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Effects of fat sources and dietary $C_{18:2}$ to $C_{18:3}$ fatty acids ratio on growth performance, ruminal fermentation and some blood components of Holstein calves

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ABSTRACT

The objective of this study was to evaluate effects of fat sources and various ratios of dietary $C_{18:2}$ to $C_{18:3}$ fatty acids, through inclusion of palm fat powder and flaxseed oil, on growth performance, ruminal fermentation, health indices and some blood components of Holstein calves during the pre and postweaning periods. A total of 65 Holstein calves (30 males and 35 females) were randomly assigned to 1 of the 5 experimental groups: 1- starter diet with no fat supplement (CON, $C_{18:2}$:$C_{18:3}$ = 18:1), 2- starter diet with 2.2% palm fat powder (PF, $C_{18:2}$:$C_{18:3}$ = 18:1), 3- starter diet with 1.5% palm fat powder and 0.8% Ca-salts of flaxseed oil (PCF, $C_{18:2}$:$C_{18:3}$ = 4:1), 4- starter diet with 2.7% Ca-salts of flaxseed oil (CFO, $C_{18:2}$:$C_{18:3}$ = 1.5:1) and 5- starter diet with 2.2% flaxseed oil (FO, $C_{18:2}$:$C_{18:3}$ = 1.5:1). Results showed that palm fat powder had no effect on feed efficiency, but increased ($P < 0.05$) BCS and decreased ($P = 0.01$) blood urea nitrogen compared to the CON. Feeding CFO increased ($P < 0.01$) ADG during postweaning period and feed efficiency over the entire study. Calves in the CFO group had better fecal scores ($P = 0.03$) and general appearance scores ($P < 0.05$) compared to the CON. Calves fed starter diet with lower $C_{18:2}$ to $C_{18:3}$ fatty acids ratio (CFO and FO groups) had greater hip height ($P = 0.01$) and serum alkaline phosphatase ($P = 0.04$) and fewer days with diarrhea ($P = 0.03$) than the CON group. Fat sources and different $C_{18:2}$ to $C_{18:3}$ fatty acids ratio had no effect on ruminal fermentation parameters. In general, our results provide evidence that different fat sources had no adverse effect on feed intake and growth performance of calf. Moreover, decreasing $C_{18:2}$:$C_{18:3}$ fatty acids ratio in calves starter diets, using Ca-salts of flaxseed oil (2.7%) or flaxseed oil (2.2%) had beneficial effects on growth performance, health indices and some blood components in Holstein dairy calves.

1. Introduction

Feeding programs contribute significantly to growth, health and future productivity of dairy calves. Neonatal calves have limited capability for dry matter intake (DMI) and the main source of their starter diet is carbohydrates. Convenient starter diets are typically low in fat and have high ratios of $C_{18:2}$ to $C_{18:3}$ fatty acids (18:1), which may affect the normal growth and weight gain of weaned calves (Hill et al., 2007c). Fat supplementation in milk replacer or starter diet has been suggested to improve the energy content of calf diets (Raven, 1970). Enrichment of milk replacer and starter diet with specific fatty acids improved average daily gain (ADG) and feed efficiency and reduced fecal scouring in Holstein calves (Hill et al., 2007b, 2007c, 2009).

Both $C_{18:2}$ and $C_{18:3}$ fatty acids are precursors for eicosanoids, the potent signaling molecules involved in the regulation of inflammation (Simopoulos, 2002). However, these fatty acids have opposing physiological functions. Eicosanoids derived from $C_{18:2}$ fatty acids have pro-inflammatory effects, whereas those derived from $C_{18:3}$ fatty acids have anti-inflammatory effects (Patterson et al., 2012). The dietary ratio of $C_{18:2}$ to $C_{18:3}$ fatty acid plays an important role in normal growth and health of mammals (Knapp, 1997). However, the effects of this ratio in starter diets or milk replacers of calves have been poorly understood. We hypothesized that, lower dietary $C_{18:2}$ to $C_{18:3}$ ratios in calves' starter diet may have beneficial effects on their growth, ruminal fermentation, health and blood components. Therefore, the aim of study was to evaluate the effects of fat sources and reducing ratios of $C_{18:2}$ to $C_{18:3}$ fatty acids (18:1 to 4:1 and to 1.5:1) using palm fat powder, flaxseed oil and Ca-salt of flaxseed oil) on growth and health

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factors and ruminal fermentation, as well as blood components of Holstein calves during pre and postweaning periods.

2. Materials and methods

2.1. Animals, housing and diets

This study was carried out on a commercial dairy farm (Behroozi Dairy Farm, Tehran district, Iran) during December 2014 to February 2015. The Animal Care Advisory Committee of the Isfahan University of Technology approved all experimental procedures and confirmed that the experiments were conducted according to the Iranian Council of Animal Care guidelines (Isfahan University of Technology, 1995). A total of 65 newborn Holstein calves were housed in individual pens (2.4 × 1.2 m), bedded with chopped wheat straw from 3 to 77 d of age. Calves were checked for health status at the start of the study according to procedures described by Heinrichs et al. (2003).

