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Milk Fatty-Acid Profile after Feeding Increasing Doses of a Mixture of Soybean and Linseed Oils to Pasture Dairy Cows

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ABSTRACT

The goal was to determine the effect of growing intake of a mixture (75:25) of soybean (SoOi) and linseed (LiOi) oils on milk production and composition and milk fatty-acid (MF-A) profile in grazing dairy cows. Twenty-four Holstein cows were assigned to 4 treatments in a completely randomized design with three weeks of adaptation to oil doses and one week of experimental measurements. On a dry matter (DM) basis, cows were fed pasture (63%), energy concentrate (37%) and the SoOi LiOi oil mixture at zero (Tr0%), 2% (Tr2%), 4% (Tr4%) and 6% (Tr6%) of total DM intake equivalent to 0, 0.36, 0.72 and 1.08 kg/cow/day of the oil mixture. The oil mixture was manually mixed-up to the concentrate (7.04 kg DM/cow/day) and supplied by halves during each milking time without refusals. Pasture ($P = 0.49$) and total DM intakes ($P = 0.31$) were similar between treatments averaging 11.27 and 18.85 kg DM/cow/day respectively. Milk output (22.71 kg/cow/day) was not affected ($P = 0.46$). Milk fat content reduced linearly ($P < 0.05$) from 3.20 (Tr0%) to 2.67 g/100g (Tr6%) without effects ($P = 0.73$) on fat or fat corrected milk (4%FCM) yields. Milk protein concentration ($P < 0.56$) or yields ($P < 0.11$) were not affected. Lactose contents tended ($P < 0.08$) to be higher in oil supplemented cows and milk urea nitrogen was not affected ($P = 0.14$). The basal (Tr0%) concentration (g/100g MF-A) of totally hypercholesterolemic MF-A ($C_{12:0}$, $C_{14:0}$ and $C_{16:0}$) averaged 38.93 and decreased linearly ($P < 0.0001$) with oil intake to 37.81 (Tr2%), 31.59 (Tr4%) and 29.18 (Tr6%). Levels of elaidic (*trans*-9 $C_{18:1}$) and *trans*-10 $C_{18:1}$ MF-A resulted low-slung in the basal (Tr0%) milk (0.21 and 0.20 g/100g MF-A, respectively) but increased linearly ($P < 0.0001$) after oil intake reaching the maximum values at Tr6% (0.73 and 2.23 g/100g MF-A, respectively). Milk concentration (g/100g MF-A) of vaccenic acid (*trans*-11 $C_{18:1}$, VA) averaged 3.63 in Tr0% and increased linearly ($P < 0.0001$) with oil intake reaching 4.97, 7.05 and 8.38 in Tr2%, Tr4% and Tr6%, respectively. Basal concentration of rumenic acid (*cis*-9, *trans*-11 $C_{18:2}$, RA) was 2.28 g/100g MF-A and increased linearly ($P < 0.0001$) with increased oil dose resulting in maximal plateau in Tr4% (3.88) and Tr6% (3.89). The basal atherogenic index (AI) of milk was 1.87 and linearly decreased ($P < 0.01$) to 1.64 (Tr2%), 1.18 (Tr4%) and 0.95 (Tr6%) after oil intake. The basal Ω_6/Ω_3 ratio (3.57) was no different ($P > 0.05$) from Tr2% (3.37) but was upper ($P < 0.05$) in Tr4% (4.41) and Tr6% (4.63) remaining under the recommended value of 5:1. Taken together the results suggest that feeding a blend (75:25; SoOi) of SoOi an LiOi oils at 4% of total DM intake to grazing dairy cows maximize the milk RA content with a concomitant decrease in the hypercholesterolemic MF-A of milk maintaining a beneficial for health Ω_6/Ω_3 ratio with low levels of the detrimental *trans*-9 $C_{18:1}$ and *trans*-10 $C_{18:1}$.

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1. Introduction

Milk F-A composition is a determinant factor of its healthy properties due to the potential effects that certain specific MF-A have on human health. In human diets, dairy fat can account up to 75% of total consumption of fat from ruminant origin and although dairy products (which have a very low cholesterol content) provide only 15-25% of the total fat, they provide about 25 to 35% of total milk saturated fat (MSF) consumed daily^[1].

Some saturated FA present in milk just like lauric (C_{12:0}), myristic (C_{14:0}) and palmitic (C_{16:0}) are potentially atherogenic when consumed in excess^[2,3] and related with the increased risk of cardiovascular diseases^[2,4]. Feeding oils high in polyunsaturated PUFA is an effective and natural tool to inhibit *de novo* mammary synthesis of milk saturated FA (MSF-A) reducing the presence of the pro-atherogenic MF-A of milk fat^[5,6].

A special interest has been placed in RA, the *cis*-9-*trans*-11C_{18:2} isomer of conjugated linoleic acid (CLA), for its potential healthy role on the levels and composition of circulating lipids, cardiovascular health^[7,8] and the reduction in the incidence of some types of cancer^[4,9,12] and immune response^[13,14]. On the other hand, VA (*trans*-11 C_{18:1}) is the main trans MF-A being the most important precursor of RA^[1]. It showed antiproliferative properties itself or after being converted to RA in human tissues at an estimated rate of 20%^[15].

Dairy fat is the most important natural source of RA and its concentration in milk fat is highly dependent on type of diet and lipid supplementation^[1,5,6,16]. A pasture-based diet allows to obtain a milk with a high basal level of RA which can be amplified feeding vegetable oils high in PUFA^[1,5,6,17]. A mixture (75:25) of SoOi and LiOi showed to be very effective^[6] but the optimal level of oil-blend supply has not yet been well defined. This is a subject of concern taking into account oils costs, deviations towards unhealthy *trans*-MF-A (*trans*-9 and *trans*-10 C_{18:1}) synthesis owing to oil overdoses and the potential deleterious effects of free oils on ruminal function and digestion. Despite the practical importance of knowing what is the most adequate quantity of oils to be supplied, in our knowledge experimental results are still very scarce or directly non-existent. A linear effect of oil intake on milk RA was postulated reaching a plateau at an oil dose of 4% of total DM intake^[1]. The objective of the study was to define the most adequate quantity of the SoOi LiOi mixture (75:25) to be supplied to grazing dairy cows in order to obtain milk with up high level of CLA and reduced concentrations of unhealthy fatty acids at the lowest cost.

2. Materials and Methods

2.1 Treatments, Animals and Experimental Design

The experiment was carried out at the National Institute of Agricultural Technology (INTA) in Balcarce (37°45'S, 58°18'W) during September and October of 2014. The experimental period lasted 4 weeks (wk) with the first three wk as adaptation to oils intake and the fourth wk for data collection. Twenty-four multiparous Holstein cows (552 ± 50 kg, body weight, BW) in mid lactation (244 ± 69 days postpartum) and producing 20.5 ± 1.8 kg milk/day were allocated to four treatments (6 cows per treatment) in a completely randomized design. Treatments were defined by the increasing intake of a blend (w/w) of 75% (SoOi) and 25% (LiOi). The oil blend was consumed at 0% (Tr0%), 2% (Tr2%), 4% (Tr4%) and 6% (Tr6%) of total DM intake of the dairy cow (18 kg DM/cow/day) measured during the first wk prior to the start of the trial. Procedures and animal care were approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAE, INTA CERBAS).

