J. Dairy Sci. 92:670–676
doi:10.3168/jds.2008-1368

Effects of changing the essential and functional fatty acid intake of dairy calves

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ABSTRACT

There is limited information on the effects and requirements of specific fatty acids for dairy calves. The starter diet based on corn and soybean meal, which is typical in the United States, is low in $C_{18:3}$, and the ratio of $C_{18:2}$ to $C_{18:3}$ is quite high relative to recommendations for human infants. Additionally, other functional fatty acids ($C_{20:4}$, $C_{20:5}$, $C_{22:6}$) elongated from $C_{18:2}$ and $C_{18:3}$ have proven benefits in monogastric species. Thus, the effect of adding Ca salts of flax oil (high in $C_{18:3}$) or fish oil (high in $C_{20:4}$, $C_{20:5}$, $C_{22:6}$) to the starter diet of calves less than 3 mo old was investigated. In trial 1, 48 Holstein bull calves [43.2 ± 1.4 kg of body weight (BW); 12/treatment] that were 2 to 3 d of age were fed 1 of 4 starter treatments containing A) no flax or fish oil (control), B) 0.125% Ca salt of flax oil, C) 0.250% Ca salt of flax oil, or D) 0.250% Ca salt of fish oil. Starters and water were fed free-choice to calves. During the first 56 d, calves were individually penned. From arrival until d 28, calves were fed a 26% crude protein, 17% fat milk replacer. From 56 to 84 d, calves were penned in groups of 6 and maintained on their same starter blended with 5% chopped grass hay. Trial 2 used 96 Holstein steer calves (66.3 ± 3.11 kg of BW; 24/treatment) that were 59 to 60 d old in a 28-d trial. These calves had been managed for their first 56 d in the same way as the calves from trial 1 before starting trial 2. Trial 2 evaluated increasing concentrations of Ca salt of flax oil within a starter blended with 5% chopped grass hay and fed with water free-choice. The 4 treatments were A) 0%, B) 0.083%, C) 0.167%, and D) 0.250% Ca salt of flax oil. In trial 1, there were no differences among calves fed the control diet and calves fed the diet supplemented with flax oil. In trial 1, average daily gain (ADG) increased linearly as flax oil increased in the starter from d 0 to 56 and from d 56 to 84, and hip width change increased linearly as flax oil increased in the starter. Serum urea nitrogen and serum glucose concentrations decreased as flax oil increased in the diet. In trial 2, ADG and feed efficiency increased linearly as flax oil increased in the starter. Serum alkaline phosphatase concentrations increased as flax oil increased in the diet. Supplementing a Ca salt of fish oil had no effect on any variables measured. Supplementing $C_{18:3}$ (linolenic acid) as a Ca salt of flax oil to the corn and soybean meal-based diet of dairy calves less than 3 mo old resulted in increased ADG and feed efficiency.
Key words: fatty acid, calf, starter

INTRODUCTION

There is limited information on the effects and requirements of specific fatty acids for the calf. Although calves consume high-fat milk or milk replacer-based diets (≥20% fat, DM basis) initially, their diet is typically very low in fat once weaned (NRC, 2001). No estimates of requirements exist for specific fatty acids or groups of fatty acids (NRC, 2001). Recently, it was demonstrated that supplementing the milk replacer with sources of $C_{18:2}$ and $C_{18:3}$ increased ADG and reduced days with scours (Hill et al., 2007a,b). Additionally, supplementing the starter with sources of $C_{18:2}$ and $C_{18:3}$ was shown to increase ADG and feed efficiency in calves up to 4 mo old (Hill et al., 2007c).

There are only 2 essential fatty acids ($C_{18:2}$ and $C_{18:3}$), yet these essential fatty acids have been shown to elongate into other functional fatty acids (i.e., $C_{20:4}$, $C_{20:5}$, $C_{22:6}$) that are important for neural development and production of hormones (Klein, 2002). Expert panel guidelines are in place for the diets of infants and preterm infants to contain $C_{18:2}$ and $C_{18:3}$; however, those guidelines do not include requirements for $C_{20:4}$, $C_{20:5}$, and $C_{22:6}$, the longer chain fatty acids derived from $C_{18:2}$ and $C_{18:3}$ (Klein, 2002). There could be a critical balance between over- and underconsumption and the ratio of polyunsaturated fatty acids with a stimulation or depression of immune function (Maki and Newberne, 1992). Specific suggestions have been made by an expert panel to keep ratios of polyunsaturated fatty acids in balance in infant diets (Klein, 2002).