Calves received colostrum (10% of birth weight) for 3 d and then were fed 3, 4, 5, 6, 5, 4, 3 and 1–2 l of milk per day during weeks 1 to 8, respectively. The milk (38 °C) was provided twice a day (at 07:00 and 16:00 h) using plastic buckets and calves had *ad libitum* access to water throughout the experimental period. Calves were randomly allocated to the experimental groups at 3 d of age, with 13 replicates for each (6 males and 7 females). Calves had *ad libitum* access to one of the five starter diets: 1- starter diet with no fat supplement (CON, $C_{18:2}$:$C_{18:3}$ = 18:1), 2- starter diet with 2.2% palm fat powder (PF, $C_{18:2}$:$C_{18:3}$ = 18:1), 3- starter diet with 1.5% palm fat powder and 0.8% Ca-salts of flaxseed oil (PCF, $C_{18:2}$:$C_{18:3}$ = 4:1), 4- starter diet with 2.7% Ca-salts of flaxseed oil (CFO, $C_{18:2}$:$C_{18:3}$ = 1.5:1) and 5- starter diet with 2.2% flaxseed oil (FO, $C_{18:2}$:$C_{18:3}$ = 1.5:1).

All calves were weaned at 56 d in a 4 d period and the feeding program continued until 77 d. Diets were iso-nitrogenous and the ingredients and composition are shown in Table 1. Samples of the diets were analyzed for dry matter (934.01; AOAC, 1990), ether extract (920.39; AOAC, 1990), crude protein (988.05; AOAC, 1990), NDF and ADF (Van Soest et al., 1991). The Ca-salts of flaxseed oil (Persiafat) kindly provided by Kimiya Danesh Alvand Co. (Tehran, Iran), contained 84% fat and 9% Ca as fed. Chopped high quality alfalfa hay (5% of starter diet) was included in the starter diets beginning at 30 d. The starter diets were offered at 09:00 on daily basis and the refusals were weighed daily throughout the experimental period to calculate feed intake (3 to 77 d).

Table 1
Ingredients and nutrient composition (DM basis) of the starter feeds.

Item	Treatment[a]				
	CON	PF	PCF	CFO	FO
Ingredient, %					
Corn	56.2	53.9	53.8	53.5	53.9
Soybean meal	32.1	32.5	32.5	32.5	32.5
Beet pulp dry	6.8	6.6	6.6	6.6	6.6
Limestone	1.2	1.2	1.1	1.0	1.2
White salt	0.3	0.3	0.3	0.3	0.3
Sodium bicarbonate	0.8	0.8	0.8	0.8	0.8
Sodium bentonite	2.0	2.0	2.1	2.2	2.0
Mineral vitamin mix[b]	0.5	0.5	0.5	0.5	0.5
Palm fat powder[c]	-	2.2	1.5	-	-
Ca-salts of flaxseed oil[d]	-	-	0.8	2.7	-
Flaxseed oil	-	-	-	-	2.2
Nutrient composition, DM basis					
CP, %	20.1	20.0	20.0	19.9	20.01
NDF, %	14.1	13.9	13.8	13.8	13.83
ADF, %	7.77	7.68	7.67	7.66	7.68
NFC, %	56.5	54.7	54.8	54.8	54.75
Ether extract, %	3.0	5.1	5.0	5.1	5.07
Ash, %	8.4	8.4	8.8	8.3	8.35
ME, MJ/kg[e]	11.2	11.5	11.5	11.4	11.4
NEm, MJ/kg	7.3	7.7	7.6	7.6	7.5
NEg, MJ/kg	4.7	5.0	5.0	4.9	4.9
Fatty acid,[f]%					
$C_{14:0}$	1.9	1.7	1.6	1.2	1.2
$C_{16:0}$	13.2	40.8	32.0	10.1	10.1
$C_{16:1}$	0.1	0.1	0.1	0.1	0.1
$C_{18:0}$	2.3	4.6	4.2	3.1	3.2
$C_{18:1}$	23.1	15.5	17.2	21.3	21.3
$C_{18:2}$	55.3	30.8	33.1	38.2	38.3
$C_{18:3}$	3.1	1.7	8.3	25.2	25.0
Other	1.0	4.8	3.7	0.8	0.8
$C_{18:2}$:$C_{18:3}$	18	18	4	1.5	1.5