A perennial pasture of brome grass (*Bromus unioloides*) and red clover (*Trifolium pratense*) was offered using a daily-strip grazing system. The area of each strip was regulated using a temporary electric fence to provide an herbage allowance (HA) of 27 kg DM/cow/day. The available total biomass (kg DM/ha) was estimated every week to adjust the size of the daily grazing strip by the double sampling method using the relationship between the height of the forage (x) and the available biomass (y) as described previously^[18]. The equations were adjusted for both, initial availability and for the remaining forage after grazing. The concentrate included ground corn grain (35%), malt brewery waste (10%), pelletized sunflower meal (20%), soybean grains (10%), wheatgrass (21.48%), calcium carbonate (2%), magnesium oxide (0.4%), salt (1%), rumensin (0.02%) and a vitamin-mineral mix (0.1%). It was offered at a rate of 8 Kg/cow/day in two equal feedings during each milking time (06.00 and 16.00).

According to treatments, the daily dose of the oil-blend was manually mixed to the concentrate during each milking time and thoroughly consumed by cows. The effective quantities of the oil-blend consumed (kg/cow/day) were 0.36 (Tr2%), 0.72 (Tr4%) and 1.08 kg (Tr6%). Adaptation to oil intake proceeded gradually by feeding by halves the target daily dose at each milking time starting with 0.1 (Tr2%), 0.2 (Tr4%) and 0.3 (Tr6%) Kg/cow/day during the first day, 0.2, 0.3 and 0.4 Kg/cow/day for the next 2 days, 0.2, 0.3 and 0.5 Kg/cow/day at day 4 and full dose

according to treatment from day 5 until the end of the trial.

The animals were milked twice a day at 6:00 a.m. and 4:00 p.m. and after each milking they were conducted to the pasture with fresh and clean water available *ad libitum*.

2.2 Sampling Measurements and Laboratory Procedures

Representative samples (0.5 kg) of pasture and concentrate were taken weekly. Pasture samples were collected from the grazing horizon by hand-plucking^[19]. All samples were dried at 60°C for 48 hours in an oven with forced air circulation to determine DM content and then milled in a Willey mill (1 mm mesh). They were assayed for organic matter (OM) (muffle at 550-600°C for 4 hours), crude protein (CP)^[20] with a LECO FP-528 analyzer), water soluble carbohydrates (WSC)^[21], neutral (NDF) and acid (ADF) detergent fiber (using the filter bag technique^[22] and^[23] respectively) with an autoanalyzer (ANKOM Corp. Fairport. New York. USA 1970). Ether extract (EE) was determined by the solvent extraction technique^[24] with an autoanalyzer (ANKOM Corp. Fairport. New York. USA). The *in vitro* DM digestibility (IVDMD) was estimated after 48 hours of incubation in a Daisy II ANKOM equipment. Starch content was determined as described in^[25].

Pasture DM intake was individually estimated by the difference method^[19] during 3 consecutive days in the week prior to the start of the experiment and during the last 3 days of week 4th. The average DM intake of the three consecutive days of measurements from each cow was computed for the statistical analysis.

Milk production was daily recorded over the whole experiment. Milk samples (50 ml) were collected at a.m. and p.m. milkings twice a week on non-consecutive days. The two samples were pooled according to the corresponding volume measured at each milking time and analyzed for fat, total protein, lactose, total and not-fat solids by mid-infrared spectrophotometry (Milko Scan-Minor, Foss Electric, Hillerod, Denmark). Milk urea nitrogen (MUN) was determined using a commercial enzymatic kit (Wiener Laboratories., Rosario, Argentina).

The cows were weighed on two consecutive days after the morning milking on days 6th, 7th, 27th and 28th of the trial. Body weight (BW) change was calculated as the difference between the final minus the initial BW (average of two days) divided by the number of days elapsed. During the last two weeks of the trial, blood samples were taken by jugular vein puncture after the a.m. milking. Blood was collected in tubes containing EDTA (7-8 drops/tube, 0.342 mol/l, pH 7.2, Wiener Laboratory, Rosario, Argentina)

and centrifuged (2000 × g for 15 min at 4°C). Plasma was collected and stored at -24°C until analysis for glucose, plasma urea nitrogen (PUN), triglycerides, and cholesterol using enzymatic kits (Wiener Laboratories, Rosario, Argentina). Non-esterified fatty acids (NEFA) were assayed using the enzymatic kit from Randox Laboratories Ltd (UK).

At day 21st of oil-blend supplementation and from each composite sample collected to determine the chemical composition of milk, aliquots of 50 ml were frozen (-24°C) to obtain a single pool sample per cow for the determination of MF-A composition by gas liquid chromatography (GLC) as previously described^[6]. Total milk fat (TMF) was determined gravimetrically by extraction with petroleum ether at 65-80°C^[26]. The lipids were extracted with a mixture of hexane: isopropanol (3: 2) and 6% sodium sulfate at room temperature^[27]. The lipid residue was dried at 40°C under a stream of nitrogen. For FA methyl esters (FAME) preparation, a cold method with hexane and 2N KOH in methanol was used^[28]. The FAME were quantified using a gas chromatograph (GLC-Shimadzu GC-2014. Shimadzu Corporation. Kyoto. Japan) equipped with a CP-Sil 88 capillary column (100 mx 0.25 mm id., Varian. Lake Forrest. CA. USA) and a flame ionization detector. Injector and detector temperatures were maintained at 250°C, the flow rate at 1:100 and 1 µl of standard or milk sample using an automatic sampling device at each run of the GLC^[29]. The hydrogen flow was fixed at 1 ml/min and the nitrogen flow (compression gas) at 25 ml/min. Maximum retention times and area percentages of total FA were identified by injecting known patterns. Internal standards [(Tritridecanoin [13: 0-triacylglycerol (TAG)], external reference standards GLC-463 (mixtures of 52 EMAG (purity> 99%) and *trans*-mix GLC 481 (purity> 99%) were purchased from Nu-Chek (Nu-Chek Prep. Inc., Elysian. MN. USA). Methyl esters of linoleic acid, *cis/trans* mixture (Catalog No. 47791), mixtures of unsaturated C₄-C₂₄ chain length methyl esters (Catalog N° 18919) and of the individual chain length FAMEs from C_{4:0} to C_{24:1} saturated and unsaturated were obtained from Supelco (Bellefonte. PA. USA). Mixtures of positional and geometric FA isomers were provided by the CYT-ED International Network (208RTR0343). The FAME were identified by comparing their retention times with commercial standards. The values were expressed as a percentage of the total FAME. The lower limit of quantification for the FAMEs identified varied from 0.01% to 0.03%. To convert g FAME/100g methyl esters to equivalents of triacylglycerides (TAG) (g of FA as TAG/100g of total TAG) the respective Conversion Factors tabulated in the AOCS Method Ce 1j-07^[29] were used. To estimate the

g of MF-A 100g of sample, the g of MF-A 100g of TAG were multiplied by the total fat content (%). The results are expressed in g/100g of total MF-A.

