Original Research Article

Comparison between linseed expeller and canola expeller on concentrate intake, and circulating inflammatory mediators in Holstein calves

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ABSTRACT

The objective of this study was to compare the effect of linseed and canola expeller on average daily weight gain (ADG), concentrate intakes, incidence of diarrhea, serum haptoglobin, interleukin (IL)-1, and resolvin-E1 in female Holstein calves from birth to weaning. A sample size of 20 calves per group was calculated and were randomly allocated at the time of birth. Linseed group (LIN) was fed a starter with linseed expeller, while canola group (CAN) received a similar concentrate, but with canola expeller. Both expellers were included at a rate of 25% dry matter (DM) basis of the starter. Pasteurized waste milk was fed twice a day until weaning. Calves were weighed at birth, 30, and 60 d of age. Starter intake was evaluated daily from 5 to 60 d. A blood sample was obtained at birth, 14, 28, 35, and 49 d of age, and bovine serum resolvin-E1, haptoglobin, and IL-1 were assayed by commercial enzyme-linked immuno-sorbent assay (ELISA) kits. Incidence of diarrhea and the duration of the events were also recorded. The effect of the interaction group by time on body weight (BW) and starter intake was not significant ($P > 0.05$). Average daily gains (ADG) from 0 to 60 d for CAN and LIN groups were 0.680 and 0.675 kg/d ($P > 0.05$), respectively. Incidences of diarrhea were 25% and 45% for CAN and LIN groups, respectively ($P = 0.18$). LIN group had greater concentrations of IL-1 at d 21, haptoglobin at d 7, and resolving-E1 at d 14 and 49 than CAN group, respectively. It is concluded that BW at weaning, ADG, and concentrate intakes were not different between groups fed starters containing linseed or canola expeller (25% in-clusion). The concentrations of cytokines and haptoglobin were the greatest in LIN group.

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1. Introduction

Optimizing growth and decreasing mortality rates in calves are important goals in modern dairy operations. Consequently, adequate colostrum management, a well-organized feed manage-ment program, appropriate housing, and disease prevention stra-tegies are essential to an efficient transition from monogastric to ruminant stage (Donovan et al., 1998; Godden, 2008; McGuirk, 2008; Gelsinger et al., 2016; Khan et al., 2016).

Lipids are highly energetic nutrients provided by milk and concentrates during the first months of life (Khan et al., 2016). Linoleic and alpha-linolenic fatty acids (FA) are 18 carbon poly-unsaturated fatty acids (PUFA) considered essential for animals. As

they cannot be synthesized in the body, they must be obtained from vegetable sources (Jenkins and Harvatine, 2014). Linoleic acid, an n-6 fatty acid (FA), and linolenic acid, an n-3 FA, have critical roles on performance and immunity of ruminants (Sordillo, 2016) and have received considerable attention in calves, as they are born with small reserves of linoleic and linolenic acids. In addition, essential FA should be minimally affected by the biohydrogenation process in the rumen of unweaned dairy calves (Hill et al., 2011; Khan et al., 2016) to be incorporated into cellular membranes to participate in the transport of lipids and lipoprotein enzymes and in the synthesis of prostaglandins, prostacyclines, and thromboxanes (Calder, 2010, 2012; Worden et al., 2018).

In general, n-6 FA have roles as pro-inflammatory precursors, and n-3 FA act as anti-inflammatory precursors (Sordillo, 2016). Indeed, reduced inflammatory responses occur when oxylipid profiles change after n-3 FA supplementation because of the enhanced expression of pro-resolving oxylipids, such as resolvins, protectins, and lipoxins (Contreras et al., 2012).

The use of seeds containing oils with high content of linolenic acid, such as linseed (*Linum usitatissimum*), is one interesting approach to increase the contribution of n-3 FA in the diet of calves. Linseed contains 45% to 48% oil with more than 50% of its total FA being n-3 FA (Lessard et al., 2003). Linseeds can be used as whole seeds, pure oil, meals, and expellers. Meals and expellers can be obtained through the use of organic solvents and pressure, respectively. On the other hand, canola, as either meal or expeller, is another interesting nutritional byproduct for ruminants; however, its oil composition is richer in n-6 and n-9 than in n-3 FA (Lewinska et al., 2015). Canola and linseed expellers are common byproducts used in animal nutrition in Chile. They are similar in protein (30% to 34%) and ether extract content (7% to 10%), but differ in their FA profile (Lewinska et al., 2015).

