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# Improving Concentration of Healthy Fatty Acids in Milk, Cheese and Yogurt by Adding a Blend of Soybean and Fish Oils to the Ration of Confined Dairy Cows

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## Abstract

Compared to pasture based sistems, milk produced in confined dairy systems is characterized by a high saturated fat (SF) content with a lower concentration of healthy fatty acids (FA) such as vaccenic (VA, *trans*-11$C_{18:1}$), conjugated linoleic cid (*cis*-9, *trans*-11 $C_{18:2}$, CLA), *α*-linolenic (*cis*-9, *cis*-12, *cis*-15$C_{18:3}$), eicosapentaenoic (EPA, $C_{20:5}$) and docosahexaenoic (DHA, $C_{22:6}$) whose presence in milk and dairy products can be increased by feeding polyunsaturated FA (PUFA). The aim of the study was to determine the differences in milk composition and FA profile between a regular (Reg) milk (Reg-Milk), a Reg-Dambo type cheese (Reg-DCh) and a Reg yogurt (Reg-Yog) with that obtained after including a blend (7:1) of soybean (SO) and fish (FO) oils in the total mixed ration (TMR) of lactating dairy cows. The experiment was carried out at the Talar Farm located at Laguna del Sauce, Maldonado Department, Punta del Este, Uruguay Republic during a period of 30 experimental days using a single production batch of 29 Holstein cows. Within this batch, one group of 8 cows (1.88 ± 0.99 calves) in early lactation (135 ± 19 days postpartum) was selected to individually measure milk yield and composition. During the first 7 days of the experiment, cows were fed a TMR without oil-blend inclusion to obtain the Reg-Milk, Reg-DCh and Reg-Yog. From the 8$^{th}$ day onwards, the oil blend was added to the TMR at 4% DM (1.0 kg oil blend cow$^{-1}$ day$^{-1}$) and after 23 days of feeding, the modified milk

(Mod-Milk) was analyzed and collected to elaborate the modified Dambo-type cheese (Mod-DCh) and Mod-yogurt (Mod-Yog). Milk yield was recorded daily in the selected 8 cows and milk composition was determined over two consecutive days prior to the start of blend-oil supply (Reg-Milk) and at the end of oil supplementation (Mod-Milk) on days 29$^{th}$ and 30$^{th}$. Milk-tank samples of Reg-Mi and Mod-Mi were also collected and analyzed for chemical composition and milk FA profile. Cheese and yogurt were assayed for its FA profile. Differences in milk yield and composition and in the FA profile before and after oil-feeding were stated using the Student T-test for paired observations. Milk production (kg·cow$^{-1}$·day$^{-1}$) slightly (−6.7%) decreased ($P < 0.03$) from 36.89 (before) to 34.69 after oil feeding. Milk fat content decreased ($P < 0.05$) from 3.28 to 3.02 g 100 g$^{-1}$ g leading to a lower ($P < 0.02$) yield (kg·cow$^{-1}$·day$^{-1}$) of fat corrected milk (4%FCM) from 32.83 (before) to 29.63 after oil. Milk protein content (g 100 g$^{-1}$) increased ($P < 0.04$) from 2.89 (before) to 3.08 (after) oil feeding (+5.92%) a result confirmed ($P < 0.01$) in samples taken from the tank. Milk protein output (1.07 kg·cow$^{-1}$·day$^{-1}$) ($P < 0.96$), lactose ($P < 0.65$) and total solid ($P < 0.38$) contents were not affected. Concentration of non-fat solids (NFS) tended ($P < 0.08$) to increase from 8.50 in Reg-Mi, to 8.68 g 100 g$^{-1}$ in Mod-Mi as it was observed ($P < 0.001$) in samples taken from the tank (8.78 vs. 9.93 g 100 g$^{-1}$). Yield of NFS tended ($P < 0.07$) to decrease from 3.14 to 3.01 kg·cow$^{-1}$·day$^{-1}$ after oil supply. Content of atherogenic FA ($C_{12:0}$ to $C_{16:0}$) was significantly ($P < 0.064$) reduced (−10.29%) from 44.50 (Reg-Mi) to 39.92 g 100 g$^{-1}$ (Mod-Mi) with a concomitant decrease ($P < 0.10$) in the atherogenic index (AI) from 2.45 (Reg-Mi) to 2.03 (Mod-Mi). Concentration (g 100 g$^{-1}$ FA) of elaidic (*trans*-9 $C_{18:1}$) and *trans*-10 $C_{18:1}$ FA was low in Reg-Mi (0.22 and 0.34 respectively) but tended ($P < 0.11$) to increase in Mod-Mi (0.43 and 0.95). Concentration (g 100 g$^{-1}$ FA) of VA resulted higher (+110%, $P < 0.039$) in Mod-Mi (2.42) compared to Reg-Mi (1.15). Total CLA content (g 100 g$^{-1}$ FA) increased ($P < 0.036$) from 0.66 in Reg-Mi to 1.36 in Mod-Mi (+106%). Levels (g 100 g$^{-1}$) of $\alpha$-linolenic were higher ($P < 0.004$) in Reg-Mi (0.34) compared to Mod-Mi (0.29). The omega-6/omega-3 ratio was not changed ($P < 0.13$) averaging 5.98 in R-Mi and 7.18 in M-Mi. Oleic acid (*cis*-9 $C_{18:1}$) content (g 100 g$^{-1}$) remained unchanged ($P < 0.504$) averaging 21.99 in Reg-Mi and 22.86 in Mod-Mi. Composition of FA of the Mod-DCh was highly correlated ($R^2 = 0.999$) with FA profile from its original Mod-Mi. A serving of the M-DCh (30 g) theoretically involved a 12.1% reduction in total fat consumption with 16.9% less in SF intake compared to the Reg-Ch. A serving of the M-DCh could putatively increase total CLA consumption by 72.7% compared to the Reg-DCh. Compared to Reg-Yo, a portion (178 g) of the Mod-Yo could increase (69.4%) total CLA intake. The nutritional value of milk fat was improved by feeding a blend of PUFA oils to confined dairy cows and the consumption of the mofified dairy products obtained could promote a lower intake of the potentially atherogenic saturated FA and some increase in healthy FA ingestion.

## Keywords

Dairy Cows, Oil, Saturated Fat, Conjugated Linoleic Acid, Cheese, Yogurt

# 1. Introduction

The growing incidence in the modern society of noncommunicable cardiovascular and chronic diseases plus the increase of cases of childhood obesity implies a significant economic and social burden for the states and creates the need to produce food capable of mitigating these risks. Milk and its derivatives represent the largest contribution in the consumption of saturated fatty acids (SFA), which represents a potential risk of chronic cardiovascular diseases, obesity and metabolic syndrome [1] [2] [3]. The potentially unhealthy milk SFA are lauric ($C_{12:0}$), myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) for their putative atherogenic role when consumed in excess [4]. Those SFA have been associated with the risk of cardiovascular disease [5] [6]. As a counterpart, milk and dairy are the main natural foods containing the conjugated linoleic acids (CLA) with promising cardioprotective [3] and antitumor [7] [8] [9] properties.

