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Improving the Quality of Milk Fatty Acid in Dairy Cows Supplemented with Soybean Oil and DHA-Micro Algae in a Confined Production System

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Abstract

The objective was to reduce saturated fatty acids (SFA) and increase conjugated linoleic acid (CLA, *cis*-9, *trans*-11 $C_{18:2}$), α-linolenic (*cis*-9, *cis*-12, *cis*-15 $C_{18:3}$) and docosahexaenoic (DHA, $C_{22:6}$) contents in milk from confined dairy cows in order to promote a healthier option. The work was carried out in a commercial farm (Talar) located in Laguna del Sauce, Maldonado (Uruguay). Twenty four cows were assigned to one of two treatments (12 cows per treatment) over a 6 weeks experimental period. Treatments consisted in a control total mixed ration (C-TMR) without supplementary lipids (L) or the same TMR with the addition of 0.144 kg/cow·day of algae and 0.72 kg/cow·day of soybean oil (L-TMR). Chemical composition of the TMR (44.27% DM) averaged 15.94% for crude protein (CP), 38.20% neutral detergent fiber (NDF), 20.36% acid detergent fiber (ADF), 5.56% fat, 5.30% ash and 28.6% nonstructural carbohydrate (NSCH) with 1.81 Mcal/kg of net energy for lactation (NE_L). After 39 days of feeding, individual milk samples were collected during three consecutive days. From the total milk collected, 20 ml were immediately used for chemical composition (Milko Scan) and 80 ml for analysis for milk FA profile. From week 3 onwards, milk production (kg/cow·day) resulted higher ($P < 0.001$) in L-TMR (36.9) compared to C-TMR (35.2). At week 6 of trial, the difference in milk production averaged 5.14% for L-TMR. Supplementary lipids reduced ($P < 0.002$) milk fat concentration (g/100g) from 3.36 in C-TMR to 2.40 in L-TMR without effect ($P = 0.43$) on milk protein content

(C-TMR = 3.20; L-TMR = 3.07 g/100g). Milk lactose (C-TMR = 4.86, L-TMR = 4.69 g/100g) and urea nitrogen contents (C-TMR = 21.18, L-TMR = 17.33 g/100g) tended ($P < 0.056$) to decrease in L-TMR as well as fat corrected milk output (C-TMR = 30.89, L-TMR = 29.49 kg/cow·day, $P < 0.098$). Lipid supplementation reduced (−23%) milk content of $C_{12:0}$ to $C_{16:0}$ FA averaging 45.19 in C-TMR and 34.74 g/100g in L-TMR ($P < 0.001$). The atherogenic index (AI) of milk decreased ($P < 0.001$) from 2.69 in C-TMR to 1.50 in L-TMR (−44.2%). Concentration (g/100g) of elaidic ($C_{18:1}$ *trans*-9) (0.23) and $C_{18:1}$ *trans*-10 (0.44) FA increased ($P < 0.001$) in L-TMR milk. Milk vaccenic acid (*trans*-11 $C_{18:1}$, **VA**) increased from 1.08 in C-TMR to 2.56 g/100g of FA in L-TMR ($P < 0.001$). Milk CLA content (*cis*-9, *trans*-11 $C_{18:2}$) increased (127%) from 0.62 in C-TMR to 1.41 g/100g FA in L-TMR milk. Content of *α*-linolenic acid resulted 20% higher ($P < 0.001$) in L-TMR milk (0.35 g/100g FA) compared to C-TMR (0.30 g/100g FA). Milk DHA increased from 0 in C-TMR to 0.14 g/100g FA in L-TMR. The omega-6/-3 ratio in C-TMR milk (9.61) was reduced ($P < 0.001$) to 6.78 in L-TMR milk. Milk oleic acid (*cis*-9 $C_{18:1}$) resulted higher ($P < 0.001$) in L-TMR (23.65) than in C-TMR (19.75 g/100g FA). The nutritional value of milk fat from confined cows was naturally improved by feeding polyunsaturated FA in the ration, obtaining a reduction of saturated FA and increased levels of healthy FA (CLA, DHA and *α*-linolenic).