The hypothesis of this study was that a starter concentrate composed of linseed expeller (25% of inclusion dry matter [DM] basis) would have a better effect on health and immunity of Holstein calves than a starter concentrate including canola expeller, as linseed oil is richer in n-3 FA. Consequently, a positive immunomodulation and, indirectly, a better calf performance is expected in calves fed a linseed expeller starter concentrate. Therefore, the objective of the present study was to compare the effect of linseed expeller and canola expeller as part of the concentrate (25% inclusion as DM basis) on average daily weight gain (ADG), concentrate intakes, feed efficiency (FE), incidence of diarrhea, and concentrations of serum haptoglobin, interleukin (IL)-1, and resolvin-E1 in female Holstein calves from birth to weaning.

2. Materials and methods

This study was carried out under the protocols of the animal welfare committee of the University Santo Tomás, Viña del Mar, Chile.

2.1. Farm and management

The study was conducted at a commercial dairy farm located in the central area of Chile (32.76° S; 70.83° W). The farm consisted of 750 lactating Holstein cows handled in a dry-lot system. Cows were milked 3 times a day and fed a total mixed ration based on corn silage, alfalfa hay and concentrate. Dry cows were moved to a prepartum lot 30 d before expected parturition. Cows calved in individual pens inside of a maternity barn. After calving, the offspring was immediately separated from the dam. Calves received high quality pasteurized colostrum (evaluated by a Brix refractometer, > 24%), within the first 4 h of life. Then, each calf was moved to individual metal hutches bedded with wheat straw over

concrete in a calf barn. Calves were fed pasteurized waste whole milk (12.5% solids, 3.6% fat, 3.3% crude protein) as follow: 2 L in the morning and 2 L in the afternoons during the first week of life; 3 L in the mornings and 3 L afternoons from week 2 to 7; and only 3 L in the mornings from week 8 to 9. Beginning at 3 d of age, water and concentrate were offered. Any health event was recorded and treated immediately, according to established standard protocols.

2.2. Experimental design

The study was carried out between April and September of 2017 (fall to winter season) with average minimum and maximum temperatures of 5 and 15 °C, respectively. Mean rainfall for the period was 200 to 300 mm (Chilean Weather Center, 2017). To determine a difference of 1.5 kg of live weight at weaning between a group receiving linseed expeller (LIN) and a group receiving canola expeller (CAN), considering a SD = 3.5 kg, 95% confidence, and 80% statistical power, a sample size of 20 female Holstein calves per group was calculated. The study used a completely randomized design, where animals were randomly allocated at the time of birth. Eligible animals had to be born from cows that experienced a normal and standard dry period (45 to 70 d in length) and a normal parturition (no dystocia). Calves from LIN group were fed a starter with an inclusion of 25% DM basis of linseed expeller (Terraflax, SpA, La Unión, Región de los Ríos, Chile. www.terraflax.com) (Table 1). Calves from CAN group received a similar concentrate but with canola expeller (Oleotop, Ruta 5 Sur 2735, Padre Las Casas, IX Region, Chile) (25% DM basis) (Table 1) instead of linseed expeller. Both starters were isonitrogenous and isoenergetic (Table 2), but differed in their content of n-3 and n-6 FA, based on feed reference library (Lewinska et al., 2015). The diet was formulated for a daily gain from birth to weaning of 0.67 kg/d, using the commercial ration formulator and evaluator software (NDS Professional, RUM&N, Reggio Emilia, Italy) based on the Cornell Net Carbohydrate and Protein System (CNCPS) version 6.55 (Van Amburgh et al., 2015). Assumption for the diet formulation was an average calf weighing 55 kg at 30 d old and consuming an amount of 2.45 kg/d of DM. The starter was prepared and mixed on the farm,

Table 1
Chemical composition of canola and linseed expeller analyzed by near infra-red spectrometry methodology (dry matter basis).

Item	Canola expeller	Linseed expeller
Nutrient levels, %		
Dry matter	91.0	90.0
Crude protein	32.2	32.0
Acid detergent fiber	20.2	18.3
Neutral detergent fiber$_{om}$[1]	25.5	31.4
Starch	8.5	8.9
Sugars	9.0	9.1
Fat	10.9	9.8
Fatty acids	8.1	7.8
Ash	5.1	6.5
Ca	0.75	0.40
P	1.24	0.80
Mg	0.51	0.57
K	1.31	1.19
S	0.63	0.33
Na	0.09	0.04
Cl	0.03	0.04
Fatty acid profile, g/100 g of fatty acid[2]		
C16:0	9.6	5.7
C18:0	2.2	4.3
C18:1	45.3	18.9
C18:2	31.1	14.1
C18:3	7.6	55.9

[1] Neutral detergent fiber from organic matter.
[2] From NDS Professional software (CNCPS Model 6.55) and Lewinska et al. (2015).

