



Effect of feed supplementation based on extruded linseed meal and fish oil on composition and sensory properties of raw milk and ultra-high temperature treated milk

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ABSTRACT

The objectives of this study were to test the influence of a recently developed rumen protected feed supplement containing extruded linseed meal and fish oil (LFO), which was fed to lactating Holstein-Friesian cows for 10 weeks at the rate of 800 g day⁻¹ per animal, on the chemical and fatty acid (FA) composition of raw and ultra-high temperature (UHT) treated milks and to evaluate changes in sensory properties of UHT milk by both instrumental analysis and a panel of human assessors. Inclusion of LFO in the diet did not affect milk yield or the protein and fat contents of raw and UHT milks; however, it improved the FA composition of the milk fat by increasing the concentrations of health-enhancing polyunsaturated FA and beneficially decreasing the n-6/n-3 FA ratio without adversely affecting the sensory properties of the final product (UHT milk).

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1. Introduction

Over the last 100 or so years, milk has become one of the staples of Western diets (McCollum, 1918). The fatty acid (FA) composition of milk fat affects the nutritional value, sensory properties, and overall quality of milk. Some of the FAs in bovine milk fat are known to have beneficial effects on human health. These include α -linolenic acid (ALA, C18:3n-3), docosahexaenoic acid (DHA, C22:6n-3), and eicosapentaenoic acid (EPA, C20:5n-3), all three belonging to the n-3 polyunsaturated FA (PUFA) group, and the biologically active *cis*-9, *trans*-11 isomer of conjugated linoleic acid (CLA) (Ferlay, Doreau, Martin, & Chilliard, 2013; Kairenius et al., 2015). In contrast, excessive intakes of *trans* FAs and medium-chain saturated FAs (MCSFAs) are implicated as risk factors for various diseases (Kairenius et al., 2015; WHO, 2003). The FA profile of milk fat is influenced in part by the diet the cow consumes (Collomb et al., 2004; Ferlay et al., 2013; Tóth, Viszket, Csavajda, Tanai, & Varga, 2012). Therefore, it is important to develop ways to beneficially

alter the FA composition of milk through nutrition of lactating cows (Gebreyowhans, Lu, Zhang, Pang, & Lv, 2019).

Certain supplemental fat sources used in dairy cow diets may negatively affect ruminal metabolism and dry matter (DM) intake, thereby increasing body fat mobilisation and decreasing milk production and milk fat content (Juchem et al., 2008; Kairenius et al., 2015). To offset such adverse impacts of fat supplements rich in unsaturated FAs (UFAs), use of rumen inert fats (e.g., Ca salts of fish and palm oils) has been explored as an alternative to improve the FA profile and nutritional value of milk fat (Juchem et al., 2008).

Several authors have suggested that linseed (Ferlay et al., 2013; Hurtaud, Faucon, Couvreur, & Peyraud, 2010) or fish oil (Chilliard, Ferlay, & Doreau, 2001; Gulati, McGrath, Wynn, & Scott, 2003; Juchem et al., 2008; Rego et al., 2005) should be fed, preferably in a rumen protected form, to decrease the levels of MCSFAs and increase the concentrations of health-enhancing *cis*-9, *trans*-11 CLA, ALA, EPA, and DHA in milk fat. However, at higher rates of inclusion of n-3 PUFAs, possible negative effects on oxidative stability and organoleptic properties of raw milk may be observed (Dubroeuq et al., 2002; Let, Jacobsen, & Meyer, 2005; Palmquist, 2009). In contrast, linseed (Lerch et al., 2015; Saliba et al., 2014) or fish oil

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(Allred et al., 2006; Lynch et al., 2005) supplementation at levels not exceeding 5% of DM and 1% of DM, respectively, has been reported to result in no flavour differences in either raw or pasteurised bovine milk.

Because the objective evaluation of organoleptic properties of foods by human sensory panels requires highly qualified assessors whose sensations may be impaired after several tests in a row, there is a growing need for rapid and cost effective instrumental methods, preferably automated ones, to measure the aroma profile of products (Nagy et al., 2014). Numerous studies have shown the applicability of various electronic noses (ENs) in the field of odour profiling of milk and dairy foods (Peris & Escuder-Gilbert, 2009). EN systems based on metal oxide semiconductor (MOS) gas sensor array technologies combined with multivariate data analysis have been used in milk production and processing for a range of purposes, including detection of mastitic milk (Eriksson et al., 2005), recognition and classification of milk samples according to their heat treatment and fat content (Brudzewski, Osowski, & Markiewicz, 2004), shelf life determination of fluid milks (Labreche, Bazzo, Cade, & Chanie, 2005), and identification of milk flavourings (Wang, Xu, & Sun, 2010). Falchero et al. (2009) even used this technology to identify milks produced by cows grazing on different Alpine vegetation types.

The aims of this research were to analyse the effect of a rumen protected extruded linseed meal and fish oil (LFO) supplement on major chemical composition and FA profile of raw and ultra-high temperature (UHT) treated cow milk and to evaluate changes in organoleptic properties of UHT milk using both human sensory tests and electronic sensor based olfaction.

2. Materials and methods

2.1. Feeding experiments

The feeding trials were performed at the dairy farm of Solum Zrt. in Komárom (Hungary). In a randomised complete block design, a total of 70 multiparous Holstein-Friesian dairy cows were used in the control ($n = 35$) and experimental ($n = 35$) groups. All the animals included in the trials were in their second or third lactation. Average daily milk production of the control and experimental cows in the 2-week adaptation period prior to the start of the trials was 42.5 kg and 42.6 kg, respectively. The mean days in milk was 71 in both groups at the beginning of the adjustment period. The average DM intake of the control and experimental animals was 24.7 kg day⁻¹ and 25.4 kg day⁻¹, respectively. The diet consisted of a total mixed ration (TMR) based on corn silage, alfalfa haylage, and dried corn meal. The feed for the two groups was identical except for fat supplementation (LFO: 800 g day⁻¹ per cow). Cows were fed ad libitum twice daily at 11 a.m. and 5 p.m. Water and mineral salts were freely available.

After 2 weeks of pre-feeding, the experimental period lasted 10 weeks. The individual milk production of each cow was recorded, and the chemical composition of the morning and evening milk was determined once a week ($n = 70 \times 10 \times 2$). In addition to individual milk samples, pooled samples were also collected from both the control and experimental groups, in the morning and evening, on each sampling day ($n = 2 \times 2 \times 10$), and the FA profile of these samples was determined.

2.2. Manufacturing of the experimental feed supplement

LFO was manufactured according to a patented technology (Tóth, Grosz, Szilvássy, & Jurányi, 2018) using commercially available fish oil (Inve DHA Oil 9/9; Inve, Dendermonde, Belgium), extruded linseed meal (Nutex 68; Dumoulin, Kortrijk, Belgium),

carriers, and a hydrogenated fat product (Optima-100; UBM, Pílisvörösvár, Hungary).

2.3. In sacco experiments

Cattle used in the trials were fitted with ruminal cannulae (permission number: 17.1/00997/006/Győr/2007). Degradability of LFO crude fat in the rumen was examined with four Holstein-Friesian steers (550–600 kg of body weight) using an in situ method. The experimental animals consumed 10 kg day⁻¹ of corn silage, 3 kg day⁻¹ of grass straw, 2 kg day⁻¹ of wheat straw, and 3 kg day⁻¹ of concentrate (890 g kg⁻¹ of corn meal, 80 g kg⁻¹ of extracted sunflower meal, 5 g kg⁻¹ of standard premix, 10 g kg⁻¹ of feed salt, and 15 g kg⁻¹ of calcium carbonate).