Experimental evidence of the CLA's contribution to cancer patients is still insufficient but both, *cis*-9, *trans*-11$C_{18:2}$ CLA (rumenic acid) and *trans*-10, *cis*-12$C_{18:2}$ CLA, have shown promising effects in animal studies or in *in vitro* cell cultures with different tumor lines [7] [8] [9].

Feeding with conventional foods would not allow sufficient daily intake of CLA to attain expression of its potential biochemical, molecular and physiologically preventive effects on cardiovascular pathologies [8], diabetes [10] [11], atherosclerosis [12], different types of cancer [13] [14], hypertension [8], obesity [8] [15] or anti-inflammatory effects [16] [17]. From the analysis of nine studies in humans, it was concluded that the chronic consumption of a milk with a modified FA profile was beneficial for the cardiovascular health of normal and hypercholesterolemic human individuals [3]. In another study, the consumption of CLA from dairy products was associated with a lower risk of myocardial infarction in a population of 1813 cases of first non-fatal acute infarction and 1813 control individuals grouped by age, sex and area of residence [18].

The inclusion of oils rich in polyunsaturated FA (PUFA) in the ration of dairy cows allows modifying the milk and dairy FA profile in a sense that may be favorable to human health [19] [20]. In dairy cows, intake of linoleic acid (*cis*-9, *cis*-12 $C_{18:2}$) contained in SO is a quick and effective tool to inhibit the mammary synthesis of the potentially atherogenic FA and to increase the milk CLA content [21].

Inclusion of FO in the ration as a source of EPA and DHA inhibits the biohydrogenation of VA (the main CLA precursor) to stearic acid [21] and moderate amounts of FO do not appear to affect the rumen environment or fiber digestión [22]. The aim of the study was to determine the effect of feeding a blend of PUFA oils on FA composition of milk, Dambo type cheese and yogurt specifically concerning those FA that are beneficial to human health.

# 2. Materials and Methods

## 2.1. Cows and Diets

The trial lasted 30 days, from June 24 to July 23, 2019 and was carried out at the

Agroindustrial Complex located in Laguna del Sauce, Route 12 km 10, Department of Maldonado, Punta del Este (Uruguay Republic). A single production-batch composed by 29 confined Holstein cows was used from which one subgroup of 8 cows (1.88 (±0.99) calves) in early lactation (135 ± 19 days postpartum) were selected for individual measurements of milk yield and composition.

During a pre-experimental period of 7 days, the cows were fed a basal TMR (Table 1) without supplementary oils in order to asses milk yield and composition at the beginning of the trial (Reg-Milk). A regular Dambo type cheese (R-DCh) and yogurt (R-Yo) were manufactured using the Reg-Milk.

From the 8th day onwards, a blend (7:1 w/w) of SO and FO was added to the TMR at 4% DM delivering about 0.875 and 0.125 $kg \cdot cow^{-1} \cdot day^{-1}$ respectively. The oil-blend was mixed with the non-forage components of the TMR prior to the incorporation into the mixer and subsequent mixed with the ryegrass silage. The deodorized FO (99.5% DM, AD-1, Omega Sur Laboratories, Mar del Plata, Argentina) presented a density of 0.925 $g/cm^3$ (IRAM 5504), a peroxide index of 1.91 meq. $O^2$/kg of oil (AOCS Cd 8-53) and absence of Salmonella. The FA profile of the FO was determined by gas chromatography with FID detection and is presented in Table 2.

**Table 1.** Type and cost of ingredients used to formulate the total mixed ration of the experiment.

| Ingredient | % DM(1) | % as fed | Cost(2) |
|---|---|---|---|
| Cracked corn grain | 16.84 | 8.22 | 180 |
| Ryegrass silage | 47.45 | 74.65 | 35 |
| Pelletized soybean meal | 15.49 | 7.61 | 360 |
| Mineral-vitamin premix (Nutral™) | 2.72 | 1.18 | 500 |
| Dried distillery grains (DDGS) | 9.36 | 4.26 | 215 |
| Cracked sorghum grain | 8.14 | 4.09 | 140 |

(1)DM = dry matter. (2)Values are expressed in US dollars per Ton "as fed".

**Table 2.** Fatty acid (FA) composition of the fish oil used in the experiment.

| FA | $g\ 100\ g^{-1}$ FA |
|---|---|
| $C_{18:3\ n3}$ | 0.88 |
| $C_{18:4\ n3}$ | 0.40 |
| $C_{20:3\ n3}$ | 0.39 |
| $C_{20:4\ n3}$ | 0.71 |
| $C_{20:5\ n3\ \mathbf{EPA}}$ | 7 |
| $C_{21:5\ n3}$ | 0.62 |
| $C_{22:5\ n3\ DPA}$ | 1.01 |
| $C_{22:6\ n3\ \mathbf{DHA}}$ | 16.99 |
| Total n-3 FA | 28 |
| Total n-6 FA | 2.05 |
| Polyunsaturated FA | 30.05 |
| Free FA | 0.24 |

### 2.2. Samples Collection and Analysis

In the subgroup of 8 selected cows, milk production was daily and individually recorded during the whole trial. Milk composition was measured on the individual milk samples (8 cows) and in samples taken from the collecting tank (29 cows) on days 6th and 7th (Regular) and 29th and 30th of the trial after oil delivery to obtain the modified products (Mod-Milk, Mod-Dch and Mof-Yo).

On each day, a sample of milk was taken from the morning (50 ml) and the afternoon (50 ml) milkings to analyze chemical composition (fat, protein, lactose, non-fat solids (NFS) and total solids (TS)) by infrared spectrophotometry (MilkoScanTM Minor; FOSS Electric, Hilleroed Hillerod, Denmark) according to ISO 9622 IDF 141 (2013). Milk samples collected from the tank were also assayed for FA composition. After 23 days of oil-blend feeding, the Mod-Milk was analyzed for chemical composition an collected to manufacture the Mof-DCh and Mod-Yo.