2.3 Statistical Analyses

The effect of increased levels of the oil-blend intake on milk production and composition, MF-A profile and BW changes was analyzed by orthogonal contrasts taking into account the linear, quadratic and cubic effects using the PROCEDURE MIXED^[30]. Results of DM intake and plasma metabolites were analyzed by PROCEDURE GLM^[30] using the following model:

$$Y_{ij} = \mu + T_i + E_{(ij)}$$

Where: Y_{ij} = the dependent variable, μ : overall mean, T_i = treatment effects and $E_{(ij)}$ = the residual error associated with the ij observation. The threshold of statistical significance was stated at $P < 0.05$.

3. Results and Discussion

3.1 Forage and Oil Characteristics

In the pre-grazing strips, pasture biomass averaged 2100 (± 308) kg DM/ha being above the critical value of 2000 kg DM/ha below which DM intake could be restricted^[31]. The daily-strip grazing system used allowed to maintain the target HA of 27 (± 2) kg DM/cow/day adequate to obtain *ad libitum* pasture intake^[31]. It has also been postulated that the maximum pasture DM intake would be achieved when HA ranges from 45 to 55 g DM/kg BW/day^[32]. For the average BW of cows (554 kg, Table 6), the HA range would be 25 to 30 kg DM/cow/day and therefore the 27 kg DM/cow/day obtained fell inside the proposed range. Pasture intake may also be affected if forage DM content is less than 18% following the linear relationship observed between pasture DM content and intake in the range of 13-22% DM content^[33]. In our trial, pasture DM content averaged 20.5% (Table 1) therefore exceeding the critical range reported^[33]. In the same way, pasture CP (24.1%) and NDF (36.4%) contents (Table 1) were found within the range of 15-25% (CP) and 36-54% (NDF) proposed by^[31] to obtain a high forage digestibility as observed (70.93%) *in vitro* (Table 1). The WSC and EE contents may be considered normal for good quality pastures. It can be concluded that both, the quality and the amount of the pasture offered to cows were sufficient to achieve adequate DM and energy intakes. The chemical composition of the concentrate (Table 1) was normal for a good quality energy concentrate.

Table 1. Chemical composition and *in vitro* dry matter (DM) digestibility of pasture and concentrate⁽¹⁾

Parameter	Pasture ²	Concentrate
Dry matter, %	20.50 \pm 0.70	89.6 \pm 0.65
Organic matter, % DM	91.66 \pm 0.55	92.80 \pm 0.46
Crude Protein, % DM	24.10 \pm 1.56	17.32 \pm 1.02
NDF, % DM	36.40 \pm 1.00	23.97 \pm 2.00
ADF, % DM	18.23 \pm 1.11	11.51 \pm 1.41
<i>In vitro</i> DM digestibility, %	70.93 \pm 0.40	75.14 \pm 1.88
Starch, % DM	1.57 \pm 0.40	32.59 \pm 4.05
Ether Extract, % DM	3.23 \pm 0.31	4.47 \pm 0.77
Metabolizable Energy, Mcal/kg DM	2.89 \pm 0.01	2.71 \pm 0.07
Water Soluble Carbohydrates, % DM	12.00 \pm 3.40	20.80 \pm 1.51

Note: ¹ Values expressed as the mean \pm standard deviation. Pasture and concentrate (n = 4). ² Consociated pasture containing *Bromus unioloides* and *Trifolium pratense*.

The MF-A composition of feedstuffs and oils used in the experiment is shown in Table 2.

Table 2. Fatty acids composition of feedstuffs and oils

Fatty acid	Pasture ¹	SoOi ²	LiOi ³	Concentrate
	g/100g FA			
C _{16:0}	14.08	10.67	6.71	13.91
C _{18:0}	1.31	4.31	5.38	1.92
<i>cis</i> -9 C _{18:1}	1.53	17.43	18.28	26.89
<i>cis</i> -11 C _{18:1}	0.18	2.01	3.16	4.13
<i>cis</i> -9 <i>cis</i> -12 C _{18:2}	14.58	52.87	16.35	49.75
<i>cis</i> -9 <i>cis</i> -12 <i>cis</i> -15 C _{18:3}	62.76	11.36	49.87	2.29

Note: ¹ Consociated pasture containing *Bromus unioloides* and *Trifolium pratense*. ² Soybean oil. ³ Linseed oil.

As expected, pasture and LiOi were rich in linolenic acid (*cis*-9 *cis*-12 *cis*-15 C_{18:3}). The average linoleic acid content of the pasture resulted higher than reported in other experiments^[34,35] probably due to the high quality of the pasture used in the present trial. The SoOi was characterized by its high linoleic acid (*cis*-9 *cis*-12 C_{18:2}) content (52.87%) and by a low SFA concentration. Concentrate and oils were a good source of oleic acid (*cis*-9 C_{18:1}) as reported previously^[36,39].

When a high quality pasture is included in cow's diet, rumen lipid metabolism is oriented to healthy changes in MF-A composition mainly concerning PUFA of the Ω 3 series and CLA. A significant reduction of MSF-A-content and the increase of oleic acid are also expected^[40]. Intake of high quality fresh pastures also prevents the shift and increase in concentration of unhealthy MF-A like *trans*-9 and *trans*-10 C_{18:1} isomers.

3.2 Dry Matter Intake, Milk Yield and Composition

Feeding increased doses of the oil-blend mixed to the con-

concentrate did not affect ($P > 0.05$) concentrate, pasture or total DM intake of cows (Table 3).

Table 3. Intake of pasture, concentrate and oil in dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of total DM intake

Parameter	Treatment ¹				SEM	P - value ²
	Control	Tr2%	Tr4%	Tr6%		
Intake, kg/DM/cow						
Pasture ³	12.00	10.83	10.67	11.57	0.59	0.49
Concentrate	7.04	7.04	7.04	7.04	-	-
Oil-blend	0.00	0.36	0.72	1.08	-	-
Total DM	19.04	18.23	18.43	19.69	0.68	0.31

Note: ¹ Values are expressed as LS Means and standard error of least squares means (SEM). ² Treatment effect. ³ Consociated pasture containing Bromus unioloides and Trifolium pratense.

Feeding the free oil-blend could adversely affect ruminal NDF digestion [41] and reduces DM intake [42] and milk production [43,45]. Effects of free oil feeding on ruminal digestion are variable, including negative [42], neutral [16,46,49] or even positive effects [50,51]. Inclusion of LiOi at 3.2% (± 1.7) or SoOi at 2.9% (± 1.2) of total DM did not affect DM intake [34,36,37]. The forage concentrate ratio (F:C) seems to interact with effects of free oil supplementation on ruminal digestion [52]. When LiOi was included at 3% of DM in a F:C ratio of 65:35, positive effects on NDF digestion were observed with an opposite result when the F:C ratio was 35:65 [51]. In the present trial, the F:C ratio averaged 61:39 (Table 3) and DM intake was not affected.

Estimated intake of linoleic and linolenic acids from the oil-blend was 149.7 and 71.9 g/cow/day in Tr2%, 299.4 and 143.8 g/cow/day in Tr4% and 449.1 and 215.7 g/cow/day in Tr6%, respectively. As negative effects on total DM intake were not observed (Table 3), energy intake would have been higher in oil-supplemented cows but yields of milk or 4%FCM remained unchanged (Table 4).