Keywords

Dairy Cows, Milk Fatty Acids, Conjugated Linoleic Acid, Docosahexaenoic Acid

1. Introduction

Bovine milk fat represents up to 75% of total fat consumption from ruminant animals and dairy products provide about 15% - 25% of the total saturated fat (SF) in the human diet [1]. Attention on milk fat composition has been to reduce SF and increase polyunsaturated fatty acids (PUFA) to promote a healthier option. Reduction of SF has been identified as a priority since a high intake is associated with raised blood cholesterol levels and hence to an increased risk of developing heart disease a major public concern across the world [2]. Compared to milk produced from pasture-based diets, that obtained in confined feeding systems leads to higher levels of SF [3] and lower concentrations of healthier fatty acids (FA) such as conjugated linoleic acid (*cis*-9, *trans*-11 $C_{18:2}$) also called rumenic acid (RA) and FA from the omega-3 series like the *α*-linolenic (*cis*-9, *cis*-12, *cis*-15 $C_{18:3}$), eicosapentaenoic ($C_{20:5n3}$, EPA) and docosahexaenoic ($C_{22:6n3}$, DHA). There is evidence that substituting SF with PUFA reduces the risk of coronary heart disease and so, consumers are interested in dairy products with more PUFA including DHA. Dietary PUFA increases beneficial cholesterol and high density lipoprotein cholesterol while decreasing circulating triglycerides

and low density lipoprotein cholesterol. PUFA may also act as a nutritional prevention and treatment of neuro-degenerative processes [4].

Feeding PUFA rich supplements to dairy cows is an effective tool to inhibit *de novo* mammary synthesis of SF and reduce the potentially atherogenic FA of milk [1]. When consumed in excess, some milk FA such as lauric ($C_{12:0}$), myristic ($C_{14:0}$) and palmitic ($C_{16:0}$) FA are classed as potentially atherogenic [5] and associated to increased risk of heart disease [6] [7] [8]. Any attempt to reduce concentration of those FA might lead to health benefits for the consumers.

A current special interest exists on RA because it plays an important role regulating levels of plasma lipids and cardiovascular functions, reducing cancer incidence, as well as blocking tumor growth and metastasis from breasts [9]. The main precursor of RA in the cows mammary gland is vaccenic acid (*trans*-11$C_{18:1}$, VA) which showed anticarcinogenic properties itself and conversion to RA by human tissues [6] at an average rate of 20% [10]. Milk fat is considered the main natural source of VA and RA and their concentration in milk is highly dependent on diet and lipid supplementation [1]. Another strategy to enhance milk RA content is feeding long chain PUFA such as EPA and DHA which may reduce microbial activity associated with the biohydrogenation pathway of CLA precursors [1]. In addition to its intrinsic beneficial effects on human health, DHA and EPA inhibit biohydrogenation of VA in the rumen leading to a higher availability of this precursor for the synthesis of RA at the mammary level [1].

Supplementation with DHA and EPA from fish oil did not affect rumen environment nor fiber digestion [11] but may affect the palatability of the animal ration as well as the taste, smell and rancidity of the final dairy products [12]. This can be prevented by using other organisms of marine origin such as algae and plankton that are rich in EPA and DHA [13] and also heterotrophic microalgae such as *Schizochytrium limacinum* (All-G-Rich, Alltech Inc.). Its combination with soybean oil as a source of linoleic acid ($C_{18:2}$) would produce an improved milk FA profile when included in the ration of confined dairy cows an aspect that was evaluated in the present work.

2. Materials and Methods

2.1. Cows and Treatments Diets

The work was carried out at the Talar Agroindustrial Complex located in Laguna del Sauce, (Route 12 km 10, Department of Maldonado, Uruguay). Two lots of 80 multiparous Holstein cows (80 - 100 days in lactation) were used. During a pre-experimental period of 14 days all cows received the control ration (C-TMR) composed on a DM basis by ryegrass silage (18.26%), sorghum silage (23.24%), concentrate (56.43%) and cheese whey (2.07%). Concentrate included corn grain (51.95%), soybean meal (31.17%), dry distillers grains (12.99%) and a commercial premix (3.90%). The C-TMR averaged 44.27% DM with 15.94% crude protein (CP), 38.20% neutral detergent fiber (NDF), 20.36% acid detergent fiber