### 2.3. Analysis of Fatty Acid Profile in Milk and Dairy Products

Milk fat was extracted following the method described in [23]. Methyl esters of FA (FAME) were prepared by base-catalysed methanolysis of the glycerides according to the ISO-IDF procedure (2002). Analysis of FAME in hexane was performed on a gas-liquid chromatograph (Varian CP3800, Walnut Creek, CA, USA) fitted with a flame ionization detector. The FAME profile was determined by split injection (1:100) onto a CP-Sil 88 fused silica capillary column (100 m × 0.25 mm i.d., 0.20 μm film thickness, Varian CP7489) using a gradient temperature programme. The column oven was held at 45˚C for 4 min, then increased from 45˚C to 165˚C at 13˚C/min and held for 35 min and finally from 165˚C to 215˚C at 4˚C/min and held for 30 min. The total run time was 90 min. The carrier gas was helium and was held at a constant flow of 1.0 mL/min. The injector and detector temperatura were 250˚C. Fatty acids were identified by comparing relative retention times with individual fatty acids standard (PUFA-2 Animal Source; Grain Fatty acid Methyl Ester Mix; Octadecadienoic acid conjugated methyl ester; trans-11-Vaccenic Methyl Ester; cis-11-Vaccenic Methyl Ester; trans-9-Elaidic Methyl Ester; 37-Component FAME mix (Sigma-Aldrich, USA) and GLC 481B (NuChek Prep. Inc. Elysian, MN, USA). Analytical results are expressed as percentages of total FA. The tank-milk samples were also collected during days 6th and 7th (Basal) and 29th and 30th (Final) and the same procedure was applied.

### 2.4. Statistical Analysis

The difference in milk production, chemical composition and milk FA profile was analyzed through the Student's T test for paired observations.

## 3. Results and Discussion

The TMR averaged 43.26% DM with 17.62% crude protein, 35.91% neutral detergent fiber (NDF), 2.74 Mcal·kg$^{-1}$ DM of estimated metabolizable energy content and a forage:concentrate ratio of 47:53. It was offered at 4.55% of the average live weight of the production-batch (29 cows) and thoroughly consumed by cows which implied a daily allowance of 25 kg DM cow$^{-1}$·day$^{-1}$ equivalent to 57.8 kg TMR as fed.

### 3.1. Milk Yield and Composition

The yield of 4%FCM decreased after oil supplementation (−11.9%) as the combined effects of both, a slight decrease ($P < 0.03$) in milk production and a lower milk fat content (Table 3). Supplementation with unsaturated lipids generally has neutral effects on the production of 4%FCM both in confined [25] and in pasture based diets [26]. Feeding unsaturated lipids to lactating dairy cows neither increase nor negatively affect milk production [25] [26]. Negative effects on milk production were also not observed after feeding unprotected vegetable oils to confined dairy cows with a high frequency of favorable effects on milk yield

**Table 3.** Milk yield and composition in confined dairy cows before (Initial) and 23 days after (Final) including a blend of soybean oil (0.875 kg·cow$^{-1}$·day$^{-1}$) and fish oil (0.125 kg·cow$^{-1}$·day$^{-1}$) in the total mixed ration.

| **Selected cows**(1) | Initial (a) | Final (b) | Difference (a)-(b) | Δ%(2) | $P <$(3) |
|---|---|---|---|---|---|
| Milk yield, kg·cow$^{-1}$·day$^{-1}$ | 36.89 (±5.49) | 34.69 (±5.48) | 2.20 (±2.13) | −6.66 | 0.03 |
| 4% FCM yield, kg·cow$^{-1}$·day$^{-1}$ | 32.83 (±4.39) | 29.63 (±5.28) | 3.21 (±2.73) | −11.86 | 0.02 |
| Milk fat content, g 100 g$^{-1}$ | 3.28 (±0.42) | 3.02 (±0.39) | 0.27 (±0.29) | −9.24 | 0.05 |
| Milk fat yield, , kg·cow$^{-1}$·day$^{-1}$ | 1.21 (±0.18) | 1.05 (±0.22) | 0.16 (±0.14) | −16.68 | 0.03 |
| Milk protein content, g 100 g$^{-1}$ | 2.89 (±0.12) | 3.08 (±0.10) | 0.10 (±0.25) | 5.92 | 0.04 |
| Milk protein yield, kg·cow$^{-1}$·day$^{-1}$ | 1.07 (±0.16) | 1.07 (±0.15) | - | - | 0.96 |
| Lactose content, g 100 g$^{-1}$ | 4.87 (±0.10) | 4.86 (±0.10) | 0.02 (±0.08) | −0.32 | 0.65 |
| Lactose yield, kg·cow$^{-1}$·day$^{-1}$ | 1.80 (±0.28) | 1.69 (±0.29) | 0.11 (±0.10) | −6.97 | 0.02 |
| Total solid content, g 100 g$^{-1}$ | 11.78(±0.47) | 12.01 (±0.81) | −0.23 (0.64) | +1.66 | 0.38 |
| Total solid yield, kg·cow$^{-1}$·day$^{-1}$ | 4.34 (±0.60) | 4.17 (±0.75) | 0.17 (±0.46) | −5.09 | 0.37 |
| Non-fat solid content, g 100 g$^{-1}$ | 8.50 (±0.12) | 8.68 (±0.11) | 0.18 (±0.22) | +2.06 | 0.08 |
| Non-fat solid yield, kg·cow$^{-1}$·day$^{-1}$ | 3.14 (±0.48) | 3.01 (±0.47) | 0.13 (±0.15) | −4.38 | 0.07 |
| **Milk tank samples**(4) | | | | | |
| Milk fat content, g 100 g$^{-1}$ | 3.43 (±0.03) | 3.26 (±0.08) | 0.17 (±0.11) | −5.40 | 0.11 |
| Milk protein content, g 100 g$^{-1}$ g | 3.15 (±0.01) | 3.28 (±0.03) | 0.12 (±0.02) | +3.76 | 0.01 |
| Lactose content, g 100 g$^{-1}$ | 4.88 (±0.01) | 4.90 (±0.01) | 0.01 (±0.01) | +0.27 | 0.06 |
| Total solid content, g 100 g$^{-1}$ g | 12.23(±0.03) | 12.23 (±0.07) | - | +0.27 | 0.94 |
| Non-fat solid content, g 100 g$^{-1}$ g | 8.78 (±0.01) | 8.93 (±0.03) | 0.14 (±0.02) | +1.61 | 0.00 |

(1)Eight animals individually monitored; (2)Relative changes (%) compared to Initial values. (3)Statistical significance of the difference (a – b), Student's t-test for paired differences. (4)Obtained from the production-batch of 29 milked cows. 4%FCM = milk corrected at 4% fat.

[27]. Feeding SO at 2.9% (±1.2) of total DM intake did not affect milk production in the experiments reviewed by [28] and also when oil was fed at 3.5% to 5% of DM intake [29] [30] [31].

