Title: Effect of supplementation fat during the last weeks of uterine life and preweaning time on performance, ruminal fermentation, blood metabolites, passive immunity and health of the newborn calf

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Running title: Strategic supplementation of essential fatty acids on calves' performance

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Abstract

The objective of this study was to evaluate the effects of supplementing essential fatty acids (FA), during late gestation and the preweaning and early weaning periods on passive immunity, growth, health, rumen fermentation parameters, blood metabolites, and behavior of dairy calves. During the last 3 weeks of pregnancy, cattle (n = 120), within parity, were randomly assigned to 1 of 3 diets (DD) with different fat supplements, no supplemental fat (CON), supplement rich in C18:2n-6 (CSO), or supplement rich in eicosapentaenoic and docosahexaenoic acid (CFO). Eighty-four newborn Holstein calves were randomly assigned, within the prepartum diets, to one of 2 calf starter (CS) diets: no fat supplement (FC-0) or 2% Ca-Salt of unsaturated FA (FC-2). Overall, the interaction between DD and CS did not affect calf performance and other measured parameters. Plasma concentrations of IgG and apparent efficiency of IgG absorption were improved ($P < 0.01$) in calves born from dams fed fat (n-6 or n-3) compared with those not fed fat. Calves born from cattle fed fat prepartum had greater average daily gain (ADG) compared with calves born from cattle fed no fat supplement prepartum (597 vs. 558 g/d, $P = 0.02$). Calves fed the FC-2 CS had greater ($P < 0.01$) ADG, feed efficiency, and weaning weight compared with those fed the FC-0 CS. Prepartum supplementation with fat reduced rectal temperature (RT) during pre-weaning time, but calves fed FC-2 CS had lower ($P \leq 0.04$) RT during pre- and postweaning periods. Calves in the FC-2 CS groups had fewer ($P < 0.001$) days with diarrhea. Time spent on eating, ruminating, standing, lying, and nonnutritive oral behavior exhibited no differences across treatments. Similarly, DD and CS did not affect ruminal fermentation parameters. Calves fed FC-2 CS had greater hip and wither heights ($P = 0.01$) during both pre- and postweaning periods. At 28 and 77 d of life, concentrations of plasma albumin and cholesterol ($P \leq 0.02$) were increased but, urea N decreased at the same time and alkaline phosphatase was greater only at the end of the study for calves fed FC-2. The findings suggest that moderate feeding of long-chain polyunsaturated FA during the last weeks of uterine life or during the preweaning time improve growth performance, health indices, and some blood components of calves.

Keywords: Holstein calf: Performance: Fatty acid: Peripartum diet

Abbreviations: ADF, acid detergent fiber; ADG, average daily gain; AEA, apparent efficiency of IgG; APT, Adequate passive transfer; BUN, blood urea nitrogen; BW, body weight; CFO, Ca-salts of fish oil; CP, crude protein; CS, calf starter; CSO, Ca-salts of soybean oil; DD, dam diet; DHA, docosahexaenoic acid; DM, dry matter; DMI, dry matter intake; EFA, essential FA; EPA, eicosapentaenoic acid; FA, fatty acids; FAT, contrast of fat supplement versus no fat supplement; (CSO + CFO vs. CON); FC, fat Ca-salts; FE, feed efficiency; NEFA, non-esterified fatty acid; MR, milk replacer; PUFA, polyunsaturated fatty acids; NDF, neutral detergent fiber; NH3-N, ruminal ammonia nitrogen; SFO, contrast of fatty acid source (CSO vs. CFO);VFA, volatile fatty acids.

INTRODUCTION

The cost of rearing replacement heifers is one of the highest investments in the dairy industry. Thus, proper nutritional and management practices must be placed to maximize the return of investment. Particularly, during the preweaning and early weaning period, the most critical goals are to minimize the incidence of diseases and mortality and maximize growth. Several studies have concluded that the benefits of proper preweaning management, leading to maximize average daily gain (ADG), have not only short- but also life-long impacts on heifers' performance[1,2].

The discovery of the essentiality of the fatty acids (FA) linoleic[3], and α-linolenic acids[4,5,6] led the livestock scientific community to further investigate the role of these essential FA (EFA) and their derivatives arachidonic acid (C:20:4 n-6) and eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), respectively. Fatty acids, are more than structural components of triglycerides, as components of cell membranes FA regulate signal transduction[7]. Several studies in humans and rodents have reported that n-3 compared with n-6 FA are more potent activators of genes involved in lipid oxidation, more potent reducers of genes encoding enzymes for lipid synthesis, and important regulators of immune response[8,9]. In dairy calves, the supplementation of a specific type of FA to late gestation cows have increased its concentrations and/or that of its derivatives in colostrum[10,11] and plasma of newborn

calves([12,13]). Nevertheless, preweaned calves fed milk replacer (MR) supplemented with EFA, regardless of in uterus diet, also reported increased concentrations of the corresponding EFA and/or that of its derivatives in plasma[13,14,15] and liver[15,16].

Previous studies have reported benefits of feeding diets enriched with EFA to late gestation cows on performance of newborn calves, Salehi *et al.*[17] reported ,to better concentrations of colostrum IgG and Garcia *et al.*[13] reported better efficiency of IgG absorption of calves fed supplemental enriched in EFA. Adequate passive transfer (APT) of IgG is crucial to minimize neonatal morbidity and mortality and strengthen calf immunity[18]. Supplementing long-chain FA prepartum in saturated or unsaturated forms may influence the FA profile of enterocytes that could affect the transfer of immunoglobulin to plasma of calves[10]. Furthermore, strategic supplementation of EFA in MR[13,19] or starter[20,21] of preweaned calves have improved growth, feed efficiency (FE), health, and immune status.

Therefore, the hypothesis of this study was that supplementing prepartum diets with Ca-salts of soybean oil (CSO) or Ca-salts of fish oil (CFO) would improve immune status, health, and growth of newborn calves to a greater extent than calves born to dams not supplemented with EFA or their derivatives. Another hypothesis was that calves born from dams supplemented with EFA or non-EFA would benefit in a greater extend from eating a starter grain mix supplemented with calcium salts of unsaturated FA. Therefore, the objective was to evaluate the effect of supplementing CSO enriched in C18:2n-6 and CFO enriched in EPA and DHA to Holstein cows in late gestation on metabolic profile, immune status, health, and growth of calves consuming starter diets enriched in unsaturated FA during pre- and postweaning periods.

Methods

Dam Management and Dietary Treatments

The experiment was conducted from July 2016 through October 2016, at a commercial dairy farm (Zarin Khoshe Dairy Farm, Arak district, Iran). All experimental procedures were conducted according to The Care and Use of Agricultural Animals in Research and Teaching[22] guidelines. The Animal Experiment Committee at Tehran University, Karaj, Iran approved all procedures and guidelines involving animals. The present study was part of a large research

project, with a portion of the results about the performance of the dams of calves enrolled in the present study were presented elsewhere[11]. Briefly, pregnant nulliparous (n = 42) and previously parous (n = 78) Holstein animals were blocked by parity and enrolled in the study starting at 3 wk before their calculated parturition date. The basal prepartum diet was formulated to have low concentrations of total FA (1.87% of dry matter (DM)), linoleic acid (0.84% of DM), and α-linolenic acid (0.36% of DM). Prepartum cattle were fed 1 of the following 3 diets: (1) no fat supplement (CON), (2) 1.15% of dietary DM as Ca-salts of soybean oil (CSO, 140 g/cow/daily) supplement (Persiafat, Kimiya Danesh Alvand Co. Tehran, Iran), or (3) 1.15% of dietary DM as Ca salts of fish oil (CFO, 140 g/cow/daily) supplement (Persiafat, Kimiya Danesh Alvand Co. Tehran, Iran). The CSO and SFO diets contained 1.30 and 0.87% of linoleic acid and 0 and 0.15% of EPA and EPA, respectively; and were isocaloric and greater in energy concentration than that of the CON diet. All diets were isonitrogenous. Ingredient and chemical composition of the prepartum diets and specific details on prepartum feeding and management were reported previously[11].

Calving Management and Colostrum, Milk and Starter Feeding

Calves were born in a sod-based pen. All dams were monitored for signs of calving initiation every 30 min between 05:30 to 15:30 h and then every 2 h between 15:30 and 05:30 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 in individual pens (1 × 1 m) equipped with a heat lamp before moving to individual wired pens (1 × 1.5 m) bedded with sand between 6 to 16 h of age. Cows were milked with a cow-side vacuum pump within 1 h after calving. Colostrum from each cow (4 L) was fed to their corresponding calf, regardless of IgG concentration. Remnant colostrum (>1 L and >50 g of IgG/L) was stored at -20°C to fed calves born from dams fed the same dietary treatment but producing low amounts of colostrum. Calves were considered to have APT if the plasma concentration of total IgG was ≥10 g/L after 24 h of colostrum feeding[23]. Whole colostrum samples were collected and frozen (−20° C) for later analyses of IgG concentration and FA profile, and results were reported elsewhere[11]. Plasma samples of newborn calves (at birth and 24 h after colostrum feeding) were measured for bovine total IgG concentration using