(ADF), 5.56% fat, 5.30% ash, 28.6% non-structural carbohydrates with an estimated net energy of lactation content of 1.81 Mcal/kg DM. TMR in take in the pre-experimental period averaged 24 kg DM/cow·day. After the pre-experimental period cows were fed treatment diets for an extra period of 6 weeks. Cows in the control group (80) continued with the C-TMR while cows in the lipid supplemented group (80) were fed the same TMR in which supplementary soybean oil (0.72 kg/cow·day) and DHA-micro algae (0.144 kg/cow·day) were added. Micro-algae (*Schizochytrium limacinum,* about 14% DHA, All-G Rich, All Tech Inc.) were grown heterotrophically in a unique process on a fresh water, low sodium media and fed at 6 g/kg DM intake as suggested in [14] and included into a mineral-vitamin premix (Nutral™) as carrier. For its part, soybean oil (53.1%) linoleic (*cis*-9 *cis*-12$C_{18:2}$, 8.50% linolenic FA) was mixed with the concentrate and fibrous components of the TRM.

2.2. Samples Collection and Analysis

Within each lot of 80 animals, two groups of 12 cows/treatment were selected for experimental measurements. Milk production was recorded individually during the whole trial and milk samples were collected from each cow during the last 3 days of the study for milk chemical and FA composition. Samples were obtained from the morning (50 ml) and the afternoon (50 ml) milkings. Of the total (100 ml) milk collected, 20 ml were immediately analyzed for fat, protein, lactose, total solids and non-fat solids by mid-infrared spectrophotometry (Milko Scan, Foss Electric, Hillerod, Denmark) and the remaining 80 ml were frozen (−20˚C) until analysis for milk FA composition. Milk fat was extracted following the method described in [15]. Fatty acid methyl esters (FAME) were prepared by base-catalysed methanolysis of the glycerides according to the ISO-IDF procedure [16]. 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 temperature was 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.

At the end of the experimental period, additional milk was obtained from the

storage tanks of control and supplemented groups to make two cheeses and analyze its FA profile.

2.3. Statistical Analysis

Milk production was analyzed using a model with repeated observations over time adjusted for covariate with cow (C = 12), treatment (T = 2), week (W = 6) and T*W interaction. The difference in the milk quality parameters and milk FA profile was analyzed using the Student t test for independent observations.

3. Results and Discussion

3.1. Milk Yield and Composition

At the start of the experiment, milk yield averaged 35.9 kg/cow·day in C-TMR and 36.4 kg/cow·day in L-TMR ($P < 0.88$). The treatment x week interaction ($P < 0.018$) showed that from week 3 onwards, cows fed the L-TMR produced more milk ($P < 0.001$) than cows fed the C-TMR (Figure 1). By the end of the experiment milk production resulted 5.14% higher in lipid supplemented cows.

Feeding soybean oil at 2.9% (±1.2) of DM intake did not affect milk production in the experiments reviewed by [17] and also when oil was fed at 3.5% to 5% of DM intake [18] [19] [20]. These results were not consistent with what was observed in the present work since the inclusion of soybean oil at about 3% of DM intake increased milk production. Feeding protected unsaturated lipids does not seem to increase milk yield in confined dairy cows [21] whereas in grazing trials the observed increase resulted higher when saturated rather than unsaturated lipids were fed [22]. Negative effects on milk production were also not observed with a high frequency of favorable effects after the inclusion of unprotected vegetable oils in the ration of confined dairy cows [23].

Before lipid supplementation, milk fat content averaged 3.71 and 3.53 g/100g in C-TMR and L-TMR treatments respectively ($P = 0.48$). Addition of soybean oil and DHA-micro algae to the TMR strongly reduced ($P < 0.002$) milk fat concentration to an average of 3.36 g/100g in C-TMR and 2.40 g/100g in L-TMR milk. Milk fat content resulted very low in L-TMR treatment if compared to the average pre-trial record of 3.53 g/100g. The inhibition of the *de novo* mammary synthesis of FA with the corresponding reduction in the total concentration of SFA in milk (Table 1) explained milk fat content reduction. In L-TMR group, it seems that the decrease in FA synthesized de novo (Table 1) was apparently not compensated by a correlative increase in the mammary uptake of supplementary preformed FA contained in soybean oil and DHA-micro algae.