These results were not confirmed in the present work since the inclusion of SO at 3.5% of DM intake decreased milk production. In our previous trial using corn-silage as forage source in the TMR, the inclusion of SO at 3% of DM intake increased milk production [20]. In the present experiment, the shift from corn to pasture-silage could probably explain the observed slight decrease (−6.7%) in milk production (**Table 3**) owing to a lower palatability of the ryegras silage linked to its little bitter taste.

Intake of supplementary PUFA contained in the SO-FO blend reduced ($P < 0.05$) milk fat concentration by 9.24%. A lower milk fat content (−5.4%) was also detected ($P < 0.11$) in samples taken from the tank reflecting an overall negative effect of oil intake over the total herd. Milk fat content decrease (9.24% to 5.4%) was close to the average value of 8% reported for grazing dairy cows [26] but lower than the 28.6% observed in our previous work using the SO (0.72 $kg \cdot cow^{-1} \cdot day^{-1}$), micro-algae (0.144 $kg \cdot cow^{-1} \cdot day^{-1}$) combination [20].

The presence of DHA in the FO (a potent inhibitor of *de novo* mammary lipid synthesis) plus the ruminal generation of certain FA such as *trans*-10 $C_{18:1}$ and its subsequent transfer to the mammary gland contribute to explain the fall in milk fat content (**Table 3**). A direct relationship between increasing levels of *trans*-10 $C_{18:1}$ in milk and the reduction of *de novo* mammary synthesis has been reported [32]. The presence of *trans*-10 $C_{18:1}$ has been associated with dysfunctions in the activity of the lipoprotein lipase (LPL) and stearyl CoA desaturase (SCD) enzymes that are involved in fat synthesis thus causing a decrease in milk fat content [33]. In the present work, both the concentration of *trans*-10 $C_{18:1}$ and that of DHA in milk were low, contributing in part to explain the moderate depressant effect observed on milk fat concentration. In a previous grazing experiment, feeding SO combined or not with FO strongly reduced milk fat content (−19 to −27%) compared to the pre-supplementation record [34].

The inhibition of the *de novo* FA mammary synthesis with the lower total concentration of SFA in milk (**Table 4**) partially contributes to explaining the milk fat content reduction. The decrease in FA synthesized *de novo* (**Table 4**) was not apparently compensated by a correlative increase in the mammary uptake of supplementary preformed FA contained in SO and FO and milk fat content decreased. It is worth noting that the observed fat reduction (**Table 3**) occurred in part at the expense of the amount of the hypercholesterolemic FA (**Table 4**). This fact potentially improves the healthy value of milk and contributes to decrease its atherogenic potential and the atherogenicity index of the Mod-Milk (**Table 4**).

After oil-blend intake, milk protein concentration slightly (+5.92%) increased ($P < 0.04$) an effect that was also observed in milk samples taken from the tank (**Table 3**). Synthesis of milk protein can be limited by energy availability and a

**Table 4.** Milk fatty acid (FA) composition in regular (Reg-Milk) and modified (Mod-Milk) milks after including a blend of soybean (0.875 $kg \cdot cow^{-1} \cdot day^{-1}$) and fish (0.125 $kg \cdot cow^{-1} \cdot day^{-1}$) oils to the ration of confined dairy cows.

| Fatty Acid g/100g FA | Reg-Milk | Mod-Milk | Δ%(1) | $P <$(2) |
|---|---|---|---|---|
| $C_{4:0}$ | 2.21 | 2.04 | −8.04 | 0.194 |
| $C_{6:0}$ | 1.81 | 1.55 | −14.36 | 0.104 |
| $C_{8:0}$ | 1.20 | 1.04 | −13.80 | 0.063 |
| $C_{10:0}$ | 2.74 | 2.41 | −12.11 | 0.073 |
| $C_{12:0}$ | 3.12 | 2.89 | −7.26 | 0.158 |
| $C_{14:0}$ | 10.68 | 10.05 | −5.93 | 0.230 |
| $C_{16:0}$ | 30.70 | 26.98 | −12.12 | 0.029 |
| $\sum C_{12:0\text{-}C16:0}$ | 44.50 | 39.92 | −10.29 | 0.064 |
| $C_{18:0}$ | 9.64 | 10.17 | +5.44 | 0.321 |
| $C_{18:1\ t9}$ | 0.22 | 0.43 | +100.23 | 0.015 |
| $C_{18:1\ t10}$ | 0.34 | 0.95 | +178.53 | 0.109 |
| $C_{18:1\ t11\ \text{(vaccenic acid)}}$ | 1.15 | 2.42 | +110.23 | 0.004 |
| $C_{18:1\ c9\ \text{(oleic acid)}}$ | 21.99 | 22.86 | +3.97 | 0.504 |
| $C_{18:2\ c9\ c12\ \text{(linoleic acid)}}$ | 2.33 | 2.42 | +3.64 | 0.392 |
| $C_{18:3\ c89\ c12\ c15\ \text{(linolenic acid)}}$ | 0.34 | 0.29 | −14.90 | 0.004 |
| $C_{18:2\ cis\text{-}9,\ trans\text{-}11}$, CLA | 0.66 | 1.34 | +102.85 | 0.039 |
| $C_{18:2\ cis\text{-}12,\ trans\text{-}10}$, CLA | - | 0.02 | | |
| Total CLA | 0.66 | 1.36 | +106.36 | 0.036 |
| Saturated FA | 65.27 | 60.55 | −7.24 | 0.046 |
| Monounsaturated FA | 28.26 | 31.65 | +12.00 | 0.102 |
| Polyunsaturated FA | 3.68 | 4.32 | +17.57 | 0.016 |
| Total Omega 3 (n-3) | 0.43 | 0.36 | −16.26 | 0.058 |
| Total Omega 6 (n-6) | 2.58 | 2.60 | +0.59 | 0.847 |
| Atherogenic index | 2.45 | 2.03 | −17.23 | 0.102 |
| n-6/n-3 | 5.98 | 7.18 | +20.10 | 0.129 |

(1)Relative FA changes (%) compared to values observed in the Reg-Milk, (−) = decrease, (+) = increase. (2)Student t Test *t* for paired observations.

reduced milk fat content (**Table 3**) could have spared energy improving the energy status of the cows. As the consequence of a lower milk production after oil supplementation, milk protein output remained constant ($P < 0.96$) averaging 1.07 kg protein $cow^{-1} \cdot day^{-1}$ (**Table 3**). The increase in milk protein content is a desirable effect not observed in our previous work using a mix of SO and microalgae [20]. A higher protein concentration improves the industrial aptitude of milk for cheese making and determines the speed and quality of coagulation. Under grazing conditions, lipid supplementation does not usually affect milk protein content [26] [35] but in confined feeding systems, milk protein concentration is systematically affected [25] [34]. The effect of supplementation with unprotected lipids on milk protein was unfavorable in 71% of the cases analyzed by [27] and is associated with a reduction in casein synthesis [37] [38]. The negative effect is more consistent using SF supplements (−0.18 g 100 $g^{-1}$) and calcium salts of FA (−0.12 g 100 $g^{-1}$) with respect to PUFA rich oils [25].

