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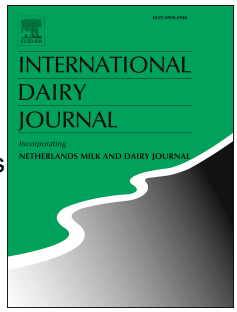

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Effect of olive oil in dairy cow diets on the fatty acid profile and sensory characteristics of cheese

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ABSTRACT

The effect of dietary unrefined olive oil (OO) residues and hydrogenated vegetable oil (HVO) on the fatty acid profiles of milk and cheese and the sensory characteristics of cheeses was determined. For 9 weeks, animals were fed a control diet with no added lipid (n = 5 cows), or fat-supplemented diets containing OO or HVO (in both cases n = 5 cows; 30 g kg^{-1} dry matter). Compared with control and HVO, OO increased C18:1 cis-9, and C18:3 cis-9, cis-12, cis-15 fatty acids in milk; and also increased C18:1 trans-10, C18:1 trans-11, C18:1 cis-9, C18:2 cis-9, trans-11 and C18:3 cis-9, cis-12, cis-15 fatty acids in cheeses. OO reduced the number of holes, overall odour and acidity of cheeses, whereas HVO increased the cow milk odour, bitterness and acidity of cheeses. Overall, OO can improve the cheese fatty acid profile, but with adverse effects on sensory attributes.

1. Introduction

Consumers are becoming increasingly aware that food components such as dietary fatty acids (FAs) have the potential to influence human health maintenance and disease prevention (Halmemies-Beauchet-Filleau et al., 2017). The diet of dairy cows is a major factor affecting the FA composition of milk fat and improving the content of C18:1 isomers (i.e., C18:1 trans-11 FA or vaccenic acid) and other polyunsaturated FAs (i.e., C18:2 cis-9, trans-11 FA or rumenic acid). This has led to extensive research in which the dairy cow diet has been supplemented with different ingredients such as dry olive pomace (Castelleni et al., 2017); extruded soybeans (Khanal et al., 2005), extruded linseeds (Lerch et al., 2015), fish oil (Vargas-Bello-Perez, Iniguez-Gonzalez, Fehrmann-Cartes, Toro-Mujica, & Garnsworthy, 2015b), soybean oil and hydrogenated vegetable oil (Vargas-Bello-Perez, Fehrmann-Cartes, Iniguez-Gonzalez, Toro-Mujica, & Garnsworthy, 2015a) and calcium salts of palm and fish oil in combination with soybean products (Allred et al., 2006). These studies have reported no effects on the sensory properties of experimental cheeses.

Olives are a major crop in Mediterranean South American countries such as Argentina and Chile and olive oil plantations co-exist with dairy farms in Central Chile. Olive oil extraction is associated with production of large quantities of residues (unrefined olive oil) that require extra processing to convert them into virgin olive oil (Beltran-Ortega, Martínez Gila, Aguilera Puerto, Gámez García, & Gómez Ortega, 2016). Feeding crude (unrefined) olive oil to dairy cows is not common and is rarely cited in reports, contrary to the case with sheep (Vargas-Bello-Perez et al., 2013b).

Crude olive oil residues and other olive oil by-products, however, represent a potentially valuable high energy feed source for dairy cows, which might enhance the FA composition of milk and dairy products (Castelleni et al., 2017). Hydrogenated vegetable oils (HVO) are used to increase the energy density of the diets for high-production dairy cows without negative effects on milk yield and composition and they are readily available for animal use in Chile (Vargas-Bello-Perez et al., 2015a). To our knowledge, no study has been published reporting the effects of dietary supplementation with unrefined olive oil residues (OO; as a monounsaturated FA source) and HVO (as a saturated FA source) on the sensorial properties of cheeses. This study aimed to enhance the FA composition of milk and cheeses while maintaining milk production, milk composition, cheese chemical composition and cheese sensory characteristics. The main hypothesis tested in this study was that the degree of saturation (monounsaturated versus saturated FAs) of dietary lipids can affect the FA profile of milk and cheese, thus influencing the organoleptic properties of the cheese produced.

2. Materials and methods

2.1. Animals and treatments

The study was conducted at the Estación Experimental Pirque of the Pontificia Universidad Católica de Chile (33°38′28″S, 70°34′27″W). Animals were housed in individual stalls (2.4 × 6 m) and had continuous access to water. Animal care and

procedures were carried out according to the guidelines of the Animal Care Committee of the Pontificia Universidad Católica de Chile.