Table 4. Milk production and composition from grazing dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of DM intake.

Parameter	Treatment ¹				SEM	P-value		
	Control	Tr2%	Tr4%	Tr6%		Treat ²	Lin ³	Quad ³
Milk, kg/cow/day	21.96	20.58	23.99	24.30	1.90	0.46	NS	NS
4%FCM4, kg/cow/day	19.32	17.93	20.61	19.45	1.62	0.73	NS	NS
Fat, kg/cow/day	0.706	0.641	0.724	0.652	0.06	0.73	NS	NS
Fat, g/100g	3.20 ^a	3.14 ^a	3.06 ^{ab}	2.67 ^b	0.14	0.06	0.05	NS
Protein, kg/cow/day	0.831	0.774	0.919	0.963	0.06	0.11	NS	NS
Protein, g/100g	3.81	3.80	3.87	3.99	0.11	0.56	NS	NS
Lactose, g/100g	4.80	4.85	4.93	5.06	0.07	0.08	NS	NS
Total solids, g/100g	12.74	12.85	12.82	12.61	0.23	0.85	NS	NS
Urea, mg/dl	37.83	37.11	33.30	33.12	1.76	0.14	NS	NS

Note: ¹ Values expressed as least squares means and standard error of least squares means (SEM). ² Treatment effect. ³ Contrasts: linear (Lin) and quadratic (Quad). a,b Means in the same row with different super-scripts differ significantly ($P < 0.05$). NS = Not significant effect.

Supplementation at 4% of total DM intake with SoOi or LiOi alone or in combination (50-50) increased milk production (+16.7%) without differences between both oils [36]. In our previous trial, the average increase in milk production after oil feeding over unsupplemented cows was moderate (9.4%) and mainly explained by SoOi-LiOi mixtures at a ratio of 75-25 [6]. In non-grazing trials, a high frequency of favorable effects on milk production after the inclusion of unprotected vegetable oils in the diet was reported [53]. Feeding LiOi at 3 or 4% of DM increased milk yield [30] a result that was not observed in other experiments [34,54]. When SoOi was fed at 2.9 (± 1.3)% of the DM ration (0.533 \pm 0.228 kg/cow/day) milk yield was not affected in the experiments reviewed by [37] and also when SoOi was supplied at 3.5 to 5% of total DM intake [55,57]. Supplementation with SoOi (1 to 7% of total DM) did not affect milk production [34,35,37,55].

Milk fat content decreased linearly ($P < 0.05$) as intake of the oil-blend increased (Table 4) an effect mainly explained by the significant decrease (-13%) observed in Tr6%. The significant reduction in the concentration of *de novo* synthesized MF-A (-100g/kg) in Tr6% compared to Control (Table 7) was not apparently compensated by a concomitant increase in mammary uptake of the preformed MF-A (+137 g/kg) since milk fat content was lower (Table 4). The result was in turn consistent with the highest concentration of the *trans*-10 C_{18:1} isomer in milk (Table 7) since both parameters correlated negatively (Figure 1).

A direct relationship between increasing levels of *trans*-10 C_{18:1} in milk and the reduction of *de novo* MF-A mammary synthesis has been reported [58] which contributes to explain the linear drop in milk fat content (Table 4). The observed fall in milk fat content was explained in part by the lower presence of the hypercholesterolemic MF-A (Table 7), which improves the healthy value of milk.

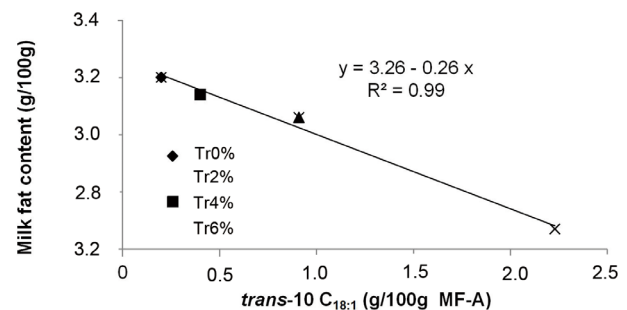


Figure 1. Relationship between milk fat content and *trans*-10 C_{18:1} in milk from cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of DM intake

The decrease in milk fat content after PUFA oil intake is a well-documented result [36,55,57,59,60]. In an extended range (0.2 to 1.0 kg/cow/day) of unsaturated lipid supplementation to grazing dairy cows, an average decrease of 8% in milk fat concentration and secretion has been reported [17]. Mammary uptake of certain preformed FA (*trans*-10 C_{18:1}, *trans*-10, *cis*-12 CLA and *trans*-8, *cis*-10 CLA) reduces the activity and/or expression of genes encoding important enzymes involved in the capture, synthesis and desaturation of MF-A [61] contributing to explain the reduction in milk fat content.

Yield of 4%FCM was not different between treatments (Table 4) suggesting that the numerical increase in milk production in Tr4% and Tr6% compensated for the reduction in milk fat content. These results were consistent with the effects of unsaturated lipid supplementation that generally shows neutral effects on 4%FCM yield in confined [62] and in pasture-based diets [17].

The lack of negative effects on milk protein concentration (Table 4) was an important result since this parameter positively affects the price of milk and determines the speed and quality of milk coagulation for cheese making. In pasture based diets, lipid supplementation does not usually affect milk protein concentration [17,63] while in confined feeding systems this parameter is systematically affected [62,64]. Inclusion of LiOi in the ration of dairy cows does not seem to affect milk protein content or yield [34,36,44,54]. In confined conditions, supplementation with unprotected lipids negatively affected milk protein content in 71% of the cases analyzed by [53] and the result was also associated with a reduction in casein synthesis [65,66]. A large number of studies demonstrated a negative effect of supplemental lipids on the protein concentration of bovine milk [56,60,62,67]. The effect seems more consistent with the use of saturated fats (-0.18 g of protein/100g of milk) and calcium salts of FA (-0.12 g of protein/100g of milk) with respect to unsaturated vegetable oils [62]. From the analysis of 8 supplementation trials feeding unsaturated lipids to grazing dairy cows, a decrease (-3.2%) in milk protein concentration (-0.11 g/100g) was reported [17]. The physiological mechanisms that explain this reduction are not fully elucidated. Some alteration in ruminal fermentation that reduces microbial protein synthesis and therefore amino-acid availability for the mammary gland for milk protein synthesis has been proposed [70]. A dilution effect after milk production increase has also been suggested [17,62,64,70]. In the present work, the increased levels of the oil-blend intake did not affect milk production, milk protein content or yield (Table 4).

Lactose content was also not affected ($P > 0.05$) after intake of the oil-mixture (Table 4). The apparent decrease

in *de novo* mammary lipogenesis (Table 7) implies some reduction in glucose oxidation for NADPH synthesis which could have been spared glucose and increased its bioavailability for lactose synthesis an effect that does not appear to have occurred in the present work. Indeed, plasma circulating glucose levels did not change after oil feeding (Table 5). The lack of changes in milk lactose content would be explained by its osmoregulatory capacity. Some authors suggest that changes in lactose content would only occur in very extreme and infrequent feeding situations [71]. Milk urea concentration was not affected by increasing oil intake, a result consistent with the absence of a depressing effect of supplemental lipids on pasture intake (Table 3).