[a] Treatmets: CON = No fat supplements, PF = 2.2% Palm fat powder, PCF = 1.5% Palm fat powder + 0.8% Ca-salts of flaxseed oil, CFO = 2.7% Ca-salts of flaxseed oil, FO = 2.2% of flaxseed oil.
[b] Mineral vitamin mix composition: 10,000,000 IU/kg of vitamin A; 2,000,000 IU/kg of vitamin D3; 6,000 IU/kg of vitamin E; 0.5 g/kg of vitamin B1; 0.5 g/kg of vitamin B2; 48 g/kg of Mg; 35 g/kg of Zn; 30 g/kg of Mn; 23 g/kg of Fe; 10 g/kg of Cu; 0.6 g/kg of I; 0.4 g/kg of Co; 0.1 g/kg of Se.
[c] Optima 100, Malaysia (1.51% $C_{14.0}$, 75.38% $C_{16:0}$; 7.54% $C_{18:0}$, 6.04% $C_{18:1}$; 9.55% other).
[d] Ca-salts of flaxseed oil, Persiafat, Kimiya Danesh Alvand Co. Iran., contained 84% fat and 9% Ca (0.16% $C_{14.0}$, 5.74% $C_{16:0}$; 0.18% $C_{16:1}$; 4.3% $C_{18:0}$, 18.88% $C_{18:1}$, 14.15% $C_{18:2}$; 55.95% $C_{18:3}$, 0.64% other).
[e] Using NRC (2001) equations.
[f] Percentage of total fatty acids analyzed.

2.2. Sampling and measurements

Starter diet intake and ADG were recorded daily and once every 2 wk, respectively. The skeletal characteristics and their changes (hip width, wither height, hip height, body length, heart girth and body barrel) were measured at the start of experiment (3 d) and 56 d using a caliper. Body condition score (BCS) and its changes were determined (1–5 scale with 0.25 increments) at 3, 28 and 56 d (Wildman et al., 1982). Rectal temperature was measured weekly before morning meal and calves with over 39.5 °C temperature were categorized as febrile. Feces samples were scored for determination of consistency based on a five-point scale (Kertz and Chester-Jones, 2004). General appearance of calves was also scored on a 1–5 scale, with 1 being normal and alert; 2 being ears drooped; 3 being head and ears drooped, dull eyes and slightly lethargic; 4 being head and ears drooped, dull eyes and lethargic and 5 being severely lethargic (Calf Health Scoring, Chart prepared by the School of Veterinary Medicine, University of Wisconsin, Madison. Accessed Jan. 11, 2017. http://www.vetmed.wisc.edu/dms/fapmtools/8calf/calf health scoring chart. pdf). A calf was considered to have diarrhea if scours were observed more than 3 times in a given day. Feeding behaviors of calves (eating starter diet, ruminating while standing or lying) as well as non-nutritive oral behaviors were recorded within two consecutive weeks at preweaning period (42–56 d) and two consecutive weeks at postweaning period (56–70 d) (Castells et al., 2012). Rumen fluid samples (30 mL) were collected (6 male calves per treatment) using a stomach tube and vacuum pump 4 h post morning feeding on the day of weaning. The first 15 mL of collected rumen fluid was discarded to prevent saliva contamination and the pH of the rest of rumen fluid was determined immediately after collection (Digital pH meter, CRISON, Spain). Four layers of cheesecloth were used for squeezing the rumen fluid and 2 mL of metaphosphoric acid (25%) was added to the filtered liquid (8 mL) before storing at −20 °C. Concentration of VFAs (acetate, propionate, butyrate, isobutyrate and valerate) (Bal et al., 2000) and ammonia nitrogen (N-NH3) (Weatherburn, 1967) were determined in rumen fluid.

Blood samples were collected (8 calves per treatment) from the jugular vein of calves into 10 mL vacuum tubes containing heparin at 3, 28, 56 and 70 d. The samples were immediately centrifuged at 3000 rpm for 10 min. Plasma samples were preserved at −20 °C until further analysis. Plasma glucose, total protein, albumin, creatinine, urea nitrogen and alkaline phosphatase were analyzed using commercial kits (Pars Azmoon Kit; Pars Azmoon, Tehran, Iran) by an automated analyzer (Technicon-RA 1000 Auto analyzer; DRG Instruments GmbH, Marburg, Germany). Plasma globulin was determined by subtraction of albumin from total serum protein.

2.3. Statistical analysis

The repeated measured data (*Pj* = effect of period of data measurement*)* were analyzed as a completely randomized design with 5 treatments and 13 replicates each, by the Mixed Procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC) according to the following model:

$$Yij = \mu + Ti + Pj + \beta(Xi - X) + \varepsilon ij$$

Where Yij = the dependent variable, μ = mean, Ti = the dietary treatment effect, Pj = effect of period of data measurement, β $(Xi - X)$ = the covariate variable and εij = the error term.

Significance among treatments was determined by the LSMEANS test and results were considered as significant the *P*-value was less than 0.05; tendencies were declared at $0.05 < P < 0.10$.

3. Results

3.1. Starter diet intake and fatty acid intakes of $C_{18:2}$ and $C_{18:3}$

Means of starter diet intake and fatty acid intakes of $C_{18:2}$ and $C_{18:3}$ using different fat sources are presented in Table 2. The results showed that feeding various fat supplements with different ratio of $C_{18:2}$ and $C_{18:3}$ had no adverse effect on starter diet intake during the pre and postweaning periods, as well as during the entire study. Furthermore, the PF and PCF groups had greater ($P = 0.03$) starter diet intake than the CON group during the 42–56 d period.