Fifteen Holstein cows averaging (± SD) 189 ± 28 days in milk at the beginning of the study were assigned to three treatment groups based on body condition score (BCS; scored on a five-point scale where 1 = emaciated to 5 = overly fat; Wildman et al., 1982) and milk yield to achieve comparable groups. Before commencing the study, the average BCS for the 3 groups were 2.8 ± 0.3, 3.0 ± 0.0, and 2.8 ± 0.3. For 9 weeks all cows received a basal diet containing 65% forage (corn silage, fresh alfalfa and alfalfa hay) and 35% concentrate (malt distillers, corn grain, wheat bran, soybean grain and rapeseed meal) to satisfy the nutritional requirements of a 650 kg dairy cow in mid-lactation consuming 26.5 kg DM daily (NRC, 2001) and were isocaloric (NE_L = 1.6 Mcal kg^{-1} DM). Cows were individually fed at a fixed rate (so that cows consumed all their feed and treatment). The control or basal diet contained no added lipid (n = 5 cows); treatment diets were supplemented with OO (n = 5 cows; unrefined olive oil; 30 g kg^{-1} DM) or HVO (n = 5 cows; manufactured from palm oil; 30 g kg^{-1} DM). Oils were mixed manually into the daily ration for each cow. Dietary oils had distinct differences in their main FA contents: olive oil contained (in g 100 g^{-1} total FAs) 14 of C16:0 and 74 of C18:1 cis-9, whereas HVO contained (in g 100 g^{-1} total FAs) 58 of C16:0 and 40 of C18:0. Treatment diets were sampled every 14 days and stored at -20 °C for later chemical analyses. Standard procedures used to analyse the chemical composition of experimental diets were reported previously (Vargas-Bello-Perez et al., 2015a,b). Ingredients, chemical composition and FA profiles of the diets are shown in Table 1. BCS and body weight were measured on d 21, 42 and 63.

2.2. Milk yield and composition

Cows were milked daily at 07:00, 15:00 and 22:00 h in a 2 × 6 parallel milking parlour equipped with DELPRO™ farm manager system (DeLaval, Sweden). Milk yields were recorded electronically at each milking time and individual milk samples were taken as previously reported by Vargas-Bello-Perez et al. (2015a,b) on d 21, 42 and 63. Milk samples were analysed for fat, protein, and somatic cell count by using an infrared analyser (Milko-Scan CombiFoss 6000; Foss Electric, Hillerød, Denmark).

2.3. Cheese manufacturing and compositional analyses

Milk collected on days 21, 42 and 63 from cows on the same treatment was pooled, made into cheese (n = 9 cheeses per treatment), ripened for 7 days and later analysed in terms of FA profile and sensory characteristics. To analyse the effect of dietary lipids, milk used for cheese manufacture was not standardised for fat content. Chanco-style cheeses were made in a pilot plant as follows: 15 L of milk per treatment per period were pasteurised at 63 °C for 30 min, and cooled to 31 °C before addition of calcium chloride solution (6 g $CaCl_2$ 100 mL^{-1} H_2O) at a rate of 333 mL 100 L^{-1} of milk and equilibrated for 3 min. No starter culture was added for cheese making. A solution of commercial calf rennet [20 g 100 g^{-1} deionised water; strength of 1:10 000; Kyrein(r) (Santiago, Chile)] was added at a concentration of 100 g 100 L^{-1} of milk to aid dispersion and avoid localised destabilisation of casein micelles in milks. Once the coagulum

developed enough firmness (~45 min), the curd was cut with knives into cubes of 2 cm, healed for 5 min, cooked to 38 °C at a heating rate of 1 °C 3 min^{-1} and maintained at cooking temperature for additional 15 min. The whey was then completely drained from the vat. The curd was milled and brined-salted with NaCl solution (18 g 100 mL^{-1} H_2O) at a level of 2 L 100 L^{-1} milk over a 20 min period. The salted curds were hopped on 500 g moulds and pressed for 14 h at 20 °C. Experimental cheeses were then ripened at 10 °C and 70% relative humidity for 7 days (usual ageing days for the artisan Chanco-style cheeses; Oliveira & Brito, 2006).

On days 21, 42 and 63, three cheeses per treatment were obtained and two cores of each cheese were used for analysis of chemical composition and FAs. Cheeses were analysed for moisture content (oven drying method), fat content (Gerber method), total protein (macro-Kjeldahl method; N × 6.38) and ash (gravimetric method) as previously described (Vargas-Bello-Perez et al., 2015a,b). Cheese colour was measured with a Konica-Minolta colorimeter CR-400 (Konika Minolta Optics Inc., Osaka, Japan) based on the CIELAB colour system (CIE, 1986). Measurements were performed on six random measurements on the cheese surface and on the cheese core after removing a layer of 3 cm from the upper surface.

2.4. Fatty acid analysis

Milk fat separation was carried out using the non-solvent method according to Feng, Lock, and Garnsworthy (2004) and the transesterification of FAs according to Chouinard, Corneau, Saebo, and Bauman (1999) and Christie (1982). Lipids from

cheeses were extracted according to Bligh and Dyer (1959) and methylated as previously indicated for milk samples. A gas chromatography system (GC 2010; Shimadzu Scientific Instruments AOC-20s, Columbia, MD, USA) equipped with a 100 m column (Rtx column 100 m × 0.32 mm × 0.20 µm) was used. The GC conditions were as follows: oven temperature was initially set at 110 °C for 4 min after injection, and then ramped to 160 °C at 5 °C min^{-1} and held for 10 min. Temperature was then ramped to 225 °C at 3 °C min^{-1} and held for 10 min, and finally ramped to 240 °C at 3 °C min^{-1}; total run time, therefore, was 61 min. Inlet and flame-ionisation detector temperatures were 260 °C, the split ratio was 15:1, and a 2 µL injection volume was used. Hydrogen carrier gas flow to the detector was 25 mL min^{-1}, airflow was 400 mL min^{-1}, and flow of nitrogen makeup gas was 40 mL min^{-1}. Fatty acid GC peaks were identified using a FA methyl ester (FAME) standard (37 Component FAME mix; Supelco, Bellefonte, PA, USA), and reference standards for C18:1 trans-11 and C18:1 cis-9, trans-11 FAs (Nu-Chek-Prep Inc., Elysian, MN, USA). Atherogenic index (AI) and thrombogenic index (TI) were calculated according to equations of Ulbricht and Southgate (1991):

