFN- γ produced by lymphocytes in whole blood stimulated with LPS and PHA tended ($P = 0.09$) to be greater in male calves fed T3 (260, 357, 411, and 209 pg/mL for T1, T2, T3, and T4, respectively), whereas lymphocytes from female calves fed T2 tended to produce more IFN- γ compared with female calves fed the other treatments (256, 327, 227,

and 265 pg/mL for T1, T2, T3, and T4, respectively; Table 8). As with lymphocyte proliferation, dietary intake of linoleic acid can be helpful to the immune system but overconsumption may have inhibitory effects. Stimulated peripheral blood mononuclear cells produced more IFN- γ when Holstein calves were fed linoleic acid at 0.45 versus 0.14 g/kg of BW^{0.75} in our previous study (Garcia et al., 2014b). The pattern of cytokine production is used to verify the predominant type of Th response. An increased concentration of IFN- γ with constant or decreased production of IL-4 is indicative of Th-1 predominance (Chase et al., 2008). Greater mean production of IFN- γ by calves fed T2 or T3 might indicate an improved ability of these calves to switch to a Th-1 response. Based on the proliferation index of lymphocytes and production of IFN- γ by these proliferated lymphocytes, T3 for males and T2 for females appeared to provide optimal intakes of linoleic acid and α -linolenic acid to induce proinflammatory activity of lymphocytes under these ex vivo conditions. However, this ex vivo-enhanced effect was not evidenced by corresponding improved health measures of male and female calves due to treatments, except for an overall effect of reduced diarrhea by greater intakes of essential FA.

In vivo challenge with ovalbumin, which is reported to be a good indicator of B-cell function in calves (Foote et al., 2007), resulted in greater ($P = 0.04$) concentrations of serum anti-OVA IgG in male calves fed T2 or T3 (0.10, 0.22, 0.24, and 0.13 OD; Table 8), whereas female calves had similar mean serum concentrations across treatments (0.17, 0.18, 0.18, and 0.22 OD for T1, T2, T3, and T4, respectively; Table 8). The positive effect of T2 and T3 on ex vivo responsiveness of immune cells (greater production of IFN- γ) was verified by the in vivo measure of antibody production. Therefore, male calves appeared to have a more competent immune system due to enhanced cellular and humoral responses. Alternately, female calves did not increase production of anti-OVA IgG due to treatment although production of anti-OVA IgG in female calves was numerically greater than that in males when fed T1 (0.10 vs. 0.17 OD) and T4 (0.13 vs. 0.22 OD). Based on these results, it seems that an enhanced humoral response in females is not limited by the intake of essential FA but an enhanced cell-mediated response requires that females be provided with increased supply of essential FA, such as that of T2.

A measure of DTH evaluates the ability of immune cells to infiltrate and accumulate into regions of antigen deposition. It is strictly a cell-mediated response, not an antibody-mediated response (Bernhagen et al., 1996). The use of PHA to induce a DTH response in cows resulted in the primary infiltration of eosinophils,

Table 8. Mean concentrations of acute phase proteins in plasma, anti-ovalbumin (OVA) IgG in serum (optical density, OD), tumor necrosis factor α (TNF- α), and IFN- γ produced by whole blood cells stimulated with LPS and phytohemagglutinin, and proliferation of whole blood cells by incubation with concanavalin A in preweaned male (M) and female (F) Holstein calves fed increasing amounts of linoleic and α -linolenic acids