The typical starter fed the young calf is low in fat and almost void of sources of $C_{20:4}$, $C_{20:5}$, and $C_{22:6}$. Additionally, as the calf gets older and the rumen develops, the

Received May 15, 2008.
Accepted October 18, 2008.
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microbes in the rumen will presumably biohydrogenate the essential fatty acids of the diet. Starters containing corn, soybean meal, and oats are typical in the United States and contain relatively low concentrations of $C_{18:2}$ and $C_{18:3}$ and have a $C_{18:2}$ to $C_{18:3}$ ratio of greater than 15:1. This 15:1 ratio of $C_{18:2}$ to $C_{18:3}$ exceeds the recommended optimum ratio of 10:1 for pigs (Leskanich and Noble, 1999) and 6:1 for preterm human infants (Klein, 2002). Because of the typical low concentration of $C_{18:2}$ and $C_{18:3}$ in calf starters and the improvements in ADG and feed efficiency previously observed with supplementation of $C_{18:2}$ and $C_{18:3}$ (Hill et al., 2007c), we hypothesize that the calf will consistently respond with increased ADG and feed efficiency to supplementation of $C_{18:3}$.

The objectives of these studies were to evaluate increasing calf intake of specific essential and functional fatty acids in the form of a Ca salt that would be somewhat resistant to rumen biohydrogenation. A pilot study and a subsequent larger trial were conducted to test the effect of adding either Ca salts of flax oil (high in $C_{18:3}$) or fish oil (high in $C_{20:4}$, $C_{20:5}$, and $C_{22:6}$) to the starter diet of calves less than 3 mo old.

MATERIALS AND METHODS

In trial 1, 48 Holstein bull calves (43.2 ± 1.4 kg of BW; 12/treatment) that were initially 2 to 3 d of age from a single farm were used to evaluate different concentrations and sources of Ca salts of fatty acids in 4 starter treatments (Table 1). The starters were A) control, B) 0.125% Ca salt of flax oil (Flaxtech, Virtus Nutrition LLC, Corcoran, CA), C) 0.250% Ca salt of flax oil, and D) 0.250% Ca salt of fish oil (StrataG 113, Virtus Nutrition LLC). A Ca salt of palm fatty acids (EnerGII, Virtus Nutrition LLC) was used to equalize the fat in the diets. Each of the Ca salts contained 84% fat and 9% Ca. The other ingredients of the starters were the same, yielding 4 starters with similar nutrient concentrations. Trial 1 was a pilot study to test the effects of increased amounts of the essential fatty acid $C_{18:3}$ and the functional fatty acids in fish oil ($C_{20:4}$, $C_{20:5}$, and $C_{22:6}$). Treatment C was formulated with supplemental flax oil to provide an approximately 8:1 ratio of $C_{18:2}$ to $C_{18:3}$. This is mid way between the optimum recommendations of a 10:1 ratio for pigs (Leskanich and Noble, 1999) and a 6:1 ratio for preterm human infants (Klein, 2002). Treatment B was an intermediate concentration of flax oil compared with treatments A and C. The concentration of Ca salt of fish oil in treatment D was selected to match the concentration of the Ca salt of flax oil in treatment C.

All calves were fed a 26% milk CP, 17% fat milk replacer (Akey Pinnacle, Akey, Lewisburg, OH) at 0.681 kg of powder/d reconstituted to 0.15 kg/L, halved into a.m. and p.m. feedings for 25 d, followed by 0.227 kg of powder/d for d 26 to 28 (a.m. feeding only). Calves were maintained in individual pens (1.2 m × 2.4 m pens in a curtain sidewall barn with no added heat) for d 0 to 56 and then grouped into 4 replicate pens of 6 calves on d 56 to 84. The group pens consisted of 5.5 m^2 of outside pen space and 0.9 m^2 of inside pen space per calf. The individual pens and inside pen space in the group pens were bedded with straw. Calves were maintained on the same starter for 84 d. Five percent chopped grass hay was blended with the starters before feeding to the calves from d 56 to 84 in the group pens. Starters and water were offered ad libitum and refused starter was weighed daily.