Daily calculated intake of $C_{18:2}$ was not statistically different among experimental groups during the preweaning period (3 to 56 d). However, the CFO and FO groups (during the postweaning period) and FO group (entire study) had greater ($P < 0.01$) $C_{18:2}$ intake than the other groups. On the other hand, daily calculated intake of $C_{18:3}$ revealed that the CFO and FO groups had the greatest ($P < 0.01$) $C_{18:3}$ intake during the preweaning (3.64 and 4.21 g/d, respectively), postweaning (20.41 and 19.65 g/d, respectively) and entire study (7.08 and 8.32 g/d, respectively) periods. The starter diet intake and fatty acids ($C_{18:2}$ and $C_{18:3)}$ intake were affected by period. However, there was no interaction between the period and treatment.

Table 3
Effect of starter supplementation with different fat sources on average daily gain (ADG), body weight and feed efficiency of Holstein calves during a 77 d period.

Item	Treatment[1]					SEM	P-value
	CON	PF	PCF	CFO	FO		
Calves, n	13	13	13	13	13		
ADG (g/d)							
3 to 14 d	228	258	271	201	140	82.55	0.11
14 to 28 d	494	522	521	581	545	80.88	0.28
28 to 42 d	629	721	577	692	627	85.26	0.09
42 to 56 d	734^{b}	831^{ab}	863^{ab}	912^{a}	934^{a}	83.54	0.01
3 to 56 d (preweaning)	521	583	558	586	564	83.54	0.44
56 to 77 d (postweaning)	698^{b}	837^{ab}	782^{b}	936^{a}	826^{ab}	80.88	< 0.01
3 to 77 d (entire study)	569	652	620	681	635	80.88	0.17
Body weight (kg)							
3 d	37.9	38.1	37.4	38.0	38.4	3.75	0.79
56 d (weaning date)	67.1	70.8	68.7	70.8	70.0	3.67	0.31
77 d	81.7^{b}	88.4^{ab}	85.1^{ab}	90.5^{a}	87.3^{ab}	3.67	0.01
Feed efficiency[2]							
3 to 56 d (preweaning)	0.50	0.52	0.52	0.58	0.52	0.089	0.35
56 to 77 d (postweaning)	0.43	0.44	0.44	0.51	0.43	0.040	0.08
3 to 77 d (entire study)	0.42^{b}	0.45^{b}	0.44^{b}	0.52^{a}	0.43^{b}	0.025	< 0.01

a-b Means within a row with different superscripts differ ($P < 0.05$).
1 Treatmets: CON = No fat supplements, PF = 2.2% Palm fat powder, PCF = 1.5% Palm fat powder + 0.8% Ca-salts of flaxseed oil, CFO = 2.7% Ca-salts of flaxseed oil, FO = 2.2% of flaxseed oil.
2 Gain divided by milk plus starter intake.

3.2. Growth performance

The ADG, body weight and feed efficiency data are presented in Table 3. We observed that calves fed flaxseed oil (CFO and FO) had better ($P = 0.01$) ADG during the last weeks of preweaning (42–56 d) and this condition continued postweaning in the CFO group ($P < 0.01$). The experimental treatments had no effect on weaning weight. However, the final body weight (77 d) was higher ($P = 0.01$) in CFO calves than CON. The other groups had intermediate final body weight.

Table 2
Effect of starter supplementation with different fat sources on starter intake and $C_{18:2}$ and $C_{18:3}$ fatty acid intakes in Holstein calves during a 77 d period.

Item	Treatment[1]					SEM	P-value
	CON	PF	PCF	CFO	FO		
Calves, n	13	13	13	13	13		
Starter intake, g/d							
3 to 14 d	71.8	88.3	61.1	44.6	48.7	114.48	0.70
14 to 28 d	210.1	236.6	205.1	184.7	168.0	104.87	0.51
28 to 42 d	380.0	465.8	396.3	370.9	432.8	104.87	0.37
42 to 56 d	703.7^{b}	938.0^{a}	927.4^{a}	875.8^{ab}	900.8^{ab}	104.87	0.03
3 to 56 d (preweaning)	353.7	448.7	399.1	336.5	419.8	74.58	0.14
56 to 77 d (postweaning)	1659	1881	1767	1818	1870	140.3	0.12
3 to 77 d (entire study)	620	753	659	607	735	87.48	0.10
$C_{18:2}$ intake, g/d							
3 to 56 d (preweaning)	4.96	5.62	5.94	5.53	6.45	1.183	0.21
56 to 77 d (postweaning)	23.18^{b}	25.64^{b}	25.33^{b}	30.94^{a}	30.10^{a}	1.557	< 0.01
3 to 77 d (entire study)	9.93^{b}	11.47^{b}	10.83^{b}	10.73^{b}	12.74^{a}	1.379	0.04
$C_{18:3}$ intake, g/d							
3 to 56 d (preweaning)	0.27^{c}	0.31^{c}	1.5^{b}	3.64^{a}	4.21^{a}	0.533	< 0.01
56 to 77 d (postweaning)	1.27^{c}	1.43^{c}	6.39^{b}	20.41^{a}	19.65^{a}	0.672	< 0.01
3 to 77 d (entire study)	0.54^{c}	0.64^{c}	2.74^{b}	7.08^{a}	8.32^{a}	0.699	< 0.01

a-b Means within a row with different superscripts differ ($P < 0.05$).
1 Treatmets: CON = No fat supplements, PF = 2.2% Palm fat powder, PCF = 1.5% Palm fat powder + 0.8% Ca-salts of flaxseed oil, CFO = 2.7% Ca-salts of flaxseed oil, FO = 2.2% of flaxseed oil.