The DM and ether extract (EE) degradation characteristics were determined using the nylon bag technique as described by McDonald (1981) and Ørskov and McDonald (1979). LFO samples, equivalent to approximately 2 g of DM, were weighed into nylon bags (pore size: 30–50 µm) with an internal size of 100 mm × 75 mm and were incubated in the rumen of four Holstein steers for 2, 4, 8, 16, 24, and 48 h ($n = 30$ bags per animal, i.e., 5 bags × 6 incubation times per animal). The 0 h bags (5 bags per animal) were not placed in the rumen and the solubility was determined by soaking them in water. After each incubation interval, the respective bags were taken out of the rumen, dipped immediately into cold water (13 ± 2 °C) to stop further microbial activity and then rinsed by cold tap water (13 ± 2 °C) for 30 min. Residues were dried at 55 °C for 48 h, individually weighed, and duplicates were pooled by incubation time to analyse their DM and EE content. From these data, nutrient disappearance was calculated. In sacco DM and EE degradation were fitted to the first order kinetics defined by the following equation (McDonald, 1981; Ørskov & McDonald, 1979):

$$p = a + b(1 - e^{-ct}) \quad (1)$$

where p represents degradation of DM and EE at time t , $a + b$ are their potential degradation, and c is the rate of their degradation. It was assumed that 8% of the rumen content left the rumen per hour.

2.4. Production of UHT milk

Both from the control and experimental raw milks produced in the feeding trials, UHT milks were made at a dairy processing plant of Bonafarm Group in Csorna (Hungary). Two batches of UHT milk were manufactured from 3000–3500 L of raw milk each on the Tetra Pak production line (Pully, Switzerland) of the company. UHT processing involved heating the raw milk in a continuous flow system at 140 °C for 5 s.

2.5. Chemical analyses

DM, crude protein, crude fat (EE), neutral detergent fibre, acid detergent fibre, acid detergent lignin, non-structural carbohydrates, sugar, and starch contents of the feeds ($n = 3$) were determined according to the Hungarian Feedstuffs Codex (MARD, 2003). Milk samples (raw milk: $n = 284$, UHT milk: $n = 20$) were tested for fat, protein, lactose, total solids, and solids-not-fat concentrations in the Hungarian Dairy Research Institute (Mosonmagyaróvár, Hungary) by Fourier transform mid-infrared spectroscopy using an automatic milk analyser (MilkoScan FT 120; FossElectric A/S, Hillerød, Denmark).

FA profiles of feed ($n = 3$) and milk samples (raw milk: $n = 24$, UHT milk: $n = 4$) were determined with an Agilent 6890N gas chromatograph (Agilent Technologies, Wilmington, DE, USA),

equipped with an SP-2560 capillary column (100 m × 0.25 mm i.d. × 0.2 μm film thickness; Supelco, Bellefonte, PA, USA) and a flame ionisation detector, under the following instrumental conditions: sample quantity: 1 μL; carrier gas: H₂; pressure: 176.8 kPa; flow: 35 mL min⁻¹ hydrogen, 30 mL min⁻¹ nitrogen, 300 mL min⁻¹ air; injector temperature: 250 °C. Saponification of fat was performed with 1 N NaOH dissolved in methanol. Boron trifluoride dissolved in 10% methanol was used for esterification, and the samples were solved in n-hexane. Results were reported in weight percentage of total FA methyl esters.

2.6. Calculations

Fat-corrected milk (FCM) was calculated as follows:

$$\text{FCM}(\text{kg day}^{-1}) = 0.4 \times \text{milk}(\text{kg day}^{-1}) + 15 \times \text{fat}(\text{kg day}^{-1}) \quad (2)$$

Energy-corrected milk (ECM) was calculated according to the following formula:

$$\text{ECM}(\text{kg day}^{-1}) = \text{milk}(\text{kg day}^{-1}) \times [383 \times \text{fat}(\%) + 242 \times \text{protein}(\%) + 165 \times \text{lactose}(\%) + 20.7] / 3140 \quad (3)$$

One L of milk was equivalent to 1.033 kg of milk.

2.7. Electronic nose measurements

A Fox 4000 type EN system (Alpha MOS, Toulouse, France) with 18 MOS gas sensors was used to measure the types and concentrations of volatile compounds in the headspace of 20 mL sealed vials containing 1 mL of individual UHT milk samples. An HS 100 autosampler (Alpha MOS) was employed for sampling the headspace (injected volume: 1000 μL), and synthetic air was used as a permanent air flow (150 mL min⁻¹). Acquisition time and time between subsequent analyses were 120 s and 1080 s, respectively. Incubation of samples before acquisition was performed at 50 °C with an equilibration time of 300 s.

The EN system measured the relative changes in resistance of each MOS sensor over the 120 s data acquisition period following injection of headspace volatile compounds. These signals described the smell pattern of volatiles in a specific sample. All milk samples were measured accordingly, and the maximum intensities of relative resistance changes were recorded for each of the 18 gas sensors after each sniffing, resulting in a multidimensional database for the smell of the sample set.

Unlike conventional analytical chemistry methods, EN is a comparative device rather than a separate analytical technique (O'Riordan & Delahunty, 2003). Comparisons were made between the experimental UHT milk and five commercial UHT milk brands, with all samples being kept in identical containers. One of the five commercial UHT milks contained 1.5% (w/w) fat, whereas the other four products had a fat content of 2.8% (w/w).

2.8. Human sensory evaluation

Organoleptic analysis of UHT milks was performed in the sensory testing laboratory of Kaposvár University (Kaposvár, Hungary) by a panel of six women and four men trained according to international standards (ISO, 2009, 2017). The panellists had over 30 h of experience in descriptive sensory analysis of dairy and other foods (Drake, 2007) and were between the ages of 27 and 50 years. They

discussed the sensory properties of milks during two training sessions (1 h per session) within 1 week prior to the test.

The assessors were provided with 100 mL of the control (2.85% w/w, fat) and experimental (2.78% w/w, fat) UHT milks, at 10 °C, in transparent glass cups with coded identification numbers. Sensory evaluation of all samples was performed by all panellists in two sessions on two consecutive days. The sessions were conducted under natural illumination. In addition to consistency, the intensities of ivory white colour, yellow colour, overall smell, sweet smell, cooked smell, sour smell, off-smell, overall taste, sweet taste, cooked taste, sour taste, and off-taste of samples were assessed using a scale of 0–9 (from less to more intense).

2.9. Colour measurements

Colour of milks was measured through a flat glass surface using an RM200QC imaging spectrophotometer (X-Rite, Grand Rapids, MI, USA). The L* [lightness, ranging from 0 (black) to 100 (white) along a grey scale], a* (redness, with positive values being red and negative values being green), b* (yellowness, with positive values being yellow and negative values being blue), C* (saturation), and h (hue) values were recorded according to the colour system of the International Commission on Illumination (CIE, Vienna, Austria). D65 illumination was applied (average daily light, 6500 K colour temperature). The difference between groups was described by the spatial distance of colour points, i.e., total colour difference (ΔE):

$$\Delta E = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2 \right]^{1/2} \quad (4)$$

The categories of relationship between total colour difference and visual colour perception are as follows: ΔE = 0–0.5: no difference, ΔE = 0.5–1.5: slight difference, ΔE = 1.5–3.0: detectable difference, ΔE = 3.0–6.0: distinct difference, ΔE = 6.0–12.0: big difference (Abril et al., 2001).