3.3 Concentration of Plasma Metabolites and Changes in Body Weight

Plasma circulating levels of urea, triglycerides, cholesterol, glucose and NEFA were not affected (Table 5).

Table 5. Plasma metabolite concentration in grazing dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of DM intake.

Parameter	Treatment ¹				SEM	P-value ²
	Control	Tr2%	Tr4%	Tr6%		
Urea, mg/dl	45.87	39.50	44.24	48.05	3.88	0.45
Triacylglycerides, Mmol/L	0.25	0.30	0.30	0.31	0.02	0.41
Cholesterol, mg/dl	220.97	235.44	257.1	252.31	14.54	0.29
Glucose, mg/dl	71.92	70.60	69.13	69.38	3.12	0.91
NEFA, µeq/L	276.71	274.93	280.89	335.38	30.94	0.48

Note: ¹Values expressed as least squares means and standard error of least squares means (SEM). ²Treatment effect. NEFA= non-esterified fatty acids.

The absence of negative effects on glycemia suggests that the availability of gluconeogenic precursors was not affected by lipid intake which is compatible with the absence of an isoenergetic replacement of carbohydrates by oil in the concentrate and with the lack of negative effects of the oil-blend on concentrate or total DM intakes (Table 3). When total intake was not affected plasma glucose levels remained constant after protected lipid intake, [72]. Even when DM intake was decreased by duodenal infusion of rapeseed oil, glycemia remained unchanged in early or mid-lactation dairy cows [73]. The linear reduction observed in concentration of *de novo* synthesized FA (Table 7) suggests a lower mammary lipogenesis which may have contributed to maintain plasma glucose levels due to a lower oxidation of glucose for NADPH production at the mammary level [73].

Increases in the circulating levels of all plasma lipids after lipid supplementation is a well-documented result

^[74,75] explained by the increase in all fractions of plasma lipoproteins ^[65,74]. The only exception are triacylglycerides due to their high turnover rate ^[74] which could explain the lack of effect of the increasing supply of the oil mixture on triglyceridemia (Table 5).

Plasma NEFA was not affected by supplemental lipids a result that was consistent with the positive LW changes observed in all treatments (Table 6) and also with other experiments ^[77,80].

Table 6. Bodyweight (BW) changes in grazing dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of DM intake.

Parameter	Treatments ¹				SEM	P-value			
	Control	Tr2%	Tr4%	Tr6%		Trat ²	Lin ³	Quad ³	Cub ³
Initial BW, kg	599.00a	521.50b	556.40ab	538.71b	18.29	0.04	0.50	0.12	0.07
Final BW, kg	647.83a	554.83b	598.80ab	584.00bc	18.78	0.02	0.60	0.05	0.04
Daily BW gain, kg	1.62a	1.12b	1.42b	1.51ab	0.11	0.02	0.40	0.03	0.07
ΔBW, kg	48.83a	33.33b	42.30ab	45.29a	3.41	0.03	0.43	0.01	0.07

Note: ¹Values expressed as least squares means and standard error of least squares means (SEM). ²Treatment effect. ³Contrasts: linear, quadratic and cubic. ^{ab} Means in the same row with different superscripts differ significantly for treatments effect with P-value as mentioned in column for significance at $p < 0.05$ (Test Tukey-Kramer). ΔBW=final BW – initial BW.

3.4 Milk Fatty Acid Profile

Compared to Control, milk concentration of butyric acid ($C_{4:0}$) resulted lower ($P < 0.05$) only after the maximum oil-blend dose at Tr6% (Table 7). Concentration of $C_{4:0}$ is generally not affected by lipid intake since it is synthesized by an independent malonyl-CoA pathway and therefore not associated with the acetyl-CoA carboxylase activity that is inhibited by the uptake of exogenous FA from oils ^[1,81]. Any reduction in the levels of $C_{4:0}$ in milk is undesirable for its beneficial effects on human health ^[3]. In this context, intake of the oil-blend at 4% of the total DM would be the maximum recommended dose.

With respect to Control, the decrease in the concentration of *de novo* synthesized MF-A ($C_{4:0}$ to $C_{15:1}$) was significant only from the Tr4% dose (Table 7). The results reported in ^[6] also showed a reduction (-22.4%) in the total *de novo* synthesized MF-A from 21.07 to 16.35 g/100g when the same oil mixture was fed at 0.8 Kg/cow/day to grazing dairy cows. A reduction in total concentration of *de novo* synthesized FA from 22.49 to 18.48 g/100g of total MF-A (-18.8%) was also reported in grazing dairy cows that consumed 0.7 kg/cow/day of a 70:30 blend of

SoOi and LiOi ^[80]. These effects are explained by the inhibition of the activity of the mammary lipogenic enzymes such as acetyl-CoA carboxylase ^[65,76] and are normally reported when dairy cows are supplemented with sources of PUFA ^[82,83]. The inhibitory effect becomes more potent as the length of the PUFA chain and the degree of unsaturation increases and with the presence of double bonds of *trans* configuration ^[43].

Since at the higher dose (Tr6%) of the oil-blend intake milk fat content was affected (Table 4), the inclusion of the Tr4% dose would be suitable in order to maintain the commercial milk value in a context of payment by quantity of useful (fat-protein) milk solids. At higher oil doses, the decrease in mammary *de novo* MF-A synthesis did not appear to be compensated by a proportional increase in preformed exogenous FA uptake and milk fat content decreased (Table 4). Milk fat depression was maximal in Tr6% with the highest *trans*-10 $C_{18:1}$ concentration in milk fat (Table 7). This *trans*-10 isomer has showed deleterious effects on human health ^[84] and is negatively correlated with milk fat concentration (Figure 1). A high *trans*-10 $C_{18:1}$ content or the isomer *trans*-10, *cis*-12 $C_{18:2}$ CLA in milk has been related to dysfunctions in the activity of the lipoprotein lipase (LPL) and stearyl CoA desaturase (SCD) enzymes involved in fat synthesis thus causing a decrease in milk fat content ^[82].

Concentration of total hypercholesterolemic MF-A ($C_{12:0}$ to $C_{16:0}$) decreased significantly with the Tr4% oil mixture without an additional reduction at the higher Tr6% dose (Table 7). At the same time, the atherogenicity index (AI) of milk decreased at the Tr4% dose without additional detriments ($P > 0.05$) between the Tr4% (1.18) and the Tr6% (0.95) doses. Feeding 0.8 Kg/cow/day of the SoOi75:LiOi25 mix showed a 41% decrease in the AI compared to the basal value of 1.93 recorded in the Control treatment in ^[6]. In the present work, the basal AI was 1.87 (Table 7) being thus comparable to that observed in ^[6].