radioimmunodiffusion[10]. Intake of IgG was calculated using the concentration of IgG previously reported[11]. The apparent efficiency of IgG absorption (AEA, %) was calculated according to Quigley *et al.*[24] using the following equation: IgG concentration in plasma at 24 h of life (g/L) × [0.099 × BW (kg) at birth]/IgG intake (g). Plasma volume was estimated at 9.9% of birth weight[24]. Eighthly four Holstein calves were used in this study (n = 14 calves per factorial treatment, 10 males and 4 females) in a completely randomized design, with dietary treatments in a 3 × 2 factorial arrangement (3 dam diets (DD) and 2 calf starters; n = 6). Calves were fed 4 L from d 4 to 7 and then, 5, 6, 7, 6.5, 5.5, 4 and 2 L of warm (38°C) pasteurized tank milk per day, during weeks 2 to 8, respectively. The average nutritional composition of milk was (11.50 ± 0.2% DM, 2.98 ± 0.1% CP, 3.35 ± 0.1% fat and 4.51 ± 0.1% lactose). Calves were fed warm pasteurized milk twice per day (at 08:00 and 17:00 h) using steel buckets, starting at 3 d of age. Animals drank all offered milk. Calves had ad libitum access to one of two calf starter (CS) 1) no fat supplement (FC-0) or 2) supplemented with 2% Fat Ca-Salt rich in unsaturated FA (Persiafat, Kimiya Danesh Alvand Co. Tehran, Iran, Table 2). All calves were weaned at 56 d but the feeding of the experimental starter continued until 77 d of age. Starters were iso-nitrogenous, and the ingredients and composition are shown in Table 1. All calves had free access to clean and fresh water at all times. The offered starter was adjusted daily to achieve 5 to 10% orts (i.e., the portion of the starter not consumed over a 24 h period); orts were collected and weighed daily at 08:00 h. Chopped high-quality alfalfa hay (5% of starter grain mix) was included in the starter diets beginning at 30 d of age. The starter diets were offered once daily at 09:00 h.

Data Collection and Sampling

All calves in each group were used to sample calculations. Calves were weighed at birth, at 56 d, and at 77 d of age to measure changes in BW and determine ADG. In addition, calves were weighed once a week to observe growth patterns as calves aged. Body measurements were taken at 3 d of age, at weaning (d 56), and at the end of the study (d 77), in the morning and before feeding according to Lesmeister and Heinrichs[25]. Body measurements included heart girth (circumference of the chest), hip width (distance between the points of hook bones), body length (distance between the points of shoulder and rump), body barrel (circumference of the

belly before feeding), wither height (distance from the base of the front feet to wither), and hip height (distance from the base of the rear feet to the hook bones).

Starter offered and refused were recorded daily throughout the experiment. Feed samples were collected weekly and stored at -20°C for chemical analyses. Subsamples of feeds and refusals were mixed thoroughly, dried at 55°C for 48 h and ground to pass through a 1 mm screen in a hammer mill (Arthur Hill Thomas Co., Philadelphia, PA) before chemical analyses for DM (method 934.01), crude protein (method 988.05), ether extract (method 920.39)[26]. The neutral detergent fiber contents were analyzed according to Van Soest *et al.*[27], assayed with sodium sulfite, without alpha-amylase, and was expressed with residual ash. Eight representative samples of milk were collected throughout the study and analyzed for DM, fat, CP, and lactose content by infrared spectroscopy (Foss Electric, Hillerod, Denmark). Feed efficiency (kg of BW gain/kg of total dry matter intake (DMI)) was calculated for the preweaning period, d 57 to 77, and for the whole study. Total DMI corresponds to the amount of DM intake contributed by the starter diet (grain mix and alfalfa hay) and by the DMI contributed by the fixed amounts of milk provide to calves.

Total-tract apparent digestibility of DM, organic matter, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and ether extract were determined using acid-insoluble ash as an internal marker[28]. Fecal samples were obtained twice a day (in the morning and the afternoon) during 4 consecutive days (from d 74 to 77). Fecal samples were dried at 55 °C in a forced-air oven, ground to pass a 1-mm screen in a Wiley mill (Arthur Hill Thomas Co., Philadelphia, PA), and then mixed thoroughly.

Samples of ruminal fluid (approximately 150 ml) were collected from each calf on d 30 and 70, about 3 h after the morning feeding, using a stomach tube attached to an Erlenmeyer flask and vacuum pump; the first 50 mL was discarded to avoid possible saliva contamination. The ruminal fluid was filtered through four layers of cheesecloth, and pH was determined immediately using a portable pH meter (Sentron, model A102-003). Approximately 25 ml of filtrate was then preserved by adding 5 ml of 25% metaphosphoric acid solution for later determination of volatile FA (VFA), and approximately 20 ml of filtrate was combined with 20

ml 0.2 N HCl for later measurement of ammonia N concentration. The prepared samples were stored at -20°C, and then thawed for analysis of respective compounds. Stored samples were centrifuged at 1200 × *g* for 10 min, and the supernatant fluid was analyzed for VFA by gas chromatography (Hewlett-Packard, model 5890, Avondale, PA). Ruminal ammonia nitrogen (NH3-N) concentration was determined according to procedures described by Crooke and Simpson[29]. Blood samples were collected immediately after birth, 24 h after colostrum feeding, and then 3 h after the morning meal on d 28, 56, and 77 of age. Blood was taken from the jugular vein by evacuated tubes containing K2 EDTA (10.5 mg, Monoject, Sherwood Medical, St. Louis, MO). Samples were immediately chilled, and then plasma was obtained by centrifugation at 3000 × *g* for 15 min and stored at -20°C. Plasma concentrations of glucose, total protein, albumin, alkaline phosphatase (ALP), cholesterol, and triglycerides were determined using an automatic microplate Reader (EON-BIOTEK, America) and commercial kits (Pars Azmoon, Tehran, Iran). Enzymatic assays were employed for analyses of plasma insulin (DRG, Marburg, Germany), blood urea nitrogen (BUN) (Pars Azmoon, Tehran, Iran) and non-esterified fatty acids (NEFA) (Randox Laboratories Ltd., London, UK) in an automatic spectrophotometer (Clima Plus, RAL, Madrid, Spain) following the manufacturer's instructions. Behavioral data (eating, ruminating, standing, lying, and nonnutritive oral behavior) were monitored by direct observations of all the calves over the total time (min) devoted to each monitored behavior once at the last week of the preweaning period (35-42 d) and once at the last week of the postweaning period (70–77 d)[30]. Calves were observed 8 h immediately following the morning milk feeding (at 08:00 h) during the preweaning week and 8 h after the solid feed was fed (09:00 h), during the postweaning week. Thus, the total time for each calf behavior was equal to 16 h (consisting of 8 h before and 8 h after weaning).

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 CS 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). Attitude scores recorded were (0) responsive, (1) nonactive, (2) depressed, or (3) moribund. Fecal consistency scores recorded were (0) firm, no diarrhea; (1) moderate, soft, no diarrhea; (2)

runny feces, mild diarrhea; or (3) watery feces, diarrhea. Nasal score scale of calves was also scored on a 0–3 scale, with 0 being normal serous discharge; 1 being a small amount of unilateral cloudy discharge; 2 being bilateral cloudy or excessive mucus discharge, and 3 being copious bilateral mucopurulent discharge. 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. Respiration rate (RR) was measured at 14:00 h on d 30, 45, and 70, by visual observation for three 1-min periods (not consecutive) according to the methods described by Yari *et al.*[31]. Rectal temperature (RT) was measured weekly before morning meals using a rectal thermometer (Yasa Teb Co., Isfahan, Iran) and calves with rectal temperature over 39.5 °C were categorized as febrile. 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. All calves remained healthy and exhibited no signs of illness during the experiment.

Statistical Analyses

Statistical analyses were conducted for 4 periods: the first day of life (passive immunity parameters), preweaning (d 0 to 56), postweaning (d 57 to 77), and the entire period (d 0 to 77). Except for starter intake and measures of structural growth which the first measure was performed at d 3 of life. The study was a complete randomized design, with dietary treatments in a factorial arrangement 3 × 2 (3 dam diets and 2 calf starter; n = 6 treatments). AThe mixed model equation for passive immune variables was defined as: $Yijk = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$, where Y*ijk* is the dependent variable, μ is the population mean, αi is the treatment effect, βj is the fixed effect of parity, $(\alpha\beta)_{ij}$ is the interaction effect of treatment and parity, and e_{ijk} is the residual error. For passive immunity variables, preplanned contrast analysis was used to compare means for fat supplement versus no fat supplement (FAT) and CSO versus CFO (SFO). Single degree of freedom contrasts of the 2 treatment contrasts (FAT and SFO) with parity were performed.

For other continuous variables (rumen fermentation parameters, plasma metabolites, BW, skeletal growth, behavior data, rectal temperature, respiratory rate, and apparent nutrient

digestibility) the data were analyzed using the PROC MIXED procedure of SAS[32]. Fecal and health scores were analyzed using a multivariable logistic mixed model (GLIMMIX procedure of SAS). The following model was used for variables with repeated measures, for those with non-repeated measures the effect of time and its interaction were omitted:

$$Y_{ijklm} = \mu + D_i + C_j + T_k + DC_{ij} + DT_{ik} + CW_{jk} + DCT_{ijk} + + \varepsilon_{ijk},$$

Where Y_{ijklm} is the observation; μ is overall mean; D_i is the fixed effect of dam diet (CON, CSO, or CFO); C_j is the fixed effect of CS (FC-0 or FC-2); T_k is the effect of time; DC_{ij} is the interaction of dam diet × CS; Dt_{ik} is the effect of the interaction between DD and week; Ct_{jk} is the effect of the interaction between CS and week; DCt_{ijk} is the 3-way interaction of DD, CS, and time; and ε_{ijk} is the residual error. Five orthogonal contrasts of dam diet, CS, and interactions were tested for variables measured after initiation of starter feeding. Contrasts testing the effects of dam diet were (1) FAT: control versus CFO + SFO and (2) SFO: CSO versus CFO, contrast for the effect of calf starter 3) FC-0 versus FC-2, and the contrasts testing the interactions of dam diet and calf starter (4) FAT × CS (contrasts 1 and 3) and (5) SFO × CS (contrasts 2 and 3).

