Conjugated linoleic acid content and fatty acids profile of milk from grazing dairy cows in southern Chile fed varying amounts of concentrate

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ABSTRACT
Conjugated linoleic acids (CLAs) are fatty acids found in bovine milk which possess benefits for human health. This study aimed to compare fatty acid profiles, particularly CLA, of milk from grazing cows from south Chile receiving varying levels of concentrate. The study was conducted during spring of 2012 in two dairies from southern Chile. Dairy A was under low grazing intensity with high levels of concentrate (6–8 kg/cow/day); Dairy B combined low levels of concentrate (1–2 kg/cow/day) with more intensive grazing activity. Milk and blood samples were collected from 15 cows at each dairy at 30 days postpartum. Milk was analysed for fatty acid profiles, and blood for beta-hydroxybutyrate. Body condition score, parity, and daily milk yields were recorded. Pasture and concentrates were sampled for nutritional analysis. The proportion of CLA isomers in milk was 0.91% and 1.66% for Dairy A and Dairy B, respectively (p < .05). Dairy B had a lower ratio of saturated to unsaturated fatty acid and higher levels of omega-3 fatty acids than Dairy A. In conclusion, cows with increased grazing and low concentrate presented higher levels of CLA and omega-3 fatty acids than cows with less grazing and greater concentrate.

1. Introduction
Bovine milk represents an important source of nutrients rich in energy, high-quality protein, minerals, and vitamins for human nutrition (Gurbuz 2009). Fat represents the greatest energy source provided by this milk, and fat composition is determined primarily by the nutritional composition of a cow’s diet (Bauman & Griinari 2003).

Milk fat encompasses primarily saturated fatty acids (SFAs) (Jenkins et al. 2008). Because of the relationship between SFA and cardiovascular disease, milk fat is considered a risk factor for human health. Nevertheless, the presence of essential polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs) and conjugated linoleic acid (CLA) has been associated with positive impacts on human health (Belury 2002; Benjamin et al. 2015). CLA has been shown to possess hypocholesterolemic and anti-atherogenic properties (reducing the amount of cholesterol and low-density lipoprotein); to have an immunomodulant effect (increasing immune response by promoting T-cell proliferation and increasing the IgA secretion and macrophage function against certain cancers); and to have an antioxidant role and participate in the reduction of body weight by increasing lipolysis and reducing fat deposits in tissues (Benjamin et al. 2015).

CLAs occur in varying isomers but predominantly present with cis-9, trans-11 structure. Although CLA occurs in small amounts in vegetable oils, the meat and milk of ruminants contain particularly high concentrations, varying between 0.5% and 2% of total lipids (Bauman & Griinari 2003; Jenkins et al. 2008). CLAs are mainly synthesized in the rumen, representing an intermediary product of linolenic acid biohydrogenation; consequently, it is also called rumenic acid. Vaccenic acid (18:1, trans-11) is synthesized in the rumen and is subsequently transported through the bloodstream and converted to CLA in the liver and mammary gland. The process consists of the desaturation of carbon 9 by the desaturase enzyme present in the cells of the mammary gland and/or liver of ruminants, and its transformation to CLA (cis-9, trans-11 isomer) (Jenkins & McGuire 2006; Harvatine et al. 2008).

The best natural sources of CLA for human consumption are ruminant meat and dairy products, and the most important factor influencing CLA concentration in these products is the animals’ diet (Dhiman et al. 2005). Pastures are rich in linolenic acid, which represents 48–56% of total fatty acid content; corn silage is rich in linoleic acid. Alfalfa hay is also high in linoleic acid; however, a significant portion of unsaturated fatty acid is oxidized during the drying process. As a result, grazing animals should produce milk with higher CLA concentrations as compared to animals with diets rich in silage, hay, and concentrate supplements (Dannenberger et al. 2005; Harvatine et al. 2008). Dhiman et al. (2005) reported that grazing animals presented 500% greater CLA concentrations in their milk as compared to animals fed diets containing 50% concentrate and 50% conserved forage. Another factor increasing the amount of CLA in milk is the incorporation of oilseed crops in animals’ diets (Dhiman et al. 2005; Jenkins & Harvatine 2014). These seeds and their oils (soybean, sunflower, canola, peanut, etc.) are
rich in linoleic acid and linolenic acid (linseed), precursors to CLA in the rumen (Jenkins & McGuire 2006; Slots et al. 2009).