Feeding 0.8 Kg/cow/day of the 75% (SoOi)-25% (LiOi) mix induced significant reductions in the $C_{12:0}$ (-30.6%), $C_{14:0}$ (-28.8%) and $C_{16:0}$ (-21.9%) in the experiment by ^[6]. In the present trial, concentration of $C_{12:0}$ and $C_{16:0}$ showed the same response pattern (Table 7). Compared to Control, concentration of myristic ($C_{14:0}$) acid showed a 21.6% reduction at the T4% dose (Table 7) a result of concern due to the putative atherogenic role the $C_{14:0}$ MF-A ^[2] when consumed in excess. The observed reductions registered in Tr4% dose for milk content of $C_{12:0}$ (35.9%), $C_{14:0}$ (21.7%) and $C_{16:0}$ (15.5%) were slightly lower than the range estimated in the meta-analysis by ^[37] when supplementing with SoOi and LiOi. They reported values of 42-37% for $C_{12:0}$, 23-24% for $C_{14:0}$ and 30-17% for $C_{16:0}$.

Table 7. Milk fatty acid (MF-A) composition from grazing dairy cows supplemented or not (Control) with a blend of soybean (75%) and linseed (25%) oils at 2%, 4% and 6% of Total DM.

MF-A g/100g MF-A	Treatment ¹				SEM	P-value			
	Control	Tr2%	Tr4%	Tr6%		Treat ²	Lin ³	Quad ³	Cub ³
C _{4:0}	2.81 ^a	2.36 ^{ab}	2.33 ^{ab}	1.84 ^b	0.21	0.02	0.02	0.92	0.35
C _{6:0}	1.73 ^a	1.40 ^a	1.21 ^{ab}	0.91 ^b	0.13	0.001	0.001	0.91	0.65
C _{8:0}	1.03 ^a	0.85 ^a	0.64 ^b	0.45 ^b	0.08	<.0001	<.0001	0.96	0.85
C _{10:0}	2.49 ^a	2.12 ^a	1.46 ^b	1.08 ^b	0.18	<.0001	<.0001	0.97	0.51
C _{12:0}	3.04 ^a	2.77 ^a	1.95 ^b	1.58 ^b	0.19	<.0001	<.0001	0.81	0.28
C _{14:0}	10.90 ^a	10.50 ^a	8.54 ^b	7.09 ^c	0.40	<.0001	<.0001	0.20	0.26
C _{14:1 cis-9}	1.21 ^a	1.35 ^a	0.92 ^{ab}	0.95 ^{ab}	0.12	0.03	0.01	0.96	0.05
C _{16:0}	24.99 ^a	24.55 ^a	21.11 ^b	20.51 ^b	0.72	0.0002	<.0001	0.91	0.08
C _{16:1}	0.89	1.23	0.95	1.17	0.13	0.22	0.97	0.67	0.07
C _{17:0}	0.45 ^a	0.59 ^a	0.53 ^{ab}	0.29 ^{bc}	0.06	0.01	0.09	0.005	0.94
C _{18:0}	11.50	9.67	11.58	11.06	0.69	0.20	0.29	0.34	0.06
C _{18:1} Isomers									
trans-9	0.21 ^a	0.40 ^b	0.64 ^c	0.73 ^d	0.05	<.0001	<.0001	0.32	0.43
trans-10	0.20 ^d	0.40 ^b	0.91 ^c	2.23 ^d	0.17	<.0001	<.0001	0.003	0.52
trans-11 (VA)	3.63 ^a	4.97 ^b	7.05 ^c	8.38 ^d	0.24	<.0001	<.0001	0.98	0.18
Total trans	4.04 ^d	5.77 ^b	8.60 ^c	11.35 ^d	0.34	<.0001	<.0001	0.15	0.45
cis-9 C _{18:1}	25.74 ^a	26.04 ^a	27.90 ^a	30.06 ^b	0.20	0.005	0.002	0.28	0.74
cis-11 C _{18:1}	1.57 ^a	1.36 ^a	1.94 ^{ab}	1.91 ^{ab}	0.170	0.06	0.01	0.60	0.07
C _{18:2 (n-6)}	1.98 ^a	2.60 ^b	2.81 ^b	2.78 ^b	0.20	0.03	0.02	0.12	0.88
C _{18:3 (n-3)}	0.58	0.78	0.66	0.64	0.06	0.40	0.73	0.18	0.28
cis-9, trans-11 C _{18:2} CLA (RA)	2.28 ^a	3.16 ^b	3.88 ^c	3.89 ^c	0.22	<.0001	<.0001	0.05	0.57
Short chain MF-A ⁴	8.06 ^a	6.72 ^a	5.63 ^{ab}	4.27 ^{bc}	0.55	0.0005	0.0002	0.98	0.83
Medium chain MF-A ⁵	43.33 ^a	42.67 ^a	34.99 ^b	32.48 ^b	1.09	<.0001	<.0001	0.40	0.02
Long chain MF-A ⁶	48.14 ^a	50.03 ^a	58.40 ^b	62.33 ^c	1.32	<.0001	<.0001	0.44	0.08
Saturated MF-A (SMF-A)	60.69 ^a	52.38 ^b	47.59 ^c	43.38 ^d	1.15	<.0001	<.0001	0.08	0.57
Unsaturated MF-A (UMF-A)	38.52 ^a	42.93 ^b	48.69 ^c	53.39 ^d	1.04	<.0001	<.0001	0.90	0.64
SMF-A/UMF-A	1.59 ^a	1.23 ^b	0.98 ^c	0.82 ^{cd}	0.06	<.0001	<.0001	0.12	0.96
AI ⁷	1.87 ^a	1.64 ^a	1.18 ^b	0.95 ^{bc}	0.09	<.0001	<.0001	0.99	0.26
Δ9D products	34.96 ^a	38.22 ^b	44.30 ^c	49.02 ^d	1.06	<.0001	<.0001	0.49	0.40
Substrates ⁸	55.65 ^a	54.41 ^a	52.51 ^b	52.29 ^{bc}	0.65	0.003	0.0005	0.44	0.43
Index ⁹	0.38 ^a	0.41 ^b	0.46 ^c	0.48 ^d	0.009	<.0001	<.0001	0.95	0.40
De novo MF-A (C _{4:0} to C _{15:1})	24.76 ^a	22.82 ^a	17.90 ^b	14.70 ^c	1.01	<.0001	<.0001	0.53	0.31
Preformed MF-A (>C _{17:0})	48.89 ^a	50.61 ^a	58.93 ^b	62.62 ^c	1.33	<.0001	<.0001	0.46	0.07
Ω6/Ω3	3.57 ^a	3.37 ^a	4.41 ^b	4.63 ^c	0.32	0.02	0.003	0.52	0.16
RA/VA	0.56 ^a	0.59 ^a	0.49 ^b	0.37 ^c	0.03	<.0001	<.0001	0.01	0.32
Σ(C _{12:0} to C _{16:0})	38.93 ^a	37.81 ^a	31.59 ^b	29.18 ^b	1.15	<.0001	<.0001	0.57	0.10

Notes: ¹ Values expressed as least squares means and standard error of least squares means (SEM). ²Treatment effect. ³Contrasts: linear y quadratic and cubic. ^{ab} Means in the same row with different superscripts differ significantly for treatments effect with P-value as mentioned in column for significance at P<0.05 (Test Tukey-Kramer). ⁴Short chain MF-A (C_{6:0} to C_{10:0}). ⁵Medium chain MF-A (C_{12:0} to C_{17:1}). ⁶Long Chain MF-A (C_{18:0} to C_{22:6}). ⁷Atherogenicity index (C_{12:0} + 4*C_{14:0} + C_{16:0})/(ΣUMF-A). UMF-A= cis-9 C_{14:1}; C_{16:1}; cis-9 C_{18:1}; cis-11 C_{18:1}; trans-11 C_{18:1}; C_{18:3}; C_{18:2}; cis-9, trans-11 C_{18:2} (CLA). The detrimental MF-A trans-6, 8, 9, 10 C_{18:1} was excluded. ⁸Substrates: C_{14:0} + C_{15:0} + C_{16:0} + C_{17:0} + C_{18:0} + trans-11 C_{18:1}. ⁹Index: ((Σ Δ9D products)/ [Σ Δ9D products + Substrates]).