Table 2
Diet ingredients and nutrient composition of starter concentrates.

Item	Canola based starter	Linseed based starter
Diet ingredients (% of starter, dry matter basis)		
Corn grain	46.1	46.1
Soybean meal	20.2	20.2
Linseed expeller	0	25.2
Canola expeller	25.2	0
Wheat bran	7.0	7.0
Mineral and vitamin premix[1]	1.5	1.5
Nutrient composition (% of starter, dry matter basis)[2]		
Dry matter	89.1	89.1
Neutral detergent fiber	16.9	17.3
Crude protein	24.1	24.5
Starch	36.0	37.6
Fat	4.7	4.6
Fatty acids	4.3	4.2
ME, Mcal/kg dry matter[3]	2.91	2.93
Ca	0.53	0.40
P	0.50	0.43
Mg	0.25	0.27
K	1.31	1.23
Na	0.19	0.16
Cl	0.35	0.37
S	0.29	0.24
Fatty acids profile, g/100 g[4]		
C16:0	12.6	11.6
C18:0	2.3	3.0
C18:1	30.3	20.4
C18:2	46.4	41.6
C18:3	4.9	20.8

[1] Composition of mineral and vitamin premix: Ca 18%, P 1%, Mg 1.5%, K 0.2%, Na 10%, Cl 15%, S 0.1%, Mn 50 mg/kg, Cu 500 mg/kg, Fe 1,500 mg/kg, Zn 1,000 mg/kg, I 70 mg/kg, Co 4 mg/kg, Se 50 mg/kg, vitamin A 5,000,000 IU/kg, vitamin D 2,000,000 IU/kg, vitamin E 35,000 IU/kg.
[2] Laboratory analysis by near infra-red spectrometry methodology.
[3] Metabolizable energy estimation by formula from NDS Professional software.
[4] Fatty acid content based on NDS Professional software (CNCPS Model 6.55).

therefore the physical form of the concentrate was ground. The starter was weighed and offered *ad libitum* from d 3. Residual was obtained the following day, weighed and replaced with fresh starter.

Calves were weighed at birth, 30, and 60 d of age. Starter intake was evaluated daily from 5 to 60 d of age. Feed efficiency was calculated as the amount of starter consumed (kg) to gain 1 kg of BW.

A blood sample from the jugular vein was obtained at 2, 14, 28, 35, and 49 d of age for serum collection at 08:00 before feeding the calves. Blood samples were centrifuged at 3,000 ×g for 5 min. Sera were separated and placed in plastic vials and stored at - 20 °C until analysis. Serum sample from d 2 was also used to assess total protein concentrations through a clinical refractometer as an indicator of colostrum management and immunoglobulins absorption (Godden, 2008). Because there are several inflammatory and anti-inflammatory mediators participating in the inflammation process, key molecules were selected as an indicator of an acute inflammatory process (haptoglobin), inflammation due to a response to an infection (interleukin [IL]-1), and restoration of normal cellular function after an inflammation process (resolving-E1, a cytokine synthesized from n-3 FA).

One of our major interest for this study was to measure the immune mediators under the normal "steady-state" rearing process of calves; consequently, there was no experimental challenge to pathogens. Calves were evaluated under their regular management and natural exposure to typical pathogens present in any calf rearing system. Haptoglobin was selected as outcome variable because is produced predominantly in response to a bacterial or viral infection. Haptoglobin concentrations increased substantially

during acute inflammation (Andersen et al., 2017) in response to IL-1. Therefore, haptoglobin is an effective marker in the diagnosis and prognosis of several inflammatory processes in cattle (Eckersall and Bell, 2010). In addition, IL-1β is expressed rapidly during the initial stages of infection and studies have demonstrated IL-1β is a very important inflammatory mediator in cattle in the presence of several viruses and bacteria (Vrentas et al., 2018). Resolvin-E1 has not been well characterized in cattle; therefore, we decided to assess this cytokine as a potential indicator of resolution of an inflammation process (Calder, 2010, 2012).

The incidence of diarrhea and the duration of each event were also recorded. For this purpose, a system of visual evaluation of feces, based on a fecal score was used. Scores were defined as follows: 0 = normal consistency; 1 = semi-formed or pasty consistency; 2 = loose but enough consistency to remain on bedding; 3 = watery feces that sift through bedding material with or without blood (McGuirk, 2008). Diarrhea was defined as any animal presenting a score 2 or 3. Cases were treated according to standard farm protocols.