The denominator degrees of freedom were computed using the approximation described by Kenward and Roger[33]. Significance was declared at $P \leq 0.05$ and trends at $0.05 < P < 0.10$. Tukey's adjustment was used for multiple comparison tests.

Results

Calf Absorption of Colostral IgG

Total IgG intake was greater in calves born for dams feed fat (CSO or CFO) compared with calves born from dams fed CON (504 vs. 392 g, $P < 0.01$, Table 3). Concentrations of total IgG (+43%) and total IgG, when expressed as a proportion of TP (+30%), were also greater ($P < 0.01$) in calves born from dams fed fat compared with those born from CON-fed dams. Calves born from dams fed fat had better ($P < 0.01$) AEA than calves born from control dams (27.7 vs. 22.2%).

Starter Intake and Growth Performance

Body weight at birth was not impacted by prepartum diets, but BW at weaning (56 d) and 77 d were impacted by prepartum diets (Table 4). Weekly BW, as affected by prepartum diets,

started to differentiate at 8 wk of age, and indeed, calves born from dams fed either CSO or CFO were heavier than those born from dams fed CON (Figure 1A). For ADG, calves born from dams fed fat supplement prepartum had greater ADG over the pre- (542 vs. 497 g/d; $P = 0.01$, Table 4) but not during the post-weaning period. The benefit of prepartum fat feeding observed during the preweaning period had a direct impact on the overall benefit of diets in ADG for the whole experimental period (597 vs. 558 g/d; $P = 0.02$). Regardless of dam diet, calves fed FC-2 had consistently greater ADG compared with calves fed the FC-0 CS during the pre and post-weaning periods, and the overall period. The better AGD is consistent with the heavier BW observed in calves fed FC-2 starting at 7 wk of age (Figure 1B). Starter intake was not affected by prepartum or starter diets at any evaluated period. Feed efficiency tended ($P = 0.06$) to be better for preweaned calves born from dams fed fat supplements compared with those born from CON-fed dams. Regardless of dam diets, calves fed FC-2 had better FE compared with calves fed FC-0 CS, during the preweaning, post-weaning, and the overall experimental period. Neither dam diets, not calf starter affected total-tract apparent digestibility of DM, CP, OM, EE, ADF, and NDF, measured from 74 to 77 d of age.

Body measurements data are presented in Table 5. At 3 d of age, none of the parameters of structural growth were affected by diets or their interaction. Body length, heart girth, hip width, and body barrel were not affected by dam diet, calf starter, or their interaction. At weaning and the end of the study, wither and hip heights were greater ($P \leq 0.05$) in calves born from dam fed the fat compared with calves born from dam fed the CON diet prepartum. Regardless of dam diet, calves fed the FC-2 CS had greater ($P < 0.01$) wither and hip heights compared with calves fed the FC-0 CS at weaning and the end of the study.

Animal Behavior

The total times devoted to different behaviors in an 8-h evaluation period are presented in Table 6. Neither prepartum diets nor calf starter affected time spent eating, ruminating, standing, lying, or on any nonnutritive oral behavior.

Incidence of Diarrhea and Diseases

During the pre-weaning period, calves born from dams fed fat had a slightly lower ($P < 0.01$) rectal temperatures than those born from CON-fed dams (39.0 vs. 39.2 °C, respectively, Table 7), but the effect did not extend to the post-weaning period. Regardless of dam diet, calves fed FC-2 had lower ($P \leq 0.04$) rectal temperature compared with calves fed the FC-0 CS during both the pre- and post-weaning periods. Mean scores for attitude and nasal discharge were 0.28 and 0.10 and were not affected by experimental treatments. Fecal score and days with diarrhea were lower ($P < 0.01$) in calves fed FC-2 compared with those fed FC-0 CS.

Blood Metabolites

Dam diet did not affect the mean plasma concentrations of any measured metabolite (Table 8), except cholesterol for which concentrations were greater ($P = 0.02$) in calves born from dams fed fat compared with CON. Mean plasma concentrations of glucose, total protein, NEFA, insulin, and triglycerides were not different between calves fed FC-0 or FC-2 ($P > 0.10$). However, concentrations of albumin and cholesterol in calves fed FC-2 were greater ($P < 0.001$) at 28 and 77 but not at 56 d of age compared with calves fed FC-0 CS. Plasma urea N was lower ($P < 0.001$) in calves fed FC-2 at 28 and 77 d of age. Alkaline phosphatase was greater (600 vs. 537 units/L) $P < 0.001$) in calves fed FC-2 in plasma measured at the end of the study only.

Rumen Fermentation Parameters

Concentrations of ruminal pH, ammonia, and individual and total VFA were not affected by dam diet, calf starter, or their interactions (Table 9).

Discussion

Effect of Prepartum Diets

Contrary to our hypothesis, the type of FA fed prepartum did not have any impact on calves' performance, neither affected the responses to the different CS provided during the preweaning period (lack of interactions DD × CS). However, the supplementation of fat regardless of the type of FA (rich in linoleic acid [CSO] or EPA and DHA [CFO]) had a notorious impact in calf immune status and performance.

As expected, plasma concentrations of total IgG at birth were very low (≤ 0.32 g/L) and in most cases undetectable. Only 1 calf in this study failed to attain at least 10 g/L IgG after colostrum feeding, and it was born from a CON-fed dam. Twenty-four hours after colostrum feeding, plasma IgG concentrations were higher in calves born from dams fed fat supplement, due to more IgG intake, which reflected the greater IgG content in colostrum of cows fed fat compared with CON[11], which also resulted in calves born from dams fed fat having better AEA. Our findings support those who reported that calves born from dams fed fat (saturated or rich in n-6 FA) tended to have greater serum concentrations of total IgG and better AEA after colostrum feeding than calves born from dams fed no-supplemental fat[10]. Factors that may influence AEA include a) time after birth when colostrum is fed, b) amount of IgG fed, c) rate of gastric emptying, and d) absorptive capacity of enterocytes for IgG [10]. In the current study, feeding time was constant and adequate. Quigley *et al.*[34] recommended the ingestion of 150 to 200 g of IgG in the first 24 h to reduce the risk of failure of passive transfer. In the current study calves born from dams fed fat received 28% more IgG than those born from dams fed CON. The present study did not aim to investigate the specific absorptive capacity of enterocytes but a potential benefit of the FA profile in their membrane, affected by the prepartum dietary composition of FA, should not be ruled out.

In the present study adding fat supplements prepartum did not affect starter intake but calves born from dams fed fat compared with those born from CON-dams had a better ADG preweaning, and it was maintained across the 77 d of the study. Therefore, the better BW gain observed in calves born from dams fed fat resulted in a trend for an improved FE. The improved ADG and FE observed in calves born from dams fed fat may be due to meeting metabolic demands for elongated polyunsaturated fatty acids (PUFA) in those calves[16], and may not be due to a better digestibility of nutrients, as dam diets did not impact apparent nutrient digestibility and VFA production. Although hip and wither heights at birth were similar among calves born from dams fed any prepartum diet, calves born from dams fed fat were taller than those born from dams fed CON at weaning (56 d) and the end of the study (77d). This delayed impact of in uterus exposure on skeletal growth, coupled with better ADG also observed in calves born from dams fed fat, maybe a reflect of a better health and immune status of calves

born from dams fed fat, as they received more IgG from their dams, resulting in greater plasma IgG after colostrum intake, and nevertheless had lower rectal temperatures during the preweaning period, compared with calves born from dams fed CON. Although prepartum feeding did not alter birth weight, a potential epigenetic effect of dam diets (e.g., fat supplementation) on post-birth calf metabolism, can't be ruled out. Indeed, others have also reported this potential mechanism with strategic feeding of FA in the uterus[35,36].

Previously, we found that plasma cholesterol was greater in prepartum cows fed fat (CSO or CFO) compared with cows fed CON[11]. In the current study, cholesterol concentrations were higher in calves born from dams fed fat during the first 28 d of life. Researchers have reported the transfer of maternal cholesterol across the placenta and reaching fetal circulation, contributing substantially to the fetal cholesterol pool in animals and humans[37,38]. Similar transfer mechanism may occur in calves born from dams fed fat, and it may carry over the first weeks of life.

Effect of Preweaning Diets

In the present study adding fat supplements to the starter did not affect its intake. Hill *et al.*[21] reported that preweaning calves fed a starter diet with tallow or soybean oil had similar starter intake, but postweaning, calves fed soybean oil had reduced starter intake. These results are in accordance with Kadkhoday *et al.*[39], who found that feeding starter diets with different ratios of C18:2 and C18:3 did not affect starter intake of Holstein dairy calves. Therefore, the better BW gain observed in the current study, without changes in starter or milk intake, resulted in an improved FE of calves fed FC-2 compared with those fed FC-0. The improved ADG and FE may be due to meeting metabolic demands for elongated PUFA in FC-2 CS-fed calves[16]. Hill *et al.*[20] reported that supplementing C18:3 (linolenic acid) as Ca-salts of flaxseed oil to starter diets of dairy calves less than 3 mo old resulted in increased ADG and FE.