The level of stearic acid (C_{18:0}) did not differ ($P > 0.05$) from Control in any of the oil-blend doses used with a significant and linear ($P < 0.01$) increase of linoleic acid (C_{18:2}). These results could be explained by a possible inhibition in the biohydrogenation from C_{18:2} to C_{18:0} when high levels of linoleic acid are present in rumen [86,88]. The absence of increases in milk content of C_{18:0} after supplementation with oils rich in C_{18:2} or C_{18:3} can be considered a positive result due to its potential thrombogenic role [2]

and was consistent with other experiments [69,80].

The level of oleic acid (cis-9 C_{18:1}) increased ($P < 0.05$) over Control only in Tr6% at maximum dose of the oil mixture (Table 7). Using the same SoOi-LiOi mixture at a dose of 0.8 kg/cow/day, the oleic acid content (g/100g MF-A) in Control milk (26.14) did not differ from that observed in supplemented cows (27.50) [6]. This result was consistent with that obtained in the Tr4% treatment (Table 7). Differences in oleic acid levels were also not detected

after feeding 0.7 Kg/cow/day of a 70% (SoOi)-30% (LiOi) mixture to grazing dairy cows^[80]. However, the increase in milk oleic content after supplementation with sunflower or SoOi oils is a frequently reported result^[37,44,89] even when LiOi is fed^[35-37,90].

The linear ($P < 0.0001$) increase in the desaturation index used to estimate mammary desaturation activity (Table 7) was compatible with the increase in oleic acid, contributing in part to maintaining similar levels of $C_{18:0}$ in milk (Table 7). A higher desaturase index was reported in the milk of animals supplemented with LiOi despite the fact that PUFA feeding inhibits the activity of the $\Delta 9$ -D enzyme complex^[45]. Several authors did not observe differences in this index when comparing a control ration with those that included SoOi and LiOi or their mixtures at 50-50^[36].

Milk fat content of linoleic acid increased ($P < 0.02$) linearly from 1.98 (Control) to 2.78 g/100g MF-A in Tr6% (Table 7) thus remaining within the normal range (2-3 g/100g MF-A) reported by^[1] but lower than that observed in our previous experiment (3.25 to 3.92 g/100g MF-A) after supplementation with 0.7 Kg/cow/day of a mixture of 70% (SoOi)-30% (LiOi) in grazing cows^[80]. Likewise, an increase in milk $C_{18:2}$ content from 1.96 (Control) to 3.50 g/100g MF-A was reported after intake of the same SoOi LiOi mixture at 0.8 Kg/cow/day^[6].

On the other hand, the levels of linolenic acid ($C_{18:3}$) in milk did not differ ($P > 0.05$) between treatments (Table 7), a result consistent with that observed in^[80]. Feeding LiOi at 4% of DM intake in pure form increased (170%) the levels of $C_{18:3}$ in milk a result not observed when cows were supplemented with a 50% mixture with SoOi^[36].

The milk $\Omega 6/\Omega 3$ ratio from Control cows was low (3.57) and increased ($P < 0.02$) with the increasing supply of the oil mixture but always remaining below the recommended value of 5. When the same oil mixture was fed at 4% of total DM intake to grazing dairy cows, the $\Omega 6/\Omega 3$ ratio remained between 3.18 in control milk and 3.87 in supplemented cows^[80]. In order to achieve a low $\Omega 6/\Omega 3$ ratio, the supply of LiOi alone at 4% of total DM intake was very effective averaging 2.13 in supplemented vs. 4.25 in control cows. The effect was attenuated when using mixtures with SoOi^[6]. In that work^[6], the milk $\Omega 6/\Omega 3$ ratio after intake of 0.8 Kg/cow/day of the 75% (SoOi)-25% (LiOi) blend was greater (5.66) than observed in the present experiment.

Feeding non-protected PUFA oils increase synthesis of different isomers of $trans$ - $C_{18:1}$ MF-A in the rumen which are transferred to milk. Some of them, like the $trans$ -9 (elaidic) and $trans$ -10 $C_{18:1}$, are classed as deleterious or unhealthy^[85,91,92] and hence any excessive increase in

concentration in milk should be avoided. Basal concentration (g/100g MF-A) of $trans$ -9 (0.21) and $trans$ -10 $C_{18:1}$ (0.20) was linearly ($P < 0.01$) increased after oil-blend intake (Table 7) reaching maximum values of 0.73 for $trans$ -9 and 2.23 for $trans$ -10 $C_{18:1}$ in Tr6% that can be considered low or harmless. Indeed, a concentration of 2.28 g/100g MF-A of $trans$ -10 $C_{18:1}$ in a butter supplied at 12% of the diet of experimental rabbits subjected to a cholesterol challenge did not show deleterious effects on the plasma lipid profile or the metabolism of lipoproteins when the level of $trans$ -10 $C_{18:1}$ was accompanied by at least 7 g/100g MF-A of VA and 3 of RA^[85]. In Tr4%, the $trans$ -9 (0.64 g/100g of total MF-A) and $trans$ -10 $C_{18:1}$ (0.91 g/100g MF-A) concentrations in milk fat were lower ($P < 0.05$) than in Tr6% showing some advantage. Since in Tr4%, concentrations of $trans$ -10 $C_{18:1}$ were only 0.91 g/100g MF-A of the total MF-A with levels of VA and RA in milk of 7.05 and 3.88 g respectively (Table 7) it can be expected an athero-protective role of Tr4% milk similar (or even higher) to that obtained in^[85]. Intake of 0.7 kg/cow/day of a 70% (SoOi)-30% (LiOi) blend also induced low values of $trans$ -9 (0.58 g/100g of total MF-A) and $trans$ -10 $C_{18:1}$ (0.99 g/100g of total MF-A) in milk from grazing dairy cows^[80]. In Control milk, concentration of VA accounted for 90% of the total $trans$ - $C_{18:1}$ MF-A a value that remained high (74 to 86%) after intake of the oil-blend doses (Table 7). In Control milk, $trans$ -9 and $trans$ -10 $C_{18:1}$ represented 5.19 and 4.95% of total $trans$ - $C_{18:1}$ but the relative contribution of the $trans$ -10 $C_{18:1}$ increased after feeding the oil-blend. The relative increase expressed as % of the total $trans$ - $C_{18:1}$, resulted greater in treatments Tr4% (10.5%) and Tr6% (19.7%). Although the concentrations of these two $trans$ FA were moderate, it is convenient to avoid deviations towards its formation due to its potential atherogenic effect^[84,85]. It seems therefore advisable not to use doses greater than 4% of supplementary lipids in order to avoid non-undesirable deviations towards non-healthy trans isomers appearance.