2.10. Statistical analyses

AdWare Research Kft. (Balatonfüred, Hungary) assisted in performing statistical evaluation (i.e., Kolmogorov–Smirnov test, Levene's test, ANOVA, and Mann–Whitney U test) of milk production, milk composition, and FA profile results using the SPSS 19.0 for Windows program package (IBM, Armonk, NY, USA). The data of human sensory tests and those of colour measurements were analysed with the SPSS 21.0 for Windows statistical program package (IBM).

The recorded data of EN measurements were analysed with multivariate classification methods. Principal component analysis (PCA) as an unsupervised method was used to find outliers and describe the general multidimensional patterns of EN data (Naes, Isaksson, Fearn, & Davies, 2002). For supervised classifications, discriminant factor analysis (DFA) was applied to test the possibility of group identification based on odour properties (Naes et al., 2002). Whereas grouping of the samples is not handled as input information in the case of PCA, DFA uses these additional data and checks whether the original grouping can be reproduced using only the EN information. The first ten principal components of PCA were used as independent input variables in DFA (Bázár et al., 2015). The DFA model was tested with cross-validation when EN data of a sample were left out of the model development phase, and the generated model was used to identify the sample being left out by reading its EN data only. Within the iterative terms, data of another sample were left out for being used in testing. Performance of the DFA model was rated based on the ratio of correctly classified samples. AlphaSoft V12 (Alpha MOS), The Unscrambler 9.7 (CAMO

Software, Oslo, Norway), and SPSS for Windows 21.0 (IBM) were applied for multivariate data analysis approaches.

3. Results and discussion

3.1. Feed composition and fatty acid profile

The composition of the LFO supplement used in our trials and that of the experimental and control TMR are shown in Tables 1 and 2, respectively. It can be seen in Table 2 that the two diets were similar in their DM, crude protein, neutral detergent fibre, acid detergent fibre, and acid detergent lignin contents. However, due to LFO supplementation, the EE of the experimental diet was considerably higher than that of the control TMR.

As for FA profiles, the experimental diet proved to be a good source of PUFAs in general and both DHA and EPA in particular, whereas the control TMR contained neither EPA nor DHA (Table 3) because it was manufactured without the use of fish oil. Depending

on fish species and geographical and seasonal variations, the EPA and DHA contents of fish oils may range from 4 to 32% and from 2 to 25%, respectively (Moffat & McGill, 1993); however, the transfer efficiency of these long-chain n-3 PUFAs to milk is typically lower than 5% (Chilliard et al., 2001; Rego et al., 2005). It should also be noted that both diets had relatively high levels of linoleic acid (C18:2n-6) and ALA (Table 3).

3.2. Rumen stability of the experimental feed supplement

The ruminal stability of EE was found to be 70%, which is in good agreement with results obtained with fat products rich in UFAs (i.e., protected tuna oil and Ca soap) in both in vitro (Kitessa, Peake, Bencini, & Williams, 2003) and in situ (Schmidt, Sipőcz, & Sipőcz, 2000) trials. However, the results of Ribács and Schmidt (2006) revealed that the in situ method for determining the rumen stability of Ca soap containing elevated levels of UFAs was not always appropriate.

3.3. Milk yield and composition

Milk yields and the composition of raw and UHT milks are presented in Table 4. Inclusion of LFO in the diet had no effect ($P > 0.05$) on milk production. This was somewhat surprising because supplementation of diets with oils high in PUFAs often leads to a decline in feed intake and a subsequent reduction in milk yield (Juchem et al., 2008; Rego et al., 2005). However, AbuGhazaleh and Holmes (2007) reported results similar to ours when feeding 8 kg day⁻¹ of grain mix diet containing either 400 g of saturated animal fat (control) or 100 g of fish oil and 300 g of sunflower oil (experimental) to partially grazing dairy cows. The authors explained the comparable milk production for the two diets by the identical intake of grain mixes and the low level of PUFAs in the experimental diet. This may have also been the case in our study because there was only a difference of 2.36 percentage points in total PUFA content between the two TMR diets (Table 3), which were very similar in their ingredients and chemical composition (Table 2). As shown in Table 4, supplementing the diet of cows with LFO did not affect FCM, ECM, and the protein and fat concentrations of raw and UHT milks. Milk fat content and yield were equally low in both groups ($P > 0.05$), averaging 2.99% and 1.15 kg day⁻¹, respectively. Milk fat percentage generally decreases when dairy cows are fed fish oil or other feedstuffs rich in PUFAs (Abu-Ghazaleh, Schingoethe, Hippen, & Whitlock, 2002). UFAs are known to undergo biohydrogenation in the rumen, inhibiting rumen bacteria, altering fermentation processes, and producing *trans* FAs (Palmquist, 2009). This normally leads to the depression of milk fat secretion. However, the LFO used in our trial was rumen protected and, therefore, only partially accessible to rumen bacteria for biohydrogenation, which may explain the unchanged level of milk fat synthesis.

Milk protein content and yield were similar ($P > 0.05$) for both treatments, averaging 3.19% and 1.22 kg day⁻¹, respectively. In a study by Rego et al. (2005), however, a high dose (i.e., 320 g) of fish oil in the daily diet of cows decreased milk protein yield and content ($P < 0.05$) by 120 g day⁻¹ and 0.24 percentage points, respectively. In contrast, diets supplemented with 1% fish oil and 2% fat source high in stearic, oleic, linoleic, or linolenic acids resulted in comparable milk protein concentrations ($P > 0.05$) ranging from 3.03 to 3.10% (AbuGhazaleh, Schingoethe, Hippen, & Kalscheur, 2003). Similarly, AbuGhazaleh and Holmes (2007) reported no changes in milk protein yield and content when they fed a total of 400 g day⁻¹ of fish oil and sunflower oil to dairy cows.

Table 1

Major chemical composition and fatty acid (FA) profile of linseed meal and fish oil coated with hydrogenated fat (LFO).^a

| Parameter | Concentration |
|----------------------------------|---------------|
| Chemical composition (% w/w) | |
| Dry matter | 94.40 |
| Ether extract | 42.60 |
| Total FA | 35.30 |
| Crude protein | 11.80 |
| Crude fibre | 5.70 |
| Crude ash | 12.40 |
| FA profile (% w/w, of total FAs) | |
| Total saturated FAs | 36.06 |
| C8:0 | 0.06 |
| C10:0 | 0.02 |
| C12:0 | 0.20 |
| C13:0 | 0.01 |
| C14:0 | 3.24 |
| C15:0 | 0.22 |
| C16:0 | 19.35 |
| C17:0 | 0.19 |
| C18:0 | 12.60 |
| C20:0 | 0.09 |
| C22:0 | 0.08 |
| Total monounsaturated FAs | 21.56 |
| C14:1 | 0.01 |
| C16:1 | 2.42 |
| C17:1n-7 | 0.06 |
| C18:1 <i>trans</i> | 0.75 |
| C18:1n-9 | 13.37 |
| C18:1 <i>cis</i> | 1.16 |
| C22:1 | 3.57 |
| C24:1 | 0.22 |
| Total polyunsaturated FAs | 35.14 |
| C18:2n-6 | 8.30 |
| C18:2n-6 <i>trans</i> | 0.04 |
| C18:3n-3 | 18.32 |
| C18:3n-6 | 0.09 |
| C20:2n-6 | 0.17 |
| C20:3n-6 | 0.05 |
| C20:4n-6 | 0.22 |
| C20:5n-3 | 3.80 |
| C22:4n-6 | 0.02 |
| C22:5n-3 | 0.61 |
| C22:6n-3 | 3.52 |
| Total unsaturated FAs | 56.70 |
| Unidentified FAs | 7.24 |
| Total n-6 FAs | 8.89 |
| Total n-3 FAs | 26.25 |

^a Ingredients in LFO: extruded linseed meal (50%, w/w), fish oil (20%, w/w), and carriers (30%, w/w); values are means based on 3 observations.