Wither and hip heights at weaning and the end of the study were also greater for calves fed FC-2. Recent studies have reported that n-3 FA have a regulatory role in neonatal bone turnover in humans and different animal models[40,41]. In preweaned calves, studies feeding saturated or n-

6 FA reported no changes in skeletal growth[42]. Others feeding a lower ratio of n-6 to n-3 FA reported a greater hip height compared with calves fed with no fat supplement[39].

Many authors have reported that moderate concentrations of FA have beneficial effects on health and immune system function of dairy calves[43,44,16]. The acute inflammatory response is protective; however, an unresolved inflammation could lead to amplified inflammation and chronic diseases. Currently, it is well understood that an acute inflammation has a temporal peak and should, via chemical mediators, be resolved to enable tissue function's restorage[45]. The FC-2-fed calves had lower RT and lower overall fecal scores, which resulted in calves fed FC-2 having a shorter number of days with diarrhea (-2 d) compared with calves fed FC-0. Days with abnormal fecal scores decreased by the addition of a FA blend (butyrate, coconut oil, and flax oil) or (butyrate, medium-chain fatty acids, and linolenic acid) to the MR[44,46]. Similarly, others have reported a reduction in fecal scores when feeding starters with flaxseed oil or FA blend (coconut oil and canola oil) to the MR[39,19]. These beneficial effects of health-related parameters observed in calves fed FC-2 may have elicited a positive impact on growth rate and FE of calves fed FC-2.

Animal behavior was similar across treatments, and all calves spent similar times eating, ruminating, standing, lying, or in nonnutritive oral behavior during both pre- and post-weaning periods. To the best of our knowledge, animal behavior has not been measured in experiments where dietary treatments consisted of different fat contents in utero and pre- and early post-weaning. Nevertheless, Kadkhoday *et al.*[39] reported that adding palm fat powder or flaxseed oil or both to starter diets had no adverse effect on the feeding behaviors of Holstein calves during pre and postweaning periods. On the other hand, in mature ruminants, Benson *et al.*[47] reported that daily time spent engaged in eating, ruminating, and idling activities were not affected by abomasal infusion of a mixture of sunflower and rapeseed oils in dairy cows.

The plasma metabolic profile of calves was affected by the feeding of a calf starter rich in unsaturated FA (FC-2) compared with a starter without supplemental fat (FC-0). The liver plays a key role in nutrient metabolism and utilization due to its strategic position of integration within the circulatory system[35]. Garcia *et al.*[35] reported that calves fed diets rich in n-6 compared

with saturated FA upregulated the expression of hepatic genes involved in protein synthesis. In the current study, calves fed FC-2 had higher plasma albumin concentrations, which may represent a better hepatic function. Similarly, the addition of a FA blend to MR showed an increase in serum albumin concentration[46]. The BUN-reducing effect of feeding FC-2 compared with FC-0 may be due to an improvement in protein deposition and reduction in protein oxidation, which is supported by higher concentrations of albumin and better ADG and skeletal growth in calves fed FC-2. Moreover, Le Roith *et al.*[48] reported that lower urea N could be related to changes in protein synthesis and protein oxidation, which is confirmed by our current findings.

Cholesterol is synthesized primarily in the liver of preruminant calves as products of lipid metabolism[49]. The observed higher concentrations of cholesterol in plasma of calves fed FC-2 may simply reflect the preferential esterification of cholesterol esters with high intake of unsaturated FA (FC-2) compared with no fat supplement (FC-0). Previous studies in preweaned calves reported different impacts of FA supplemented in MR, linoleic acid-enriched MR reduced plasma cholesterol concentrations compared with lauric acid-enriched MR[13], whereas, soybean oil-enriched MR quadratically increase plasma concentrations of cholesterol[16]. Nevertheless, in studies in humans and rodents, n-3 FA supplementation elicits a hypocholesterolemic effect[50,51]. Plasma concentrations of ALP were greater in calves fed FC-2 CS at the end of the study. Hill *et al.*[52-53] reported that the increase in ALP is a good index of bone formation in young calves. Also, other researchers reported that adding flaxseed oil in the starter diet increased ALP[39,20]. These results are in accordance with our finding in skeletal growth, which indicated that hip and wither height were greater in calves fed FC-2 CS.

Feeding a starter rich in unsaturated FA compared with non-supplemental fat did not alter any ruminal parameter. The mean ruminal pH values were within normal physiological values and ranged from 5.4 to 6.2, reflecting no adverse effect of diets on ruminal fermentation. Others have also reported that extruded full-fat soybean did not alter ruminal pH in dairy calves[54]. Several authors have reported that high amounts of PUFA in the rumen of mature cattle have adverse effects on microorganism function, and led to disturbed ruminal fermentation[55,56]. In the current study, the inclusion of 2% of Ca-salts of unsaturated FA (rich in C16:1, C18:2, and

C18:3), which increased the % of ether extract to 5.1% compared with 3.35% in the non-Ca-salts supplemented FA (FC-0) did not alter ammonia, specific VFA, or total VFA concentrations, which may indicate that cellulolytic bacterial populations were not affected by feeding fat to calves. Limited information is available on the role of dietary FA on ruminal fermentation in dairy calves. Kadkhoday *et al.*[39], showed that starters supplemented with low levels of palm oil or flaxseed oil (less than 3% starter diet) had no adverse effect on ruminal fermentative parameters.

Finally, there is increased consumer awareness that foods contain fatty acids that may have beneficial effects on health maintenance and disease prevention. Both quantitative and qualitative aspects of dietary lipid intake exert important influence upon human health and the expression of chronic disease. Thus, fatty acids contents of animal production (meat and milk) could have beneficial effects on the human health.

Conclusions

Strategic feeding of diets enriched in PUFA (n-6 or n-3 FA) to pregnant nonlactating dams (1.15% of dietary DM) or a starter rich in unsaturated FA to preweaned Holstein calves (2% of CS DM) impacted calf performance. Prepartum supplementation of fat improved passive immunity, overall ADG, weaning weight, final weight, hip height and wither height of calves without changing DMI. However, prepartum diets did not influence the effect of CS on calf performance or other measured parameters in the present study. Calves fed a starter enriched in unsaturated FA compared with non-supplemented calves, had better ADG and FE, greater hip height, lower rectal temperature, fewer days with diarrhea, and higher plasma albumin and ALP. Further research is needed to increase the understanding of the effects of fat supplemented during the last weeks of uterine life and preweaning period on inflammatory status and response of immune cells of preweaned calves.

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The authors declare that there are no conflicts of interest.

References

1. Soberon F & Van Amburgh M (2013) Lactation Biology Symposium: The effect of nutrient intake from milk or milk replacer of preweaned dairy calves on lactation milk yield as adults: a meta-analysis of current data. *J Anim Sci* 91, 706-712.
2. Gelsinger S, Heinrichs A & Jones C (2016) A meta-analysis of the effects of preweaned calf nutrition and growth on first-lactation performance. *J Dairy Sci* 99, 6206-6214.
3. Burr GO & Burr MM (1930) On the nature and role of the fatty acids essential in nutrition. *J Biol Chem* 86(2), 587-621.
4. Duttaroy A K (2000) Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta. *Am J Clin Nutr* 71, 315S– 322S.
5. Neuringer M, Anderson GJ & Connor WE (1988) The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Annu Rev Nutr* 8, 517−541.
6. Anderson GJ & Connor WE (1989) On the demonstration of omega-3 essential-fatty-acid deficiency in humans. *Am J Clin Nutr* 49, 585–587.
7. Simons K & Ikonen E (1997) Functional rafts in cell membranes. nature. 387:569–572.
8. Clarke, S. D., 2000. Polyunsaturated fatty acid regulation of gene transcription: a mechanism to improve energy balance and insulin resistance. *Br J Nutr* 83, 59−S66.
9. Sampath H & Ntambi JM (2005) Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Annu Rev Nutr* 25, 317-340.
10. Garcia M, Greco LF, Favoreto MG, *et al.* (2014) Effect of supplementing fat to pregnant nonlactating cows on colostral fatty acid profile and passive immunity of the newborn calf. *J Dairy Sci* 97, 392–405.
11. Jolazadeh AR, Mohammadabadi T, Dehghan-banadaky M, *et al. (*2019) Effect of supplementing calcium salts of n-3 and n-6 fatty acid to pregnant nonlactating cows on

colostrum composition, milk yield, and reproductive performance of dairy cows. *Anim Feed Sci Technol* 247, 127–140.

