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Effects of Feeding Combinations of Soybean and Linseed Oils on Productive Performance and Milk Fatty Acid Profile in Grazing Dairy Cows

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Abstract

Thirty-six grazing dairy cows were used to determine the effect of combinations of soybean (SO), and linseed (LO) oils on milk production, composition and milk fatty acid (FA) profile. Treatments were a basal control diet (56% pasture, 44% concentrate) or the control diet supplemented with oils at 4% of estimated total dry matter (DM) intake. Oils were manually mixed to the concentrate in pure forms (SO100 or LO100) or in blends (%w/w) at SO75 - LO25, SO50 - LO50 and SO25 - LO75. Concentrate and oils were thoroughly consumed. Pasture intake (kg DM/cow·day) was 9.27 in control and decreased ($p < 0.05$) in SO25 - LO75 (8.09) and LO100 (8.98). Total DM intake (kg/cow·day) in control (16.47) increased ($p < 0.05$) to 17.04 in SO100 and 17.20 in SO75. Yield of fat corrected milk (4% FCM) averaged 20.73 kg in control resulting higher in SO75 (23.73 kg). Milk fat content (g/100g) in control averaged 3.40 and decreased to 2.79 in SO50-LO50 and to 3.06 in SO25 - LO75 treatments. Milk protein content was not affected and milk protein yield increased in SO100 (11%) and SO75 - LO25 (21%) over Control (0.729 kg/cow·day). Milk basal (Control) content (g/100g FA) of C12:0 (2.58), C14:0 (10.21) and C16:0 (25.69) was reduced ($p < 0.05$) to 1.64, 6.82 and 19.70 respectively in oil supplemented cows. Basal content of C12:0 to C16:0 averaged 38.48 g/100g FA and decreased (27.4%) after oil intake. Basal *trans*-10 C18:1 (0.46 g/100g FA) increased ($p < 0.01$) in SO100 (1.48) and SO50-LO50 (1.80). Basal level (g/100g FA) of vaccenic acid (*trans*-11 C18:1, **VA**) averaged 3.49 and increased (135%) after oil intake with maximum values observed in LO100 (8.17) and SO50 - LO50 (9.20). Rumenic acid (*cis*-9, *trans*-11 C18:2, **RA**) level (g/100g FA) in milk from Control cows (1.56) increased ($p < 0.05$) to 3.03 (SO100), 3.21 (SO75 - LO25), 3.24 (SO50 - LO50), 2.33 (SO25 - LO75)

and 2.96 (LO100). Results obtained confirmed a great milk fat plasticity in response to PUFA feeding in grazing dairy cows which constitutes a very effective and easy tool in order to improve the healthy value of milk with a potential benefit to the consumer's health. A net or conclusive response pattern over parameters that improve the healthy value of milk to soybean and linseed oils and their blends was not clearly detected. Taken together, the results suggest some advantage for the SO75:LO25 blend considering the relative costs of both oils, the positive effects on milk, fat and protein yields, the lower hypercholesterolemic FA content of milk and the increase in VA and RA content while maintaining a healthy n − 6/n − 3 ratio and very low levels of the detrimental *trans*-9 C18:1 and *trans*-10 C18:1 FA.

Keywords

Grazing Dairy Cow, Conjugated Linoleic Acid, Soybean Oil, Linseed Oil

1. Introduction

Dairy products provide about 25% - 30% of total saturated fat in the human diet and some saturated FA like lauric (C12:0), myristic (C14:0) and palmitic (C16:0) may have a potential negative effect on human health if consumed in excess [1]. Milk also contains healthy FA such as RA, the main natural conjugated linoleic acid (CLA) which showed anticarcinogenic and antiatherogenic properties and VA that can be converted to RA in human [2] and animal body tissues. Milk RA originates from ruminal biohydrogenation of linoleic acid (*cis*-9, *cis*-12 C18:2) as an intermediate product and from endogenous synthesis in the mammary gland from VA. The RA and VA content of milk from ruminant animals can be increased by dietary factors as pasture intake and feeding polyunsaturated FA **(PUFA)** contained in vegetable oils like linoleic acid in soybean oil **(SO)** and linolenic (*cis*-9, *cis*-12, *cis*-15, C18:3) acid in linseed oil **(LO)** [3] [4] [5]. This practice is also effective to reduce the saturated FA content in milk fat [6] [7].