The day after arrival, the calves were weighed, hip widths were measured, body condition was scored, blood was sampled intravenously for serum protein using a refractometer (Atago USA Inc., Bellevue, WA), and calves were randomly assigned to treatment (d 0, initial BW). The following measurements were made during the first 56 d when calves were in individual pens. Calves were weighed every 7 d. Hip widths were measured every 14 d using a caliper. Body condition was scored on a scale of 1 to 5 using 0.25-unit increments with 1 being emaciated and 5 being obese (Wildman et al., 1982). Scores were based on changes around the vertical and transverse processes of the spine as palpated by the same experienced technician and ranged from 1.5 to 3.5. Feed offered and refused was weighed daily. Fecal scores were assigned daily based on a 1 to 5 system (1 being normal, thick in consistency; 2 being normal, but less thick; 3 being abnormally thin but not watery; 4 being watery; 5 being watery with abnormal coloring). During d 56 to 84 when calves were in group pens, feed offered and refused was weighed daily. On d 84, calves were weighed, hips were measured, and BCS was estimated. Additionally, on d 14, 56, and 84, blood was sampled intravenously from 4 calves per treatment (the same calves each time), and the serum was analyzed for alkaline phosphatase, creatinine, glucose, and urea-N with methods as described in Hill et al. (2007b). A power analysis from the 96 calves sampled 3 times over time in Hill et al. (2008) and another unpublished data set of similar size was used to determine this blood sampling regimen. In trial 1, the average temperature was 22°C and ranged from 6 to 37°C based on hourly measurements during d 0 to 56. The average temperature was 17°C and ranged from 1 to 31°C based on hourly measurements during d 56 to 84.

Trial 2 used 96 Holstein steer calves (66.3 ± 3.11 kg of BW; 24/treatment) that were initially 59 to 60 d old from one farm. These calves had been managed for their first 56 d similarly to the calves from trial

Table 1. Ingredient composition and actual analysis (as-fed basis) of starters (A, B, C, and D) from trials 1 and 2

	Trial 1				Trial 2			
Item	Control (A)	0.125% Ca salt of flax oil (B)	0.250% Ca salt of flax oil (C)	0.250% Ca salt of flax oil (D)	0% Ca salt of flax oil (A)	0.083% Ca salt of flax oil (B)	0.167% Ca salt of flax oil (C)	0.250% Ca salt of flax oil (D)
Ingredient, % as-fed								
Corn, rolled coarse	40.00	40.00	40.00	40.00	40.00	40.00	40.00	40.00
Oats, whole	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Molasses, cane	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean meal,[1] 48% CP	22.96	22.96	22.96	22.96	22.96	22.96	22.96	22.96
Wheat middlings[1]	7.81	7.81	7.81	7.81	7.81	7.81	7.81	7.81
Minerals[1,2]	2.88	2.88	2.88	2.88	2.88	2.88	2.88	2.88
Vitamins, trace minerals[1,3]	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Animal fat[1]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
EnerG II (Ca salt of palm oil)[1,4]	0.25	0.125	0	0	0.25	0.167	0.083	0
Flaxtech (Ca salt of flax oil)[1,4]	0	0.125	0.25	0	0	0.083	0.167	0.25
StrataG 113 (Ca salt of fish oil)[1,4]	0	0	0	0.25	0	0	0	0
Decoquinate, 60 g/kg[1,5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Nutrient, % as-fed								
CP	18.23	18.13	18.15	18.19	18.02	18.16	18.04	18.05
Ca	0.78	0.79	0.74	0.75	0.79	0.79	0.81	0.79
P	0.58	0.57	0.59	0.58	0.57	0.6	0.59	0.56
Fat	3.89	3.87	3.85	3.81	3.94	3.9	3.88	3.92
$C_{18:2}$ (linoleic acid)[6]	1.84	1.83	1.79	1.80	1.81	1.84	1.82	1.81
$C_{18:3}$ (linolenic acid)[6]	0.10	0.17	0.23	0.13	0.11	0.15	0.19	0.24
$C_{18:2}$ to $C_{18:3}$ ratio	17.9	10.9	7.8	13.9	17.1	12.5	9.7	7.6

[1]Combined into a supplement pellet.