Supplementing PUFA to dairy cows in pasture based diets tends to reduce milk fat content by 8% [22] a value that resulted much lower than that observed in the present trial (−28.6%). In a previous work under grazing conditions [24], supplementation with soybean oil combined or not with fish oil strongly reduced milk fat content (−19% to −27%). A milk fat content reduction may be an objective in countries with fat production quotas. In this trial, it is important to
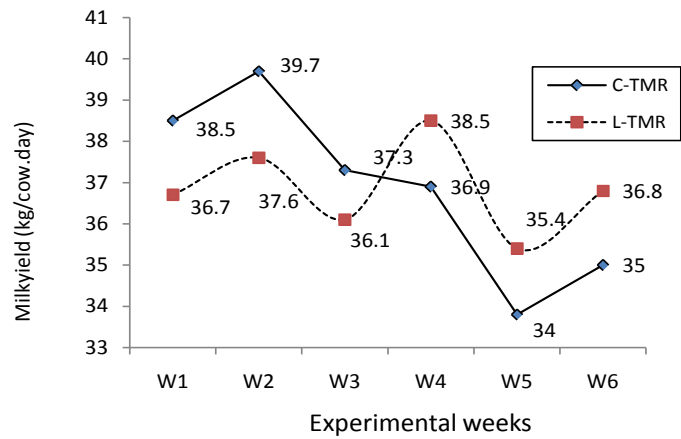
Figure 1. Milk production in cows supplemented (L-TMR) or not (C-TMR) with a combination of microalgae (0.144 kg cow·day) and soybean oil (0.72 kg/cow·day) over six experimental weeks (W).

Table 1. Milk fatty acid (FA) composition in cows fed with a control total mixed ration (C-TMR) or an L-TMR that included soybean oil (0.72 kg/cow·day) and DHA mycro-algae (0.144 kg/cow·day).

Fatty Acid g/100g FA	C-TMR	L-TMR	$P<$ [1]	$\Delta\%$ [2]
$C_{4:0}$	2.10 (±0.22)	1.86 (±0.30)	0.068	11.4
$C_{6:0}$	1.89 (±0.18)	1.32 (±0.26)	0.000	−30.2
$C_{8:0}$	1.36 (±0.12)	0.83 (±0.18)	0.000	−38.9
$C_{10:0}$	3.37 (±0.36)	1.87 (±0.40)	0.000	−44.5
$C_{12:0}$	3.91 (±0.26)	2.29 (±0.41)	0.001	−41.4
$C_{14:0}$	11.89 (±0.54)	8.61 (±1.0)	0.001	−27.6
$C_{16:0}$	29.39 (±2.60)	23.84 (±1.22)	0.001	−18.9
$\Sigma C_{12:0}$-$C_{16:0}$	45.19 (±2.69)	34.74 (±2.34)	0.001	−23.1
$C_{18:0}$	9.75 (±1.33)	11.42 (±1.89)	0.043	+17.1
$C_{18:1\ trans\text{-}9\ (elaidic\ acid)}$	0.23 (±0.02)	0.54 (±0.06)	0.001	+135
$C_{18:1\ trans\text{-}10}$	0.44 (±0.05)	3.14 (±1.86)	0.001	+614
$C_{18:1\ trans\text{-}11\ (vaccenic\ acid)}$	1.08 (±0.14)	2.56 (±0.71)	0.001	+137
$C_{18:1\ cis\text{-}9\ (oleica\ cid)}$	19.75 (±2.05)	23.65 (±1.54)	0.001	+19.7
$C_{18:2\ cis\text{-}9\ cis\text{-}12\ (linoleic\ acid)}$	3.01 (±0.34)	3.50 (±0.38)	0.01	+16.3
$C_{18:2\ cis\text{-}9.\ trans\text{-}11\ (rumenic\ acid)}$	0.62 (±0.09)	1.41 (±0.22)	0.001	+127
$C_{18:3\ cis\text{-}9\ cis\text{-}12\ cis\text{-}15\ (linolenic\ acid)}$	0.30 (±0.04)	0.36 (±0.03)	0.004	+20
$C_{20:5\ n\text{-}3\ (EPA)}$	0.017 (±0.002)	0.017 (±0.002)	0.57	−
$C_{22:6\ n\text{-}3\ (DHA)}$	---	0.14 (±0.03)	0.000	
CLA/(CLA+AV)	0.36 (±0.03)	0.36 (±0.06)	0.937	
SFA	66.76 (±2.24)	54.92 (±3.30)	0.001	−17.7
MUFA	26.60 (±2.02)	36.44 (±2.82)	0.001	+37
PUFA	4.45 (±0.48)	6.05 (±0.53)	0.001	+36
Atherogenic index	2.69 (±0.30)	1.50 (±0.20)	0.001	−44.2
n-6/n-3	9.61 (±0.50)	6.78 (±0.66)	0.001	+29.4

[1]Student t Test for independent observations. [2]Relative FA changes (%) compared to values observed in milk from C-TMR cows.

note that the reduction in milk fat occurred at the expense of the hypercholesterolemic milk fraction contributing to decrease its atherogenic index (Table 1).