Table 2
Major compositional characteristics of control and experimental total mixed rations (TMRs).^a

| Parameter | Control TMR | Experimental TMR |
|---|-------------|------------------|
| Ingredient (% w/w, of dry matter) | | |
| Corn silage | 18.44 | 17.89 |
| Rye haylage | 18.56 | 18.00 |
| Corn meal (dry) | 13.55 | 13.15 |
| Alfalfa haylage | 7.86 | 7.63 |
| Sunflower meal | 7.72 | 7.50 |
| Soybean meal | 6.10 | 5.92 |
| Wet corn grains | 5.63 | 5.46 |
| Brewer's grains | 4.96 | 4.82 |
| SoyPass | 3.97 | 3.85 |
| Molasses (liquid) | 3.22 | 3.13 |
| Glycerin (liquid) | 3.15 | 3.06 |
| Wheat meal | 2.88 | 2.80 |
| Grass hay | 1.80 | 1.75 |
| Minerals and vitamin premix | 1.76 | 1.71 |
| Lime | 0.40 | 0.39 |
| LFO | – | 2.94 |
| Chemical composition (% w/w, of dry matter) | | |
| Dry matter | 51.11 | 51.80 |
| Crude protein | 17.42 | 17.25 |
| Neutral detergent fibre | 30.68 | 30.22 |
| Acid detergent fibre | 19.35 | 18.99 |
| Acid detergent lignin | 3.47 | 3.45 |
| Ether extract | 3.04 | 4.27 |
| Non-structural carbohydrates | 40.25 | 39.45 |
| Starch | 23.28 | 22.91 |
| Sugar | 7.82 | 7.66 |
| Net energy for lactation (MJ kg ⁻¹ dry matter) | 6.65 | 6.85 |

^a Values are calculated values; dry matter is reported as a percentage (w/w) of TMR. SoyPass was from LignoTech USA (Rothschild, WI, USA). LFO, linseed meal and fish oil coated with hydrogenated fat; ingredient were: extruded linseed meal (50%, w/w), fish oil (20%, w/w), and carriers (30%, w/w).

Table 3
Fatty acid (FA) profile of total mixed rations (TMRs).^a

| FA (group) | TMR (% w/w, of total FAs) | |
|---------------------------|---------------------------|--------------|
| | Control | Experimental |
| Total saturated FAs | 28.67 | 28.15 |
| C6:0 | 0.03 | 0.02 |
| C8:0 | 0.02 | 0.01 |
| C10:0 | 0.01 | 0.01 |
| C12:0 | 0.20 | 0.05 |
| C14:0 | 0.79 | 0.71 |
| C15:0 | 0.19 | 0.17 |
| C16:0 | 19.69 | 18.72 |
| C17:0 | 0.26 | 0.22 |
| C18:0 | 6.46 | 6.40 |
| C20:0 | nd | 1.08 |
| C21:0 | nd | 0.04 |
| C22:0 | 0.45 | 0.37 |
| C23:0 | 0.15 | 0.07 |
| C24:0 | 0.42 | 0.28 |
| Total monounsaturated FAs | 18.69 | 18.52 |
| C16:1n-7 | 0.71 | 0.68 |
| C18:1n-9 | 17.05 | 16.34 |
| C18:1 cis | 0.77 | 0.88 |
| C20:1 | nd | 0.38 |
| C22:1 | 0.08 | 0.15 |
| C24:1 | 0.08 | 0.09 |
| Total polyunsaturated FAs | 50.92 | 53.28 |
| C18:2n-6 | 36.58 | 33.34 |
| C18:3n-3 | 14.03 | 16.13 |
| C20:2 cis-11, trans-14 | 0.07 | 0.08 |
| C20:3n-6 | 0.01 | 0.02 |
| C20:4n-6 | 0.23 | 0.22 |
| C20:5n-3 | nd | 0.84 |
| C22:5n-3 | nd | 0.26 |
| C22:6n-3 | nd | 2.39 |
| Unidentified FAs | 1.72 | 0.05 |

^a Values are means based on 3 observations; nd, not detectable or detected at <0.01.

Table 4
Daily milk production and major chemical composition of raw and ultra-high temperature treated milks.^a

| Parameter | Control | Experimental |
|---|--------------------------|--------------------------|
| Milk production (kg day ⁻¹) | 38.02 ± 6.58 | 38.74 ± 6.88 |
| FCM (kg day ⁻¹) | 32.32 | 32.81 |
| ECM (kg day ⁻¹) | 32.80 | 33.22 |
| Composition of raw milk (% w/w) | | |
| Total solids | 11.54 ± 0.66 | 11.57 ± 0.76 |
| Fat | 3.00 ± 0.56 | 2.98 ± 0.62 |
| Protein | 3.19 ± 0.18 | 3.18 ± 0.20 |
| Lactose | 4.65 ± 0.09 ^a | 4.61 ± 0.16 ^b |
| Composition of UHT milk (% w/w) | | |
| Total solids | 11.35 ± 0.50 | 11.01 ± 0.61 |
| Fat | 2.85 ± 0.05 | 2.78 ± 0.07 |
| Protein | 2.95 ± 0.05 | 3.17 ± 0.12 |
| Lactose | 4.54 ± 0.12 | 4.33 ± 0.09 |

^a Abbreviations are: FCM, fat-corrected milk (kg day⁻¹) [i.e., 0.4 × milk (kg day⁻¹) + 15 × fat (kg day⁻¹)]; ECM, energy-corrected milk (kg day⁻¹) [i.e., milk (kg day⁻¹) × {383 × fat (%) + 242 × protein (%) + 165 × lactose (%) + 20.7}/3140]; UHT, ultra-high temperature. For milk production, values are means ± SD based on 2904 control and 2891 experimental observations; for composition of raw milk and UHT milk, values are means ± SD based on 284 observations and 20 observations, respectively. Means within a row with different superscripts differ ($P < 0.05$).

3.4. Milk fatty acid profile

The FA profiles of raw and UHT milks are presented in Table 5. Compared with the control diet, inclusion of LFO in the TMR of cows led to decreases ($P < 0.05$) in the levels of C8 to C16 saturated FAs (SFAs) in raw milk, except for C15. Furthermore, LFO supplementation also reduced ($P < 0.05$) the concentrations of C18:0 and total SFAs both in raw and heat-treated milk samples. Feeding 100 g day⁻¹ of fish oil and 300 g day⁻¹ of sunflower oil to partially grazing dairy cows was shown to decrease the levels of C6 to C16 SFAs in milk fat (AbuGhazaleh & Holmes, 2007), and this reduction in MCSFAs improved the nutritional value of milk by lowering its hypercholesterolaemic potential (Ney, 1991). Dietary n-3 PUFAs and their rumen biohydrogenation products strongly inhibit the synthesis of short- and medium-chain SFAs in the mammary gland (Bauman, Perfield, Harvatine, & Baumgard, 2008). Supplementing grass silage based diet of cows with fish oil at 0, 75, 150, or 300 g day⁻¹ resulted in linear decreases ($P < 0.05$) in C4 to C14 SFAs and C16, C18, and total SFAs (Kairenius et al., 2015). Hurtaud et al. (2010) observed an overall decrease ($P < 0.001$) in the SFA content of milk with increasing levels of extruded linseed in the corn silage based TMR of dairy cows. More specifically, the concentrations of udder-synthesised SFAs (i.e., C6 to C14) and palmitic acid (C16:0) decreased, whereas stearic acid (C18:0) content linearly increased with linseed dose.