[2]Calcium carbonate, dicalcium phosphate, and salt.

[3]Labeled concentrations: 0.04 g of Se/kg, 800 kIU of vitamin A/kg; Akey (Lewisburg, OH).

[4]Virtus Nutrition LLC (Corcoran, CA).

[5]Concentration: 60 g/kg; Alpharma Inc. (Fort Lee, NJ).

[6]Other fatty acids within the Ca salt of fish oil treatment included 0.004% $C_{20:4}$, 0.020% $C_{20:5}$, and 0.016% $C_{22:6}$.

1 before starting trial 2. Trial 2 evaluated increasing concentrations of Ca salt of flax oil (Flaxtech) within a starter (Table 1). The 4 treatments were A) 0%, B) 0.083%, C) 0.167%, and D) 0.250% Ca salt of flax oil. A Ca salt of palm fatty acids (EnerG II) was used to equalize the concentration of fat in the diets. The significant responses observed in trial 1 were the basis for the concentrations of flax oil in the starters used in this trial with a larger number of calves per treatment.

Calves were maintained in group pens (as was described in trial 1) of 6 calves for the 28-d trial. There were 2 blocks of 48 calves beginning the trials 5 wk apart with 2 replicates per treatment in each block. Five percent chopped grass hay was blended with the starters before feeding to the calves. Starters and water were fed ad libitum. On d 0 and 28 calves were weighed, hip width was measured, and BCS was estimated. Additionally, on d 0 and 28, blood was sampled intravenously from all 48 calves within 1 block (12 calves per treatment), and serum was analyzed for alkaline phosphatase, creatinine, glucose, and urea-N. The average temperature for trial 2 was 23°C and ranged from 5 to 37°C based on hourly measurements.

Calves in both trials were cared for by acceptable practices as described in the *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS, 1999). Vaccines and health protocols were based on the recommendations of a veterinarian. Calves received an intranasal tissue sensitive respiratory disease vaccine (TSV-2, Pfizer, Exton, PA) and subcutaneous injections of vitamins A, D, E (Vital E - A + D, Schering-Plough Animal Health, Union, NJ) and Se (MU-SE, Schering-Plough Animal Health, Union, NJ) upon arrival. Calves received an intramuscular respiratory disease vaccine (Bovashield Gold 5, Pfizer) at d 7 and again at d 28. At d 14 they received an intramuscular vaccine for types C&D clostridium (Ultra Choice 7, Pfizer). A pasteurella vaccine (Presponse HM, Fort Dodge, Fort Dodge, IA) was administered intramuscularly on d 28 and 42. Calves were castrated and dehorned on d 36. Animals that required medication for infections were treated per veterinary recommendation and treatments were recorded daily. Subcutaneous ceftiofur sodium (Naxcel, Pharmacia & Upjohn, Kalamazoo, MI) was used to treat scouring. Subcutaneous penicillin G procaine (Agri-Cillin, Agri-Labs, St. Joseph, MO) was used to treat navel infections. In trial 1, scouring was diagnosed based on rectal temperatures >39.5°C, lack of vitality, and fecal scores >2. One calf (treatment D in trial 1) died suddenly from what appeared to be *Clostridium* infection and all the data from that calf was removed before analysis.

Approximately 110% of the estimated feeds needed for each study was manufactured at one time. Samples of manufactured feeds were collected from every other bag (22.7 kg) of feed at the time of manufacture. Approximately 110% of the estimated hay needed for each study was purchased at one time and samples were taken from each bale (approximately 20-kg bales). Composites were analyzed (AOAC, 1996) before the animal phase of the studies for DM (oven method; method 930.15), CP (Kjeldahl; method 988.05), fat [milk replacer using alkaline treatment with Röse-Gottlieb method (method 932.06); starters and hay using diethyl ether extraction (method 2003.05)], fatty acids (GC; method 963.22), and Ca and P (dry ashing, acid digestion, analysis by inductively coupled plasma spectrometry; method 985.01). The chopped hay fed in the group pens was mixed, mostly grass hay (timothy, *Phleum pratense* L.). In trial 1, the hay averaged 86.8% DM, 15.9% CP (DM). In trial 2, the hay averaged 87.3% DM and 16.5% CP (DM).