A direct effect on fat synthesis in the mammary gland by supplemental PUFA or *trans*-fatty acids formed during the ruminal biohydrogenation and ulterior transfer to the udder is the more likely explanation. Indeed, uptake of some specific preformed FA like *trans*-10, *cis*-12 CLA and *trans*-8, *cis*-10 CLA reduce the activity and/or expression of genes that encode important enzymes involved in uptake, synthesis and desaturation of fatty acids in the mammary gland [25]. Negative correlations between milk fat percentage and milk fat content of *trans*-$C_{18:1}$ (−0.65), CLA (−0.63), EPA (−0.67) and DHA (−0.58) were reported by [26].

The presence of DHA (inhibitor of *de novo* mammary lipogenesis) in the micro algae plus the generation of certain FA such as *trans*-10 $C_{18:1}$ and its subsequent transfer to milk (Table 1) would explain the important decrease. The *trans*-FA's produced after PUFA supplementation are powerful inhibitors of mammary synthesis of *de novo* FA [1]. A direct relationship has been reported between increasing levels of *trans*-10 $C_{18:1}$ in milk and reduction of *de novo* mammary lipogenesis [25] which helps to explain the observed drop in fat content. A high concentration 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 the synthesis of fat, thus causing a decrease in milk fat content [27].

Milk protein content was not affected ($P = 0.43$) by supplementary PUFA averaging 3.20 g/100g in C-TMR and 3.07 g/100g in L-TMR. The absence of negative effects on milk protein concentration is an important result since this parameter not only affects the price of milk but also determines the speed and quality of coagulation in the cheese making industry. Synthesis of milk protein can be limited by energy availability and the reduced milk fat content observed could improve the energy status of the cows. In pasture based diets, lipid supplementation does not usually affect milk protein concentration [22] [28], whereas in confined feeding systems this parameter is frequently affected [21] [29]. Supplementation with non-protected lipids reduced milk protein concentration in 71% of the cases analyzed by [23] and the result was associated with a reduction in casein synthesis [30] [31]. This negative effect on milk protein content resulted more consistent when using SF (−0.18 g/100g) and calcium salts of FA (−0.12 g/100g) respect to unsaturated vegetable oils [21] [32].

Lactose (C-TMR = 4.86 and L-TMR = 4.69 g/100g) and milk urea nitrogen (C-TMR = 21.18 and L-TMR = 17.33 g/100g) tended ($P < 0.056$) to decrease in PUFA supplemented cows as well as yield of 4% fat corrected milk (C-TMR = 30.89 and L-TMR = 29.49 kg/cow·day; $P = 0.098$). Supplementation with unsaturated lipids generally has neutral effects on the production of 4% fat corrected milk both in confined [20] or in pasture based diets [22].

3.2. Milk Fatty Acid Profile

Changes in milk FA composition induced by the addition of soybean oil and micro algae to the TMR are presented in Table 1. The important changes observed may be explained by the increase in the mammary uptake of plasma triglycerides after supplementary PUFA feeding and confirms the existence of a great plasticity in milk FA composition [1] [17]. The absence of a net depressant effect (P = 0.068) of supplementary PUFA on butyric acid ($C_{4:0}$) is a result frequently reported [17]. This FA is only found in ruminant milk and has shown antineoplasic effects inhibiting the development of mammary carcinoma in rats [9] showing a potential beneficial role in human health.

Except in the case of butyric acid which is synthesized by an independent malonyl-CoA pathway, milk concentration of *de novo* FA ($C_{4:0}$-$C_{15:1}$) decreased after adding PUFA supplements to the TMR. This result may be explained at secretory cell level due to the inhibition of activity of lipogenic enzymes such as acetyl-Coa carboxylase [7] [33]. The inhibitory effect increases with the FA chain-length, the degree of unsaturation and the presence of double bonds or *trans* configuration [33].