$$AI = [(12:0 + 4(14:0) + 16:0] / [(n\text{-}6 + n\text{-}3)\ PUFA + 18:1 + \sum MUFA]$$

$$TI = (14:0 + 16:0 + 18:0) / [(0.5 \times 18:1) + 0.5\ (\sum MUFA) + 0.5\ (n\text{-}6PUFA) + 3\ (n\text{-}3PUFA) + (n\text{-}3PUFA/n\text{-}6PUFA)].$$

2.5. Sensory analysis of cheeses

Three cheeses per treatment, at 7 days of ageing, were used for sensory evaluation on each sampling period (21, 42 and 63 days). The sensory panel comprised

177 twelve judges familiar with the attributes, definitions and the numerical scale used in the
178 study. Judges were not provided with any information regarding treatment of samples in
179 any testing session. Before evaluation, the panel judged commercial Chanco cheese in
180 a pre-testing session to standardise the panel´s definitions for sensorial attributes.
181 Evaluations considered the following attributes: colour homogeneity, holes, overall
182 odour, ripe cheese odour, cow milk odour, salty, acid, bitter, overall flavour, ripe cheese
183 flavour, sharpness, toughness, graininess, screeching, moisture and greasiness. The
184 sensory descriptors and definitions from the appearance, aroma, flavour and texture
185 have been previously described (Vargas-Bello-Perez et al., 2015a). Judges evaluated all
186 samples (cheese cubes of 2 × 2 × 2 cm) in a monadic sequential way, scoring attributes
187 on a continuous unstructured line intensity scale ranging from 0 to 9 and anchored at
188 both ends with extremes for each attribute.
189
190 *2.6. Statistical analyses*
191
192 All data were analysed using the MIXED procedure in SAS (SAS Institute Inc.,
193 Cary, NC). A model including diet, time, and diet × time as fixed effects and cow within
194 treatment as random effect was used to determine differences in BCS, body weight and
195 milk (performance, proximate analysis and FA profile) and cheese (proximate analysis,
196 colour, FA profile and sensory characteristics) samples. Least squares means (LSM)
197 were separated using the PDIFF (Piecewise Differentiable) statement in SAS.
198 Further analysis included a correlation matrix and a factorial analysis by principal
199 component analysis using SPSS statistical software for Windows (version 15.0.0; SPSS

Inc., Chicago, IL, USA). To determine which sensory attribute was responsible for differentiation between cheeses (that were made from different dietary treatments), a multivariate analysis was carried out using a correlation matrix between sensory attributes to discard those that showed high correlation (r = >0.9) and those without significant correlations. The factorial analysis by principal component analysis (PCA) included the Bartlett's test of sphericity which was applied to examine the hypothesis that the variables were uncorrelated in the population and the Kaiser-Meyer-Olkin index was used to measure sampling adequacy.

3. Results and discussion

3.1. Diets and animal performance

The FA composition (g 100 g^{-1} FAs) of oil supplements was reflected in the FA profile of dietary treatments (Table 1). For example, OO was composed mainly of C18:0 and C18:1 cis-9 FAs, whereas HVO contained mainly C16:0 and C18:0 FAs. In terms of animal performance, dry matter intake was not affected by treatments; this is explained in part by the amount of dietary oils supplemented to the basal diet (30 g kg^{-1} DM), which has been previously reported as being sufficient for establishing complete biohydrogenation of dietary FA without compromising feed intake, BCS and body weight (Vargas-Bello-Perez et al., 2015a,b).

3.2. Milk yield, milk composition and cheese composition

223

224 Supplementing fat through OO resulted in a 10.9% increase in milk yield
225 compared with control and HVO. This is similar to previous studies reporting increases
226 in milk yield when cow feed was supplemented with vegetable oils such as soybean oil
227 (Bu, Wang, Dhiman, & Liu, 2007; Vargas-Bello-Perez et al., 2015a) and blends of olive
228 oil, linseed oil and rapeseed oil (Lock & Garnsworthy, 2002). There was a time effect in
229 milk yield and protein content (g 100 g^{-1}); in both parameters, the higher values were
230 found in the third experimental period, possibly due to rumen microbial adaptation to
231 dietary lipid supplements, adaptation to the general animal management and the natural
232 changes in milk composition as days in milk progresses.
233 OO resulted in a 14.6% decrease in milk fat yield and a 13.7% decrease in milk
234 fat content. These differences are important for producers especially when milk income
235 is based on kilograms of solids. Because OO was added to the diet as unprotected oils,
236 an increase in ruminal biohydrogenation intermediates most likely occurred, as indicated
237 by C18:1 trans-11 and C18:2 cis-9, trans-11 FA contents in milk and cheese (Bauman,
238 Harvatine, & Lock, 2011). Some of these FAs can affect expression of several genes
239 involved in lipid metabolism in the mammary gland. Harvatine and Bauman (2006)
240 reported that the mechanisms involved in the inhibition of milk fat synthesis is a
241 coordinated downregulation of mammary gene expression of rate-limiting lipogenic
242 enzymes, including lipoprotein lipase (LPL), acetyl-CoA carboxylase (ACC), fatty acid
243 synthase (FAS), and stearoyl-CoA desaturase (SCD).
244 An interesting finding from the current study was that somatic cell count (SCC)
245 was reduced by OO. SCC in milk is important since it is inversely related to milk quality