12. Moallem U & Zachut M (2012) Short communication: The effects of supplementation of various n-3 fatty acids to late-pregnant dairy cows on plasma fatty acid composition of the newborn calves. *J Dairy Sci* 95, 4055−4058.
13. Garcia M, Greco LF, Favoreto MG, *et al.* (2014) Effect of supplementing essential fatty acids to pregnant nonlactating Holstein cows and their preweaned calves on calf performance, immune response, and health. *J Dairy Sci* 97, 5045–5064.
14. Jenkins KJ, Kramer JK. Sauer G, *et al.* (1985) Influence of triglycerides and free fatty acids in milk replacers on calf performance, blood plasma, and adipose lipids. *J Dairy Sci* 68, 669−680.
15. Jenkins KJ & Kramer JKG (1986). Influence of low linoleic and linolenic acids in milk replacer on calf performance and lipids in blood plasma, heart, and liver. *J Dairy Sci* 69, 1374−1386.
16. Garcia M, Shin JH, Schlaefli A, *et al*. (2015) Increasing intake of essential fatty acids from milk replacer benefits performance, immune responses, and health of preweaned Holstein calves. *J Dairy Sci* 98, 458-477.
17. Salehi R, Ambrose DJ & Oba M (2016) Short communication: effects of prepartum diets supplemented with rolled oilseeds on Brix values and fatty acid profile of colostrum. *J Dairy Sci* 99, 3598–3601.
18. Quigley JDIII & Drewry JJ (1998) Nutrient and immunity transfer from cow to calf pre- and post-calving. *J Dairy Sci* 81, 2779–2790.
19. Hill T, Aldrich J, Schlotterbeck R, *et al.* (2007) Effects of changing the fat and fatty acid composition of milk replacers fed to neonatal calves. *Prof Anim Sci* 23, 135–143.
20. Hill T, Bateman H, Aldrich J, *et al.* (2009) Effects of changing the essential and functional fatty acid intake of dairy calves. *J Dairy Sci* 92, 670–676.
21. Hill TM, Bateman HG, Aldrich JM, *et al.* (2015). Inclusion of tallow and soybean oil to calf starters fed to dairy calves from birth to four months of age on calf performance and digestion. *J Dairy Sci* 98, 4882-4888.

22. Federation of Animal Science Societies (2010) Guide for the care and use of agricultural animals in research and teaching, 3rd ed., Champaign, IL.
23. Tyler JW, Hancock DD, Parish SM, *et al.* (1996) Evaluation of 3 assays for failure of passive transfer in calves. *J Vet Intern Med* 10, 304−307.
24. Quigley JD, Drewry IIIJJ & Martin KR (1998) Estimation of plasma volume in Holstein and Jersey calves. *J Dairy Sci* 81, 1308−1312.
25. Lesmeister KE & Heinrichs AJ (2005) Effects of adding extra molasses to a texturized calf starter on rumen development, growth characteristics, and blood parameters in neonatal dairy calves. *J Dairy Sci* 88, 411–418.
26. Association of Official Analytical Chemists, AOAC (1990) Official Methods of Analysis, 15th ed. Washington, DC.
27. Van Soest Pv, Robertson J & Lewis B (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J Dairy Sci* 74, 3583−3597.
28. Van-Keulen J & Young BA (1977) Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *J Anim Sci* 44, 282–289.
29. Crooke W & Simpson W (1971) Determination of ammonium in Kjeldahl digests of crops by an automated procedure. *J Sci Food Agric* 22, 9-10.
30. Castells L, Bach A, Araujo G, *et al.* (2012) Effect of different forage sources on performance and feeding behavior of Holstein calves. *J Dairy Sci* 95, 286–293.
31. Yari M, Nikkhah A, Alikhani M, *et al.* (2010) Physiological calf responses to increased chromium supply in summer. *J Dairy Sci* 93, 4111-4120.
32. SAS (2003) SAS User′s Guide: Statistics. Ver 9.0. SAS Institute, Cary, NC, USA956.
33. Kenward MG & Roger JH (1997) Small sample inference for fixed effects from the restricted maximum likelihood. *Biometrics* 53, 983–997.
34. Quigley J, Kost CJ & Wolfe TM (2002) Absorption of Protein and IgG in Calves Fed a Colostrum Supplement or Replacer. *J Dairy Sci* 85, 1243-1248.

35. Garcia M, Greco LF, Lock AL, *et al*. (2016) Programming effect of dietary fatty acids on performance of Holstein heifers from birth through first lactation. *Anim Feed Sci Technol* 222, 64-74.
36. Garcia M, Greco LF, Lock AL, *et al.* (2016) Supplementation of essential fatty acids to Holstein calves during late uterine life and first month of life alters hepatic fatty acid profile and gene expression. *J Dairy Sci* 99, 7085-7101.
37. Woollett LA (2005) Maternal cholesterol in fetal development: transport of cholesterol from the maternal to the fetal circulation. *Am J Clin Nutr* 82, 1155-1161.
38. Yoshida S & Wada Y (2005) Transfer of maternal cholesterol to embryo and fetus in pregnant mice. *Lipid Res* 46, 2168-2174.
39. Kadkhoday A, Riasi A, Alikhani M, *et al*. (2017) Effects of fat sources and dietary C18: 2 to C18: 3 fatty acids ratio on growth performance, ruminal fermentation and some blood components of Holstein calves. *Livest Sci* 204, 71-77.
40. Lau BY, Cohen DJ, Ward WE, *et al.* (2013) Investigating the role of polyunsaturated fatty acids in bone development using animal models. *Molecules* 18, 14203-14227.
41. Kajarabille N, Peña M, Díaz-Castro J, *et al.* (2018) Omega-3 LCPUFA supplementation improves neonatal and maternal bone turnover: A randomized controlled trial. *J Funct Foods* 46, 167-174.
42. Ghasemi E, Azad-Shahraki M & Khorvash M (2017) Effect of different fat supplements on performance of dairy calves during cold season. *J Dairy Sci* 100, 5319-5328.
43. Maddock T, Bauer M, Koch K, *et al.* (2006) Effect of processing flax in beef feedlot diets on performance, carcass characteristics, and trained sensory panel ratings. *J Anim Sci* 84, 1544–1551.
44. Hill T, VandeHaar M, Sordillo L, *et al.* (2011) Fatty acid intake alters growth and immunity in milk-fed calves. *J Dairy Sci* 94, 3936–3948.
45. Serhan CN & Levy BD (2018) Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. *J Clin Invest* 128, 2657-2669.
46. Esselburn KM, O'Diam KM, Hill TM, *et al.* (2013) Intake of specific fatty acids and fat alters growth, health, and titers following vaccination in dairy calves. *J Dairy Sci* 96, 5826-5835.

47. Benson JA, Reynolds CK, Humphries DJ, *et al.* (2001) Effects of abomasal infusion of long-chain fatty acids on intake, feeding behavior and milk production in dairy cows. *J Dairy Sci* 84, 1182-1191.
48. Le Roith T, Hammond S, Todd S, *et al.* (2011) A modified live PRRSV vaccine and the pathogenic parent strain induce regulatory T cells in pigs naturally infected with Mycoplasma hyopneumoniae. *Vet Immunol Immunopathol* 140, 312–316.
49. Drackley JK (2005) Interorgan lipid and fatty acid metabolism in growing ruminants. Pages 323–350 in Biology of Metabolism in Growing Animals. D G, Burrin & HJ, Mersmann, ed. Elsevier Limited, Edinburgh, UK.
50. Maki KC, Bobotas G, Dicklin MR, *et al.* (2017) Effects of MAT9001 containing eicosapentaenoic acid and docosapentaenoic acid, compared to eicosapentaenoic acid ethyl esters, on triglycerides, lipoprotein cholesterol, and related variables. *J Clin Lipidol* 11, 102-109.
51. Drouin G, Catheline D, Guillocheau E, et al. (2019) Comparative effects of dietary n-3 docosapentaenoic acid (DPA), DHA and EPA on plasma lipid parameters, oxidative status and fatty acid tissue composition. *J Nutr Biochem* 63, 186-196.
52. Hill T, Aldrich J, Schlotterbeck R, *et al.* (2007) Amino acids, fatty acids, and fat sources for calf milk replacers. *Prof Anim Sci* 23, 401–408.
53. Hill T, Aldrich J, Schlotterbeck R, et al. (2007) Effects of changing the fatty acid composition of calf starters. *Prof Anim Sci* 23, 665–671.
54. ZeidAli-Nejad A, Ghorbani GR, Kargar S, *et al.* (2018) Nutrient intake, rumen fermentation and growth performance of dairy calves fed extruded full-fat soybean as a replacement for soybean meal. *animal* 12, 733-740.
55. Liu S, Bu D, Wang J, *et al.* 2012. Effect of incremental levels of fish oil supplementation on specific bacterial populations in bovine ruminal fluid. *J Anim Physiol Anim Nutr* 96, 9–16.
56. Ivan M, Petit H, Chiquette J, *et al.* 2013. Rumen fermentation and microbial population in lactating dairy cows receiving diets containing oilseeds rich in C-18 fatty acids. *Br J Nutr* 109, 1211–1218.

Table 1

Ingredients and nutrient composition (g/kg DM unless otherwise noted) of calf starters*

Item	Calf starter	
	FC-0	FC-2
Ingredient (g/kg DM)		
Corn, ground	500	480
Barley, ground	100	100
Corn gluten meal	40	40
Beet pulp dry	90	90
Extruded soybean seed	20	20
Soybean meal	200	200
Fat Ca-salts†	0	20
Calcium carbonate	7.0	7.0
Sodium bicarbonate	10	10
Vitamin–mineral premix‡	10	10
White salt	5.0	5.0
Monensin sodium 10%	1.0	1
Sodium bentonite	12	12
Dicalcium phosphate	5.0	5.0
Nutrient composition (g/kg DM)		
CP	20.10	19.92
NDF	14.85	14.62
ADF	7.82	7.71
NFC§	53.80	52.40
Ether extract	3.35	5.10
ME‖ (Mj/kg DM)	11.13	11.55
NEm‖ (Mj/kg DM)	7.24	7.65
Neg‖ (Mj/kg DM)	4.64	5.06

* Calves starters are defined as containing no fat supplement (FC-0) and supplementation with 2% Fat Ca-Salt (FC-2).

† Fat Ca-salts, Persiafat, Kimiya Danesh Alvand Co. Iran., contained 85% fat and 9% Ca (1% C14.0, 28% C16:0; 3% C16:1; 5% C18:0, 26% C18:1, 30% C18:2; 3% C18:3, 4% other).