Table 4
Effect of starter supplementation with different fat sources on BCS (at 28 and 56 d) and body size parameters (withers height, hip height, hip width, body length, heart girth and body barrel) (at 56 d) of Holstein calves.

	Treatment[1]						
Item	CON	PF	PCF	CFO	FO	SEM	P-value
Calves, n	13	13	13	13	13		
BCS[2]							
28 d	2.07[b]	2.33[a]	2.33[a]	2.10[b]	2.28[ab]	0.119	0.03
56 d	2.14[b]	2.37[a]	2.37[a]	2.27[ab]	2.34[ab]	0.117	< 0.05
28 to 56 d change	0.08	0.04	0.03	0.17	0.06	0.087	0.13
Skeletal parameters (cm)							
wither height	88.64	89.61	89.59	89.10	87.79	1.615	0.29
hip height	85.65[b]	87.21[ab]	88.41[ab]	89.75[a] 75[a]	89.90[a]	1.673	0.01
hip width	20.07	19.42	19.87	19.91	20.01	0.620	0.28
body length	43.20	42.95	41.75	43.97	42.88	1.405	0.12
heart girth	96.72	99.60	98.89	97.86	98.95	1.853	0.13
body barrel	105.42	102.14	104.40	102.88	102.83	2.767	0.24

[a-b] Means within a row with different superscripts differ ($P < 0.05$).
[1] Treatmets: CON = No fat supplements, PF = 2.2% Palm fat powder, PCF = 1.5% Palm fat powder + 0.8% Ca-salts of flaxseed oil, CFO = 2.7% Ca-salts of flaxseed oil, FO = 2.2% of flaxseed oil.
[2] BCS: 1 to 5 system based on Wildman et al. (1982).

Overall, the CFO calves had the highest ($P < 0.01$) feed efficiency.

3.3. BCS and skeletal parameters

Data related to body condition score and some skeletal parameters are presented in Table 4. Results showed that PF and PCF groups had higher BCS compared to the CON calves at 28 d ($P = 0.03$) and 56 d ($P < 0.05$). However, the experimental diets had no effect on changes of BCS during the 28–56 d period.

The experimental diets did not affect most of the skeletal parameters. However, calves fed flaxseed oil (CFO and FO) had greater ($P = 0.01$) hip height than those fed the CON.

3.4. Health parameters

Some health parameters such as rectal temperature, fecal score, days with diarrhea and general appearance score of calves are shown in Table 5. We observed that the CON group had higher ($P = 0.03$) rectal temperature (38.8 °C) than those calves fed CFO and FO (38.3 °C and 38.4 °C, respectively) at 77 d. The experimental diets did not affect mean fecal score during the preweaning periods. However, during the postweaning period calves fed CFO had lower ($P = 0.02$) fecal scores than the CON and PF groups and during the entire study calves fed CFO and FO had lower ($P = 0.04$) fecal scores than the CON and PF groups. We observed that calves fed flaxseed oil (CFO or FO) had fewer ($P = 0.03$) days with diarrhea (1.8 and 1.83 d, respectively) compared with the CON and PF groups (2.22 and 2.12 d, respectively). Calves fed CFO had lower general appearance scores during the preweaning period ($P = 0.02$) and during the entire study ($P < 0.05$) compared to the CON group. There was no difference between groups fed other supplements of fat in this respect.

3.5. Feeding behaviors

We did not observe any significant differences in feeding behaviors of calves supplemented with various fat sources and different ratios of $C_{18:2}$ to $C_{18:3}$ (Table 6). In other words, adding the palm fat powder or flaxseed oil or both had no adverse effect on the time spent eating and ruminating in calves.

3.6. Ruminal fermentative parameters

Fat supplementation had no effect on rumen pH and molar production of different volatile fatty acids and total VFAs (Table 7). The dietary treatments tended to have significant ($P = 0.10$) effect on ruminal ammonia nitrogen and the PF group had the lowest ruminal ammonia nitrogen.

Table 5
Effect of starter supplementation with different fat sources on rectal temperature, fecal score, days with diarrhea and general appearance score of Holstein calves during a 77 d period.