The concentration of VA in milk showed a linear increase ($P < 0.01$) after increasing the oil mixture intake but its apparent conversion into RA estimated by the RA/VA ratio showed an opposite ratio reaching a minimum value of 0.37 in Tr6% (Table 7). The result suggests that the increase in precursor availability (VA) for RA synthesis did not induce proportional increases in the activity Δ -9D desaturase which was also consistent with the lack ($P > 0.05$) of increase in milk RA content between treatments Tr4% (3.88 g/100g MF-A) and Tr6% (3.89 g/100g MF-A).

An average RA/VA ratio of 0.41 was proposed by^[93] a value that resulted close to the 0.49 observed in Tr4%

(Table 7). VA present in milk and dairy products can exert beneficial properties in itself through a direct anticarcinogenic effect [94] or mediated after its endogenous conversion to RA at an estimated rate of 20% in human tissues [95] via $\Delta 9$ -desaturase activity [93].

Since only 20% of the VA would be converted into RA in human tissues and until more experimental evidence of the healthy effects of VA is available, it seems advisable to avoid excess milk VA concentration and intake. In this context, our results suggest that the oil-blend fed at 4% of total DM intake (Tr4%) would be the most advisable dose since marginal increases in RA were not detected when VA increased with higher oil-blend intake up to Tr6% (Table 7 and Figure 2).

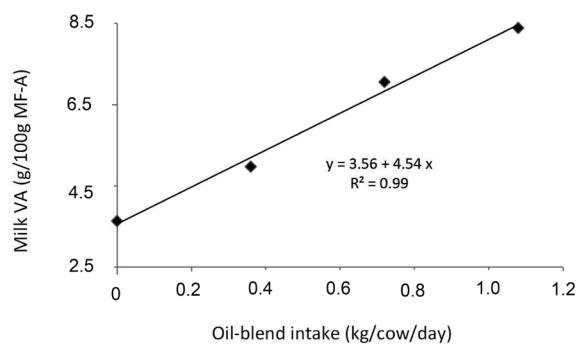


Figure 2. Concentration of vaccenic acid (*trans*-11 C_{18:1}) in milk from grazing dairy cows supplemented with increased levels of a soybean-linseed oil blend (75:25)

As expected, milk RA concentration correlated positively ($R^2 = 0.89$, $P < 0.01$) with VA (Figure 3), a frequently reported result [1,36]. The average conversion rate of VA into RA appeared to be 34.2% (Figure 3) that resulted very close to the 35-39% values estimated at [80] after supplying 700 g/cow/day of a mixture of 70% (SoOi)-30% (LiOi) blend to grazing dairy cows. Taking the RA/VA ratio as an estimator, the average conversion rate resulted somehow higher (50.25%, Table 7).

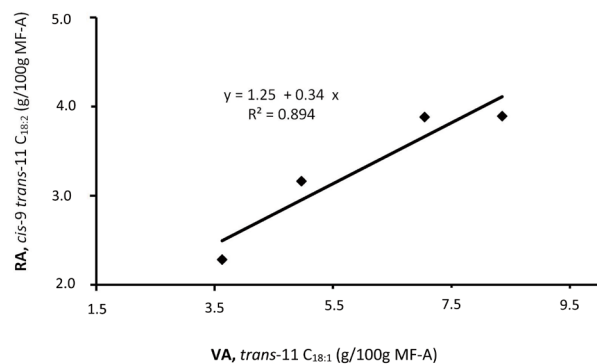


Figure 3. Concentration of vaccenic acid (*trans*-11 C_{18:1}) in milk from grazing dairy cows supplemented with increased levels of a soybean-linseed oil blend (75:25)

In the present work, the baseline (Control) concentration of RA resulted very high (2.28 g/100g MF-A) and was increased ($P < 0.05$) after oil-blend intake without differences ($P > 0.05$) in RA concentration between Tr4% and Tr6% (Table 7). This result suggests that the response in milk RA content would be linear up to a maximum of 4% of oil-blend consumption (Figure 4) and confirms what was previously suggested by [1].

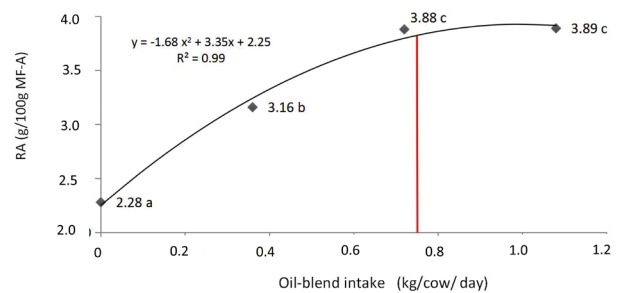


Figure 4. Concentration of rumenic acid (RA, *cis*-9 *trans*-11 C_{18:2}) in milk from grazing dairy cows supplemented with increased levels of a blend of soybean and linseed oil (75:25)

At a similar oil-dose (4% DM intake), milk concentration of RA observed in Tr4% (3.88 g/100g MF-A) resulted higher than those reported in [36] (1.60-2.39 g/100g MF-A) in rations with a high forage content (59%) and also than those obtained in [6] using the 75% (SoOi)-25% (LiOi) oil blend (3.21 g/100g MF-A). When grazing dairy cows were fed 0.7 Kg/cow/day of a mixture of 70% (SoOi)-30% (LiOi), milk concentration of RA averaged 3.13 g/100g MF-A [80].

Milk content of RA in Tr4% (Table 7) was also higher than values reported in the meta analysis by [37] after feeding SoOi (1.02 (± 0.36) g/100g MF-A) or LiOi (1.75 (± 0.84) g/100g MF-A) and that those reported in [44] when the cows were supplemented with 0.5 kg/day of sunflower or soybean oil (2.02 g/100g MF-A) or after feeding 0.9 kg/cow/day of unsaturated FA calcium salts [98]. The high baseline values of RA observed in Control milk (2.28 g/100g MF-A, Table 7 and Figure 3) could partly explain the differences between experiments.

The presence of unsaturated MF-A increased linearly ($P < 0.0001$) with oil intake reaching a maximum at Tr6% with a 38.6% increase over Control (Table 7). The SMF-A/UMF-A ratio decreased ($P < 0.0001$) with oil intake without differences between Tr4% and Tr6% (Table 7). The results obtained confirmed the existence of great response plasticity in the composition of milk fat in terms of its constitutive MF-A [1,37] which can be modulated by oil supplementation to increase the healthy value of dairy products.

4. Conclusions

Supplementation of grazing dairy cows with increasing doses of a mixture of soy and linseed oils linearly increased the rumenic and vaccenic acid content of milk without significant deviations towards unhealthy fatty acids like *trans*-9 or *trans*-10 C_{18:1} and without affecting either milk production or protein content. This nutritional strategy was also an effective tool to reduce milk content of saturated milk fatty acids and the hypercholesterolemic fraction of milk fat which improves its healthy value. Overall results shows that the optimum level of inclusion of the soybean-linseed oil mixture was around 3.91% of total DM intake of cows without additional advantages by increasing the dose of oils in the total ration.

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