Studies *in vitro* suggested that the partial replacement of linoleic by linolenic acid in the diet increased the rate of conversion of linoleic to RA and from RA to VA in ruminal fluid. The higher rate of isomerization was obtained when linoleic was combined with linolenic acid [8]. Partial ruminal biohydrogenation of linolenic acid also yields VA and the inhibition in the conversion of VA to stearic acid (C18:0) [9] [10] may also contribute to increase milk RA content avoiding a shift towards the formation of undesirable FAs like *trans*-10 C18:1 [11] which may be detrimental to human health [12]. Supplementation with LO can also reduce (or contribute to maintain) the milk n − 6/n − 3 ratio close to 5 in cows [4], goats [13], ewes [14] and buffaloes [15]. The objective of the experiment was to quantify the effectiveness of the combination of SO and LO on productive performance and milk FA profile in order to obtain a functional bovine milk

characterized by a reduction of its hypercholesterolemic FA fraction and enhanced RA content.

2. Material 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 2013. Total duration of the experiment was 38 days. Procedures and animal cares were approved by the Institutional Committee for the Care and Use of Experimental Animals (CICUAE, INTA CERBAS). Thirty-six multiparous Holstein cows (548 ± 56 Kg LW) in early lactation (77 ± 43 days postpartum) were grouped based on parity and milk production measured during the first 7 days of the experiment and randomly assigned to 1 of 6 treatments (6 cows/treatments) in a complete randomized design. The basal (**Control**) diet was composed (DM) by pasture (56%) and concentrate (44%) without supplementary oils. From day 8th of the trial, six cows remained in the Control diet while the cows in oil treatments were supplemented with SO (*Glycine max*), LO (*Linum usitatitissimum*), or their blends (%w/w) at 75 - 25 (**SO75 - LO25**), 50 - 50 (**SO50 - LO50**) and 25 - 75 (**SO25 - LO75**). The dose of supplemented materials was calculated to provide 4.0% of the total DM intake of cows [3]. Pure oils or blends (0.8 kg/cow.day) were manually-mixed to the concentrate during each milking time and thoroughly consumed by cows. Adaptation to oils proceeded gradually starting with 0.2 kg/cow.day over the first day, 0.3 kg during the following 2 days and 0.8 kg from day 4 until the end of the experiment including 28 days of full-dose oil supply. Cows were weighed on 2 consecutive days after the a.m. milking at the start (day 8th) and at the end of the period of lipid supplementation. Animals grazed together on mixed pastures of fescue (*Festuca arundinacea*), red clover (*Trifolium pratense*), white clover (*Trifolium repens*) and bromegrass (*Bromus unioloides*) in a daily-strip grazing system. The area of the strip was regulated using a temporary electric fence to provide an herbage allowance of 27 kg DM/cow.day. After grazing, each strip was clipped-out of non-grazed forage to about 6 cm to allow a lean and uniform regrowth. The concentrate (16% CP) was composed by ground corn grain (35%), malt brewery waste (10%), pelletized sunflower meal (20%), soybean (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 milking times (06.00 and 16.00).

2.2. Sampling Measurements and Laboratory Procedures

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, composited 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 Lab., Rosario, Argentina). During the last 2 weeks of oil 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 milk FA composition by gas liquid chromatography (GLC) as described in [16]. Cows were weighed on two consecutive days after the morning milking at the start (days 6 and 7) and the end (days 38 and 39) of the experiment and the mean value of the 2 records was used to calculate changes in body weight **(BW)** gain.

The quality of the concentrate and herbage was estimated from samples taken weekly. Each sample was dried in a forced-air oven (60˚C, 48 hs), ground through a 1-mm screen (Willey mill, Philadelphia, PA) and analyzed for organic matter **(OM)**, neutral detergent fiber **(NDF)** [17], acid detergent fiber (**ADF**) [18], crude protein **(CP)** [19] using an autoanalyzer (LECO FP-528, Leco Corp., Saint Joseph, MI, EE.UU.), water-soluble carbohydrate (**WSC**) [20], ether extract (**EE**) [21] using an autoanalyzer (ANKOM Corp., Fairtport, NY, EE.UU.), *In vitro* DM digestibility **(IVDMD)** was estimated using the Ankom Tech. Daisy II incubator for 48 h and starch as described in [22]. Pasture DM intake was individually estimated during the last 3 days of weeks 4 and 5 of the trial by the difference method [23]. The average DM intake of the three consecutive days from each cow was computed for the statistical analysis.