Inclusion of LFO in the dairy cow diet increased the total monounsaturated FA level in milk (Table 5), which is consistent with results reported by others (AbuGhazaleh & Holmes, 2007; Murphy, Coakley, & Stanton, 2008).

With regard to PUFAs, concentrations of linoleic acid (C18:2n-6) and CLA isomers (cis-9, trans-11 CLA, trans-10, cis-12 CLA, cis-9, cis-11 CLA, and trans-9, trans-11 CLA) were higher in the raw milk of the LFO-fed group compared with control cows ($P < 0.05$). Increased milk cis-9, trans-11 CLA was reported in previous studies, where fish oil (AbuGhazaleh & Holmes, 2007) or linseed oil (Ferlay et al., 2013) was fed to dairy cows. In work by Ferlay et al. (2013), a grassland hay based diet supplemented with extruded linseed at 0–10% was shown to linearly increase ($P < 0.05$) the cis-9, trans-11 and total CLA contents of bovine milk, whereas at 10–15% a reduction ($P < 0.05$) in both the cis-9, trans-11 and total CLA levels was observed. Supplementation of dairy cows' grass silage based diet with fish oil, up to 150 g day⁻¹, was reported to increase the cis-

Table 5
Fatty acid (FA) profile of raw and ultra-high temperature (UHT) treated milks in the control and experimental groups of cows.^a

| FA (group) | Raw milk (% w/w, of total FAs) | | UHT milk (% w/w, of total FAs) | |
|---------------------------|--------------------------------|---------------------------|--------------------------------|---------------------------|
| | Control | Experimental | Control | Experimental |
| Total saturated FAs | 64.54 ± 1.09 ^a | 58.00 ± 1.09 ^b | 60.95 ± 0.23 ^a | 57.72 ± 0.41 ^b |
| C8:0 | 0.97 ± 0.05 ^a | 0.76 ± 0.02 ^b | 1.52 ± 0.03 ^a | 0.73 ± 0.12 ^b |
| C10:0 | 2.50 ± 0.19 ^a | 1.90 ± 0.08 ^b | 2.26 ± 0.01 ^a | 1.55 ± 0.17 ^b |
| C11:0 | 0.34 ± 0.05 ^a | 0.28 ± 0.05 ^b | 0.05 ± 0.01 ^b | 0.09 ± 0.02 ^a |
| C12:0 | 3.20 ± 0.25 ^a | 2.53 ± 0.08 ^b | 2.40 ± 0.03 | 2.01 ± 0.17 |
| C13:0 | 0.19 ± 0.01 ^a | 0.15 ± 0.01 ^b | 0.08 ± 0.01 | 0.07 ± 0.01 |
| C14:0 | 10.69 ± 0.48 ^a | 10.18 ± 0.17 ^b | 9.00 ± 0.17 | 9.57 ± 0.24 |
| C15:0 | 1.03 ± 0.06 | 1.00 ± 0.01 | 0.92 ± 0.05 | 1.01 ± 0.12 |
| C16:0 | 34.98 ± 0.69 ^a | 32.66 ± 0.35 ^b | 33.46 ± 0.29 ^a | 32.90 ± 0.34 ^b |
| C17:0 | 0.58 ± 0.02 ^b | 0.75 ± 0.01 ^a | 0.51 ± 0.01 | 0.59 ± 0.02 |
| C18:0 | 9.87 ± 0.53 ^a | 7.50 ± 0.80 ^b | 10.58 ± 0.51 ^a | 8.87 ± 0.62 ^b |
| C20:0 | 0.15 ± 0.01 ^b | 0.24 ± 0.03 ^a | 0.13 ± 0.01 ^b | 0.19 ± 0.03 ^a |
| C22:0 | 0.04 ± 0.00 ^b | 0.05 ± 0.00 ^a | 0.04 ± 0.00 ^b | 0.14 ± 0.05 ^a |
| Total monounsaturated FAs | 28.77 ± 1.07 ^b | 31.27 ± 0.87 ^a | 27.22 ± 0.50 ^b | 30.79 ± 0.96 ^a |
| C14:1 | 0.98 ± 0.05 ^b | 1.40 ± 0.11 ^a | 0.76 ± 0.05 ^b | 1.16 ± 0.09 ^a |
| C16:1n-7 | 2.20 ± 0.09 ^b | 2.95 ± 0.21 ^a | 1.50 ± 0.08 ^b | 2.61 ± 0.01 ^a |
| C17:1 | 0.19 ± 0.01 ^b | 0.27 ± 0.02 ^a | nd | 0.23 ± 0.00 |
| C18:1n-9 | 23.08 ± 0.95 ^a | 20.74 ± 1.15 ^b | 23.42 ± 0.14 | 23.40 ± 0.85 |
| C18:1 cis | 1.53 ± 0.09 ^b | 4.91 ± 1.12 ^a | 1.51 ± 0.01 ^b | 3.29 ± 0.88 ^a |
| C18:1 trans | 0.67 ± 0.05 ^b | 0.77 ± 0.03 ^a | nd | nd |
| C20:1 | 0.12 ± 0.01 ^b | 0.23 ± 0.02 ^a | 0.03 ± 0.01 ^b | 0.10 ± 0.01 ^a |
| Total polyunsaturated FAs | 3.87 ± 0.25 ^b | 5.46 ± 0.43 ^a | 3.31 ± 0.32 ^b | 5.48 ± 0.12 ^a |
| C18:2n-6 | 2.58 ± 0.20 ^b | 2.79 ± 0.21 ^a | 2.15 ± 0.24 ^b | 2.36 ± 0.06 ^a |
| C18:2 trans-6 | 0.02 ± 0.00 ^b | 0.05 ± 0.00 ^a | nd | nd |
| CLA cis-9, trans-11 | 0.41 ± 0.02 ^b | 1.31 ± 0.24 ^a | 0.50 ± 0.02 ^b | 2.17 ± 0.02 ^a |
| CLA trans-10, cis-12 | 0.01 ± 0.00 ^b | 0.04 ± 0.01 ^a | nd | nd |
| CLA cis-9, cis-11 | 0.02 ± 0.00 ^b | 0.06 ± 0.00 ^a | nd | nd |
| CLA trans-9, trans-11 | 0.03 ± 0.00 ^b | 0.05 ± 0.00 ^a | nd | nd |
| C18:3n-3 | 0.32 ± 0.03 ^b | 0.62 ± 0.07 ^a | 0.36 ± 0.01 ^b | 0.55 ± 0.02 ^a |
| C18:3n-6 | 0.02 ± 0.00 ^a | 0.01 ± 0.00 ^b | nd | nd |
| C20:2n-6 | 0.03 ± 0.00 ^b | 0.04 ± 0.00 ^a | 0.02 ± 0.00 ^b | 0.05 ± 0.00 ^a |
| C20:3n-6 | 0.11 ± 0.00 ^a | 0.09 ± 0.00 ^b | 0.10 ± 0.02 | 0.07 ± 0.02 |
| C20:4n-6 | 0.19 ± 0.01 ^a | 0.13 ± 0.01 ^b | 0.16 ± 0.01 ^a | 0.07 ± 0.01 ^b |
| C20:5n-3 | 0.02 ± 0.00 ^b | 0.07 ± 0.00 ^a | 0.02 ± 0.00 ^b | 0.04 ± 0.01 ^a |
| C22:2n-6 | 0.01 ± 0.00 ^b | 0.02 ± 0.00 ^a | nd | nd |
| C22:4n-6 | 0.05 ± 0.00 ^a | 0.02 ± 0.00 ^b | nd | nd |
| C22:5n-3 | 0.05 ± 0.00 ^b | 0.10 ± 0.00 ^a | nd | 0.11 ± 0.04 |
| C22:6n-3 | nd | 0.06 ± 0.01 | nd | 0.06 ± 0.01 |
| Total unsaturated FAs | 32.64 | 36.73 | 30.53 | 36.27 |
| Unidentified FAs | 2.82 ± 0.11 ^b | 5.27 ± 0.70 ^a | 8.52 | 6.01 |
| Total n-6 FAs | 3.01 | 3.15 | 2.43 | 2.55 |
| Total n-3 FAs | 0.39 | 0.85 | 0.38 | 0.76 |
| n-6:n-3 FA ratio | 7.72 | 3.71 | 6.39 | 3.36 |