‡ Vitamin–mineral premix contained per kg: Ca 160 g; Mg 40 g; Na 30 g; Fe 3 g; Cu 3 g; Zn 7 g; Mn 4 g; I 0.08 g; Co 0.05 g; Se 0.06 g; vitamin A 800,000 IU; vitamin D 150,000 IU; vitamin E 2000 IU.

§ Calculated as DM – (NDF + CP + ether extract + ash) (NRC, 2001).

‖ Estimated using NRC (2001) equations, ME = metabolizable energy, NEg = net energy for gain, NEm = net energy for maintenance.

Table 2

Fatty acid profile of calf starters and fat supplement used.

FA, g/100 g of total FA	Fat supplement	Calf starters†	
	FC*	FC-0	FC-2
C16:0	28	14.5	19.2
C16:1	3.0	0.09	1.10
C18:0	5.0	2.54	3.37
C18:1	26	21.57	23.63
C18:2n-6	30	55.30	46.60
C18:3n-3	3.0	3.80	3.60
Other	5.0	2.20	2.50
Σ SFA	33	17.04	22.57
Σ UFA	62	80.76	74.93
Σ PUFA	33	59.1	50.2

* Fat Ca-Salt rich in unsaturated FA (Persiafat, Kimiya Danesh Alvand Co. Tehran, Iran).

† Calf starters are defined as containing no fat supplement (FC-0) and supplementation with 2% Fat Ca-Salt (FC-2).

Table 3

Passive immunity in calves born from Holstein cows fed prepartum diets non-supplemented (CON) or supplemented with Ca-salts of soybean oil (CSO) or Ca-salts of fish oil (CFO) fatty acids, during the last 3 wk before expected parturition.

Item	Dietary treatment* and parity (P)						SEM	*P*-value†		
	CON		CSO		CFO					
	Null	Parous	Null	Parous	Null	Parous		FAT	SFO	P
At birth										
TP, ‡ g/dL	4.90	4.38	4.65	4.44	4.73	4.60	0.16	0.26	0.45	0.27
IgG intake, § g	381	404	481	541	461	532	31.2	<0.01	0.64	0.05
Plasma IgG, g/L	0.28	0.26	0.31	0.32	0.30	0.23	0.04	0.64	0.24	0.48
24 h after birth										
TP, ‡ g/dL	5.38	5.44	5.55	5.63	5.38	5.34	0.19	0.71	0.27	0.83
Plasma IgG, g/L	18.6	19.9	27.8	28.6	26.2	27.3	1.97	<0.01	0.47	0.50
Plasma IgG, % of TP	38.6	40.1	50.8	51.4	49.5	52.2	2.55	<0.01	0.94	0.43
AEA, ‖ %	22.6	21.8	28.5	26.8	28.6	27.2	1.86	<0.01	0.74	0.32

* CON = no fat supplement; CSO = supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil during the last 3 wk before expected parturition.

† FAT= Con vs. fat supplement; SFO = CSO vs. CFO. Interactions of FAT and SFO with parity were not significant for any parameter reported in this table (P > 0.40)

‡ TP: Total protein

§ IgG intake was calculated using IgG concentration in colostrum, reported in our pervious paper[11] and the amount offered colostrum was 4 L, which was similar for all calves.

‖ Apparent efficiency of IgG absorption, % = {IgG concentration in serum at 24 h of life (g/L) × [0.099 × BW at birth (kg)]/IgG intake (g)} ×100.

Table 4

Effects of dietary treatments on BW, starter intake, ADG, gain-to-feed ratio and apparent digestibility of Holstein calves (n = 14 per treatment) fed a calf starter (CS) containing no fat supplement (FC-0) or supplemented with 2% Fat Ca-Salt (FC-2) in pre- and post-weaning periods

Items*	Dam diet† and calf starters‡						SEM	*P*-value§		
	CON		CSO		CFO			Dam diet		
	FC-0	FC‖-2	FC-0	FC-2	FC-0	FC-2		FAT	SFO	CS
Body weight, kg										
At birth	40.73	41.20	41.95	39.60	42.13	41.33	0.91	0.72	0.29	0.24
d 56	67.08	70.52	70.50	72.10	70.14	73.75	1.19	0.03	0.58	<0.01
d 77	81.40	86.50	85.00	88.61	84.85	90.46	1.33	0.02	0.52	<0.01
ADG, g/d										
Pre-weaning	470	524	510	580	500	579	17.3	<0.01	0.74	<0.01
Post-weaning	680	760	690	786	700	796	26.5	0.33	0.70	<0.01
Overall	528	588	559	636	555	638	15.2	0.02	0.93	<0.01
Starter intake, g/d										
Pre-weaning	413.4	404.5	428.7	407.2	415.8	394.6	23.2	0.67	0.69	0.35
Post-weaning	1519	1457	1498	1533	1512	1504	34.0	0.83	0.42	0.68
Overall	754.6	736.6	760.6	755.8	757.6	755.3	29.1	0.84	0.59	0.74
Feed efficiency¶										
Pre-weaning	0.48	0.54	0.51	0.59	0.51	0.60	0.024	0.06	0.97	<0.01
Post-weaning	0.45	0.53	0.46	0.53	0.47	0.54	0.021	0.46	0.58	<0.01
Overall	0.38	0.43	0.40	0.46	0.40	0.46	0.016	0.14	0.99	<0.01
Apparent digestibility (g/kg)										
DM (g/kg DM)	816	823	818	823	838	824	10.2	0.29	0.91	0.93
CP	782	783	773	781	779	789	6.90	0.17	0.24	0.14
OM	849	855	863	859	859	861	6.30	0.52	0.16	0.74
NDF	623	617	606	621	631	623	8.50	0.17	0.45	0.92
ADF	515	521	521	531	553	532	13.0	0.14	0.55	0.84

Ether extract	934	933	936	937	934	943	4.80	0.65	0.27	0.16

[*] Starter was offered since 3 d of age. Preweaning: 0 to 56 d. Post weaning: 57 to 77 d old.

[†] CON = no fat supplement; CSO supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil during the last 3 wk before expected parturition.

[‡] Calf starters are defined as containing no fat supplement (FC-0) and supplementation with 2% Fat Ca-Salt (FC-2).

[§] *P*-values for orthogonal contrasts: FAT = control versus CSO + CFO; SFO = CSO versus CFO; CS = FC-0 versus FC-2. Interactions of FAT or SFO with CS were not significant for any parameter reported in this table ($P \geq 0.20$).

[‖] Fat Ca-salts, Persiafat, Kimiya Danesh Alvand Co. Iran., contained 85% fat and 9% Ca (1% C14.0, 28% C16:0; 3% C16:1; 5% C18:0, 26% C18:1, 30% C18:2; 3% C18:3, 4% other).

[¶] Feed efficiency as calculated from dividing ADG (g) by average daily DMI. DMI includes the DM provided by the milk and starter. The DM intake provided by milk was similar among treatments.

Table 5

Effect of dietary treatments on structural growth of Holstein calves fed a calf starter (CS) containing no fat supplement (FC-0) or supplemented with 2% Fat Ca-Salt (FC-2) during experimental periods.

Items*	Dam diet† and calf starters‡							*P*-value§		
	CON		CSO		CFO			Dam diet		
	FC-0	FC-2	FC-0	FC-2	FC-0	FC-2	SEM	FAT	SFO	CS
Hip height, cm										
d 3	82.9	83.1	83.3	83.3	82.9	83.1	0.51	0.81	0.58	0.65
d 56	91.3	93.2	92.2	94.3	92.1	94.4	0.62	0.05	0.97	<0.01
d 77	96.8	97.8	97.3	98.8	97.5	98.7	0.41	0.03	0.92	<0.01
Hip width, cm										
d 3	16.3	16.6	16.2	17.1	16.6	17.2	0.68	0.55	0.72	0.26
d 56	21.0	21.8	21.5	22.2	21.7	22.1	0.62	0.39	0.91	0.18
d 77	25.2	25.6	25.3	26.1	25.2	26.1	0.73	0.59	0.98	0.22
Heart girth, cm										
d 3	78.7	79.2	79.6	78.9	79.5	79.7	0.61	0.36	0.55	0.96
d 56	90.9	91.1	91.5	90.8	90.1	91.8	0.75	0.94	0.77	0.47
d 77	98.4	98.7	99.1	98.3	98.4	99.3	0.65	0.68	0.76	0.76
Body length, cm										
d 3	42.0	42.3	42.5	42.6	42.2	42.4	0.76	0.67	0.72	0.73
d 56	53.6	53.7	53.5	53.9	53.9	53.8	0.63	0.90	0.79	0.85
d 77	60.3	61.0	60.3	60.7	60.9	60.2	0.67	0.76	0.95	0.77
Body barrel, cm										
d 3	83.5	84.5	84.8	85.2	84.9	84.7	0.94	0.26	0.84	0.58
d 56	109.8	110.7	109.9	111.1	109.5	110.3	0.88	0.86	0.42	0.22
d 77	123.0	123.6	122.9	123.4	122.6	122.7	0.98	0.50	0.48	0.45
Wither height, cm										
d 3	77.9	78.8	79.1	78.6	79.5	80.0	0.70	0.12	0.19	0.63
d 56	83.8	85.8	84.6	86.8	84.8	86.7	0.53	0.03	0.90	<0.01
d 77	87.8	90.2	89.2	90.70	89.1	91.0	0.54	0.03	0.86	<0.01

[*] Calves were weaned at 56 d of age.