There is evidence that a dairy cow’s energy balance affects milk fatty acid profile. Marin et al. (2010) and Melendez et al. (2016) found that cows with ketosis (determined by measuring beta-hydroxybutyrate (BHB)) produced milk with lower CLA. Increasing PUFA content, mainly omega-3 and CLA in animal products, may contribute to greater dietary intake and generate positive effects on human health. Considering the variety of grazing production systems present in southern Chile, it is of paramount benefit to compare CLA content in milk from grazing cows receiving varying amounts of concentrate supplement.

This study hypothesized that cows fed diets supplemented with a greater quantity of concentrate would produce milk with lower CLA content than cows with greater grazing activity and lower concentrate consumption. The objective of this study was, therefore, to quantify the differences between fatty acid profiles, especially CLA content, in milk from southern Chilean grazing dairy cows during early postpartum (pp), supplemented with high and low levels of concentrate supplement.

2. Materials and methods

2.1. Animals

This study was conducted in two dairies located in the Union county, Ranco province, XIV region of Chile (40.33° S and 72.63° W). Both dairies consisted of Holstein cows and employ seasonal breeding (30% fall calving, 70% spring calving), milked twice a day, and were fed based on grazing (ryegrass) supplemented with two levels of concentrate consisting of corn, triticale, oat grains, soybean meal, canola meal, vitamins and minerals. Dairy A supplemented 6–8 kg of concentrate and 4 kg of grass silage per cow, per day, as a mixed ration before milking; this diet was defined as high-concentrate supplementation (Group A). Dairy B supplemented grazing with 1–2 kg of concentrate per cow, per day; this diet was defined as a low-concentrate supplementation (Group B).

High versus low grazing was defined as cows receiving 1–2 kg and 6–8 kg of concentrate, respectively. Given animals’ potential dry matter intake, the amount of pasture consumed each day during grazing was expected to be lower for the low-grazing (high concentrate) group as compared to the high-grazing (low concentrate) group.

2.2. Experimental design

Fifteen animals per group, at 30 days pp, were randomly selected from each farm. Major inclusion criterion was normal animals with no history of clinical disorders. Sample size was determined considering a 0.3% difference in CLA concentrations between groups (0.3% concentration for Group A and 0.6% for Group B) with a standard deviation of 0.2%, a 95% confidence level, and power of 80% (SAS 2001).

Sampling was conducted during October and November 2012. Data were recorded from each individual for: parity number (PN), grouping animals as primiparous and multiparous; milk production at day 30 pp; and body condition score (BCS) on a scale 1–5 in a ¼ point increment (Ferguson et al. 1994). Feed (concentrate, conserved forage, and pasture) were sampled for nutritional analysis, using near-infrared spectroscopy technology.

2.3. Laboratory analysis

A blood sample from each animal was taken at day 30 pp using a Vacutainer® system (without anticoagulant). Samples were centrifuged at 4000 rpm for 10 min, and serum was separated and stored in 1 ml Eppendorf® tubes and frozen at −20°C for subsequent BHB analysis using an enzymatic colorimetric method (Randox Laboratories Ltd. ©, UK). A composite, 200 ml milk sample from each of the four quarters was taken at day 30 pp for the analysis of milk fat composition. Samples were frozen at −20°C for subsequent analysis. For the analysis of free fatty acids, fat extraction was performed using the Blight and Dyer (1959) method and gas chromatography technique (©Shimadzu GCMS-QP2010 QP5050A Ultracombination with GC columns sp2380). The identification of fatty acids was based on the comparison of retention times for standard methyl esters, ranging from C4 to C24 (Sigma-Aldrich, St. Louis, MO, USA). Calibration standards were obtained for seven different fatty acids concentrations. Quality control samples were prepared at four different concentrations.