^a Abbreviation: CLA, conjugated linoleic acid. Values for raw milk and UHT milk are means ± SD based on 24 observations and 4 observations, respectively; nd, not detectable or detected at < 0.01. Sub-column means within row and milk type with different superscripts differ ($P < 0.05$).

9, *trans*-11 CLA percentage and, thus, total CLA content of milk in a quadratic manner (Kairenius et al., 2015). In a recent study, addition of up to 10% of extruded linseed to a corn silage based diet linearly increased ($P < 0.01$) the total n-3 FA content of cow milk (Ferlay et al., 2013).

Inclusion of fish oil in the LFO supplement increased ($P < 0.05$) the EPA and DHA percentages of raw milk (Table 5). This observation is supported by the studies of Abu-Ghazaleh et al. (2002) and Kairenius et al. (2015), who also noticed elevated EPA and DHA levels ($P < 0.01$) in milk when using fish oil in the diet of dairy cows. For lack of official recommendations, an intake of 250 mg day⁻¹ of combined DHA and EPA appears to be sufficient for a healthy adult (EFSA, 2010). The average EU citizen, who drinks and eats 62.4 kg of milk, 18.2 kg of cheese, and 3.7 kg of butter annually (IDF, 2015), could cover approximately 2.3% and 15.1% of his or her daily EPA plus DHA needs through consumption of dairy foods based on milks produced by our control and experimental groups of cows, respectively.

As shown in Table 5, the n-6:n-3 FA ratio was lowered both in the raw (3.71) and UHT milk (3.36) of experimental cows as compared with the control group (7.72 and 6.39, respectively); and

this is important because humankind is known to have evolved on a diet with a ratio of n-6 to n-3 FAs of close to 1:1. However, Western societies today are characterised by increased n-6 and *trans* FA and decreased n-3 FA intakes (Novaes et al., 2019). In other words, the current nutritional environment in developed countries largely differs from that on which our genetic patterns were established roughly forty millennia ago (Eaton & Konner, 1985). Therefore, a significant reduction in n-6 FAs is desirable because excessive doses of n-6 PUFAs and extremely high n-6 to n-3 FA ratios (i.e., 15–17:1) promote the pathogenesis of a wide range of chronic diseases, including inflammatory bowel disease, rheumatoid arthritis, cancer, and cardiovascular and autoimmune diseases; whereas decreased n-6:n-3 ratios have been shown to exert suppressive effects (Simopoulos, 2002, 2008; Szakály, 2001).

3.5. Electronic nose olfaction and odour characteristics

Fig. 1 shows the differences in the volatile profile of experimental and commercial UHT milks. Apparently, fat content was the biggest factor of variation because the 1.5% fat commercial milk was well separated from the other samples containing 2.8% fat and

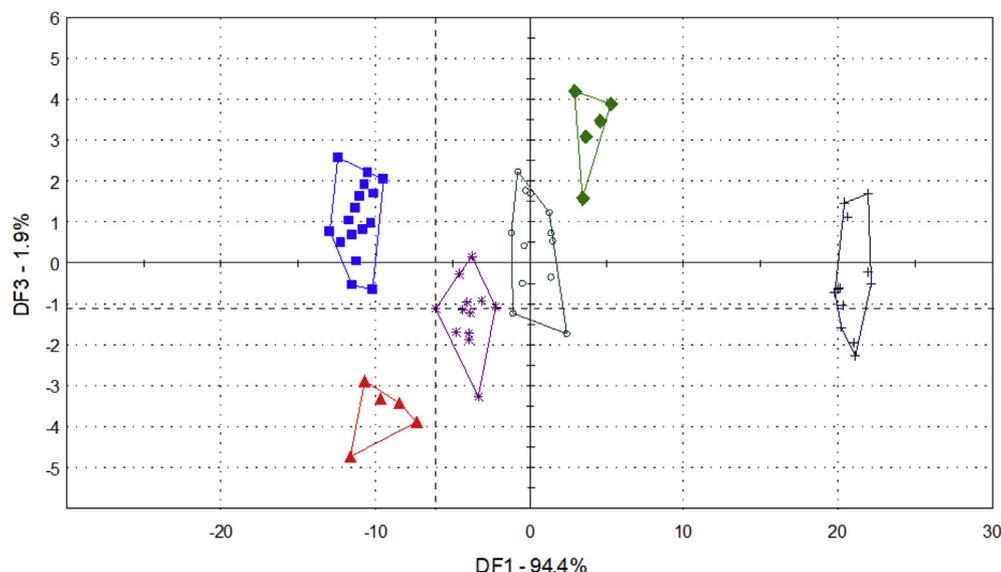


Fig. 1. Separation of experimental (E) and commercial (C) ultra-high temperature (UHT) treated milk samples by discriminant factor analysis based on their volatile profiles measured with an electronic nose: ■, E-UHT milk with 2.8% (w/w) fat; ▲, *, ○, and ◆, C-UHT milk brands with 2.8% (w/w) fat; +, C-UHT milk with 1.5% (w/w) fat.

showed less internal variation. Experimental milk samples formed a distinguishable group separated from all other milks. It is worth mentioning that processing and packaging of experimental and commercial milks were identical. The multivariate discrimination model based on odour profiles classified 89% of the samples correctly in the cross-validation test, indicating that there is a describable connection between the volatile profile and the grouping variables (i.e., experimental versus commercial products).

3.6. Human sensory evaluation and colour properties

Based on the results of human sensory analyses and colour measurements, the experimental and control UHT milks did not

differ ($P > 0.05$) in terms of organoleptic properties (Table 6). However, Campbell, Drake, and Larick (2003) described differences ($P < 0.05$) in flavour, consumer acceptability, and instrumentally measured colour values between skim milks fortified, at 1 or 2%, with either sunflower oil rich in CLA (comprised of 33.04% of *cis*-9, *trans*-11 and 33.68% of *cis*-10, *trans*-12 isomers) or regular milk fat (containing 1.51% of CLA isomers). In our study, the CLA-rich UHT milk produced with feed supplementation contained 2.17% of *cis*-9, *trans*-11 CLA, which was over four times the level measured in the control UHT milk (Table 5). Our results are in agreement with the findings of Allred et al. (2006) and Lynch et al. (2005), who reported that supplementation of cows' diets with ingredients rich in PUFAs increased milk CLA and total n-3 FA contents without negatively affecting the sensory properties of milk.