[†] Con = no fat supplement; CSO = supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil during the last 3 wk before expected parturition.

[‡] Calf starters are defined as containing no fat supplement (FC-0) and supplementation with 2% Fat Ca-Salt (FC-2).

[§] *P*-values for orthogonal contrasts: FAT = control versus CSO + CFO; SFO = CSO versus CFO; CS = FC-0 versus FC-2. Interactions of FAT or SFO with CS were not significant for any parameter reported in this table ($P \geq 0.13$).

Table 6

Total time devoted to perform different behaviors during 8 h of observation of Holstein calves fed a calf starter (CS) containing no fat supplement (FC-0) or supplemented with 2% Fat Ca-Salt (FC-2) in pre- and post-weaning periods.

Items*	Dam diet† and calf starters‡						SEM	*P*-value§		
	CON		CSO		CFO			Dam diet		
	FC-0	FC-2	FC-0	FC-2	FC-0	FC-2		FAT	SFO	CS
Eating, min										
d 35	33.5	34.8	35.2	30.2	31.6	36.3	2.86	0.73	0.66	0.89
d 70	65.2	64.4	59.7	59.7	63.2	59.4	3.11	0.14	0.62	0.55
Ruminating, min										
d 35	114.0	112.3	108.3	113.3	111.5	113.5	1.85	0.25	0.86	0.84
d 70	70.4	72.6	72.1	74.7	69.3	74.7	2.56	0.98	0.15	0.36
Standing, min										
d 35	124.3	131.1	127.7	130.1	118.2	114.4	11.20	0.60	0.26	0.84
d 70	99.85	101.2	106.0	109.6	103.8	108.5	8.15	0.33	0.97	0.50
Lying, min										
d 35	191.5	183.0	188.5	186.7	199.7	193.0	14.29	0.70	0.53	0.62
d 70	226.7	222.3	222.4	219.6	225.0	220.2	15.00	0.83	0.91	0.74
Nonnutritive oral behavior, ‖ min										
d 35	16.6	18.7	20.2	19.6	19.8	17.0	2.38	0.47	0.53	0.80
d 70	15.9	19.6	19.8	16.4	18.5	16.6	1.84	0.83	0.77	0.72

* Starter was offered since 3 d of age. Preweaning: 3 to 56 d. Post weaning: 57 to 77 d old.

† Con = no fat supplement; CSO = supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil during the last 3 wk before expected parturition.

‡ Calf starters are defined as containing no fat supplement (FC-0) and supplementation with 2% Fat Ca-Salt (FC-2).

§ *P*-values for orthogonal contrasts: FAT = control versus CSO + CFO; SFO = CSO versus CFO; CS = FC-0 versus FC-2. Interactions of FAT or SFO with CS were not significant for any parameter reported in this table ($P \geq 0.13$)

‖ Tongue rolling, licking surfaces, or eating wood shavings.

Table 7

Effect of dietary treatments on rectal temperature, respiratory rate, health scores1, and percentage of days with poor attitude, fever, diarrhea, and nasal discharge, fecal score and days with diarrhea of Holstein calves fed a calf starter (CS) containing no fat supplement (FC-0) or supplemented with 2% Fat Ca-Salt (FC-2) during experimental periods.

Items*	Dam diet† and calf starters‡						SEM	*P*-value§		
	CON		CSO		CFO			Dam diet		
	FC-0	FC-2	FC-0	FC-2	FC-0	FC-2		FAT	SFO	CS
Rectal temperature, °C										
Pre-weaning	39.22	39.18	39.10	38.92	39.08	38.82	0.09	<0.01	0.36	0.04
Post-weaning	39.32	38.95	39.27	38.93	39.22	38.98	0.08	0.64	0.95	<0.01
Respiration rate, breaths/min										
Pre-weaning	46.4	46.0	46.7	48.0	47.8	47.9	1.15	0.17	0.66	0.74
Post-weaning	55.9	57.0	57.5	54.7	58.8	58.5	2.22	0.64	0.24	0.72
Attitude score‖	0.32	0.30	0.31	0.25	0.31	0.24	0.04	0.41	0.99	0.16
Nasal discharge‖	0.14	0.11	0.10	0.08	0.11	0.08	0.03	0.34	0.89	0.45
Days with diarrhea	7.50	5.80	7.20	5.00	7.40	5.30	0.62	0.42	0.67	<0.001
Fecal score‖	0.82	0.62	0.77	0.53	0.78	0.55	0.070	0.30	0.87	<0.001
Days with: ¶%										
Poor attitude	18.4	15.4	16.6	13.4	16.5	12.3	2.80	0.35	0.82	0.14
Diarrhea	19.2	17.1	18.3	15.3	18.2	15.4	2.39	0.52	0.99	0.19
Nasal discharge	6.80	4.60	6.50	3.78	5.90	3.25	1.94	0.66	0.76	0.13
Fever, first 21 d	8.45	6.27	8.16	5.45	7.58	4.93	2.07	0.64	0.78	0.15

* Starter was offered since 3 d of age. Preweaning: 3 to 56 d. Post weaning: 57 to 77 d old.

† Con = no fat supplement; CSO = supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil during the last 3 wk before expected parturition.

‡ Calf starters are defined as containing no fat supplement (FC-0) and supplementation with 2% Fat Ca-Salt (FC-2).

§ *P*-values for orthogonal contrasts: FAT = control versus CSO + CFO; SFO = CSO versus CFO; CS = FC-0 versus FC-2. Interactions of FAT or SFO with CS were not significant for any parameter reported in this table ($P \geq 0.24$)

‖ Attitude 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.

¶ Percentage of days with health issue over a 77-d period unless indicated. Poor attitude and nasal discharge if score >0, fever if temperature ≥39.5°C, and diarrhea if score >1.

Table 8

Effect of dietary treatments on blood metabolites of Holstein calves fed a calf starter (CS) containing no fat supplement (FC-0) or supplemented with 2% Fat Ca-Salt (FC-2) during experimental periods.

Items*	Dam diet† and calf starters‡						SEM	*P*-value§		
	CON		CSO		CFO			Dam diet		
	FC-0	FC-2	FC-0	FC-2	FC-0	FC-2		FAT	SFO	CS
Glucose, mg/dL										
d 28	104.6	109.2	108.6	111.0	112.8	109.5	3.76	0.27	0.71	0.69
d 56	87.5	87.9	87.3	90.0	86.5	89.6	2.00	0.69	0.77	0.22
d 77	81.5	83.6	82.7	84.8	82.3	85.1	1.78	0.45	0.97	0.11
Total protein, g/dL										
d 28	6.00	5.98	6.05	6.35	5.95	6.26	0.23	0.45	0.72	0.28
d 56	6.14	6.43	6.30	6.52	6.33	6.57	0.21	0.44	0.82	0.15
d 77	6.43	6.51	6.63	6.89	6.60	6.75	0.24	0.27	0.74	0.41
Albumin, g/dL										
d 28	2.95	3.48	2.84	3.64	2.88	3.45	0.17	0.91	0.66	<0.001
d 56	3.32	3.47	3.45	3.73	3.36	3.54	0.16	0.37	0.39	0.15
d 77	3.12	3.88	3.26	3.95	3.20	3.91	0.17	0.63	0.80	<0.001
Urea nitrogen, mg/dL										
d 28	19.3	17.5	19.1	17.3	19.2	17.3	0.52	0.73	0.89	<0.001
d 56	18.0	17.4	17.9	17.2	17.7	17.3	0.42	0.68	0.96	0.13
d 77	22.18	20.13	22.1	20.4	22.2	20.4	0.64	0.80	0.94	<0.001
NEFA, mmol/L										
d 28	0.212	0.235	0.213	0.226	0.220	0.231	0.013	0.90	0.62	0.16
d 56	0.168	0.180	0.176	0.164	0.181	0.174	0.010	0.98	0.48	0.82
d 77	0.173	0.177	0.183	0.182	0.179	0.182	0.009	0.38	0.81	0.76
Insulin, μIU/mL										
d 28	27.0	26.7	28.1	27.9	26.7	27.4	0.70	0.25	0.16	0.90

d 56	24.9	25.1	25.4	26.4	25.1	26.3	0.78	0.26	0.77	0.20
d 77	16.8	18.1	16.4	17.1	17.5	17.2	0.87	0.50	0.51	0.41
Cholesterol, mg/dL										
d 28	118.9	122.7	123.5	129.5	123.6	129.2	2.78	0.02	0.96	0.02
d 56	112.9	114.6	113.0	115.6	111.9	115.3	1.92	0.76	0.70	0.29
d 77	81.50	86.8	81.2	86.9	82.0	87.3	1.75	0.91	0.73	<0.001
Triglycerides, mg/dL										
d 28	24.0	23.0	22.9	22.8	23.1	23.4	0.52	0.30	0.45	0.50
d 56	22.9	21.9	22.0	21.7	21.5	22.1	0.57	0.26	0.90	0.62
d 77	19.0	18.3	18.8	17.8	17.8	17.9	0.52	0.21	0.40	0.23
Alkaline phosphatase, units/L										
d 28	553	573	568	581	557	585	18.3	0.53	0.82	0.18
d 56	567	575	565	571	547	580	17.3	0.71	0.78	0.27
d 77	531	598	540	600	542	603	19.6	0.69	0.94	<0.001

* Calves were weaned at 56 d of age.

† Con = no fat supplement; CSO = supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil during the last 3 wk before expected parturition.