Data from trial 1 were analyzed using the MIXED procedure (version 8, SAS Institute Inc., Cary, NC) as a completely randomized design. Calf within treatment was included as a random effect that was used to test the main effect of treatment. Time was modeled as a repeated measurement using an autoregressive type 1 covariance structure within d 0 to 28, d 28 to 56, d 0 to 56, and d 56 to 84 periods. When the overall F test for treatment was significant ($P < 0.05$) in trial 1, the following contrast statements were used: a linear and quadratic relationship of Ca salt of flax oil among treatments A, B, and C, and a contrast of treatment A (control) versus treatment D (0.250% Ca salt of fish oil). Data reported are least squares means for the experimental unit of calf for 0 to 56 d and pen of calves from 56 to 84 d. Data from trial 2 were analyzed using the MIXED procedure as a randomized complete block design. Block and pen were random effects and treatment was a fixed effect. Time was modeled as a repeated measurement using an autoregressive type 1 covariance structure to analyze the serum measures from d 0 and 28. When the overall F test for treatment was significant ($P < 0.05$), linear, quadratic, and cubic contrasts were used to further characterize the means of treatments A, B, C, and D (increasing concentrations of Ca salt of flax oil). Data reported are least squares means for the experimental unit of pen.

RESULTS AND DISCUSSION

Performance results from trial 1 are shown in Table 2. In trial 1, there were no differences in the initial measurements ($P > 0.05$). Calf ADG increased linearly ($P < 0.05$) as flax oil increased in the starter from 28 to 56 d, 0 to 56 d, and 56 to 84 d. Hip width change increased linearly ($P < 0.05$) as flax oil increased in the starter from d 28 to 56 and d 0 to 56. Starter intake tended ($P < 0.10$) to increase from d 28 to 56 and d 0 to 56, and feed efficiency tended ($P < 0.10$) to increase from d 28 to 56 as the Ca salt of flax oil increased in the starter. Serum urea nitrogen and glucose concentrations decreased ($P < 0.05$) as flax oil increased in the diet (Table 3). Serum alkaline phosphatase concentration tended ($P < 0.10$) to increase as flax oil increased in the diet (Table 3). There were no differences ($P > 0.10$) for any measurement between the control group and the group fed fish oil.

Performance results from trial 2 are shown in Table 4. In trial 2, there were no differences in the initial measurements ($P > 0.05$). Calf ADG and feed efficiency increased linearly ($P < 0.05$) as flax oil increased in the starter. Serum alkaline phosphatase concentration increased ($P < 0.05$) as flax oil increased in the diet (Table 5). Serum urea nitrogen concentrations tended ($P < 0.10$) to decrease as flax oil increased in the diet (Table 5).

In both trials, the ADG response to supplementing flax oil was consistent. In trial 1, there was a trend for starter intake and feed efficiency to increase as flax oil supplementation increased, whereas in trial 2 there was no change in intake and feed efficiency increased. The apparent increase in intake in trial 1 only partially met the nutrient needs for the increase in ADG. When also considering the results of trial 2, where intakes were numerically and statistically similar, the ADG response was more than just a response to greater intake. Hill et al. (2007c) held intake constant when a broader blend of fatty acids, but including $C_{18:3}$, was added to starters, and reported an increase in ADG. In fact, any increase in intake from added flax oil is unlike the typical response of decreased intake and ADG when larger amounts of oil are added to starters (Caffrey et al., 1988; Doppenberg and Palmquist, 1991).