Table 6
Sensory properties of ultra-high temperature (UHT) treated milks.^a

| Parameter | UHT milk | |
|------------------------------|--------------|--------------|
| | Control | Experimental |
| Human sensory evaluation | | |
| Ivory white colour intensity | 5.52 ± 2.04 | 5.17 ± 2.11 |
| Yellow colour intensity | 3.08 ± 1.75 | 3.46 ± 2.21 |
| Smell intensity | 4.33 ± 2.57 | 5.49 ± 1.39 |
| Sweet smell intensity | 4.17 ± 2.80 | 4.44 ± 2.00 |
| Cooked smell intensity | 1.54 ± 0.54 | 1.63 ± 0.63 |
| Sour smell intensity | 1.42 ± 0.73 | 1.92 ± 0.57 |
| Off-smell intensity | 0.45 ± 0.34 | 0.29 ± 0.16 |
| Taste intensity | 4.16 ± 2.28 | 5.79 ± 1.47 |
| Sweet taste intensity | 4.04 ± 1.67 | 5.35 ± 1.56 |
| Cooked taste intensity | 1.85 ± 2.34 | 1.54 ± 2.00 |
| Sour taste intensity | 1.47 ± 0.77 | 1.24 ± 0.54 |
| Off-taste intensity | 0.61 ± 0.39 | 0.64 ± 0.58 |
| Consistency | 2.54 ± 1.25 | 3.11 ± 1.02 |
| Colour measurement | | |
| L* (lightness) | 84.24 ± 0.97 | 83.92 ± 0.64 |
| a* (redness) | 0.26 ± 0.40 | 0.03 ± 0.08 |
| b* (yellowness) | 6.67 ± 0.54 | 6.63 ± 0.08 |
| ΔE (colour difference) | 0.41 | 0.39 |
| C* (colour saturation) | 6.68 ± 0.56 | 6.63 ± 0.08 |
| h (hue) | 88.18 ± 3.20 | 89.58 ± 0.56 |

^a Values are means ± SD based on 10 observations; means within a row do not differ ($P > 0.05$).

4. Conclusions

This study has demonstrated that inclusion of rumen protected LFO in dairy cow diets is capable of improving (i.e., decreasing) the n-6 to n-3 FA ratio in milk. The UHT milk made from raw milk of LFO-fed cows has clearly differed from commercial UHT milk samples in terms of volatile profile determined by EN measurements. In contrast, no differences were detected in the organoleptic properties of UHT milks by either human sensory evaluation or spectrophotometric measurements. Our findings showed that supplementation of dairy cows' diet with LFO beneficially influences the FA composition of milk without compromising consumer acceptability of the final product.