‡ Calf starters are defined as containing no fat supplement (FC-0) and supplementation with 2% Fat Ca-Salt (FC-2).

§ *P*-values for orthogonal contrasts: FAT = control versus CSO + CFO; SFO = CSO versus CFO; CS = FC-0 versus FC-2. Interactions of FAT or SFO with CS were not significant for any parameter reported in this table ($P \geq 0.22$).

Table 9

Effect of dietary treatments on ruminal parameters of Holstein calves fed a calf starter (CS) containing no fat supplement (FC-0) or supplemented with 2% Fat Ca-Salt (FC-2) during experimental periods

Items*	Dam diet† and calf starters‡						SEM	*P*-value§		
	CON		CSO		CFO			Dam diet		
	FC-0	FC-2	FC-0	FC-2	FC-0	FC-2		FAT	SFO	CS
pH										
d 30	5.44	5.52	5.42	5.54	5.46	5.57	0.13	0.86	0.83	0.35
d 70	5.65	5.67	5.61	5.73	5.64	5.74	0.11	0.78	0.85	0.39
NH3-N (mg/dL)										
d 30	11.1	9.80	11.4	12.0	11.5	10.8	1.24	0.36	0.65	0.64
d 70	12.9	13.9	14.4	15.0	14.5	15.7	1.32	0.19	0.76	0.38
Acetate (mmol/L)										
d 30	47.0	49.0	48.7	49.9	46.5	48.3	1.65	0.80	0.24	0.19
d 70	49.6	50.6	50.7	51.3	48.3	49.9	1.85	0.97	0.31	0.47
Propionate (mmol/L)										
d 30	38.2	40.2	39.9	41.1	37.7	39.5	1.58	0.80	0.24	0.20
d 70	41.5	42.5	42.6	43.2	40.2	42.0	1.83	0.99	0.33	0.44
Butyrate (mmol/L)										
d 30	14.0	15.4	15.7	15.8	13.5	15.3	1.98	0.84	0.51	0.50
d 70	16.8	17.8	17.9	17.1	15.5	17.3	2.03	0.84	0.59	0.67
Valerate (mmol/L)										
d 30	2.70	2.20	2.92	3.84	3.01	3.60	0.73	0.16	0.92	0.57
d 70	3.15	3.30	3.23	3.62	3.32	3.00	0.73	0.92	0.72	0.90
Isovalerate (mmol/L)										
d 30	0.72	0.85	0.83	0.78	0.73	0.60	0.15	0.72	0.33	0.92
d 70	0.66	0.85	0.90	0.72	0.74	1.02	0.18	0.55	0.63	0.48
Total VFA‖ (mmol/L)										

d 30	102.7	107.8	108.1	111.5	101.5	107.5	5.07	0.66	0.29	0.25
d 70	115.6	123.0	119.0	121.0	112.0	118.2	8.18	0.75	0.54	0.51
A: P¶										
d 30	1.30	1.22	1.23	1.22	1.19	1.25	0.075	0.60	0.92	0.85
d 70	1.17	1.15	1.20	1.16	1.18	1.15	0.062	0.69	0.79	0.60

* Calves were weaned at 56 d of age.

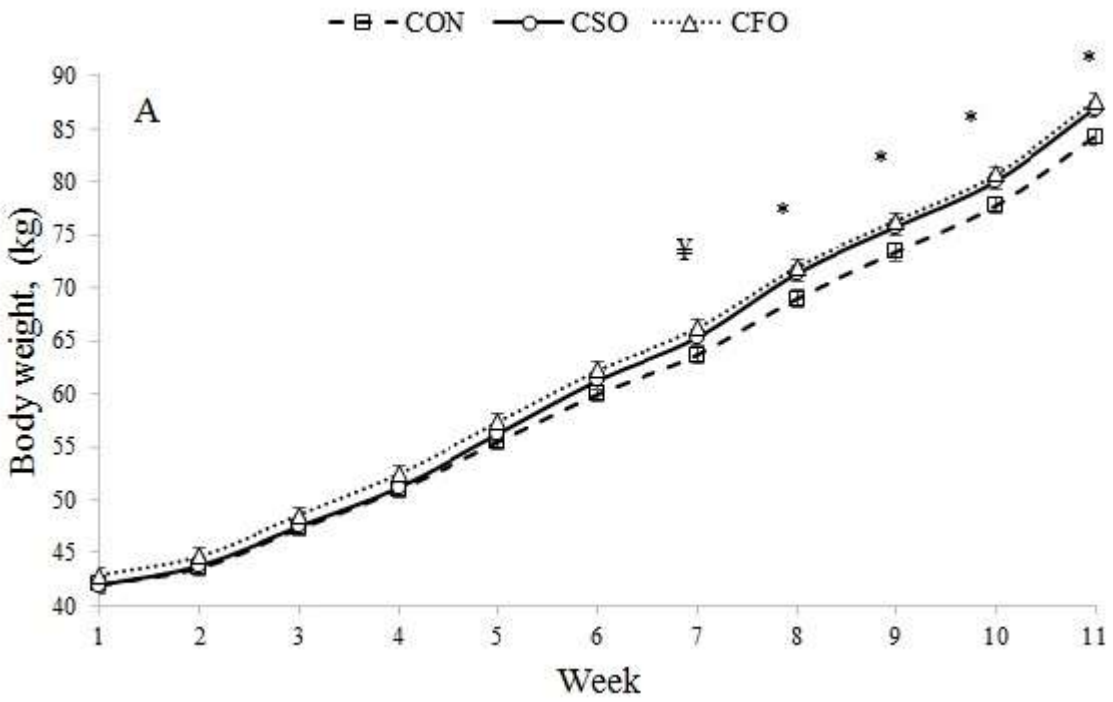
† Con = no fat supplement; CSO = supplementation with 140 g/d Ca-salts of soybean oil; CFO = supplementation with 140 g/d Ca-salts of fish oil during the last 3 wk before expected parturition.

‡ Calf starters are defined as containing no fat supplement (FC-0) and supplementation with 2% Fat Ca-Salt (FC-2).

§ *P*-values for orthogonal contrasts: FAT = control versus CSO + CFO; SFO = CSO versus CFO; CS = FC-0 versus FC-2. Interactions of FAT or SFO with CS were not significant for any parameter reported in this table ($P \geq 0.21$).

‖ VFA, volatile fatty acids.

¶ A:P, acetate to propionate ratio



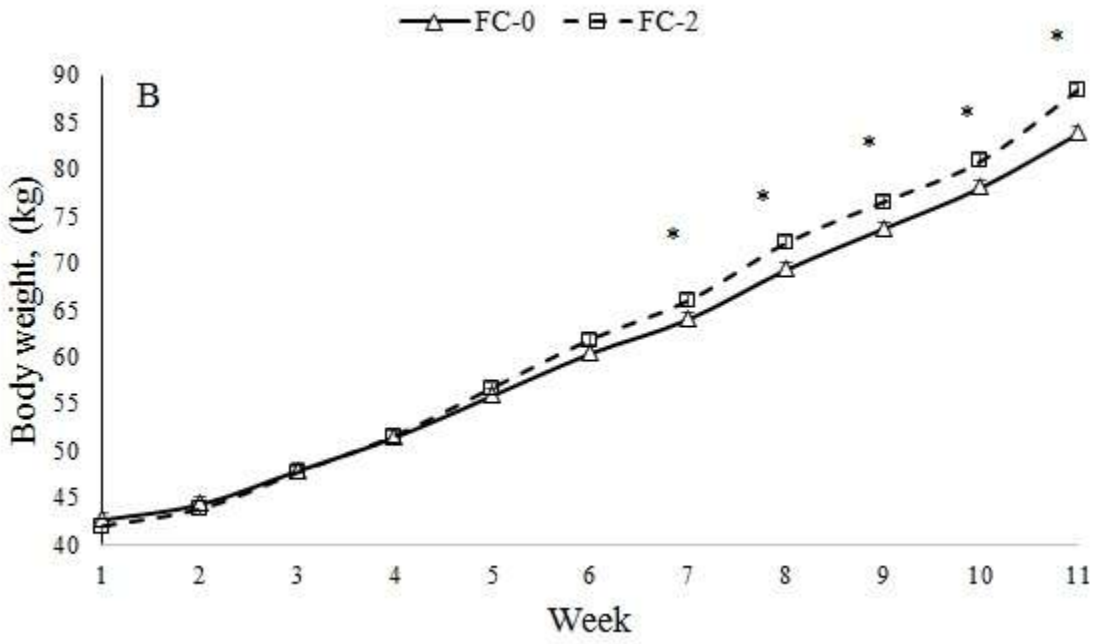


Figure 1. Effect of prepartum dam diets (A) and calf starter (B) in weekly body weights (kg) of Holstein dairy calves fed calf starter (CS) containing no fat supplement (FC-0) or supplemented with 2% Fat Ca-Salt (FC-2). Calves were born from dams fed diets supplemented with no fat (CON), Ca-salts of soybean oil (CSO), or Ca-salts of fish oil (CFO) starting at 3 wk before expected calving date. The SEM = 0.76 and 0.62 for prepartum dam diets and calf starter, respectively. Contrast of FAT = (CSO+CFO) vs. CON, SFO = CSO vs. CFO, and CS = FC-0 vs. FC-2. Effects in model: FAT: P = 0.02; CS: P <0.01; week: P <0.001; dam diets × week: P = 0.03; CS × week: P < 0.001. * means difference ($P \leq 0.05$) and ¥ means trends ($P \leq 0.10$) between treatments at a given week.