2.3. Statistical Analyses

Milk production and composition were evaluated by the PROC MIXED procedure of SAS/STAT® program [24] using the following model:

$$Y_{ijk} = \mu + T_i + A_{(i)j} + W_j + Cov + \left(T_i * W_j\right) + E_{(ijk)}$$

where: Y_{ijk} = the dependent variable, μ: overall mean, Cov = covariate (milk yield and composition over the first 7 days), T_i = treatment effects, $A_{(i)j}$ = random effects of animal within treatments, W_j = effects of week, (T_i * W_j) = interaction effects between of treatment and sampling week, $E_{(ijk)}$ = the residual error associated with the ijk observation. Data from milk FA composition, DM intake and changes in BW gain were analyzed by the PROC GLM procedure of the SAS/STAT® (2002-2010) program using the following model:

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

where: Y_{ij} = the dependent variable, μ: overall mean, T_i = treatment effects, $A_{(i)j}$ = random effects of animal within treatments, $E_{(ij)}$ = the residual error associated with the ij observation.

3. Results

Herbage mass in the pregrazing strips averaged 2253 ± 590 kg DM/ha and her-

bage allowance was 29 ± 1.1 Kg DM/cow.day. Chemical composition of the concentrate and the forage is shown in Table 1 while FA composition is presented in Table 2. On a DM basis, the estimated chemical composition for the basal Control diet was 912 g/kg OM, 161 g/kg CP, 364 g/kg NDF, 201 g/kg FDA, 157 g/kg of starch, 34 g/kg EE and 158 g/kg of water soluble carbohydrates.

As expected, the linoleic acid content in SO resulted high (53.55%) with a low level of saturated FA (SFA) and 21.55% of oleic (*cis*-9 C18:1) acid. Linolenic acid content resulted high in linseed oil (41.9%) and pasture (54.21%).

Table 1. Chemical composition and *in vitro* dry matter digestibility of pasture and concentrate[1].

Parameter	Pasture[2]	Concentrate
Dry matter,%	21.85 ± 2.70	89.6 ± 0.65
Organic matter,% DM	90.01 ± 1.35	92.80 ± 0.46
Crude protein,% DM	15.10 ± 3.68	17.32 ± 1.02
NDF,% DM	46.23 ± 8.16	23.97 ± 2.00
FDA,% DM	26.84 ± 4.42	11.51 ± 1.41
In vitro DM digestibility,%	68.62 ± 3.10	75.14 ± 1.88
Starch,% DM	2.38 ± 0.66	32.59 ± 4.05
Ether extract,% DM	2.62 ± 0.54	4.47 ± 0.77
Metabolizable energy (Mcal/kg DM)	2.48 ± 0.11	2.71 ± 0.07
Water soluble carbohydrates,% DM	11.90 ± 3.60	20.70 ± 2.30

[1]Values are expressed as the mean ± standard deviation. Pasture and concentrate n = 10. [2]Consociated pasture containing *Bromus unioloides*, *Festuca arundinacea*, *Trifolium pratense* and *Trifolium repens*.

Table 2. Fatty acid composition of feeds and oils.

Fatty acid	Pasture[1]	SO[2]	LO[3]	Concentrate
	g/100g AG			
C14:0	0.32	ND[4]	ND	ND
C16:0	17.55	10.25	7.15	15.40
cis-9 C16:1	1.16	ND	ND	ND
C18:0	1.50	4.90	5.45	3.43
cis-9 C18:1	1.87	21.55	21.05	26.95
cis-11 C18:1	ND	ND	ND	3.20
cis-12 C18:1	ND	ND	ND	0.81
cis-9 *cis*-12 C18:2	12.45	53.55	23.6	45.85
C20:0	0.69	0.40	0.25	0.33
cis-9 *cis*-12 *cis*-15 C18:3	54.21	8.50	41.90	3.21
C22:0	0.99	0.40	0.20	0.38

[1]Consociated pasture containing *Bromus unioloides*, *Festuca arundinacea*, *Trifolium pratense* and *trifolium repens*. [2]Soybean oil. [3]Linseed oil. [4]Not detected.