Measure	Treatment ¹								Contrast, ² <i>P</i> -value							
	T1		T2		T3		T4		SEM	L	Q	Cb	S	L $\times$ S	Q $\times$ S	Cb $\times$ S
	M	F	M	F	M	F	M	F								
Haptoglobin ³	0.82	0.86	0.75	0.84	0.77	0.90	0.77	0.88	0.07	0.94	0.91	0.49	0.06	0.64	0.62	0.84
ASP, ⁴ mg/L	84.6	95.6	80.6	96.5	81.2	83.9	88.8	90.0	4.49	0.97	0.08	0.61	0.02	0.17	0.81	0.28
Cell proliferation ⁵																
Control	0.50	0.52	0.56	0.56	0.55	0.58	0.59	0.65	0.08	0.23	0.81	0.65	0.71	0.75	0.93	0.87
Stimulated	12.8	14.7	23.5	20.4	14.0	18.8	16.2	19.0	2.83	0.67	0.44	0.01	0.33	0.63	0.71	0.22
Stim. index ⁶	25.5	28.4	41.7	36.5	25.3	32.7	27.6	29.4	4.47	0.47	0.51	0.01	0.47	0.80	0.61	0.22
TNF- α , pg/mL	311	362	424	321	457	390	345	389	64.2	0.72	0.20	0.94	0.68	0.66	0.26	0.40
IFN- γ , pg/mL	260	256	357	327	411	227	209	265	66.4	0.38	0.19	0.38	0.39	0.62	0.09	0.57
Anti-OVA IgG, OD	0.10	0.17	0.22	0.18	0.24	0.18	0.13	0.22	0.04	0.58	0.08	0.33	0.71	0.47	0.04	0.47

¹Targeted intakes of linoleic and α -linolenic acids were 0.119 and 0.007 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of BW^{0.75}. Milk replacer was the only feed offered during the first 30 d of life.

²*P*-value for orthogonal contrasts of treatments, sex, and treatment by sex interactions: L = linear effect of treatment, Q = quadratic effect of treatment, Cb = cubic effect of treatment, S = sex. Effect of age was always significant.

³Haptoglobin (optical density $\times$ 100), treatment by age: *P* = 0.02.

⁴Acid-soluble protein, treatment by age: *P* < 0.01.

⁵Proliferation is expressed as 1,000 counts per minute of thymidine incorporation.

⁶Stimulation index.

Figure 4. Stimulation index is calculated as the number of whole blood lymphocytes proliferated by LPS and phytohemagglutinin stimulation divided by the number of lymphocytes proliferated without stimulation of Holstein calves fed C18 linoleic and α -linolenic acids at 0.119 and 0 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of BW^{0.75} respectively, in milk replacer. Cubic effect of treatment ($P = 0.01$) and effect of age ($P < 0.01$).

macrophages, and neutrophils, but not lymphocytes (Hernández et al., 2005). Not all calves were responsive to an intradermal injection of PHA. Proportions of calves responsive to PHA challenge were 90 and 79% at 30 and 60 d of age, respectively. The risk ratio analysis at 30 and 60 d of age indicated that treatments 2, 3, or 4 did not differ from T1 ($P > 0.40$) in the number of calves responsive to PHA challenge. A second analysis that only included responsive calves (calves having an increase in skin-fold thickness after PHA injection at any of the 3 measuring times postinjection) was conducted. At 30 d of age, mean skin-fold thickness across hours postchallenge increased ($P = 0.03$) linearly with increasing intake of essential FA (7.7, 11.0, 14.4, and 15.6% for T1, T2, T3, and T4, respectively; Figure 5A). When PHA was injected at 60 d of age, skin thickness tended ($P = 0.08$) to increase linearly with increased intake of essential FA across hours postinjection. Mean increases were 6.9, 12.3, 13.5, and 15.4% for T1, T2, T3, and T4, respectively (Figure 5B). However, the treatment response to PHA challenge differed between sexes at 30 d of age (sex by cubic treatment interaction, $P = 0.01$). At 30 d of age, male calves fed T2, T3, or T4 responded similarly and to a greater degree than control calves (6.5, 16.3, 11.8, and 15.6%), whereas female calves fed T3 and T4 responded similarly and to a greater degree than control calves (8.8, 5.7, 17.0, and 15.6%). Therefore, regardless of sex, increasing intake of essential FA, potentially mediated by the proinflammatory effect of linoleic acid, resulted in increased recruitment and accumulation of mononuclear cells in response to a PHA.