2.3. Laboratory analysis

Canola expeller, linseed expeller, and starters were nutritionally analyzed by near infra-red spectrometry (NIRS) methodology at the Rock River Laboratory Inc. (Temuco, Chile), requesting the CNCPS platform. This lab has developed NIRS standardized curves for canola and linseed expeller based on several wet chemistry analyses of the ingredients carried out at the Rock River Laboratory Inc. in the USA (710 Commerce Drive, Watertown, WI 53094, USA). Consequently, NIRS technology with good quality of standard curves might be used as a proxy of nutritional composition of feed ingredients for livestock. Fatty acid profiles were obtained from the Cornell University Feed Library (NDS Professional, Italy) and from Lewinska et al. (2015). Bovine serum resolvin-E1, haptoglobin and IL-1 were assayed by commercial ELISA kits (MyBioSource, Inc., San Diego, CA, USA), according to standard protocols recommended by the company. Briefly, these kits are strip plates Sandwich ELISA for analyzing the presence of the IL-1, haptoglobin, and resolvin-E1 in serum samples. The concentration gradients of the kit standards or positive controls render a theoretical kit detection range in biological research samples containing these metabolites. The ELISA analytical biochemical technique is based on antibody–antigen interactions (immunosorbency) and a colorimetric detection system to detect antigen targets in samples. As an example, for Haptoglobin, a specific antibody has been pre-coated onto 96-well plates and blocked. Standards or test samples were added to the wells and subsequently a Haptoglobin specific biotinylated detection antibody was added and then followed by washing with wash buffer. Streptavidin–peroxidase conjugate was added and unbound conjugates were washed away with wash buffer. 3,3′,5,5′-etramethylbenzidine (TMB) was then used to visualize streptavidin-peroxidase enzymatic reaction. TMB was catalyzed by Streptavidin-Peroxidase to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the amount of haptoglobin captured in plate.

2.4. Statistical analysis

Average daily gain and FE from 0 to 30 d, 30 to 60 d, and 0 to 60 d were analyzed by a General Linear Model ANOVA. BW over time, feed intakes, haptoglobin, IL-1, and resolvin-E1 concentrations were analyzed by ANOVA for repeated measures, constructing a mixed model, considering the calf as a random effect nested within the treatment group. Independent variables were the effect of

treatment, parity number of the dam, and serum total protein concentration of calves at d 2 of life.

The model was defined as follows:

$$Y_{ijklmn} = \mu + T_i + Par_j + Calf_k\,(T_i) + TP_l + Time_m + (T \times Time)_{im} + e_{ijklmn},$$

where Y_{ijklmn} = dependent variable (BW, feed intake, blood metabolites); μ = overall population mean; T_i = effect of treatment (CAN, LIN); Par_j = effect of dam's parity number (1, 2, 3 or more); $Calf_k\,(T_i)$ = random effect of calf nested within treatment; TP_l = effect of calf serum total protein at d 2 (g/dL); $Time_m$ = effect of time; $(T \times Time)_{im}$ = interaction effect of treatment by time; e_{ijklmn} = error term.

For all models, the best goodness of fit was specified according to the Schwarz's Bayesian Criterion (SBC) (Littell et al., 1998, 2006). The model with the lowest SBC was selected. Least squares means ± SEM were reported. Significant effects were considered when P was ≤0.05. A tendency was considered when the P value was between 0.05 and 0.1.

Because the time variable is quantitative, treatment was also modeled as a polynomial function of time. This gives smoothed trends over time and yields equations than can be used for comparing treatments at specific times, even though the effect of interaction treatment by time can be not significant when time is considered as class variable and not as a regression variable in the model (Littell et al., 2006).

Incidence of diarrhea was analyzed through logistic regression considering the following model: Logit $(\pi) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3$,

where π = the odds of the event (diarrhea yes, no); α = intercept; β_1 = parameter of X_1; X_1 = effect of treatment (CAN, LIN); β_2 = parameter of X_2; X_2 = effect of dam's parity (1, 2, 3 or more); β_3 = parameter of X_3; X_3 = effect of calf serum total protein (g/dL).

Statistical analyses were conducted using the corresponding procedures (PROC MIXED and PROC LOGISTIC) of SAS 9.4 for windows 2012.