Polyunsaturated fatty acids have been shown to increase bone formation in poultry (Watkins et al., 2001), and linoleic acid has increased cell differentiation in vitro (Allen et al., 1985; Hurley et al., 2006). Arbuckle and Innis (1992) suggested that a 4:1 ratio of $C_{18:2}$ to $C_{18:3}$ was required in the diet for piglets. Later, Leskanich and Noble (1999) suggested a 10:1 ratio of $C_{18:2}$ to $C_{18:3}$ for pigs based on a review of the literature. An expert panel review for requirements of formula-fed preterm infants recommended a 6:1 ratio of $C_{18:2}$ to $C_{18:3}$ (Klein, 2002). Hill et al. (2007a,b) demonstrated that decreasing the ratio of $C_{18:2}$ to $C_{18:3}$ by increasing $C_{18:3}$ in the milk replacer fed to calves increased ADG and reduced days with scours. Subsequently, decreasing the ratio of $C_{18:2}$ to $C_{18:3}$ in the starter of calves was shown to increase the ADG of calves from birth to 4 mo of age (Hill et al., 2007c).

Trials 1 and 2 showed increased ADG and feed efficiency in responses to added fatty acids. This substantiated the results of Hill et al. (2007a,b,c). Additionally, the increases in serum nitrogen and alkaline phosphatase as indicators of protein utilization and bone formation, respectively, in the current trials concur with observations of Hill et al. (2007b,c). In trial 1, intake of $C_{18:3}$ increased from 1.9 g/d in the control group to 4.3 g/d in the group of calves fed the starter with 0.250% Ca salt of flax oil during d 28 to 56. Intake of $C_{18:3}$ increased from 3.2 g/d in the control group to 6.9 g/d in the group of calves fed the starter with 0.250% Ca salt of flax oil during d 56 to 84. In trial 2, intake of $C_{18:3}$ increased from 3.1 g/d in

Table 2. Performance of calves started at less than 7 d of age and fed starters (A, B, C, and D) with different concentrations of Ca salts of either flax or fish oil in trial 1

Item	Control (A)	0.125% Ca salt of flax oil (B)	0.250% Ca salt of flax oil (C)	0.250% Ca salt of fish oil (D)	SEM	Linear *P*-value[1]
Calves, n	12	12	12	11	—	—
Initial serum protein, mg/dL	5.3	5.2	5.2	5.4	0.13	0.39
Initial BW, kg	43.8	44.1	41.5	43.2	1.4	0.48
ADG, kg/d						
0 to 28 d	0.337	0.347	0.353	0.338	0.029	0.29
28 to 56 d	0.684	0.741	0.769	0.690	0.040	0.03
0 to 56 d	0.510	0.544	0.561	0.514	0.023	0.04
56 to 84 d[2]	0.942	0.996	1.013	0.953	0.030	0.04
Starter intake, kg/d						
0 to 28 d	0.108	0.111	0.111	0.141	0.019	0.26
28 to 56 d	1.779	1.815	1.886	1.804	0.049	0.07
0 to 56 d	0.943	0.963	0.999	0.972	0.037	0.09
56 to 84 d[2]	3.018	3.023	3.000	2.995	0.241	0.99
$C_{18:3}$ (linolenic acid) intake, g/d						
28 to 56 d	1.9	3.0	4.3	2.0	0.09	0.01
56 to 84 d[2]	3.2	5.1	6.9	3.3	0.44	0.01
Milk replacer intake, kg/d						
0 to 28 d	0.645	0.645	0.645	0.645	—	—
Gain to feed intake						
0 to 28 d	0.449	0.450	0.461	0.427	0.030	0.69
28 to 56 d	0.388	0.409	0.411	0.373	0.011	0.09
0 to 56 d	0.406	0.420	0.426	0.392	0.014	0.54
56 to 84 d[2]	0.312	0.331	0.338	0.319	0.020	0.63
Abnormal fecal score days[3]						
0 to 28 d	6.8	5.7	7.4	6.0	0.74	0.88
28 to 56 d	0.3	0.3	0.2	0.2	0.14	0.59
0 to 56 d	7.0	5.9	7.6	6.2	0.74	0.80
Average fecal score[3]						
0 to 28 d	2.4	2.4	2.4	2.4	0.03	0.50
28 to 56 d	1.9	1.9	1.9	1.9	0.06	0.89
0 to 56 d	2.2	2.1	2.2	2.2	0.04	0.66
BCS[4]						
Initial	2.3	2.3	2.2	2.3	0.05	0.76
0 to 28 d change	0.1	0.1	0.2	0.2	0.07	0.12
28 to 56 d change	0.2	0.2	0.2	0.1	0.06	0.62
0 to 56 d change	0.3	0.2	0.4	0.4	0.06	0.14
56 to 84 d change	0.4	0.4	0.4	0.4	0.1	0.99
Hip width, cm						
Initial	17.5	18.1	17.3	17.6	0.26	0.66
0 to 28 d change	1.1	1.1	1.3	1.2	0.11	0.27
28 to 56 d change	1.8	2.1	2.2	1.8	0.19	0.04
0 to 56 d change	2.9	3.2	3.5	3.1	0.20	0.04
56 to 84 d change	2.4	2.5	2.6	2.4	0.2	0.77

[1]Linear contrast for increasing concentration of Ca salt of flax oil in starters A, B, and C.