and safety and is a metric through which farmers may incur penalties. This result may be related to the deleterious effects (increase membrane fluidity and permeability) on cell membranes that unsaturated FA sources (such as OO) usually cause (Maia, Chaudhary, Figueres, & Wallace, 2007); however, further research will be needed to fully understand this effect.

On average, treatments resulted in 51.3 ± 2.2 g 100 g^{-1} moisture, 23.1 ± 1.2 g 100 g^{-1} fat, 20.7 ± 1.7 g 100 g^{-1} total protein and 2.3 ± 0.1 g 100 g^{-1} ash in cheeses (Table 2). Fat contents of cheeses were in accordance with the Chilean standard for full-fat Chanco cheese (INN, 1999), establishing minimum fat levels of 25 g 100 g^{-1} of fat.

3.3. Fatty acid composition of milk and cheeses

Compared with control and HVO, OO decreased ($P < 0.05$) C12:0 FA and increased ($P < 0.05$) C18:1 cis-9 and C18:3 cis-9, cis-12, cis-15 FAs in milk whereas HVO and OO increased ($P < 0.05$) C18:1 trans-10, C18:1 trans-11, C18:2 cis-9, trans-11 FAs and reduced ($P < 0.05$) C8:0 FA in milk (Table 3). Compared with control and HVO, OO decreased ($P < 0.05$) C4:0 and C10:0 FAs, and increased ($P < 0.05$) C18:1 trans-10, C18:1 trans-11, C18:1 cis-9, C18:2 cis-9, trans-11 and C18:3 cis-9, cis-12, cis-15 FAs in cheeses (Table 4). OO increased ($P < 0.05$) total polyunsaturated FAs (PUFAs) in milk and reduced ($P < 0.05$) total saturated FAs (SFAs) and increased ($P < 0.05$) total monounsaturated FAs (MUFAs) and total PUFAs in cheeses. OO decreased ($P < 0.05$) AI and TI in milk and cheese. There was a time effect on the following milk FAs (their contents were higher in the third experimental period): C15:0, C15:1 iso; C17:1 cis-9;

C18:1 trans-10; C18:1 trans-11; C18:2 trans-9, trans-12; C18:2 cis-9, cis-12; C18:3 cis-6, cis-9, cis-12 and C18:2 cis-9, trans-11.

The FA profiles observed in milk and cheeses are explained in part by the chemical structure of dietary lipids, since they can have an impact on ruminal microorganisms involved in the biohydrogenation process (Vargas-Bello-Perez, Cancino-Padilla, Romero, & Garnsworthy, 2016). Microbial biohydrogenation is the process whereby unsaturated FAs are chemically transformed until their conversion to saturated FAs such as C18:0 FA (Castagnino et al., 2015). The OO treatment resulted in effects similar to those reported previously in milk (increase in the content of C18:2 cis-9, trans-11 FA) when cows were supplemented with vegetable oils and oilseeds rich in C18:2 cis-9, cis-12 FA (Bu et al., 2007) and also comparable results (increase in the content of C18:3 cis-9, cis-12, cis-15 FA) in cheese FA profiles when cows were fed with linseed (Lerch et al., 2015).

OO increased contents of C18:1 cis-9 FA and decreased total contents of SFAs and the AI in milk and cheese, which is similar to results reported when dairy ewes were supplemented in their diet with olive by-products such as olive cake (Vargas-Bello-Perez et al., 2013a) and olive oil (Bodas et al., 2010; Vargas-Bello-Perez et al., 2013b). Despite the fact that dietary intake of C18:1 cis-9 FA by ruminants is low and largely biohydrogenated in the rumen, milk contents of this FA comes predominantly from delta-9-desaturase action on C18:0 in the mammary gland (Lanier & Corl, 2015). This enzyme is also responsible for production of other MUFAs in milk fat that have a cis-9 double bond (C14:1 and C16:1) and conjugated linoleic acid isomers (Bessa, Alves, & Santos-Silva, 2015). The fact that C18:1 cis-9 FA was increased by OO is relevant for

human health, since this FA has a protective role against cardiovascular disease and the early and late cellular atherosclerotic process (Perdomo et al., 2015).