3. Results

3.1. BW, concentrate intakes, incidence of diarrhea

Dry matter intake, initial BW, final BW, ADG and FE from 0 to 30 d, 30 to 60 d and 0 to 60 d are shown in Table 3. BW at birth, 30, and 60 d of age are shown in Fig. 1. The interaction treatment by time effect was not statistically significant ($P > 0.05$). In addition, there was no evidence of polynomial effects over time ($P > 0.05$); consequently, both curves were linear and parallel. Both groups doubled their BW at 60 d. Average daily gain in CAN and LIN treatment groups for 0 to 30 d, 30 to 60 d, and 0 to 60 d were 0.52 vs. 0.56 kg/d ($P > 0.05$), 0.84 vs. 0.79 kg/d ($P > 0.05$), and 0.680 vs. 0.675 kg/d ($P > 0.05$), respectively. Fig. 2 presents the least squares means (±SEM) values for daily concentrate intakes (kg/d) for CAN and LIN groups. No differences were determined for FE within periods ($P > 0.05$).

Incidences of diarrhea were 25% and 45% for CAN and LIN groups, respectively ($P = 0.18$), with all cases occurring within the first 10 d of life. Time to recovery for CAN and LIN treatment groups were 2.8 vs. 2.4 d, respectively ($P > 0.05$).

3.2. Inflammatory mediators

Least squares means for IL-1 for CAN and LIN treatment groups are shown in Fig. 3. There was no treatment by time interaction

Table 3
Performance of calves fed a starter based on linseed group (LIN) and canola group (CAN).[1]

| Item	Groups[2]		SEM	P-value
	CAN	LIN		
Calves, n	20	20		
Starter DMI, kg/d	0.506	0.491	0.11	0.82
Initial BW, kg	38.8	38.6	1.93	0.91
Final BW, kg	79.6	79.1	3.96	0.82
ADG (0 to 30 d), kg/d	0.518	0.564	0.12	0.55
ADG (30 to 60 d), kg/d	0.842	0.786	0.18	0.51
ADG (0 to 60 d), kg/d	0.680	0.675	0.15	0.88
FE (0 to 30 d)	2.47	2.63	0.35	0.35
FE (30 to 60 d)	1.08	1.08	0.21	0.99
FE (0 to 60 d)	1.34	1.37	0.25	0.88

DMI = dry matter intake (d 5 to 60); ADG = average daily gain; FE = feed efficiency (ADG/DMI).
[1] Calves from both groups had 100% of milk consumption during the study period.
[2] Calves in LIN group were fed a starter with 25% linseed expeller, while those in CAN group received a similar starter with 25% canola expeller.
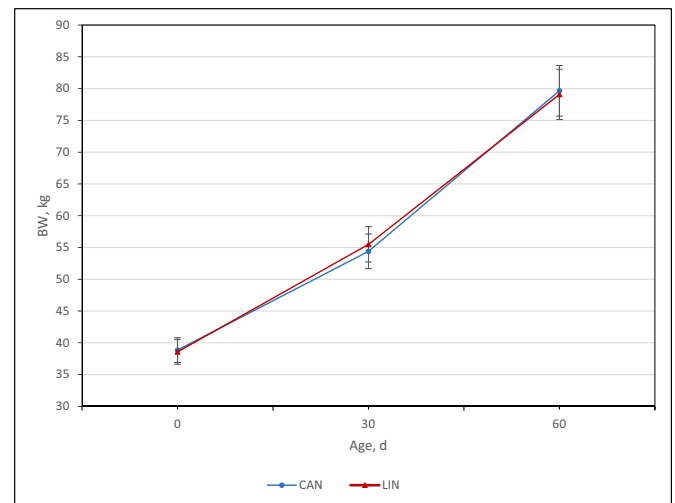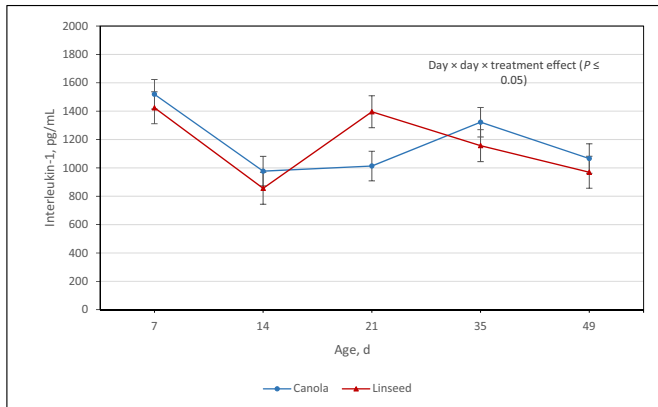