Adding soybean oil combined with DHA micro-algae to the ration decreased total SFA from 66.76 g/100g in C-TMR to 54.92 g/100g in L-TMR cows. This SFA reduction (17.7%) was coupled to a concomitant increase (36%) in total PUFA (P < 0.001) from a basal value of 4.45 g/100g in C-TMR milk to 6.05 in L-TMR milk. It has been shown that a high intake of SFA is associated with raised blood cholesterol levels which in turn can lead to an increased risk of developing heart disease. There is evidence that substituting SFA with PUFA's reduces the risk of coronary heart disease [23]. On the other hand, the level of monounsaturated FA also increased (+37%) from a basal value of 26.60 g/100g in C-TMR milk to 36.44 g/100g in L-TMR milk (Table 1).

Compared to milk from C-TMR group, level of total atherogenic FA ($C_{12:0}$ to $C_{16:0}$) was reduced (23.1%) in L-TMR promoting a healthier milk. Concentration of myristic acid ($C_{14:0}$) in C-TMR (11.89 g/100g FA) whose atherogenic role is considered to be very potent [5] was reduced to 8.61 g/100g in L-TMR milk. Milk content of palmitic ($C_{16:0}$) FA resulted high in C-TMR (29.39 g/100g) and was reduced to 23.84 g/100g in L-TMR. Reductions in PUFA supplemented cows expressed as % relative to C-TMR milk averaged 41.4% for $C_{12:0}$, 27.6% for $C_{14:0}$ and 18.9% for $C_{16:0}$. When consumed in excess, these three saturated FA raise the total plasma cholesterol and the cholesterol associated with low density (LDL) plasma lipoproteins [34]. The reduction of these FAs after PUFA intake is a frequently reported result [20] [35] [36] explained by ruminal biohydrogenation of supplementary PUFA yielding *trans*-isomers that are inhibitors of key enzymes of mammary lipogenesis such as acetyl-CoA carboxylase [7].

Another important fact, was the reduction (−44.2%, P < 0.001) in the atherogenic index (AI) of milk from a basal value of 2.69 in C-TMR to 1.50 in the L-TMR as previously observed (1.88 to 0.80) when cows were supplemented

with sunflower and fish oils [11]. Taken together, these results help avoid an excessive consumption of unhealthy FA enhancing the health benefits of milk and dairy products elaborated with it. The observed increase in milk stearic acid ($C_{18:0}$) content in L-TMR suggests that micro algae-DHA content was not high enough to attenuate the biohydrogenation of VA to stearic acid. In C-TMR milk, levels of the unhealthy *trans*-9 and *trans*-10 $C_{18:1}$ FA were normal (Table 1) and consistent with the high milk fat content observed (3.71 g/100g). Concentrations of both *trans*-$C_{18:1}$ FA increased after soy bean oil and micro-algae supplementation reaching values of 0.54 (*trans*-9) and 3.14 g/100g for *trans*-10 $C_{18:1}$ (Table 1). At the observed concentrations, those FA would not present potential risks on the degree of ischemic heart disease to humans [37]. The DHA contained in microalgae may have contributed to maintain low levels of *trans*-10$C_{18:1}$ since the concentration of this isomer in milk tended to decrease with the increasing participation of fish oil as natural source of DHA and EPA fed mixed to sunflower oil [38].

Concentration of VA in L-TMR milk averaged 2.56 g/100g FA which represented an increase of 137% over the basal value of 1.08 g/100g observed in C-TMR milk (Table 1). Natural VA contained in dairy products can exert beneficial properties itself through a direct [39] or mediated anticarcinogenic effect by its endogenous conversion to RA in human tissues at an estimated rate of 20% [10] by the Δ9-desaturase activity [40]. The metabolism of VA to RA has been shown to be an effective way to prevent chemically induced cancer in rats [41] and increases the RA bioavailability in tissues [42]. In our trial, the increase in VA was somehow moderate and should be strengthened. In C-TMR milk, VA represented about 61.7% of the total *trans*-$C_{18:1}$, a value that resulted low considering the 80.41% proportion observed in milk from cows fed pasture based diets [43]. This proportion resulted even lower in L-TMR milk decreasing up to 41% (Table 1). When grazing dairy cows were supplemented with soybean and linseed oils contribution of VA remained high (77% to 82%) whereas that of *trans*-9 and *trans*-10 $C_{18:1}$ were only 11.5% and 28.9% respectively [43]. In the present work, the proportion of *trans*-9 was low in both treatments (13.14% to 8.65%) but that of *trans*-10$C_{18:1}$ showed a significant increase in L-TMR treatment (50.32%) compared to C-TMR (25.14%). This result may be explained by a sub-optimal ruminal biohydrogenation activity of key bacteria such as *Butibibrio fibrisolvens* induced by the ration, by some TMR components and/or unknown factors. A shift towards *trans*-9 and mainly *trans*-10 $C_{18:1}$ would partially explain this result (Table 1).