[2]From 56 to 84 d calves were no longer in individual pens but in pens of 6 calves per pen with 4 pens per treatment. Calf was the experimental unit from 0 to 56. Pen was the experimental unit from 56 to 84 d.

[3]Fecal score system: 1 = normal, thick in consistency; 2 = normal, but less thick; 3 = abnormally thin but not watery; 4 = watery; 5 = watery with abnormal coloring. Abnormal fecal scores days were days with scores >2.

[4]1 to 5 system with 1 = emaciated and 5 = obese.

Table 3. Serum constituents of calves[1] started at less than 7 d of age and fed starters (A, B, C, and D) with different concentrations of Ca salts of either flax or fish oil for 84 d in trial 1

Item	Control (A)	0.125% Ca salt of flax oil (B)	0.250% Ca salt of flax oil (C)	0.250% Ca salt of fish oil (D)	SEM	Linear P-value[2]
Calves, n	4	4	4	4	—	—
Urea nitrogen, mmol/L	4.2	3.0	2.6	4.1	0.41	0.02
Creatinine, µmol/L	80.7	92.3	93.3	79.7	7.73	0.14
Alkaline phosphatase, U/L	236	257	263	238	13.3	0.09
Glucose, mmol/L	6.4	5.7	5.5	6.4	0.32	0.01

[1]Calves were in individual pens until d 56 and from 56 to 84 d calves were in pens of 6 calves per pen with 4 pens per treatment. Serum samples were collected on d 14, 56, and 84. Individual calf was used as the experimental unit.

[2]Linear contrast for increasing concentration of Ca salt of flax oil in starters A, B, and C.

the control group to 6.6 g/d in the group of calves fed the starter with 0.250% Ca salt of fish oil. The ratio of $C_{18:2}$ to $C_{18:3}$ decreased from 17 to 8 as the Ca salt of flax oil increased from 0 to 0.250% in the starter. The ratio of 8 is within the optimum range suggested by Leskanich and Noble (1999) and Klein (2002) for piglets and human infants.

The lack of response to the Ca salt of fish oil treatment in trial 1 may have been from a deficiency of $C_{18:3}$ if it was first limiting. Although $C_{18:2}$ and $C_{18:3}$ can be metabolized to $C_{20:4}$, $C_{20:5}$, and $C_{22:6}$, the reverse does not appear possible. Additionally, the metabolically active fatty acids such as $C_{20:4}$, $C_{20:5}$, and $C_{22:6}$ supplied by fish oil have utility in neural development and synthesis of particular hormones, among other things, and appear less effective at improving ADG (Klein, 2002).

The concentration of $C_{18:3}$ in the control starter was 0.11%, whereas it was 0.23% in the starter with 0.250% Ca salt of flax oil. Each starter was very low in fat and low in $C_{18:3}$. Thus, it appears that overcoming a deficiency in $C_{18:3}$ by adding approximately 0.1% $C_{18:3}$ is the priority over the ratio of $C_{18:2}$ to $C_{18:3}$. However, we are not aware of other published research to substantiate this statement or the results from trials 1 and 2.