Fig. 1. Least squared means (±SEM) for BW of female Holstein calves at birth, 30 and 60 d old. Calves in LIN group were fed a starter with 25% linseed expeller, while those in CAN group received a similar starter with 25% canola expeller. There was no group by day interaction effect ($P > 0.05$).
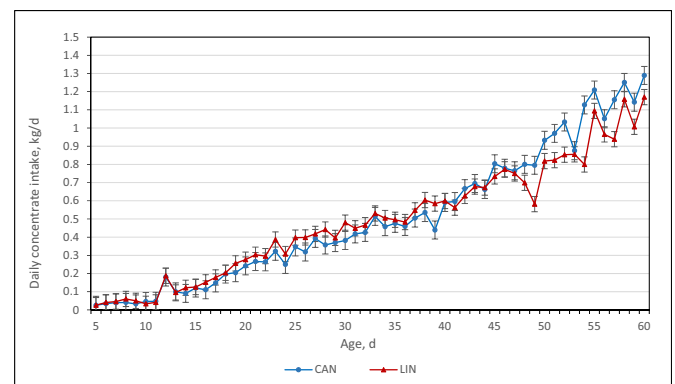
Fig. 2. Least squared means (±SEM) for daily concentrate intake in female Holstein calves. Calves in LIN group were fed a starter with 25% linseed expeller, while those in CAN group received a similar starter with 25% canola expeller. There was no group by time interaction effect ($P > 0.05$).
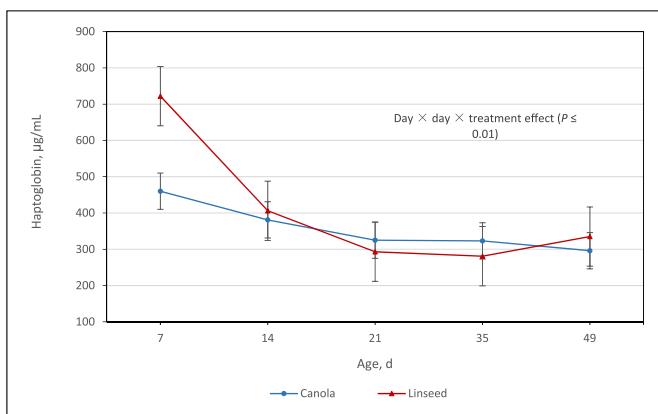
Fig. 3. Least squared means (±SEM) for interleukin-1 in female Holstein calves. Calves in LIN group were fed a starter with 25% linseed expeller, while those in CAN group received a similar starter with 25% canola expeller. There was no group by day interaction effect ($P > 0.05$). There was a quadratic polynomial by treatment effect (day × day × treatment effect; $P \leq 0.05$).

effect ($P > 0.05$); however, when time was modelled as a regression variable, there was a time by treatment interaction and a quadratic pattern for IL-1 over time ($P \leq 0.05$), with a higher concentration at d 21 in LIN group than CAN group. In Fig. 4, least squares means for haptoglobin by group are shown. There was no treatment by time interaction effect ($P > 0.05$); however, when time was modelled as a regression variable, there was a time by treatment interaction and a quadratic pattern for haptoglobin over time ($P \leq 0.01$), with a higher concentration of haptoglobin at d 7 in LIN group than in CAN group. Fig. 5 illustrates the least squares means for resolvin-E1 by treatment group. There was no treatment by time interaction effect ($P > 0.05$), but, when time was modelled as a regression variable, there was a time by treatment interaction and a cubic polynomial effect over time ($P \leq 0.05$), with a higher concentration at d 49 in LIN group than CAN group. The regression modelling for CAN group showed a linear pattern for resolvin-E1 over time.

4. Discussion

Little research has been conducted on the impact of fat sources on performance in pre-weaned dairy calves. However, it has been reported that providing more essential FA in the diet, particularly