C18:1 trans-10 FA was below 1.8% of the total FAs in milk and cheese samples, the contents of this FA were higher in OO than that of the other treatments suggesting that this FA is produced mainly by C18:1 cis-9 FA and C18:2 cis-9, cis-12 FA biohydrogenation in the rumen as reported by Bodas et al. (2010) when ewes were supplemented with OO. The relevance of C18:1 trans-10 FA is important since its rumen outflow and milk fat content is highly correlated with the reduction in the overall milk fat content (Bauman et al., 2011).

In the current study, the saturated FA nature of HVO was reflected in the SFA content in milk. Generally, inhibition of de novo mammary synthesis is more sensitive to unsaturated FA sources (Salado, Gagliostro, Becu-Villalobos, & Lacau-Mengido, 2004) such as OO. SFA concentrations of milk and cheese were reduced with OO treatment. According to Ulbricht and Southgate (1991), C12:0 and C14:0 FAs are SFAs that can promote atherosclerosis and coronary thrombosis. The reduction of SFA content in milk has been reported previously when vegetable oils are incorporated into dairy cow diets (Vargas-Bello-Perez et al., 2015a). Public health policies recommend a decrease in consumption of SFAs and an increase in PUFAs, to reduce the incidence of cardiovascular and metabolic diseases (Perk et al., 2012). However, as also found in this study, ruminant milk and cheese fat also contains several FAs with positive effects on human health (Halmemies-Beauchet-Filleau et al., 2017) including C18:1 trans-11 FA, and C18:2 cis-9, trans-11 FA. In this regard, in a hypothetical situation, drinking milk from cows consuming and OO-supplemented feed will most likely help prevent

cardiovascular problems and development of deposits of fibrous tissue and lipid on arterial walls among others health benefits (Perdomo et al., 2015).

3.4. Sensory characteristics of cheese

To our knowledge, this is the first study to analyse the effect of feeding crude OO to dairy cows on sensory characteristics of Chanco-style cheese (a semi hard and oily cheese). Because the objective of this study was to measure effects of treatments on FA profile and sensory characteristics of cheeses, no standardisation for milk fat content was used.

Colour (Table 5) of cheeses was not affected by dietary treatments. Compared with control and HVO, OO reduced ($P < 0.05$) number of holes, overall odour and acidity, whereas, compared with control, HVO increased ($P < 0.05$) cow milk odour, bitterness and acidity. Compared with control, both OO and HVO increased ($P < 0.05$) salty flavour (Table 6). PCA (Fig. 1) showed the following results: OO cheese had the lowest scores for PC1, whereas HVO cheese had the highest scores for both PC1 and PC2. Cheese made from control treatment exhibited the lowest scores for PC2 and intermediate scores for PC1.

In terms of texture, it has been shown that feeding dairy cows with extruded linseed can lead to cheeses with a less firm texture and are more meltable when ripened for 8 and 12 weeks (Lerch et al., 2015). Similarly, Ryhänen et al. (2005) reported that dietary rapeseed oil led to a softer texture in 6 week Edam cheese. These authors concluded that increased levels of unsaturated FAs in milk often result in softer cheeses.

However, Vargas-Bello-Perez et al. (2015a) found no difference in the texture of 14 d Chanco cheeses made from diet supplementation with soybean oil and HVO. In the current study, similarities in texture attributes among treatments may be due to short ripening times (1 week). However, when sensory data were analysed by PCA, OO cheese was associated with lower scores in textural and odour attributes than the Control and HVO cheeses.

In general, the major changes occurring in cheese texture are caused by a combined effect of solubilisation of colloidal calcium phosphate, led by acidification during the first month of ripening, and probably by proteolysis of the cheese matrix (Lucey, Johnson, & Horne, 2003); however, the latter should be analysed in future experiments. On the other hand, increasing unsaturation levels of FAs might not only decrease cheese toughness, but also induce other types of defects in cheese texture (sandiness, gumminess), appearance (pale colour and eye-formation problems) and flavour, since unsaturation may be prone to lipid oxidation that leads to cardboard scores (Coppa et al., 2011; Lerch et al., 2015; Ryhänen et al., 2005). It is possible that most of the changes observed in the sensory characteristics of cheeses were mainly due to the increased contents of MUFAs in milk and cheeses. Further research is needed to evaluate the effect of different times of cheese ripening because it is known that as cheese ages its flavour characteristics change, especially if the cheese is rich in unsaturated FAs (Allred et al. 2006).

4. Conclusion

Supplementing dairy cow diets with OO or HVO did not affect the main components of cheese. However, OO increased milk yield, and reduced milk fat yield, milk fat content and milk somatic cell counts. From a human nutrition standpoint, OO improved the FA profile of cheeses. Attributes related to appearance, odour, flavour and texture were adversely affected by OO and HVO. Findings reported in this study indicate that an agro industrial product such as unrefined OO residues can be used to improve the FA profile of dairy products and could be considered as an alternative feedstuff for dairy cows.

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Figure legend

Fig. 1. Principal component analyses (a, PC 1 versus PC 2; b, PC1 versus PC3; c, PC3 versus PC4) for all 16 sensory attributes of all dietary treatments. Control, no fat supplement; OO, supplemented with 30 g kg^{-1} DM olive oil; HVO, supplemented with 30 g kg^{-1} DM hydrogenated vegetable oil.