Methodology consisted of the following steps: 0.5 ml of milk was placed in test tubes with Teflon-lined screw caps, and 750 μl of n-butanol was added to each tube. The samples were vortexed at low speed while 75 μl of acetyl chloride was added. The samples were then gassed with N2, capped tightly, and heated at 100°C for 1.5 h. After samples cooled to room temperature, 5 ml of 6% K2CO3 and 1 ml of hexane were added, and the samples were vortexed for an additional 30 s. Then the samples were centrifuged for 20 min at 2500 g, and the bottom layer was aspirated and discarded. The remaining layer was washed four times (20 min at 2500 g) with distilled, deionized water. The upper layer was removed and placed in injection vials for analysis. Results were expressed in relative percentage of total fatty acids.

2.4. Statistical analysis

Fatty acid concentrations were analysed using descriptive statistics, and a comparison between the two groups was performed by ANOVA, using a mixed model, correcting for variables of greater biological interest (Littell et al. 1996). Mixed model:

\[ y_{ijklmn} = \mu + G_i + cow(G_i) + PN_j + BCS_k + MY_m + BHB_n + e_{ijklmn} \]

where \( y_{ijklmn} \) = fatty acid in grams/100 g of fat; \( G_i \) = fixed effect of group (high and low concentrate); \( cow(G_i) \) = random effect of cow nested in group; \( PN_j \) = fixed effect of parity number (primiparous and multiparous); \( BCS_k \) = random effect of body condition score at calving; \( MY_m \) = random effect of milk yield at 30 days pp; \( BHB_n \) = random effect of BHB concentration; \( e_{ijklmn} \) = error term.

The best fitting model was identified as the model with the highest value of Schwarz Bayesian Criterion (Littell et al. 1996).
3. Results

Table 1 presents descriptive statistics for average PN, MY, BCS and BHB for each group. Milk production was statistically different (35.2 ± 1.5 vs. 17.8 ± 0.22 l/day for Groups A and B, respectively) (p ≤ .05). Table 2 presents the average fatty acid profile values for Groups A and B, expressed in grams/100 g of fat. The model for each fatty acid was corrected for BCS, PN, MY, and BHB concentration at the time of sampling. In general, for palmitic acid and greater number of carbons, the r² ranged from 50% to 75%.

No significant differences were observed between groups for total values of SFA, though palmitic acid (C 16:0) was higher in Group A. Total concentrations of MUFA were significantly higher in Group A as compared to Group B (p ≤ .05), and oleic acid (C 18:1) and palmitoleic acid (C 16:1) contents were also higher in Group A than Group B.

PUFA did not vary significantly, though values of linoleic acid (C 18:3) were significantly higher in Group B (p ≤ .05) than Group A. With respect to CLA, values were again significantly higher in Group B as compared to Group A (0.91 ± 0.29 and 1.67 ± 0.45 g/100 g fat, respectively); average values were 46% higher for animals with increased levels of grazing and low supplementation of concentrate than low levels of grazing and increased supplementation of concentrate (p ≤ .01). Additionally, the ratio of omega-6: omega-3 fatty acids was significantly lower in Group B than Group A (p ≤ .05).

4. Discussion

This study aimed to compare milk fatty acid profiles, with special emphasis on CLA content of milk from cows receiving varying levels of concentrate supplement and grazing. The study hypothesized that animals receiving increased level of grazing and low concentrate supplementation would produce milk with higher concentrations of CLA, as compared to animals receiving higher levels of concentrate. The hypothesis was confirmed by the results of this study: Group B (high grazing, lower concentrate) produced milk with 46% higher CLA as compared to Group A (lower grazing, higher concentrate). Although the association between diet and CLA milk content has been well established in many other studies worldwide (Dannenberger et al. 2005; Dhiman et al. 2005; Morales et al. 2010), this report is one of the first in Chile to find that increased grazing produces higher milk CLA content. The results of this study support those reported by Morales et al. (2015), who found that cows in south Chile produced milk with 50% higher CLA content when grazing and receiving mixed feed, as compared to those receiving total mixed ration diets.

All animals were sampled at the same time of the year and received similar quality of grazing and concentrate (Table 3). Statistical models for each fatty acid corrected for BCS at calving, PN, milk production, and BHB concentration. The model for CLA content explained 53% of the variability of this fatty acid in milk.