Lactose and TS contents were not significantly affected after oil intake (**Table 3**). As a consequence of the increase in the milk protein content, concentration of NFS tended ($P < 0.08$) to increase after oil intake and resulted higher (+1.61%) in milk samples obtained from the tank (**Table 3**).

### 3.2. Milk Fatty Acid Profile

The changes observed in milk FA composition (**Table 4**) after feeding the oil blend may be explained by the increase in the mammary uptake of plasma triglycerides when adding supplementary PUFA to the ration and confirms the existence of a great plasticity in milk FA composition [21] [28].

The absence of a net depressant effect (P = 0.19) of supplementary PUFA on butyric acid ($C_{4:0}$) content of Mod-Milk (**Table 4**) was also observed in our previous study [20] being a result frequently reported [28]. This FA is only found in ruminant milk and has shown antineoplasic effects inhibiting the development of mammary carcinoma in rats [39] and hence is considered to play a potential beneficial role in human health.

The total concentration of SFA in the Reg-Milk (65.27 g 100 $g^{-1}$ FA) was similar to that observed (66.76 g 100 $g^{-1}$ FA) in our previous work [20] and decreased ($P < 0.046$) to 60.55 g 100 $g^{-1}$ FA in the Mod-Milk obtained after adding the SO-FO blend to the TMR (**Table 4**). This reduction in SFA was however less than the 17.7% obtained when SO was combined with microalgae [20]. The result can be considered of interest since the excessive consumption of SFA is considered unhealthy and associated with raised blood cholesterol levels increasing the risk of developing heart disease. There is evidence that substituting SFA with PUFA's reduces the risk of coronary heart disease. The SFA reduction was coupled to a concomitant increase (+12%) in concentration of monounsaturated FA (MUFA) from a value of 28.26 g 100 $g^{-1}$ FA in the Reg-Milk to 31.65 g 100 $g^{-1}$ FA in the Mod-Milk (**Table 4**). The increase was however less than obtained in our previous trial (37%) when a mix of SO-microalgae was included in the TMR [20].

Concentration of PUFA (g 100 $g^{-1}$ FA) was also increased ($P < 0.016$) by 17.6% from a basal value of 3.68 in Reg-Milk to 4.32 in the Mod-Milk. This increase resulted lower than the 36% obtained in our previous work using the SO-microalgae mix [20].

Compared to Reg-Milk, the Mod-Milk showed a moderate (−10.29%) but significant ($P < 0.064$) decrease (−4.58 g, **Table 4**) in total concentration of the potentially atherogenic FA ($C_{12:0}$ to $C_{16:0}$) promoting a healthier milk [4]. The reduction in the levels of myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) acids observed in Mod-Milk resulted much lower (−5.93 and −12.125) than that obtained using a combination of SO and microalgae (27.6% for $C_{14\ 0}$ and 18.9% for $C_{16:0}$) [20]. Feeding FO to grazing dairy cows (160 or 320 g·day$^{-1}$) reduced the $C_{12:0}$ concentration in milk without any effect on $C_{14:0}$ and $C_{16:0}$ [40]. When consumed in excess, these three SFA raise the levels of total plasma cholesterol and the cholesterol associated with low density (LDL) plasma lipoproteins [41].

The reduction of these FA's after PUFA intake is a frequently reported result [31] [42] [43] explained by ruminal biohydrogenation of supplementary PUFA that yields *trans*-isomers that are inhibitors of key enzymes of mammary lipogenesis such as acetyl-CoA carboxylase [6]. As an associated result, the atherogenic index decreased ($P < 0.102$) from a value of 2.45 in the Reg-Milk to 2.03 in the Mod-Milk (**Table 4**). However, this 17.23% reduction resulted much lower than the 44.2% obtained using a SO-microalgae combination [20] or the 57% reduction in the AI when grazing cows were supplemented with sunflower and fish oils [22]. Taken together, results obtained help to avoid an excessive consumption of unhealthy FA enhancing the health benefits of Mod-Milk and its dairy products compared to the Reg-Milk.

In the present experiment, milk content of stearic ($C_{18:0}$) and oleic (*cis*-9 $C_{18:1}$) acids was not affected by oil supply (**Table 4**) but in the meta-analysis by [28] all PUFA supplements generates similar increases in the content of both FA. In grazing conditions, feeding sunflower seed or sunflower oil enhanced (+51%) the concentration and secretion of $C_{18:0}$ in milk probably reflecting increased ruminal biohydrogenation of supplementary $C_{18:2}$ and VA [22].

In the present experiment (**Table 4**), milk concentration of stearic acid remained unchanged ($P < 0.321$) reaching a value of 10.17 g 100 g$^{-1}$ FA in Mod-Milk and close to the concentration of 11.42 g 100 g$^{-1}$ FA reported when the SO-microalgae supplement was included in the TMR [20].

An increase in milk oleic acid content after the addition of sunflower or SO oils to the ration is a well-documented result [28] [44] [45] also observed when supplementing with linseed oil [28] [46] [47] [48]. In our trial, the presence of this FA remained unchanged ($P < 0.504$) in both milks (**Table 4**) suggesting that the EPA and DHA contained in FO contributed to attenuate the biohydrogenation of VA to stearic as proposed by [21]. The oleic acid is a component of the so-called "Mediterranean diet" and is fundamentally present in olive oil with beneficial effects on the blood lipid profile and risk factors for cardiovascular diseases [49].

Concentration of MUFA increased by 12% in the Mod-Milk (**Table 4**) and those FA has been described to modulate blood pressure, improve insulin sensitivity and regulate circulating glucose levels [49].

In the Reg-Milk, concentration of the unhealthy *trans* FA such as *trans*-9 $C_{18:1}$ (0.22 g/100 g) and *trans*-10 $C_{18:1}$ (0.34 g 100 g$^{-1}$ FA) were comparable to those registered in [20]. In the Mod-Milk, concentration (g 100 g$^{-1}$ FA) of *trans*-9 $C_{18:1}$

(0.43) was comparable to the value recorded in the previous experiment (0.54) but that of *trans*-10 $C_{18:1}$ (0.95) resulted much lower than the 3.14 value observed with the combination of SO and microalgae [20]. The replacement of corn silage for pasture silage in the present trial could partially explain the difference. The DHA contained in FO (**Table 2**) may have also contributed to maintaining low levels of *trans*-10 $C_{18:1}$ since the concentration of this *trans* isomer in milk tended to decrease with the increasing participation of FO mixed to sunflower oil [50]. It can be stated that at the observed concentrations, those *trans* FA would not present potential risks on the degree of ischemic heart disease to humans [51]. It was postulated that ruminant *trans* fatty acids, especially concerning the effect on cardiovascular risk, do not possess the same unfavorable effects as industrially produced *trans* fatty acids [52].