Compared to Control records (23.03 kg/cow·day), supplementation with 4% oils increased ($p < 0.05$) milk yield (25.19 kg/cow·day). Production of fat corrected milk **(FCM)** resulted greater for cows in AS75 - AL25 (Table 3). Milk fat content was reduced ($p < 0.05$) only in treatments that included 50% and 75% of LO with the lowest value in the AS50 - AL50 treatment. Milk protein content was not affected ($p > 0.05$). Compared to Control, the SO75 - LO25 blend was also the most effective to increase milk fat (0.886 kg/cow·day) and milk protein (0.882 kg/cow.day) yields ($p < 0.05$). The result may be relevant in a context of the payment of milk per kg of fat and protein produced. Concentration of total solids resulted also higher ($p < 0.05$) in SO75 - LO25 (Table 3).

No significant differences were observed in BW gain of cows (Table 4).

Table 3. Milk production and composition in grazing dairy cows supplemented or not (Control) with combinations of soybean (SO) and linseed (LO) oils at different percentages (w/w).

Parameter	Treatment[1]									
	Control	SO100	SO75 - LO25	SO50 - LO50	SO25 - LO75	LO100	SEM	T	W	T * W
								P[2]		
Milk yield, kg/d	23.03^{c}	24.59^{bc}	26.12^{ab}	24.53^{c}	26.85^{a}	23.86^{c}	0.55	0.0004	0.44	NS[3]
4%FCM%, kg/d	20.73^{b}	22.87^{ab}	23.73^{a}	20.45^{b}	22.90^{ab}	21.21^{b}	0.92	0.08	0.0008	NS
Fat, g/100 g	3.40^{a}	3.47^{a}	3.33^{ab}	2.79^{c}	3.06^{bc}	3.40^{a}	0.12	0.002	<0.0001	NS
Protein, g/100 g	3.34	3.29	3.34	3.25	3.16	3.29	0.09	NS	<0.0001	NS
Lactose, g/100 g	4.82	4.90	4.89	4.89	4.88	4.96	0.04	NS	<0.0001	0.001
Solids, g/100g	12.29^{a}	12.37^{a}	12.41^{a}	11.80^{bc}	11.74^{c}	12.24^{ab}	0.15	0.006	<0.0001	NS
Fat yield, kg/día	0.748^{bc}	0.857^{ab}	0.886^{a}	0.709^{c}	0.820^{abc}	0.791^{abc}	0.04	0.04	<0.0001	NS
Protein yield, kg/día	0.729^{d}	0.811^{abc}	0.882^{a}	0.806^{bcd}	0.850^{ab}	0.773^{cd}	0.03	0.007	0.001	NS

[1]Values are expressed as least squares means and standard error of least squares means. Cows were fed a basal diet (Control) without oils or basal diet supplemented with pure oils or blends at 4% of total DM intake as follows: 0.8 kg SO, 0.6 kg SO and 0.2 kg LO (SO75 - LO25), 0.4 kg SO and 0.4 kg LO (SO50 - LO50), 0.2 kg SO and 0.6 kg LO (SO25 - LO75) and 0.8kg LO. [2]Treatment effect. [3]Not significant effects. [4]FCM% = 4% Fat Corrected Milk. T = treatment effect. W = week effect. TxW = treatment for week interaction. [a,d]Means in the same row with different superscripts differ significantly for treatment effect with *P*-value as mentioned in column for significance at $p < 0.05$ (Test Tukey-Kramer).

Table 4. Bodyweight (BW) changes in grazing dairy cows supplemented or not (Control) with combinations of soybean (SO) and linseed (LO) oils at different percentages (w/w).