Fig. 4. Least squared means (±SEM) for haptoglobin in female Holstein calves. Calves in LIN group were fed a starter with 25% linseed expeller, while those in CAN group received a similar starter with 25% canola expeller. There was no group by day interaction effect ($P > 0.05$). There was a quadratic polynomial by treatment effect (day × day × treatment effect; $P \leq 0.01$).
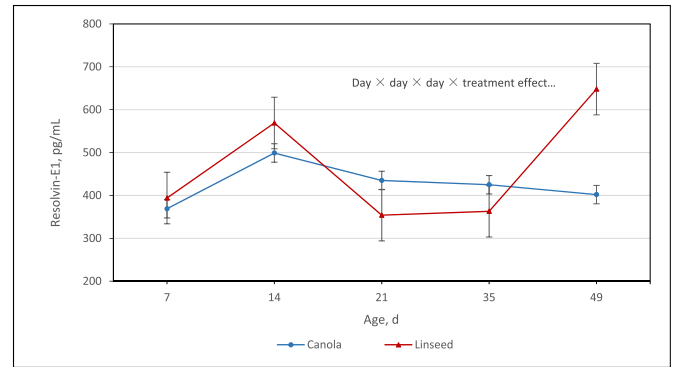
Fig. 5. Least squared means (±SEM) for Resolvin-E1 in female Holstein calves. There was no group by day interaction effect ($P > 0.05$). Calves in LIN group were fed a starter with 25% linseed expeller, while those in CAN group received a similar starter with 25% canola expeller. There was a cubic polynomial treatment effect (day × day × day × treatment effect; $P \leq 0.05$).

the n-3 FA may improve health and growth performance of young calves (Kertz et al., 2017). This study was carried out under field conditions, therefore, the results are a reflex of the real management of commercial dairy farms in Chile.

In this study, it was hypothesized that female Holstein calves fed a starter concentrate composed with linseed expeller at an inclusion level of 25% DM will have an improved growth performance and health than a group fed a starter concentrate composed with canola expeller at the same inclusion level. This hypothesis was rejected because the incorporation of linseed in the starter concentrate neither improved BW at weaning nor positively affected ADG between birth and 60 d of age. The 2 diets were isonitrogenous and isoenergetic and both experimental groups had similar ADG and average starter intake, which are acceptable values for pre-weaned dairy calves. Indeed, Gelsinger et al. (2016) in a meta-analysis study, found that calves gaining weight above 0.50 kg/d had enhanced first lactation performance when pre-weaned management is combined with good practices after weaning.

There are few studies evaluating linseed-based products on pre-weaned calf performance. A study using a pork fat-based milk replacer supplemented with 2% of linseed oil (Karcher et al., 2014) reported an ADG of 0.42 kg/d from 0 to 28 d of age, and while in our study, calves between 0 and 30 d of age gained 0.56 kg/d. In another study using male Holstein calves with an average BW at birth of 46 kg and the diet was supplemented with a commercial product containing butyrate, coconut and flax oil (Hill et al., 2011), the ADG between birth and 56 d of age was 0.72 kg/d. In our study, female Holstein calves weighed in average between 38.6 and 38.8 kg at birth and gained between 0.675 and 0.680 kg/d. Thus, the ADG obtained in the present study are similar or even greater than other studies that also used linseed byproducts as part of the experimental design and are within the recommended values for the Holstein breed during the first 60 d of age.

Partial explanations for the lack of significant differences between both treatments might be related to the higher number of calves that developed diarrhea during the first 10 d of life: 9 in LIN group, and 5 in CAN group. Although the incidence of diarrhea was not statistically different between these treatment groups, more animals in fact developed diarrhea in LIN than in CAN group, which might be sufficient to produce differences in continuous variables, such as the concentration of blood immunological metabolites.

Calves from LIN group had a higher concentration of haptoglobin at 7 d of age, higher concentration of IL-1 at d 21, and higher concentration of resolvin-E1 at 49 d of age than calves from CAN