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References

- Abril, M., Campo, M. M., Öneç, A., Sañudo, C., Albertí, P., & Negueruela, A. I. (2001). Beef colour evolution as a function of ultimate pH. *Meat Science*, 58, 69–78.
- Abu-Ghazaleh, A. A., Schingoethe, D. J., Hippen, A. R., & Whitlock, L. A. (2002). Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) content of milk. *Journal of Dairy Science*, 85, 624–631.
- AbuGhazaleh, A. A., & Holmes, L. D. (2007). Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. *Journal of Dairy Science*, 90, 2897–2904.
- AbuGhazaleh, A. A., Schingoethe, D. J., Hippen, A. R., & Kalscheur, K. F. (2003). Milk conjugated linoleic acid response to fish oil supplementation of diets differing in fatty acid profiles. *Journal of Dairy Science*, 86, 944–953.
- Allred, S. L., Dhiman, T. R., Brennand, C. P., Khanal, R. C., McMahon, D. J., & Luchini, N. D. (2006). Milk and cheese from cows fed calcium salts of palm and fish oil alone or in combination with soybean products. *Journal of Dairy Science*, 89, 234–248.
- Bauman, D. E., Perfield, J. W., II, Harvatine, K. J., & Baumgard, L. H. (2008). Regulation of fat synthesis by conjugated linoleic acid: Lactation and the ruminant model. *Journal of Nutrition*, 138, 403–409.
- Bázár, G., Kovacs, Z., Tanaka, M., Furukawa, A., Nagai, A., Osawa, M., et al. (2015). Water revealed as molecular mirror when measuring low concentrations of sugar with near infrared light. *Analytica Chimica Acta*, 896, 52–62.
- Bruzewski, K., Osowski, S., & Markiewicz, T. (2004). Classification of milk by means of an electronic nose and SVM neural network. *Sensors and Actuators B*, 98, 291–298.
- Campbell, W., Drake, M. A., & Larick, D. K. (2003). The impact of fortification with conjugated linoleic acid (CLA) on the quality of fluid milk. *Journal of Dairy Science*, 86, 43–51.
- Chilliard, Y., Ferlay, A., & Doreau, M. (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. *Livestock Production Science*, 70, 31–48.
- Collomb, M., Sollberger, H., Bütikofer, U., Sieber, R., Stoll, W., & Schaeren, W. (2004). Impact of a basal diet of hay and fodder beet supplemented with rapeseed, linseed and sunflowerseed on the fatty acid composition of milk fat. *International Dairy Journal*, 14, 549–559.
- Drake, M. A. (2007). Sensory analysis of dairy foods. *Journal of Dairy Science*, 90, 4925–4937.
- Dubroeuq, H., Martin, B., Ferlay, A., Pradel, P., Verdier-Metz, I., Chilliard, Y., et al. (2002). L'alimentation des vaches peut modifier les caractéristiques sensorielles du lait (Cows' feeding may modify sensory properties of milk). *Recontres Recherches Ruminants*, 9, 351–354.
- Eaton, S. B., & Konner, M. (1985). Paleolithic nutrition: A consideration of its nature and current implications. *New England Journal of Medicine*, 312, 283–289.
- EFSA. (2010). Scientific opinion of the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition, and Allergies (NDA) on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, 8, 1461.
- Eriksson, A., Persson-Waller, K., Svennersten-Sjaunja, K., Haugen, J.-E., Lundby, F., & Lind, O. (2005). Detection of mastitic milk using a gas-sensor array system (electronic nose). *International Dairy Journal*, 15, 1193–1201.
- Falchero, L., Sala, G., Gorlier, A., Lombardi, G., Lonati, M., & Masoero, G. (2009). Electronic nose analysis of milk from cows grazing on two different Alpine vegetation types. *Journal of Dairy Research*, 76, 365–371.
- Ferlay, A., Doreau, M., Martin, C., & Chilliard, Y. (2013). Effects of incremental amounts of extruded linseed on the milk fatty acid composition of dairy cows receiving hay or corn silage. *Journal of Dairy Science*, 96, 6577–6595.
- Gebreywhans, S., Lu, J., Zhang, S. W., Pang, X. Y., & Lv, J. P. (2019). Dietary enrichment of milk and dairy products with n-3 fatty acids: A review. *International Dairy Journal*, 97, 158–166.
- Gulati, S. K., McGrath, S., Wynn, P. C., & Scott, T. W. (2003). Preliminary results on the relative incorporation of docosahexaenoic and eicosapentaenoic acids into cows' milk from two types of rumen protected fish oil. *International Dairy Journal*, 13, 339–343.
- Hurtaud, C., Faucon, F., Couvreur, S., & Peyraud, J. L. (2010). Linear relationship between increasing amounts of extruded linseed in dairy cow diet and milk fatty acid composition and butter properties. *Journal of Dairy Science*, 93, 1429–1443.
- IDF. (2015). *The world dairy situation 2015*. IDF bulletin N° 481. Brussels, Belgium: International Dairy Federation.
- ISO. (2009). *Milk and milk products – sensory analysis – Part 1: General guidance for the recruitment, selection, training and monitoring of assessors*. International standard ISO 22935-1:2009. Geneva, Switzerland: International Organisation for Standardisation.
- ISO. (2017). *Sensory analysis – methodology – General guidance*. International standard ISO 6658:2017. Geneva, Switzerland: International Organisation for Standardisation.
- Juchem, S. O., Santos, J. E. P., Cerri, R. L. A., Chebel, R. C., Galvão, K. N., Bruno, R., et al. (2008). Effect of calcium salts of fish and palm oils on lactational performance of Holstein cows. *Animal Feed Science and Technology*, 140, 18–38.
- Kairenius, P., Ärolä, A., Leskinen, H., Toivonen, V., Ahvenjärvi, S., Vanhatalo, A., et al. (2015). Dietary fish oil supplements depress milk fat yield and alter milk fatty acid composition in lactating cows fed grass silage-based diets. *Journal of Dairy Science*, 98, 5653–5671.
- Kitessa, S. M., Peake, D., Bencini, R., & Williams, A. J. (2003). Fish oil metabolism in ruminants III. Transfer of n-3 polyunsaturated fatty acids (PUFA) from tuna oil into sheep's milk. *Animal Feed Science and Technology*, 108, 1–14.
- Labreche, S., Bazzo, S., Cade, S., & Chanie, E. (2005). Shelf life determination by electronic nose: Application to milk. *Sensors and Actuators B*, 106, 199–206.
- Lerch, S., Ferlay, A., Graulet, B., Cirié, C., Verdier-Metz, I., Montel, M. C., et al. (2015). Extruded linseeds, vitamin E and plant extracts in corn silage-based diets of dairy cows: Effects on sensory properties of raw milk and uncooked pressed cheese. *International Dairy Journal*, 51, 65–74.
- Let, M. B., Jacobsen, C., & Meyer, A. S. (2005). Sensory stability and oxidation of fish oil enriched milk is affected by milk storage temperature and oil quality. *International Dairy Journal*, 15, 173–182.
- Lynch, J. M., Lock, A. L., Dwyer, D. A., Noorbakhsh, R., Barbano, D. M., & Bauman, D. E. (2005). Flavor and stability of pasteurized milk with elevated levels of conjugated linoleic acid and vaccenic acid. *Journal of Dairy Science*, 88, 489–498.
- McCullum, E. V. (1918). *The newer knowledge of nutrition: The use of food for the preservation of vitality and health*. New York, NY, USA: Macmillan Company.
- McDonald, I. (1981). A revised model for the estimation of protein degradability in the rumen. *Journal of Agricultural Science*, 96, 251–252.
- Ministry of Agriculture and Rural Development. (2003). A földművelésügyi és vidékfejlesztési miniszter 44/2003. (IV. 26.) FVM rendelete a Magyar Takarmányködök kötelező előírásairól [Decree no. 44 of 2003 (April 26) FVM of the Ministry of Agriculture and rural development laying down the binding provisions of the Hungarian feedstuffs Codex (Codex Pubularis Hungaricus)]. *Magyar Közlöny (Official Journal of the Republic of Hungary)*, 59, 3204–3555.
- Moffat, C. F., & McGill, A. S. (1993). Variability of the composition of fish oils: Significance for the diet. *Proceedings of the Nutrition Society*, 52, 441–456.
- Murphy, J. J., Coakley, M., & Stanton, C. (2008). Supplementation of dairy cows with a fish oil containing supplement and sunflower oil to increase the CLA content of milk produced at pasture. *Livestock Science*, 116, 332–337.
- Naes, T., Isaksson, T., Fearn, T., & Davies, T. (2002). *A user friendly guide to multivariate calibration and classification*. Chichester, UK: NIR Publications.
- Nagy, J., Bázár, G., Horn, P., Andrassy-Baka, G., Mezöszentgyörgyi, D., Szabó, A., et al. (2014). Investigating venison sausages by means of human sensory panel and electronic nose. *Acta Agraria Kaposváriensis*, 18, 76–86.
- Ney, D. M. (1991). Potential for enhancing the nutritional properties of milk fat. *Journal of Dairy Science*, 74, 4002–4012.
- Novaes, S. S. C., Dantas, F. B. H., Alvim, I. D., Miguel, A. M. R. O., Dantas, S. T., & Alves, R. M. V. (2019). Stability of omega-3 enriched milk powder in different commercial packages stored under accelerated conditions of temperature and relative humidity. *International Dairy Journal*, 88, 1–9.
- Ørskov, E. R., & McDonald, I. (1979). The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *Journal of Agricultural Science*, 92, 499–503.
- O'Riordan, P. J., & Delahunty, C. M. (2003). Characterisation of commercial Cheddar cheese flavour. 1: Traditional and electronic nose approach to quality assessment and market classification. *International Dairy Journal*, 13, 355–370.
- Palmquist, D. L. (2009). Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. *Professional Animal Scientist*, 25, 207–249.
- Peris, M., & Escuder-Gilbert, L. (2009). A 21st century technique for food control: Electronic noses. *Analytica Chimica Acta*, 638, 1–15.
- Rego, O. A., Rosa, H. J. D., Portugal, P., Cordeiro, R., Borba, A. E. S., Vouzela, C. M., et al. (2005). Influence of dietary fish oil on conjugated linoleic acid, omega-3 and other fatty acids in milk fat from grazing dairy cows. *Livestock Production Science*, 95, 27–33.
- Ribács, A., & Schmidt, J. (2006). Lenolajalapú Ca-szappan felhasználása a tehéntej zsírsav-összetételének módosítására (Use of a linseed oil based calcium soap to modify the fatty acid composition of cows' milk). *Acta Agronomica Óváriensis*, 48, 73–86.
- Saliba, L., Gervais, R., Lebeuf, Y., Vuilleumard, J. C., Fortin, J., & Chouinard, P. Y. (2014). Effect of feeding linseed oil in diets differing in forage to concentrate ratio: 2. Milk lactone profile. *Journal of Dairy Research*, 81, 91–97.
- Schmidt, J., Sipőcz, P., & Sipőcz, J. (2000). Védett zsír hatása a bendőfermentációra és felhasználása a nagy tejtermelésű tehének takarmányozásában (Effect of bypass fat on rumen fermentation and its use in nutrition of high lactating cows). *Állattenyésztés és Takarmányozás (Hungarian Journal of Animal Production)*, 49, 139–154.
- Simopoulos, A. P. (2002). Omega-3 fatty acids in inflammation and autoimmune diseases. *Journal of the American College of Nutrition*, 21, 495–505.
- Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. *Experimental Biology and Medicine*, 233, 674–688.

- Szakály, S. (2001). Tej és tejtermékek a táplálkozásban (Milk and dairy products in nutrition). In S. Szakály (Ed.), *Tejgazdaságtan (Dairy science)* (pp. 425–446). Budapest, Hungary: Dinasztia Kiadó.
- Tóth, T., Grosz, G., Szilvássy, Z., & Jurányi, Z. (2018). *Takarmánykiegészítő készítmények és eljárás előállításukra (Animal feed supplements and process for their production)*. Hungarian Patent No. P 12 00741 Adexgo Kft., Balatonfüred, Hungary.
- Tóth, T., Viszket, E., Csavajda, É., Tanai, A., & Varga, L. (2012). Seasonal changes in the fatty acid composition of raw bovine milk produced in the western part of Hungary. *Milchwissenschaft*, 67, 261–264.
- Wang, B., Xu, S. Y., & Sun, D. W. (2010). Application of the electronic nose to the identification of different milk flavorings. *Food Research International*, 43, 255–262.
- WHO. (2003). *Diet, nutrition and the prevention of chronic diseases*. WHO technical report series N° 916. Geneva, Switzerland: World Health Organisation.