	Treatment[1]						
Parameter	CON	PF	PCF	CFO	FO	SEM	P-value
Calves, n	13	13	13	13	13		
Rectal temperature (°C)							
56 d	38.7	38.6	38.5	38.4	38.5	0.19	0.31
77 d	38.8[a]	38.5[ab]	38.5[ab]	38.3[b]	38.4[b]	0.14	0.03
Fecal score[2]							
3 to 56 d (preweaning)	1.53	1.55	1.49	1.50	1.53	0.051	0.25
56 to 77 d (postweaning)	1.40[a]	1.38[a]	1.34[ab]	1.24[b]	1.35[ab]	0.060	0.02
3 to 77 d (entire study)	1.46[a]	1.47[a]	1.42[ab]	1.37[b]	1.45[ab]	0.044	0.04
Days with diarrhea	2.22[a]	2.12[a]	1.97[ab]	1.80[b]	1.83[b]	0.189	0.03
General appearance score[3]							
3 to 56 d (preweaning)	1.52[a]	1.44[ab]	1.43[ab]	1.34[b]	1.48[ab]	0.074	0.02
56 to 77 d (postweaning)	1.53	1.29	1.33	1.34	1.44	0.147	0.12
3 to 77 d (entire study)	1.50[a]	1.37[ab]	1.38[ab]	1.34[b]	1.47[ab]	0.085	< 0.05

[a-b] Means within a row with different superscripts differ ($P < 0.05$).
[1] Treatmets: CON = No fat supplements, PF = 2.2% Palm fat powder, PCF = 1.5% Palm fat powder + 0.8% Ca-salts of flaxseed oil, CFO = 2.7% Ca-salts of flaxseed oil, FO = 2.2% of flaxseed oil.
[2] Fecal score: 1 = normal, 2 = soft to loose, 3 = loose to watery 4 = watery, mucous and slightly bloody, 5 = watery, mucous and bloody.
[3] General appearance score: 1 = normal and alert, 2 = ears drooped, 3 = head and ears drooped, dull eyes, slightly lethargic, 4 = head and ears drooped, dull eyes, lethargic, 5 = severely lethargic.

3.7. Blood components

Blood components of calves fed different fat supplements are shown in Table 8. The experimental diets did not affect blood components such as glucose, total protein and creatinine at different stages of sampling.

Table 6
Effect of starter supplementation with different fat sources on feeding behavior in Holstein calves during 8 h of observations.

	Treatment[a]						
Item	CON	PF	PCF	CFO	FO	SEM	P-value
Calves, n	13	13	13	13	13		
Lying (min)	161.9	157.0	164.4	167.2	172.5	12.71	0.32
Standing (min)	147.3	138.9	145.1	130.6	133.0	14.54	0.47
Eating starter (min)	27.6	28.4	32.2	40.8	48.9	7.63	0.26
Ruminating (min)	47.8	36.7	50.8	46.4	46.3	5.11	0.44
NNB[b] (min)	95.6	108.9	87.2	94.9	89.4	11.56	0.51

[a] Treatmets: CON = No fat supplements, PF = 2.2% Palm fat powder, PCF = 1.5% Palm fat powder + 0.8% Ca-salts of flaxseed oil, CFO = 2.7% Ca-salts of flaxseed oil, FO = 2.2% of flaxseed oil.
[b] NNB: Non-Nutritional Behavior.

Table 7
Effect of starter supplementation with different fat sources on ruminal volatile fatty acids (VFAs) and ammonia nitrogen (at 56 d), and pH (at 56 and 77 d) of Holstein calves.

	Treatment[a]						
Item	CON	PF	PCF	CFO	FO	SEM	P-value
Calves, n	7	7	7	7	7		
Total VFAs, mmol/L	142.12	144.9	146.3	145.7	144.3	8.06	0.61
Individual, mol/100 mol							
Acetate	47.6	45.1	46.9	48.0	44.7	0.72	0.52
Propionate + Isobutyrate	28.5	31.0	32.3	24.7	30.6	1.62	0.34
Butyrate	19.3	19.3	15.8	21.9	18.3	0.63	0.14
Valerate	2.68	2.97	2.11	3.01	2.79	0.09	0.25
N-NH3, mg/100 mL	12.23	7.13	12.13	9.67	10.04	2.95	0.10
pH							
56 d	6.32	6.33	6.41	6.61	6.43	0.25	0.27
77 d	6.21	6.19	6.35	6.49	6.23	0.23	0.20

[a] Treatmets: CON = No fat supplements, PF = 2.2% Palm fat powder, PCF = 1.5% Palm fat powder + 0.8% Ca-salts of flaxseed oil, CFO = 2.7% Ca-salts of flaxseed oil, FO = 2.2% of flaxseed oil.

Fat supplementation affected some other factors such as albumin ($P < 0.01$), globular protein ($P < 0.01$), urea nitrogen ($P = 0.01$) and alkaline phosphatase ($P = 0.04$) at the 70 d of age. Our results showed that calves fed starter diet with lower $C_{18:2}$ to $C_{18:3}$ fatty acid ratios (1.5:1) had greater ($P < 0.01$) albumin at d 70. The globular protein was lower ($P < 0.01$) in groups fed flaxseed oil, especially FO calves. Urea nitrogen in CON groups was higher ($P = 0.01$) than the PF at 28 d and PF and FO at 70 d. The calves fed different sources of fat supplement and various ratios of $C_{18:2}$ to $C_{18:3}$ fatty acids had higher ($P = 0.04$) alkaline phosphatase than the CON group at 70 d.