The higher concentration of CLA in milk from cows with increased grazing activity may be partially attributed to the alpha linolenic acid (C 18:3) content of grasses during spring (Jenkins & Havartine 2014). Cows were sampled during October and November 2012, a time when normal precipitation rates were observed for the area and grasslands showed typical growth and chemical composition for the season. Cows with increased grazing activity and therefore consuming proportionately more linolenic acid may have been able to biohydrogenate one of the carbons from the three double bonds in the rumen to produce a fatty acid with only two double bonds (linoleic acid) and, via isomerization, produce a fatty acid with a conjugated structure (two double bonds separated by a single bond). This process of biohydrogenation and conjugation occurs exclusively in the rumen and thus represents the only source of CLA that can be deposited in both body fat and milk (Bauman & Grilini 2003; Jenkins et al. 2008; Jenkins &
Harvatine 2014). CLA is considered an essential nutrient for human beings. The human body is unable to synthesize it and can only obtain this fatty acid from animal products such as those of ruminant species (cattle, sheep, goats, buffalo) (Benjamin et al. 2015).

Fatty acids found in pastures can therefore be considered a primary precursor of CLA for bovine milk, given that ryegrass (the primary forage consumed by the animals in this study) is high in linoleic and linolenic acid (20.4% and 55.9%, respectively) (Dhiman et al. 2005). Biohydrogenation in the rumen increases as unsaturation increases. Butyrivibrio fibrisolvens bacterium performs linoleic acid biohydrogenation and transforms it into a MUFA, generating a variety of CLA isomers (Bauman & Griinari 2003; Jenkins et al. 2008). The rumen biohydrogenation rate typically increases with increased unsaturation, and linoleic and linolenic acid are generally hydrogenated at 70–95% and 85–100%, respectively (Bauman & Griinari 2003).

Animals at Dairy A consuming corn silage, which has a low percentage of linolenic acid (6.1%) (Dhiman et al. 2005), would experience lower rates of bacterial biohydrogenation and subsequent transformation into CLA precursors as compared to animals at Dairy B. Dhiman et al. (2005) also indicate that corn silage has a higher proportion of C18: 0 (stearic acid) as compared to pasture. This fatty acid is important because it is a precursor to the synthesis of oleic acid in the mammary gland. Stearic acid is absorbed in the rumen, mobilized via the bloodstream to the mammary gland, and converted to cis-9 C18: 1 by stearoyl CoA desaturase (SCD) present in the mammary tissue (Bauman & Griinari 2003). Corn silage also contains a high percentage of linoleic acid (40.9%), which is converted to vaccenic trans acid and can then be absorbed and converted to cis-9, trans-11 CLA by SCD in the mammary gland, or biohydrogenated to C18: 0, which is absorbed in the rumen and transformed into oleic acid C18: 1 by the SCD enzyme in the mammary gland (Jenkins et al. 2008). This type of diet may consequently explain the differences between the two groups in milk oleic acid content (C18: 1) (21.85% higher in Dairy A, high concentrate consumption (26.14% and 24.02% for Groups A and B, respectively). Other studies have reported palmitic acid values greater than 28%. This is of nutritional importance because palmitic acid, together with myristic acid (C14: 0), is considered hypercholesterolemic in humans (Benjamin et al. 2015).

Significant differences were also observed for the ratio of omega-6:omega-3 (p ≤ .05). Group B presented more favourable values from the point of view of human nutrition. Milk from grazing systems, in addition to providing a source of energy, provides essential fatty acids, which, through various metabolic processes are transformed and deposited within cell membranes, where they serve as precursors to eicosanoids (prostaglandins, prostacyclins, thromboxanes, and leukotrienes) involved in physiological processes, such as blood clotting and inflammatory and immune responses (Benjamin et al. 2015).

In the light of these results, dairies using intensive grazing will produce milk with higher CLA content and a more favourable ratio of omega-6:omega-3 but will also produce less milk per cow per day. Despite the potential positive effect of CLA and omega-3 fatty acid on human health, the dairy market does not reward higher milk fatty acid content. Worldwide guidelines only consider total fat and protein content. In the future, the fatty acid content might receive a higher commercial value. In anticipation of such a transition, the dairy business should be prepared with evidence, such as that provided by this study, demonstrating how nutritional strategies affect the nutritional content of milk.

5. Conclusion

Cows with intensive grazing and reduced concentrate supplementation produced milk with higher CLA and omega-3 fatty acid content as compared to cows receiving higher levels of concentrate and low grazing activity.

Disclosure statement

No potential conflict of interest was reported by the authors.

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