The low content of *trans*-10 $C_{18:1}$ in Mod-Milk is also compatible with the low decrease in milk fat concentration observed (**Table 3**) and with the lowest reduction of $C_{12:0}$ to $C_{16:0}$ respect to our previous trial [20]. It was shown that the decrease in milk fat content is negatively correlated to *trans*-10 $C_{18:1}$ levels [32] [53]. A high *trans*-10 $C_{18:1}$ concentration, or its related metabolites like *trans*-10, *cis*-12$C_{18:2}$ in milk, has been associated with dysfunctions in lipoprotein lipase (LPL) and stearoyl CoA desaturase (SCD) enzymes involved in milk fat uptake (LPL) and synthesis explaining the decrease in the fatty content of milk [54].

Concentration of VA in Mod-Milk averaged 2.42 g 100 $g^{-1}$ FA representing an increase of 110% ($P < 0.004$) over the baseline value of 1.15 g 100 $g^{-1}$ FA observed in the Ref-Milk (**Table 4**). Those values were close to previously reported in [20]. Natural VA contained in dairy products can exert beneficial anticarcinogenic properties by itself through a direct effect [55] or a mediated effect by its endogenous conversion to rumenic acid (RA) in human tissues at an estimated rate of 20% [56] by the Δ9-desaturase activity [57]. The metabolism of VA to RA has been shown to be an effective way to prevent chemically induced cancer in rats [58] and increases the RA bioavailability in tissues [59]. In this and our previous trial [20], the increase in VA induced by PUFA feeding was somehow moderate and should be strengthened.

In the present experiment, FO was included at a low dose (0.5% of total DM intake). As it was stated that FO can be reduced at 1% of total DM intake when it is combined with other sources of lipid substrate [60], the optimal doses of precursors ($C_{18:2}$ or $C_{18:3}$) for CLA synthesis and its combination with FO needs to be explored in grazing and confined dairy cows in order to maximize the milk CLA content and the CLA/*trans*-11$C_{18:1}$ ratio.

Concentration (g 100 $g^{-1}$ FA) of *cis*-9, *trans*-11 $C_{18:2}$ (CLA) increased ($P < 0.039$) from a baseline value of 0.66 in the Reg-Milk to 1.34 in the Mod-Milk (+103%) a value close to that reported in [20] and higher than the average value (1.02 ± 0.36 g/100g) informed in the meta analysis by [28] when dairy cows were supplemented with SO alone.

In the present trial, the CLA/VA ratio (product/precursor) was 0.36 in both, Reg-Milk and Mod-Milk (**Table 4**) suggesting that the activity of *Δ*-9 mammary desaturase (generator of CLA from AV) was not changed by oil supply. The average relationship obtained was close to the value of 0.33% reported by other authors when cows were supplemented with PUFA [33] [54].

The concentration of linoleic acid (*cis*-9, *cis*-12 $C_{18:2}$) remained unchanged ($P < 0.392$) ranging from 2.33 in the Reg-Milk to 2.42 g 100 $g^{-1}$ FA in the Mod-Milk (**Table 4**) values that were within the normal range (2% - 3%) reported by [19].

A decrease ($P < 0.004$) of 14.9% in the concentration of the $\alpha$-linolenic acid (*cis*-9, *cis*-12, *cis*-15 $C_{18:3}$) was observed in the Mod-Milk (**Table 4**) but the observed values (0.34 and 0.29 g 100 $g^{-1}$ FA) were within the range (0.28 - 0.33 g 100 $g^{-1}$ FA) reported in [61].

The omega-6/omega-3 ratio in the Mod-Milk (7.18) resulted similar ($P < 0.129$) to the 5.98 value observed in the Reg-Milk (**Table 4**). In Western diets, increased consumption of omega-6 and decreased levels of omega-3 has left dietary omega ratios drastically out of balance (15 - 20:1) instead of an optimal of 4:1 [62]. The results obtained suggest that the consumption of Reg-Milk or Mod-Milk (or their derivatives) can contribute to lowering this ratio in the human diet.

Concentration of EPA and DHA in milk did not increase after including FO in the TMR a result that may be explained in part by the low dose of FO used as well as by a high biohydrogenation of these FA in the rumen [6] [21]. It was also postulated that EPA and DHA are present in the cholesteryl esthers and plasmatic phospholipids fractions that are poorly utilized by the mammary gland [21] [63]. The low transfer effectiveness of EPA and DHA from the diet to the milk is consistent with previous findings [21] [40].

## 3.3. Nutritional Facts of the Dambo Mofified Type Cheese Compared to the Regular Cheese

The FA composition of the Mod-DCh was highly correlated ($R^2 = 0.999$) with that Mod-Milk of origin. The Mod-DCh showed differences in FA composition that resulted equivalent to those described for Mod-Milk.

As observed in milk (**Table 4**), the concentration of total atherogenic FAs ($\Sigma C_{12:0}$-$C_{16:0}$) tended ($P < 0.11$) to decrease (−4.65%) in the Mod-DCh as well as its SF content (−5.45, $P < 0.10$) and AI (−13.18%, $P < 0.046$). These results were coupled to a significant increase in healthy FA such as VA, *cis*-9. *trans*-11 CLA, MUFA and PUFA with no changes in the n-6/n3 ratio (**Table 5**). The nutritional information of the Dambo type Cheese per a 30 g serving is presented in **Table 6**.