In previous work on pasture-based diets, VA represented 73.3% of the total *trans*-$C_{18:1}$ and remained constant after the supply of a soybean-fish oil based supplement representing 73.2% of total *trans*-$C_{18:1}$ in cows that received increasing amounts of the supplement [44]. In that work, supplementary oils induced an average VA increase of 146% and therefore slightly higher than the value of 137% observed in the present trial (Table 1).

Concentration of RA increased from a basal value of 0.62 g/100g FA in C-TMR to 1.41 g/100g FA in L-TMR milk (+127%). The RA levels observed in the L-TMR milk were higher than the 1.02 (±0.36) g/100g FA reported in the meta analysis of [17] for dairy cows supplemented with soybean oil alone. In the present work, the RA:VA ratio averaged 0.57 in C-TMR milk remaining unchanged (0.55) in supplemented cows (Table 1). This result suggests that mammary Δ-9 desaturase activity was adequate in cows of both treatments. This ratio resulted higher than the value of 0.33 reported for supplemented PUFA cows [27] [43] suggesting a substrate (VA) deficiency.

Considering the different sources that may influence on microbial ruminal biohydrogenation activity (intake and interactions of precursors with basal diet, forage:concentrate ratio), an average RA:VA ratio of 0.41 has been proposed as the most frequently observed [45] which represents a lower value than that observed in the C- and L-TMR treatments (Table 1). Since RA and VA were positively correlated (r = 0.85) ruminal VA synthesis should be improved.

The concentration of linoleic acid (*cis*-9 *cis*-12 $C_{18:2}$) was increased from a basal value of 3.01 in C- to 3.50 g/100g in L-TMR treatment (Table 1). These values are in the upper limit of the normal range of linoleic concentration (2% - 3%) reported by [45]. The increase of linoleic acid in L-TMR milk (16.3%) was close to the 19.69% observed in a previous study [11] and suggests that ruminal availability of this FA for biohydrogenation was attenuated. This phenomenon is not prompting to the generation of VA and hence RA and therefore maintaining a low omega-6 omega-3 ratio in milk.

In C-TMR milk, the concentration of *cis*-9, *cis*-12, *cis*-15 $C_{18:3}$ or *α*-linolenic acid (0.30 g/100g) was within the range (0.28 - 0.33 g/100g) obtained in a previous trial [46]. After lipid feeding, the concentration of this FA was increased by 17% reaching a concentration value of 0.35 g/100g in L-TMR milk (Table 1). In their meta-analysis [17] suggested absence of increase in *α*-linolenic acid even after feeding linseed oil (613 ± 299 g/d) at 3.2% of DM consumption of the cow. In our trial, the result could be explained in part by the protective action of DHA contained in microalgae on linolenic acid biohydrogenation [47]. The increase of linolenic acid is interesting for human and skin health and also as a building block for endogenous synthesis of EPA and DHA.

In humans, epidemiological and experimental studies have shown that the omega-3 FA have shown hypocholesterolemic, antithrombotic, anti-inflammatory and immune suppressive properties [48] [49] [50] [51]. In addition to the observed increase in linoleic acid, it is of great interest to highlight the increase in milk DHA content (0.14 g/100g FA) in cows fed the L-TMR diet. Current intakes of DHA (and EPA) have been identified as sub-optimal and considered a major public health concern [51]. This essential omega 3 FA improves cognitive health [52] and visual development, cardiovascular function, reduces blood pressure and triglyceride levels and improves general immunity [53] [54]. Considering these healthy properties, the need to increase DHA consumption

Table 2. Main fatty acid (FA) composition in cheese made with the milk collected from cows supplemented (L-TMR) or not (C-TMR) with a combination of microalgae and soybean oil.