Table 1

Ingredient and chemical composition of control, olive oil (OO), and hydrogenated vegetable oil (HVO) dietary treatments. [a]

Component	Diet		
	Control	OO	HVO
Ingredient composition (% DM)			
Fresh alfalfa	28.9	28.9	28.9
Corn silage	27.0	27.0	27.0
Malt distillers	23.1	23.1	23.1
Corn grain	8.3	8.3	8.3
Wheat bran	6.2	6.2	6.2
Alfalfa hay	2.6	2.6	2.6
Soybean grain	2.0	2.0	2.0
Rapeseed meal	1.5	1.5	1.5
Vitamin and mineral premix[a]	0.4	0.4	0.4
Olive oil	0	3.0	0
Hydrogenated vegetable oil	0	0	3.0
Chemical composition (% DM)			
Dry matter	38.4	38.9	38.4
Crude protein	14.4	13.4	14.3
Ether extract	4.6	7.7	7.1
Neural detergent fibre	33.5	31.1	33.4
Acid detergent fibre	19.8	23.1	19.4
Lignin	4.2	4.5	4.5
Ash	6.2	5.0	6.0
Fatty acid composition (g 100 g^{-1} FA)			
C6:0	0.9	0.1	nd
C10:0	0.8	0.1	nd
C12:0	1.1	0.2	nd
C14:0	3.7	0.2	0.3
C16:0	23.7	12	39.2
C18 :0	32.3	26.3	30.8
C18:1 cis-9	1.0	32.8	nd
C18:2 cis-9, cis-12	26.3	19.0	20.0
C18:3 cis-6, cis-9, cis-12	0.5	0.2	0.4
C18:3 cis-9, cis-12, cis-15	9.7	9.1	9.3

[a] Vitamin and mineral premix contained (per kg): 25 g P; 80 g Ca; 25 g Mg; 1.6 g S; 300 000 IU vitamin A; 50 000 IU vitamin D_3 and 1 600 IU vitamin E. nd, not detected.

Table 2

Performance and proximate analysis of milk and cheese from cows fed control, olive oil (OO), and vegetable hydrogenated oil (HVO) dietary treatments. [a]

Parameter	Diet			SEM	*P*-value	
	Control	OO	HVO		Diet	Time
Production						
Dry matter intake (kg DM day^{-1})	26.5	26.5	26.5			
Milk yield, kg day^{-1}	31.1^{b}	34.9^{a}	31.8^{b}	3.13	0.04	<0.001
Fat, kg day^{-1}	1.02^{a}	0.88^{b}	1.04^{a}	0.12	0.05	0.57
Protein, kg day^{-1}	1.05	0.97	1.08	0.25	0.58	0.48
Milk composition, g 100 g^{-1}						
Fat	3.28^{a}	2.83^{b}	3.28^{a}	0.31	0.04	0.94
Protein	3.39	3.16	3.41	0.36	0.29	<0.001
Somatic cell count, × 10^{3} mL^{-1}	358^{a}	145^{c}	254^{b}	82.0	0.02	0.61
Body weight, kg	662	636	700	79.0	0.23	0.07
Body condition score	2.97	2.77	2.98	0.33	0.34	0.04
Cheese composition, g 100 g^{-1}						
Fat	23.7	22.6	23.1	1.74	0.82	0.70
Protein	20.9	22.0	19.2	2.41	0.54	0.25
Moisture	55.1	49.1	49.8	3.14	0.19	0.81
Ash	2.61	2.32	2.16	0.19	0.14	0.47

[a] Control, no fat supplement; OO, supplemented with 30 g kg^{-1} DM olive oil; HVO, supplemented with 30 g kg^{-1} DM hydrogenated vegetable oil; SEM, standard error of the mean; BCS, scored on a five-point scale where 1 = emaciated to 5 = overly fat (Wildman et al., 1982). Means in the same row with different superscript letters are significantly different (diet P<0.05). Cows were individually fed at a fixed rate and did not show feed refusal.

Table 3

Milk fatty acid profile from cows fed control, olive oil (OO), and hydrogenated vegetable oil (HVO) dietary treatments. [a]