Consuming a portion (30 grams) of the Mod-DCh cheese theoretically implies a 12.1% lower intake of total fat with a 16.9% lower consumption of SF compared to the Reg-DCh with a concomitant putative increase (+72.7%) in total CLA consumption. As it was already stated, the potential increase in *trans* fats intake by serving of Mod-DCh is explained by a higher concentration of healthy *trans* fats such as VA and CLA in the Mod-Milk of origin (**Table 4**) and in the

**Table 5.** Differences in fatty acid (FA) composition between the regular Dambo type cheese (Reg-DCh) and that elaborated with mofified milk (Mod-DCh).

| Fatty Acid g 100 g$^{-1}$ FA | Reg-DCh | Mod-DCh | Δ%[1] | $P <$[2] |
|---|---|---|---|---|
| $C_{4:0}$ | 1.99 | 1.88 | −5.96 | 0.309 |
| $C_{6:0}$ | 1.59 | 1.44 | −9.87 | 0.149 |
| $C_{8:0}$ | 1.11 | 0.99 | −10.36 | 0.098 |
| $C_{10:0}$ | 2.58 | 2.31 | −10.38 | 0.102 |
| $C_{12:0}$ | 3.05 | 2.82 | −7.38 | 0.133 |
| $C_{14:0}$ | 10.80 | 10.07 | −6.75 | 0.119 |
| $C_{16:0}$ | 28.72 | 27.70 | −3.57 | 0.107 |
| $\Sigma C_{12:0\text{-}C16:0}$ | 42.57 | 40.59 | −4.65 | 0.114 |
| $C_{18:0}$ | 11.96 | 11.01 | −7.92 | 0.010 |
| $C_{18:1\ t9}$ | 0.29 | 0.43 | +52.00 | 0.039 |
| $C_{18:1\ t10}$ | 0.51 | 0.91 | +76.21 | 0.041 |
| $C_{18:1\ t11\ (vaccenic\ acid)}$ | 1.43 | 2.42 | +69.32 | 0.001 |
| $C_{18:1\ c9\ (oleic\ acid)}$ | 21.83 | 22.42 | +2.73 | 0.087 |
| $C_{18:2\ c9\ c12\ (linoleic\ acid)}$ | 2.25 | 2.27 | +1.02 | 0.301 |
| $C_{18:3\ c89\ c12\ c15\ (linolenic\ acid)}$ | 0.25 | 0.28 | +9.57 | 0.226 |
| $C_{18:2\ cis\text{-}9.\ trans\text{-}11}$, CLA | 0.72 | 1.24 | +72.69 | 0.013 |
| $C_{18:2\ cis\text{-}12,\ trans\text{-}10}$, CLA | nd | 0.02 | | |
| Total CLA | 0.72 | 1.26 | +72.69 | 0.014 |
| SFA | 65.31 | 61.75 | −5.45 | 0.104 |
| MUFA | 28.34 | 31.09 | +9.69 | 0.010 |
| PUFA | 3.48 | 4.01 | +15.24 | 0.005 |
| Total Omega 3 (n-3) | 0.31 | 0.32 | +5.70 | 0.356 |
| Total Omega 6 (n-6) | 2.46 | 2.43 | −1.11 | 0.061 |
| Atherogenic index | 2.41 | 2.09 | −13.18 | 0.046 |
| n-6/n-3 | 8.06 | 7.55 | −6.39 | 0.309 |

[1]Relative FA changes (%) compared to values observed in the R-Ch. (−) = decrease, (+) = increase. [2]Student t Test t for paired observations.

**Table 6.** Parameters of nutritional interest in the regular Dambo type cheese (Reg-DCh) and the cheese elaboreted with the modifidied milk (Mod-DCh).

| Parameter | Reg-DCh | Mod-DCh | Change (%) | Change (g per serving) |
|---|---|---|---|---|
| Serving | 30 grams | | - | - |
| Total fat[1] | 5.973 | 5.250 | −12.1 | −0.723 |
| Total SF[1] | 3.673 | 3.052 | −16.9 | −0.621 |
| Total *Trans* fat[1] | 0.197 | 0.272 | +37.9 | +0.075 |
| Total CLA[1] | 0.040 | 0.070 | +72.7 | +0.029 |
| Total omega-3[1] | 0.017 | 0.018 | +3.2 | +0.001 |
| Total omega-6[1] | 0.138 | 0.120 | −13.2 | −0.018 |

[1]Values are expressed in grams per a cheese serving of 30 g; +, − = increase or decrease compared to the regular Dambo cheese.

Mod-DCh (Table 5). It is worth mentioning that the Argentine Alimentary Code declare that the limits for *trans* FA do not apply to ruminant fat including milk fat.

### 3.4. Nutritional Facts of the Modified Yogurt (Mod-Yog) Compared to the Regular Product (Reg-Yog)

Compared to the Reg-Yog the Mod-Yog made with the Mod-Milk showed differences in its FA composition and in paramaters of nutritional interest (Table 7).

The main differences between the Reg-Yog and the Mod-Yog (Table 7) and

Table 7. Differences in fatty acid (FA) composition between the regular yogurt (Reg-Yog) and that elaborated with the mofified milk (Mod-Yog).

| Fatty Acid ($g\ 100\ g^{-1}$ FA) | Reg-Yog | Mod-Yog | Δ%[(1)] | $P <$[(2)] |
|---|---|---|---|---|
| $C_{4:0}$ | Nd | Nd | - | - |
| $C_{6:0}$ | nd | nd | - | - |
| $C_{8:0}$ | 0.82 | 0.05 | −93.50 | 0.042 |
| $C_{10:0}$ | 2.55 | 1.70 | −33.27 | 0.225 |
| $C_{12:0}$ | 3.20 | 3.02 | −5.62 | 0.669 |
| $C_{14:0}$ | 11.28 | 10.80 | −4.24 | 0.408 |
| $C_{16:0}$ | 29.69 | 29.01 | −2.31 | 0.285 |
| $\Sigma C_{12:0\text{-}C16:0}$ | 44.17 | 42.83 | −3.04 | 0.159 |
| $C_{18:0}$ | 12.33 | 11.39 | −7.66 | 0.196 |
| $C_{18:1\ t9}$ | 0.34 | 0.52 | +53.50 | 0.142 |
| $C_{18:1\ t10}$ | 0.52 | 1.00 | +92.73 | 0.037 |
| $C_{18:1\ t11\ (vaccenic\ acid)}$ | 1.66 | 2.54 | +52.71 | 0.069 |
| $C_{18:1\ c9\ (oleic\ acid)}$ | 23.00 | 23.73 | +3.20 | 0.371 |
| $C_{18:2\ c9\ c12\ (linoleic\ acid)}$ | 2.35 | 2.54 | +7.85 | 0.013 |
| $C_{18:3\ c89\ c12\ c15\ (linolenic\ acid)}$ | 0.30 | 0.31 | +3.07 | 0.664 |
| $C_{18:2\ cis\text{-}9,\ trans\text{-}11}$, CLA | 0.72 | 1.20 | +66.14 | 0.031 |
| $C_{18:2\ cis\text{-}12,\ trans\text{-}10}$, CLA | Nd | 0.02 | | |
| Total CLA | 0.72 | 1.22 | +68.73 | 0.032 |
| SFA | 63.37 | 59.49 | −6.12 | 0.067 |
| MUFA | 30.14 | 33.03 | +9.59 | 0.082 |
| PUFA | 3.65 | 4.33 | +18.76 | 0.022 |
| Total Omega 3 (n-3) | 0.36 | 0.39 | +6.52 | 0.198 |
| Total Omega 6 (n-6) | 2.56 | 2.73 | +6.42 | 0.025 |
| Atherogenic index | 2.36 | 2.08 | −11.78 | 0.145 |
| n-6/n-3 | 7.04 | 7.03 | −0.07 | 0.98 |