group. A potential explanation for these differences might be related to the higher number of calves with diarrhea in LIN group ($n = 9$) than in CAN group ($n = 5$). Certainly, a higher number of calves developing diarrhea could trigger a stronger inflammatory response from the intestine, reflected by high levels of haptoglobin at d 7, and IL-1 at 21 d of age. Indeed, calves developing respiratory disease and digestive conditions during the first week of life responded with fever, depression, and high concentrations of haptoglobin. In addition, high haptoglobin concentrations during the first week of life were associated with increased mortality up to 4 months of age (Murray et al., 2014). Consequently, in calves and adult cattle, disease has been linked with a significant rise in levels of serum haptoglobin, which can be used as biomarker for inflammatory conditions in cattle (Gånheim et al., 2007; Eckersall and Bell, 2010). The potential beneficial impact of n-3 FA as anti-inflammatory precursor in LIN group during the first 14 d of age would be negligible, as the level of starter consumption was minimal at that time. However, the increase in concentrations of resolvin-E1 relative to calves in CAN group at d 49, might be explained by the higher levels of n-3 FA in LIN group and by the response to the higher number of calves with digestive disorders. At this point, the starter intake might be greater enough to trigger a better immunological response in calves from the LIN group. The n-3 FA are related to oxylipids synthesis, which are of another group of important immune signaling molecules that are derived from cellular lipids and contribute to innate immunity by regulating the onset, magnitude, and duration of the inflammatory response. Oxylipids are synthesized from PUFA substrates primarily found in the cellular membrane, including n-6 linoleic and arachidonic acids or n-3 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Sordillo, 2016). Depending on the timing and magnitude of expression, certain oxylipids can either enhance or resolve the inflammatory response (Sordillo and Raphael, 2013). Therefore, the balance between production of pro- and anti-inflammatory oxylipids is one factor that determines the inflammatory phenotype of a cell and the surrounding microenvironment (Sordillo, 2016). In this sense, n-3 FA are the substrates for the synthesis of resolvins and protectins acting through multiple interconnected mechanisms to reduce the production of inflammatory eicosanoids and cytokines. These mediators, such as resolvin-E1, have been demonstrated to exhibit anti-inflammatory effects, for example, inhibiting transendothelial migration of neutrophils, consequently preventing neutrophilic infiltration at sites of inflammation (Calder, 2010, 2012). In our study, the concentration of resolvin-E1 in LIN group was particularly higher than in CAN calves at 49 d of age, suggesting that calves fed the linseed-based starter had a better response to an inflammatory process suffered by the calves during the trial. However, it is known the responses of cytokines to antigens are short-lasting in time, then, we acknowledge that the differences found in immune responses between the 2 groups (weekly samples) may not only be explained by the type of supplemented FA, but also by other more complex mechanisms that are challenging to explain.

Although the previous assumptions regarding the anti-inflammatory effects of n-3 FA on tissue healing process might be true, we need to consider that the potential positive impact of linolenic acid from the linseed oil might be partially reduced by the biohydrogenation process that might be occurring in early stages of the rumen of calves. It is well-known that by increasing PUFA in the diet, the extent of 18:2 and 18:3 biohydrogenation in the rumen is increased (Harvatine and Allen, 2006). However, we can argue this point, as young calves (0 to 2 months of age) are transitioning from monogastrics to ruminants. The calf rumen is immature and lacks sufficient biohydrogenating-bacteria, especially when only milk and concentrates are fed, with no access to forage (Khan et al.,

2016). At early stages of life, anaerobic acidophilus, coliforms, lactobacilli, lactose fermenters, and some anaerobes predominate in the rumen, and, with age, there is a slow transition to the microflora typically found in the fully developed rumen (Fonty et al., 1989). *Butiryvibrio fibrisolvens* (a *Firmicutes*) is a major bacterium in adult ruminants involved in the degradation of structural carbohydrates, protein breakdown, and biohydrogenation of lipids (Jenkins et al., 2008). Fewer *Firmicutes* have been described in the rumen of pre-weaned calves compared with adult ruminants, particularly if they have been fed only concentrate and milk (Li et al., 2012; Malmuthuge et al., 2014). Consequently, we can suggest that young pre-weaned dairy calves have marginal activity of FA biohydrogentation in the rumen. Then, n-3 FA are minimally altered and can be absorbed without modifications, serving as anti-inflammatory precursors. This statement is supported by González et al. (2014), who found that n-3 FA provided by linseed oil was increased in muscular tissue of young ruminants, implying that either low rumen pH induced by starch fermentation (amilotytic bacteria) or the presence of fewer colonies of cellulolytic bacteria in young ruminants is very likely. The evaluation of n-3 FA in the blood of experimental calves would have helped to support our statement. Unfortunately, one of the drawback of this study was the lack of assessment of n-3 FA in serum of calves fed both sources of FA, but this subject is well considered for further studies in our research group.

Finally, a major high point of the current study is that the use of both linseed expeller and canola expeller at the inclusion level of 25% DM of the concentrate was sufficient to reach acceptable growing rates of calves from birth to weaning. This finding is extremely valuable for the dairy industry, especially for calf rearing programs, where the availability of non-conventional ingredients for calf starter, such as linseed and canola byproducts, might be a cost-effective alternative.

5. Conclusions

BW at weaning, ADG, concentrate intakes, and FE were within acceptable responses and not different between calves fed starters containing linseed or canola expeller (25% inclusion). Concentrations of haptoglobin at d 7, IL-1 at d 21 and resolvin-E1 at d 14 and 49 were higher in LIN group than in CAN group calves. In the light of these results, either canola expeller or linseed expeller can be used as ingredients of calf starter at an inclusion of 25% DM.

Conflict of interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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