Fatty acid	Diet			SEM	*P*-value		
	Control	OO	HVO		Diet	Time	Diet × Time
C4:0	4.52	4.06	4.24	0.28	0.27	0.93	0.75
C6:0	2.82	2.49	2.93	0.26	0.24	0.57	0.98
C8:0	2.00[b]	1.31[a]	1.68[b]	0.23	0.02	0.41	0.68
C10:0	4.20	3.44	4.40	0.48	0.12	0.59	0.86
C11:0	0.26[a]	0.17[b]	0.27[a]	0.02	<0.001	0.87	0.99
C12:0	5.27[a]	4.14[b]	5.06[a]	0.34	<0.001	0.29	0.92
C13:0	0.16	0.11	0.10	0.04	0.38	0.67	0.45
C14:0	15.8	15.1	15.3	0.50	0.57	0.12	0.57
C14:1 cis-9	0.85	0.96	0.82	0.13	0.50	0.48	0.70
C15:0	0.95	0.73	0.67	0.17	0.22	<0.001	0.06
C15:1 iso	0.91	0.72	0.68	0.16	0.34	<0.001	0.45
C16:0	40.7	40.1	40.6	1.53	0.92	0.65	0.42
C17:0	0.61	0.43	0.84	0.19	0.10	0.11	0.64
C17:1 cis-9	0.41	0.47	0.41	0.06	0.50	0.02	0.10
C18:0	5.05	3.70	4.20	0.81	0.25	0.18	0.41
C18:1 trans-10	0.59[b]	0.73[a]	0.54[b]	0.11	0.25	0.05	0.06
C18:1 trans-11	1.86[b]	2.51[a]	1.16[b]	0.37	0.03	0.02	0.45
C18:1 cis-9	8.39[b]	12.2[a]	8.89[b]	1.22	0.04	0.25	0.79
C18:2 trans-9, trans-12	0.58	1.12	1.07	0.25	0.07	<0.001	0.06
C18:2 cis-9, cis-12	0.23	0.35	0.35	0.19	0.75	<0.001	0.08
C18:3 cis-9, cis-12, cis-15	0.95[b]	1.21[a]	0.72[b]	0.19	0.05	0.61	0.91
C18:3 cis-6, cis-9, cis-12	0.13	0.35	0.20	0.13	0.24	0.51	0.76
C18:2 cis-9, trans-11	0.11[b]	0.37[a]	0.42[a]	0.08	0.03	0.01	0.12
C20:1n-9	0.11	0.05	0.12	0.09	0.71	0.07	0.40
C20:2	0.02	nd	nd	0.01	0.30	0.28	0.28
C20:3n-3	0.16	0.29	0.29	0.06	0.07	0.65	0.16
C20:3n-6	0.22	0.39	0.35	0.11	0.28	0.99	0.19
Σ Saturated fatty acids	82.6	77.4	82.3	2.92	0.20	0.76	0.62
Σ Monounsaturated fatty acids	12.2[b]	16.2[a]	12.2[b]	1.24	0.03	0.20	0.83
Σ Polyunsaturated fatty acids	5.17[b]	6.43[a]	5.06[b]	0.78	0.01	0.09	0.25
Atherogenicity index	4.29[a]	3.41[b]	4.86[a]	0.42	<0.001	0.82	0.69
Thrombogenicity index	4.70[a]	3.90[b]	5.04[a]	0.38	0.02	0.89	0.95
C14:1 cis-9 / C14:0	0.05	0.06	0.06	0.01	0.35	0.19	0.98
C18:2 cis-9, trans-11 / C18:1 trans-11	0.20	1.04	1.96	0.77	0.110	0.21	0.65

[a] FA values are in g 100 g^{-1} fatty acid; means in the same row with different superscript letter are significantly different (Diet $P < 0.05$). Control, no fat supplement; OO, supplemented with 30 g kg^{-1} DM olive oil; HVO, supplemented with 30 g kg^{-1} DM hydrogenated vegetable oil. SEM: Standard error of the mean; nd, not detected.

Table 4

Cheese fatty acid profile from cows fed control, olive oil (OO), and hydrogenated vegetable oil (HVO) dietary treatments. [a]

Fatty acid (g 100g^{-1} of fatty acid)	Diets			SEM	*P*-value	
	Control	OO	HVO		Diet	Time
C4:0	3.72^{a}	2.27^{b}	3.43^{a}	0.10	<0.001	0.25
C6:0	1.67^{b}	1.21^{c}	5.09^{a}	0.11	<0.001	0.43
C8:0	0.82^{b}	1.07^{a}	0.64^{c}	0.03	<0.001	0.38
C10:0	2.35^{a}	1.39^{b}	2.45^{a}	0.24	0.01	0.42
C11:0	0.18	0.20	0.16	0.04	0.66	0.47
C12:0	1.96^{b}	2.79^{a}	2.11^{b}	0.24	0.03	0.36
C13:0	0.06	0.13	0.09	0.09	0.77	0.51
C14:0	12.9^{a}	8.49^{c}	9.33^{b}	0.78	<0.001	0.24
C14:1 cis-9	0.94^{a}	0.65^{b}	0.42^{c}	0.14	0.02	0.96
C15:0	1.03^{a}	0.87^{b}	0.62^{c}	0.12	0.04	0.49
C16:0	33.9^{a}	26.0^{c}	28.0^{b}	1.02	<0.001	0.19
C17:0	0.37	0.56	0.41	0.12	0.32	0.19
C17:1 cis-9	0.45^{a}	nd	0.34^{b}	0.06	<0.001	0.58
C18:0	17.1^{a}	11.8^{b}	12.4^{b}	0.78	<0.001	0.02
C18:1 trans-10	0.83^{c}	1.78^{a}	1.52^{b}	0.15	0.05	0.05
C18:1 trans-11	0.62^{b}	1.18^{a}	0.52^{b}	0.22	0.05	0.69
C18:1 cis-9	15.1^{c}	28.2^{a}	24.5^{b}	0.88	<0.001	0.13
C18:2 trans-9, trans-12	0.85	0.84	0.79	0.09	0.77	0.19
C18:2 cis-9, cis-12	0.21	0.52	0.44	0.16	0.21	0.71
C18:3 cis-6, cis-9, cis-12	0.55^{b}	0.99^{b}	2.07^{a}	0.57	<0.001	0.44
C18:3 cis-9, cis-12, cis-15	0.36^{b}	0.98^{a}	0.27^{b}	0.16	0.01	0.27
C18:2 cis-9, trans-11	1.17^{b}	4.31^{a}	0.19^{b}	1.22	<0.001	0.42
C20:0	0.32	nd	0.33	0.13	0.08	0.61
C20:1n-9	0.07	nd	0.13	0.11	0.54	0.44
C20:2	nd	0.04	0.07	0.04	0.39	0.50
C20:3n-6	0.52	0.73	0.60	0.10	0.26	0.86
Σ Saturated fatty acids	76.5^{a}	55.6^{c}	63.6^{b}	1.61	<0.001	0.19
Σ Monounsaturated fatty acids	18.9^{c}	30.8^{a}	27.3^{b}	1.33	<0.001	0.17
Σ Polyunsaturated fatty acids	4.61^{c}	13.4^{a}	9.04^{b}	2.04	0.01	0.18
Atherogenicity index	1.38^{a}	0.60^{c}	0.76^{b}	0.08	<0.001	0.13
Thrombogenicity index	5.01^{a}	2.00^{c}	2.55^{b}	0.25	<0.001	0.32