[(1)]Relative FA changes (%) compared to values observed in the Reference yogurt, (−) = decrease, (+) = increase. [(2)]Student t Test *t* for paired observations.

their healthy implications are equivalent to those described for the Mod-Milk of origin (Table 4). The decrease in the total SF content ($P < 0.067$) and the tendency to a lower atherogenic index (−11.78%, $P < 0.15$) coupled with the observed increase in VA (+52.7%), total CLA (+68.7%), MUFA (+9.6%) and PUFA (+18.8%) improved the healthy value of the Mod-Yog. The nutritional information per serving of 178 grams of yogurt is presented in Table 8.

Consuming a portion of the Mod-Yog (178 grams) theoretically corresponds to a 13% increase in total fat consumption with a concomitant increase of SF intake of 6.3% compared to a serving of the Reg-Yog but total CLA intake would result enhanced in 69.4%. As observed for the modified Dambo type cheese (Table 7), the increase in the consumption of *trans* fats was at the expense of a higher concentration of healthy *trans* FA such as VA and CLA in both, mofified milk (Table 4) and Mod-Yog (Table 7). As it was already mentioned, the Argentine Alimentary Code excludes trans fats from ruminants, including milk fat.

### 3.5. Simple Economic Analysis

Fixing a value of US$0.30 per liter of milk produced for the farmer and at the average milk yield obtained of 34.69 liters $cow^{-1} \cdot day^{-1}$ (Table 3), the direct income is US$10.41 $cow^{-1} \cdot day^{-1}$ being the cost without oil of US$4.942. Under these conditions, the cost of feeding represents 47.49% of the milk produced with a margin over food expenses estimated at US$5.46 $cow^{-1} \cdot day^{-1}$. Taken into account the SO (US$0.755 $kg^{-1}$) and FO (US$4.114 $kg^{-1}$) prices, it can be estimated that the feeding-cost to produce the Mod-Milk increases up to US$6.07 $cow^{-1} \cdot day^{-1}$ (+22.8%) at the oil-dose used. Under these conditions, the cost of feeding represented 58.33% of the produced milk with a margin over food expenses estimated in US$4.34 $cow^{-1}$. The feeding costs after the inclusion of soybean and fish oils to the ration of cows at different levels of milk production is shown in Table 9.

Using inputs-prices from Table 1, it can be estimated that below a production of 20 liters $cow^{-1} \cdot day^{-1}$ the margin over food expenses to produce the Mod-Milk begins to be negative (Figure 1).

Table 8. Parameters of nutritional interest in the regular yogurt (Reg-Yog) and the yogurt elaborated with the modifidied milk (Mod-Yog).

| Parameter | Reg-Yog | Mod-Yog | Change (%) | Change (g per serving) |
|---|---|---|---|---|
| Serving | 178 grams | | - | - |
| Total fat[(1)] | 5.676 | 6.426 | +13.21 | +0.75 |
| Total SF[(1)] | 3.387 | 3.599 | +6.28 | +0.213 |
| Total *Trans* fat[(1)] | 0.207 | 0.346 | +67.32 | +1.139 |
| Total CLA[(1)] | 0.038 | 0.065 | +69.44 | +0.027 |
| Total omega-3[(1)] | 0.019 | 0.021 | +8.33 | 0.002 |
| Total omega-6[(1)] | 0.137 | 0.165 | +20.75 | 0.028 |

[(1)]Values are expressed in grams per yogurt serving; +, − = increase or decrease compared to the Reg-Yog.

**Table 9.** Economic analysis of feeding costs following the inclusion of soybean oils (0.840 kg cow$^{-1}$·day$^{-1}$) and fish oils (0.115 kg/cow/day) in the ration at different levels of milk production.

| Milk cow$^{-1}$·day$^{-1}$ | Income[(1)] (US$ cow$^{-1}$·day$^{-1}$ | Feeding cost without oils (% produced milk) | Feeding cost including oils (% produced milk) |
|---|---|---|---|
| 15 | 4.5 | 109.83 | 134.89 |
| 20 | 6 | 82.37 | 101.17 |
| 25 | 7.5 | 65.90 | 80.94 |
| 30 | 9 | 54.91 | 67.45 |
| 35 | 10.5 | 47.07 | 57.81 |

[(1)]One liter of milk = 0.30 US$.

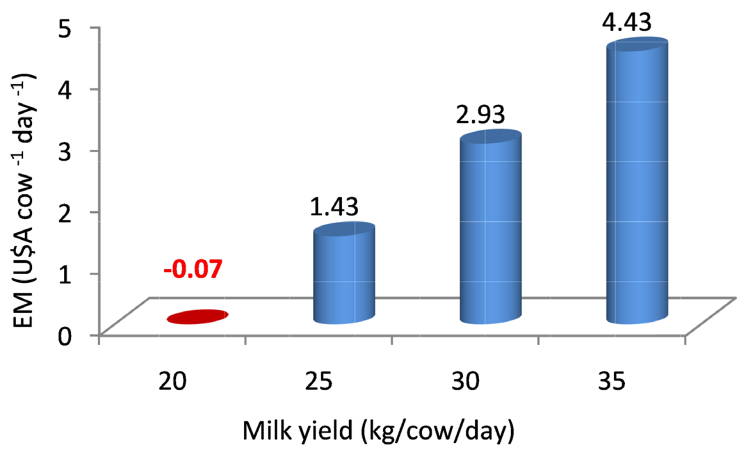


**Figure 1.** Economic margin above food costs (EM) to produce the modified milk according to milk production.

## 4. Conclusion

Supplementation with PUFA contained in SO and FO induced healthy differences in milk FA profile between the regular milk and the modified milk which improves its nutritional value and that of Dambo type cheese and yogurt made with it. This improvement involved a reduction in SF content and the increase in healthy fatty acids such as VA and natural CLA. Raw milk, Dambo type cheese and yogurt showed adequate values for the omega 6/3 ratio. The properties observed in the modified CLA milk were recovered in the cheese and yogurt made with it. The increase in total *trans* fat values was the consequence of the higher concentrations of healthy *trans* FA like VA and CLA whereas the unhealthy (*trans*-9 and *trans*-10 $C_{18:1}$) remained in values considered harmless to human health.

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## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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