What is the optimum feeding rate of essential FA for unweaned Holstein calves based upon the results

Figure 5. Proportional change in skin-fold thickness after phytohemagglutinin injection in responsive Holstein calves fed linoleic and α -linolenic acids at 0.119 and 0 (T1), 0.187 and 0.017 (T2), 0.321 and 0.036 (T3), or 0.593 and 0.076 (T4) g/kg of BW^{0.75} in milk replacer. (A) Injection at 30 d of age; linear effect of treatment ($P = 0.03$) and effect of hours postchallenge ($P < 0.01$). (B) Injection at 60 d of age; linear effect of treatment ($P = 0.08$) and effect of hours postchallenge ($P < 0.01$).

of this study? Many measurements, such as BW gain, FE, skeletal growth, concentration, and phagocytosis of neutrophils in blood, plasma concentrations of acute phase proteins, and proliferation and production of IFN- γ by blood lymphocytes, appeared to be optimized when calves were assigned to T2 (0.187 and 0.017 g of linoleic and α -linolenic acids per kg of BW^{0.75}, respectively) or to T3 (0.321 and 0.036 g of linoleic and α -linolenic acids per kg of BW^{0.75}, respectively). This would represent an intake of 3 to 5 g/d of linoleic acid and 0.3 to 0.6 g/d of α -linolenic acid for a 40-kg calf. In

addition to MR, dairy calves are offered a grain-based mixture during their preweaning period in nearly all management systems. Intake of grain during the first week of life is negligible so that MR supplies almost all of the nutrients consumed, meaning that the nutrient composition of MR is critically important. If during the second week of life, calves consumed, on average, 100 g of grain mix (DM basis), calculated intake of linoleic and α -linolenic acids from the grain mix would approximate 1.0 and 0.09 g/d, respectively based upon the FA concentration and profile of the grain mix in Tables 2 and 3. These amounts consumed are 30%, at best, of the calves' estimated essential FA requirement from this study. A calf consuming 500 g of grain DM daily (≥ 3 wk of age; Davis and Drackley, 1998) likely would be consuming almost the required daily amounts of essential FA from the grain mix. As the calf grows, its requirement for essential FA also increases. Considering that dairy calves managed under typical industry conditions attain greater BW gains than did calves in the current study, the requirement of essential FA may be greater than documented here and the calf would need to rely upon both MR and grain mix to consume sufficient amounts of essential FA.

The intake of linoleic acid by calves fed T3 was intermediate of that recommended for growing female and male rats (0.212 and 0.551 g of linoleic acid per kg of BW^{0.75}, respectively; NRC, 1995) and that of α -linolenic acid was about 50% of the intake recommended for young women (DRI, 2005). Some responses such as mean fecal score, number of days to first diarrhea, hypersensitivity of skin to PHA injections, and ADG (females only) were improved at the greatest feeding rate of the essential FA. The concentration of C20:3n-9 in liver decreased linearly with increasing intake of essential FA, becoming almost undetectable when T4 was fed. Synthesis of C20:3n-9 from C18:1n-9 is uncommon unless essential FA are present in very low amounts. Under such deficiency conditions, competition among n-9, n-6, and n-3 FA for Δ^6 - and Δ^5 -desaturase enzymes is greatly reduced, allowing C20:3n-9 to be synthesized from C18:1n-9 (Cook, 1996). Ratios of C20:3n-9 to C20:4n-6 in liver tissue at 30 d of age was <0.2 , indicating that calves in the current study were not clinically deficient in linoleic acid based on traditional guidelines. Whether or not this ratio is applicable to growing ruminants, growth and immunity of preweaned dairy calves benefited from an increasing supply of essential FA to a CCO-based MR.

CONCLUSIONS

Partial replacement of CCO with SBO in MR formulation increased daily intake of linoleic and α -linolenic

acids by preweaned Holstein calves. As a result, hepatic concentrations of these 2 essential FA also increased linearly and that of C20:3n-9 decreased linearly. During the first 30 d of life, when MR was the only feedstuff consumed, ADG and FE were optimized when males were fed 0.187 and 0.017 g of linoleic and α -linolenic acids per kg of BW^{0.75}, respectively, whereas ADG of female calves tended to increase linearly with increasing intake of linoleic and α -linolenic acids up to 0.593 and 0.076 g of linoleic and α -linolenic acids per kg of BW^{0.75}, respectively. Over the 60-d preweaning period, skeletal growth was improved across sexes as intake of essential FA increased to 0.321 and 0.036 g of linoleic and α -linolenic acids per kg of BW^{0.75}, respectively. Several measures of immune function were increased when T2 or T3 were fed. Based upon growth performance and immune responses, unweaned Holstein calves benefited from consuming 3 to 5 g/d of linoleic acid and 0.3 to 0.6 g/d of α -linolenic acid.

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