In practice, there may be several ways to manipulate the fatty acid profile of the diet. Starters in the US dairy industry are sometimes made with some portion of roasted soybeans, added soy oil, molasses/oil blends, and corn distillers grains. These feeds contain considerable concentrations of both $C_{18:2}$ and $C_{18:3}$ and could add significant amounts of $C_{18:2}$ and $C_{18:3}$ to the starter with moderate inclusions. The ratios of $C_{18:2}$ to $C_{18:3}$ have a considerable range from approximately 7 for corn products to 34 for soy products. For example, a starter with 15% roasted soybeans would contain approximately 0.2% $C_{18:3}$ or approaching 2 times the concentration in the control treatment of 0.10 to 0.11% $C_{18:3}$. However, Kuehn et al. (1994) reported reduced intake and ADG when roasted soybeans replaced soybean meal in diets not equal in calories or fat, unlike the results in trials 1 and 2. Canola and flax, less popular ingredients in the United States but widely used worldwide, have much greater concentrations of $C_{18:3}$ and narrower ratios of $C_{18:2}$ to $C_{18:3}$ (approximately 3 for canola and 0.25 for

Table 4. Performance (28 d) of calves started at approximately 59 d of age and fed starters with increasing concentrations of calcium salt of flax oil in trial 2

	Ca salt of flax oil, % as-fed					
Item	0	0.083	0.167	0.250	SEM	Linear P-value[1]
Pens,[2] n	4	4	4	4	—	—
Initial BW, kg	65.8	67.2	65.8	66.2	3.11	0.90
ADG, kg/d	1.001	1.023	1.058	1.075	0.021	0.02
Starter intake, kg/d	2.917	2.920	2.900	2.898	0.0765	0.46
$C_{18:3}$ (linolenic acid) intake, g/d	3.1	4.3	5.5	6.6	0.15	0.01
Gain to feed intake	0.343	0.351	0.365	0.371	0.0084	0.03
Initial hip width, cm	20.3	20.5	20.0	19.8	0.85	0.61
Hip width change, cm	3.1	3.3	3.5	3.7	0.23	0.29
Initial BCS[3]	2.4	2.6	2.4	2.4	0.10	0.36
BCS change[3]	0.4	0.3	0.3	0.4	0.05	0.26

[1]Linear contrast for increasing concentration of Ca salt of flax oil in starters.

[2]Six calves per pen.

[3]1 to 5 system with 1 = emaciated and 5 = obese.

Table 5. Serum constituents of calves[1] for 28 d that were started at 2 mo of age and fed starters with different concentrations of Ca salts of flax oil in trial 2

	Ca salt of flax oil, % as-fed					
Item	0	0.083	0.167	0.250	SEM	Linear *P*-value[2]
Calves, n	12	12	12	12	—	—
Urea nitrogen, mmol/L	4.3	4.6	3.5	3.0	0.54	0.06
Creatinine, μmol/L	53.2	55.2	58.2	60.2	6.48	0.24
Alkaline phosphatase, U/L	178	180	183	205	11.3	0.03
Glucose, mmol/L	5.7	5.5	5.4	5.3	0.28	0.26

[1]From 1 block of 48 calves using pen (6 calves per pen, 2 pens per treatment) as the experimental unit.

[2]Linear contrast for increasing concentration of Ca salt of flax oil in starters. Serum was sampled on d 0 and 28.

flax). However, using canola meal and whole seed to replace soybean meal in diets not equal in calories or fat reduced ADG in calves (Fiems et al., 1985; Schrama et al., 1986).

The method chosen to manipulate the fatty acid profile of the diet should minimize the total amount of fat added to the diet and consider if a fat source also alters the CP quality of the diet (i.e., oil seeds). Added oil has been shown to reduce intake and ADG in calves (Caffrey et al., 1988; Doppenberg and Palmquist, 1991). Kuehn et al. (1994) reported reduced intake and ADG when roasted soybeans replaced soybean meal. Similarly, McCoy et al. (2003) reported reduced intake and ADG when an extruded soybean product replaced soybean meal in a starter. Additionally, the use of canola meal and whole seed to replace soybean meal reduced ADG in calves (Fiems et al., 1985; Schrama et al., 1986). These studies using oilseeds and extruded oilseed products suggest that dietary CP quality was reduced when the oilseeds replaced soybean meal and reduced ADG; however, interpretation is difficult because fat intake and CP source were confounded.

CONCLUSIONS

Supplementing a Ca salt of fish oil did not change any measurements. Supplementing $C_{18:3}$ (linolenic acid), an essential fatty acid, as a Ca salt of flax oil to the corn and soybean meal-based diet of dairy calves less than 3 mo old resulted in increased ADG and feed efficiency.

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