Fatty acid, g/100g of total FA	C-TMR cheese	L-TMR cheese	Δ %[1]
$C_{4:0}$	2.09 (±0.09)	1.83 (±0.02)	−12.44
$C_{6:0}$	1.81 (±0.07)	1.29 (±0.01)	−28.73
$C_{8:0}$	1.25 (±0.02)	0.83 (±0.00)	−33.60
$C_{10:0}$	2.92 (±0.01)	1.85 (±0.03)	−36.64
$C_{12:0}$	3.36 (±0.04)	2.28 (±0.04)	−32.14
$C_{14:0}$	11.20 (±0.07)	8.94 (±0.03)	−20.18
$C_{16:0}$	29.33 (±0.09)	25.01 (±0.09)	−14.73
$C_{18:0}$	11.09 (±0.03)	11.74 (±0.01)	+5.86
trans-9 $C_{18:1}$	0.22 (±0.01)	0.59 (±0.01)	+168.18
trans-10 $C_{18:1}$	0.33 (±0.04)	2.55 (±0.30)	+672.73
trans-11 $C_{18:1}$	1.28 (±0.04)	2.82 (±0.16)	+120.31
cis-9 $C_{18:1}$	21.43 (±0.08)	22.92 (±0.04)	+6.95
cis-9,-12 $C_{18:2:n-6}$	2.52 (±0.03)	3.04 (±0.02)	+20.63
cis-9, *trans*-11 $C_{18:2}$ (RA)	0.69 (±0.01)	1.39 (±0.04)	+101.45
cis-9,-12,-15 $C_{18:3}$ (*α*-linolenic)	0.28 (±0.02)	0.33 (±0.04)	+17.86
$C_{22:6:n-3}$ (DHA)	0.00	0.09 (±0.00)	
SFA	66.16 (±0.14)	56.96 (±0.42)	−13.91
MUFA	28.25 (±0.42)	35.30 (±0.07)	+5.86
PUFAFA	3.93 (±0.19)	5.47 (±0.04)	+168.18
Total omega-6	2.69 (±0.33)	3.17 (±0.03)	+672.73
Total omega-3	0.33 (±0.03)	0.46 (±0.03)	+120.31
Omega 6/3 ratio	8.08 (±0.72)	6.92 (±0.45)	−14.36
Atherogenic Index	2.48 (±0.05)	1.42 (±0.01)	−42.74

[1]Relative FA changes (%) compared to values observed in milk from C-TMR cows. (−) = decrease, (+) = increase.

through alternative foods to fish like dairy products has been considered of interest in several countries (United States, Korea, Canada, Thailand, Australia, China and Singapore).

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 1-4:1 [55]. In the C-TMR milk, this ratio resulted high (9.61) and decreased ($P < 0.001$) to 6.78 in the L-TMR milk (Table 1) contributing to lowering this parameter.

Finally, it is worth nothing that the presence of oleic acid (*cis*-9 $C_{18:1}$) was higher ($P < 0.001$) in the L-TMR milk (+19.7%) than in the control milk (Table 1). This omega-9 FA is a component of the so-called "Mediterranean diet" and is fundamentally present in olive oil with beneficial effects on the blood lipid pro-

file and risk factors for cardiovascular diseases [56]. Mono unsaturated FA has been described to modulate blood pressure, improve insulin sensitivity and regulate circulating glucose levels [56]. The increase of oleic acid after supplementation with sunflower or soybean oils is a well-documented fact [17] [45] [57].

The FA composition of cheese made with the milk collected from cows fed the L-TMR diet showed differences compared to the cheese made with the C-TMR (standard) milk (**Table 2**) equivalent to those described for milk (**Table 1**).

4. Conclusion

Taken together, the results indicated a positive effect of lipid supplementation on milk production and milk healthy value in dairy cows fed in a confined feeding system. There is an opportunity to increase the nutritional value of milk in a rapid and natural way by dietary factors including polyunsaturated fatty acids in a confined total mixed ration system in the form of soybean oil and microalgae. This practice resulted in an effective tool to reduce saturated fat content and increase levels of healthy fatty acids like rumenic, docosahexaenoic and α-linolenic. Given the promising health benefits of these fatty acids and the importance of health and nutrition related to fat quality, the opportunity to provide milk and dairy products with increased levels of PUFA and DHA should be explored further as a way of prevention of the onset of many chronic diseases. The induced changes observed in milk fatty acid composition were recovered in cheese elaborated with it.

Acknowledgements

Conflicts of Interest

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

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