[a] FA values are in g 100 g^{-1} fatty acid; means in the same row with different superscript letters are significantly different (Diet P<0.05). Control, no fat supplement; OO, supplemented with 30 g kg^{-1} DM olive oil; HVO, supplemented with 30 g kg^{-1} DM hydrogenated vegetable oil. SEM: standard error of the mean; nd = not detected.

Table 5

Colour of cheeses from cows fed control, olive oil (OO), and hydrogenated vegetable oil (HVO) dietary treatments.[a]

Colour	Diet			SEM	*P*-value	
	Control	OO	HVO		Diet	Time
Surface						
L*	80.6	81.1	83.0	4.83	0.88	0.42
a*	-2.85	-1.08	-2.19	0.92	0.23	0.03
b*	24.8	25.8	23.0	5.78	0.88	0.45
Inner						
L*	92.0	90.1	92.3	1.19	0.28	0.14
a*	-1.49	-1.12	-1.58	0.28	0.31	0.37
b*	10.6	10.7	10.3	0.84	0.88	0.68

[a] Control, no fat supplement; OO, supplemented with 30 g kg^{-1} DM olive oil; HVO, supplemented with 30 g kg^{-1} DM hydrogenated vegetable oil. SEM, standard error of the mean. L*, lightness or whiteness (L* = 0 for black; L* = 100 for white); a*, red-green components (-a* = greenness; +a* = redness); b*, yellow-blue components (-b* = blueness; +b* = yellowness).

Table 6

Sensory evaluation of Chanco-style cheese from cows fed control, olive oil (OO), and hydrogenated vegetable oil (HVO) dietary treatments. [a]

Attributes	Diet			SEM	*P*-value		
	Control	OO	HVO		Diet	Time	Diet × Time
Appearance							
Colour homogeneity	6.1	6.3	6.3	0.20	0.60	0.23	<0.01
Holes	5.5[a]	4.4[b]	5.7[a]	0.23	<0.01	0.20	<0.01
Odour							
Overall odour	4.0[a]	3.9[b]	4.8[a]	0.19	<0.01	0.45	0.09
Ripe cheese odour	2.8	2.9	3.4	0.17	0.07	0.03	0.15
Cow milk odour	3.4[b]	3.5[b]	4.3[a]	0.18	<0.01	<0.01	0.03
Flavour							
Salty	2.5[b]	2.6[ab]	3.0[a]	0.15	0.03	<0.01	0.38
Acid	3.0[c]	3.5[b]	4.5[a]	0.17	<0.01	<0.01	0.09
Bitter	3.1[b]	3.2[b]	3.7[a]	0.17	0.03	0.97	0.30
Overall flavour	4.1	4.2	4.4	0.18	0.50	0.54	0.58
Ripe cheese flavour	2.8	2.8	2.9	0.15	0.88	<0.01	0.48
Texture							
Sharpness	3.3	3.2	3.4	0.17	0.55	0.91	0.98
Toughness	4.1	3.9	4.1	0.17	0.60	0.23	0.27
Graininess	4.3	4.1	4.6	0.20	0.20	0.01	0.86
Screeching	3.9	3.8	4.3	0.20	0.20	0.77	0.55
Moisture	4.3[b]	4.6[ab]	5.0[a]	0.18	0.02	<0.01	0.67
Greasiness	3.9	3.6	3.9	0.21	0.56	0.53	0.85

[a] Means in the same row with different superscript letters are significantly different (Diet $P<0.05$); control, no fat supplement; OO, supplemented with 30 g kg^{-1} DM olive oil; HVO, supplemented with 30 g kg^{-1} DM hydrogenated vegetable oil. SEM, standard error of the mean.