4. Discussion

4.1. Starter diet intake and fatty acid intakes of $C_{18:2}$ and $C_{18:3}$

Doppenberg and Palmquist (1991) reported that adding fat to starter diets reduced intake and ADG of calves. However, in the present study feeding various fat supplements which caused different ratio of $C_{18:2}$ and $C_{18:3}$ did not affect the starter diet intake. Our results showed that starter diet intake was higher in PF and PCF groups compared to the CON group during the 42–56 d period, which was consistent with Hill et al. (2009). It is demonstrated that effect of fat supplementation on feed intake could be dependent on age, total fat of diet, fatty acid profile and $C_{18:2}$ to $C_{18:3}$ fatty acids ratio (Hill et al., 2011a, b).

Results of the present study showed that the intake of $C_{18:3}$ was greatest in CFO and FO groups during the preweaning, postweaning and entire the study. This finding is consistent with Hill et al. (2009) who showed that $C_{18:3}$ (linolenic acid) intake increased by inclusion of 0.25% Ca-salts of flaxseed oil in dairy calf starter diets compared to 0.25% of Ca-salts of fish oil.

4.2. Growth performance

Results showed that feeding flaxseed oil (CFO and FO) had positive effect on ADG during the period of 42–56 d and this state was continued at postweaning period just for calves fed Ca-salts flaxseed oil (CFO). On the other hand, the CFO group had higher final body weight (77 d) compared to the CON group and their feed efficiency was highest. These results could be due to the higher ADG during the postweaning period and final body weight of CFO calves, without significant change in their average starter diet intake. Hill et al. (2009) reported that supplementing $C_{18:3}$ (linolenic acid) as Ca-salts of flaxseed oil to starter diet of dairy calves less than 3 mo old resulted in increased ADG and feed efficiency. It seems that reducing the $C_{18:2}$ to $C_{18:3}$ fatty acids ratio has beneficial effects on health and immune system function of dairy calves (Maddock et al., 2006; Hill et al., 2011b; Garcia et al., 2015) which therefore improved the ADG and feed efficiency in the present study.

4.3. BCS and skeletal parameters

Fat supplementation affected the calves' BCS at 28 d and 56 d. However, in accordance with the Hill et al. (2009), the experimental diet had no effect on BCS change during the 28–56 d and also the most of skeletal parameters. Calves fed flaxseed oil (CFO and FO) had greater hip height than those CON group. Previous reports showed positive (Hill et al., 2007a, 2007b, 2007c), futile (Ballou and DePeters, 2008) or even negative (Fokkink et al., 2009) effects of different fat supplements on skeletal growth of dairy calves. Watkins et al. (2001) reported that polyunsaturated fatty acids increased bone formation in poultry. The discrepancy could be attributed the linolenic acid content and the $C_{18:2}$ to $C_{18:3}$ fatty acid ratios of the diets.

4.4. Health parameters

Our results showed that calves fed CFO and FO had lower rectal temperature than the CON group at the final day of study. The relatively lower rectal temperatures could be attributed to the lesser ratio of $C_{18:2}$ to $C_{18:3}$ fatty acids in CFO and FO groups and the probability of inflammatory effects of linoleic acid (Garcia Orellana, 2012). In line with this study, Hill et al. (2011b) reported that calves fed a blend of fatty acid supplements had lower rectal temperature in the normal range.

Hill et al. (2007a, 2011b) demonstrated that feeding linolenic acid has beneficial effects on health and immune system of calves. Our data showed that calves fed flaxseed oil (CFO or FO) had better fecal scores entire the study. Moreover, flaxseed oil supplementation (CFO or FO) reduced the days with diarrhea compared to the CON and PF groups. The CON and PF groups had higher overall fecal scores, which confirms the data of days with diarrhea. In accordance with Hill et al. (2007a, 2011b), our results indicated that calves with lower ratio of $C_{18:2}$ to $C_{18:3}$ in their starter diet experienced fewer days with diarrhea and consequently had greater final body weight and better feed efficiency.

4.5. Feeding behaviors

In the present study, feeding behaviors of calves was not affected by the different sources of fat and different ratios of $C_{18:2}$ to $C_{18:3}$. This finding was unexpected based on the results reported about the effect of fat supplements on dairy cows feeding behavior (Shingfield et al., 2003; Mattos et al., 2004).

Table 8
Effect of starter supplementation with different fat sources on concentration of plasma metabolites and enzyme of Holstein calves at three different ages (28, 56, and 70 d).

Item	Treatment[1]					SEM	P-value
	CON	PF	PCF	CFO	FO		
Calves, n	8	8	8	8	8		
Glucose, mg/dl							
28 d	101.00	114.13	99.20	115.00	100.12	9.745	0.31
56 d	86.58	82.19	79.38	80.75	86.83	6.882	0.28
70 d	87.95	90.43	87.75	87.37	90.45	7.247	0.67
Total protein, g/dl							
28 d	6.15	6.15	5.90	6.19	6.11	0.285	0.36
56 d	6.26	6.29	6.33	6.15	6.41	0.297	0.38
70 d	6.44	6.56	6.50	6.60	6.48	0.288	0.58
Albumin, g/dl							
28 d	3.02	3.15	3.08	3.18	3.04	0.167	0.32
56 d	3.19	3.17	3.45	3.23	3.20	0.172	0.13
70 d	3.17[c]	3.36[bc]	3.25[c]	3.60[b]	3.97[a]	0.172	< 0.01
Globular protein (total protein – albumin) (estimated), g/dl							
28 d	3.14	3.01	2.82	3.01	3.07	0.257	0.22
56 d	3.08	3.14	2.90	3.09	3.25	0.285	0.21
70 d	3.28[a]	3.29[a]	3.26[a]	2.86[ab]	2.52[b]	0.284	< 0.01
Creatinine, mg/dl							
28 d	0.91	0.87	0.85	0.87	0.95	0.092	0.30
56 d	0.78	0.75	0.72	0.74	0.68	0.092	0.27
70 d	0.80	0.78	0.73	0.77	0.71	0.092	0.32
Urea nitrogen, mg/dl							
28 d	25.91[a]	18.02[b]	21.54[ab]	21.34[ab]	21.01[ab]	3.193	0.01
56 d	19.91	16.77	21.92	20.87	18.88	3.318	0.11
70 d	30.29[a]	23.53[b]	27.05[ab]	27.74[ab]	22.13[b]	3.214	0.01
Alkaline phosphatase, units/l							
28 d	587	524	619	585	479	105.8	0.19
56 d	522	531	635	637	687	107.2	0.13
70 d	545[b]	758[a]	719[a]	617[a]	708[a]	106.3	0.04

[a-c] Means within a row with different superscripts differ ($P < 0.05$).
[1] Treatmets: CON = No fat supplements, PF = 2.2% Palm fat powder, PCF = 1.5% Palm fat powder + 0.8% Ca-salts of flaxseed oil, CFO = 2.7% Ca-salts of flaxseed oil, FO = 2.2% of flaxseed oil.

4.6. Ruminal fermentative parameters

It is believed that excessive amounts of polyunsaturated fatty acids in the rumen have adverse effects on microorganism function, and, therefore, have the potential to disturb ruminal fermentation (Maia et al., 2010; Liu et al., 2012; Ivan et al., 2013). However, our results showed that starter diet supplementation with low levels of palm oil and flaxseed oil (less than 3% starter diet) had no adverse effect on ruminal fermentative parameters.

4.7. Blood components

Our data indicated that glucose, total protein and creatinine at different stages of sampling were in the normal ranges proposed for young calves (Klinkon and Jezek, 2012) and it appears that the fat supplements had no adverse effect on body metabolism and renal function. The concentration of albumin, globular protein, urea nitrogen and alkaline phosphatase were affected by fat supplementation at the final day of blood sampling. Lower $C_{18:2}$ to $C_{18:3}$ (1.5:1) fatty acid ratios caused greater albumin and this could be attributed to better function of liver hepatocytes for albumin synthesis. It is well known that, during acute phases of inflammation, proteins (e.g. haptogobin, serum amyloid A) are a part of total protein which may be reduced by feeding starter diets with lower $C_{18:2}$ to $C_{18:3}$ fatty acid ratios (Klinkon and Jezek, 2012; Patterson et al., 2012). Measurement of urea concentration is very helpful for assessment of dehydration and disturbances of acid-base balance in calves with diarrhea (Klinkon and Jezek, 2012). Moreover, LeRoith et al. (2011) reported that urea nitrogen could be related to the protein synthesis and protein oxidation in the body, which confirms our finding about lower final body weight in the CON group (Table 3) with higher urea nitrogen. Calves grouped as CON had lower alkaline phosphatase compared to the calves fed different fat sources. Hill et al. (2007b, 2007c) demonstrated that the increase in alkaline phosphatase is an indicator of bone formation in young calves. Also they reported that increasing level of flaxseed oil in the starter diet linearly increased alkaline phosphatase which confirm our finding.

5. Conclusions

In the present study we used flaxseed oil as a potent supplement of linolenic acid to decrease $C_{18:2}$: $C_{18:3}$ ratios from 18:1 to 1.5:1 in a corn and soybean based starter diet. The findings of present study revealed that various sources of fat supplement (palm or flaxseed oil) and decreasing ratios of $C_{18:2}$ to $C_{18:3}$ fatty acids had some positive effects for Holstein calves. Moreover, there was no adverse effect on rumen fermentation parameters. Supplementing starter diets with Ca-salts of flaxseed oil (2.7%) or flaxseed oil (2.2%) decreased the $C_{18:2}$:$C_{18:3}$ ratios and had some beneficial effects on feed intake and intake of $C_{18:3}$ fatty acids, hip height, rectal temperature, fecal score, plasma albumin and alkaline phosphatase. However, determining the best and most appropriate concentration of Ca-salts of flaxseed oil or flaxseed oil in dairy calves' starter diet needs more investigation.

Conflicts of interest

None.

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