Parameter	Treatment[1]						SEM	*P*<[2]
Kg	Control	SO100	SO75 - LO25	SO50 - LO50	SO25 - LO75	LO100		
Initial BW	542.2	547.8	562.3	528.5	554.2	554.8	23.9	0.94
Final BW	568.6	584.7	588.3	559.2	592.2	584.2	22.6	0.88
Daily BW gain	0.690	0.960	0.680	1.00	0.810	0.780	0.14	0.47
BW change	26.5	36.8	26.0	30.8	38.7	29.7	5.55	0.45

[1]Values are expressed as least squares means and standard error of least squares means (SEM). Cows were fed a basal diet (Control) without oils or basal diet supplemented with pure oils or blends at 4% of total DM intake as follows: 0.8 kg SO, 0.6 kg SO and 0.2 kg LO (SO75 - LO25), 0.4 kg SO and 0.4 kg LO (SO50 - LO50), 0.2 kg SO and 0.6 kg LO (SO25 - LO75) and 0.8 kg LO. [2]Treatment effect.

Pasture DM intake increased by 6% and 8% in SO100 and S75 - LO25 while it was reduced by 0.3%, 13% and 9% in SO50 - LO50, SO25 - LO75 and LO100 respectively (Table 5). Total DM intake was higher in SO100 and SO75 - LO25 while energy intake resulted higher in SO100, SO75, SO50 - LO50 and LO100 (Table 5). Feeding oils mixed with the concentrate (10% as fed) was an effective way to obtain the target lipid consumption avoiding refusals. Estimated intakes of linoleic and linolenic acids from supplementary oils were 407 - 179 g/d in SO100, 322 - 213 g/d in SO75 - LO25, 236 - 246 g/d in SO50 - SO50, 150 - 280 g/d in SO25 - LO75 and 65-313 g/day in LO100.

Milk content of butyric (C4:0) acid was not affected after oil intake (Table 6). The decrease in levels of *de novo* synthesized FA (C4:0 to C15:1) was not different between pure oils and their combinations. In SO50 - LO50 and SO25 - LO75 treatments, the lower synthesis of *de novo* FA was not apparently compensated for a correlative increase in the mammary uptake of preformed FA since milk fat content decreased when compared to Control (Table 3). Milk fat depression was maximum in the SO50 - LO50 treatment where the highest content of *trans*-10 C18:1 was also observed (Table 6). Content of the hypercholesterolemic FA of milk (C12:0 to C16:0) was reduced by oil intake (−27%) without differences between treatments (Table 6). The basal (1.85) atherogenic index **(AI)** and milk content of myristic acid (10.21 g/100g FA) were reduced by oil intake (40 and 33% respectively) without differences between SO-LO blends. Similar results were observed for lauric (−35%) and palmitic (−24%) acids (Table 6). After oil intake, content of stearic acid increased only when LO represented 75% and 100% of the supplementary blend suggesting a higher biohydrogenation because the estimated activity of the Δ9 desaturase enzyme did not differ between blends

Table 5. Pasture, concentrate and energy intake in grazing dairy cows supplemented or not (Control) with combinations of soybean (SO) and linseed (LO) oils at different percentages (w/w).

Parameter	Treatment[1]						SEM	P[2]
	Control	SO100	SO75 - LO25	SO50 - LO50	SO25 - LO75	LO100		
Intake (kg DM/d)								
Pasture[3]	9.27^{ab}	9.84^{ab}	10.00^{a}	9.24^{ab}	8.09^{c}	8.98^{bc}	0.35	0.007
Concentrate	7.2	7.20	7.20	7.20	7.20	7.20	-	-
Oil	-	0.80	0.80	0.80	0.80	0.80	-	-
Total DM	16.47^{b}	17.84^{a}	18^{a}	17.24^{ab}	16.09^{c}	16.98^{bc}	0.39	<0.0001
Milk/DM intake	1.40^{a}	1.38^{a}	1.45^{b}	1.42^{b}	1.67^{c}	1.41^{a}	0.11	0.0006
ME, Mcal/d	43.11^{c}	48.65^{a}	49.05^{a}	47.17^{ab}	44.07^{bc}	46.48^{abc}	0.91	<0.0001
NEl(Mcal/d)	27.59^{c}	31.14^{a}	31.40^{a}	30.18^{a}	28.20^{bc}	29.75^{ab}	0.58	<0.0001

[1]Values are expressed as least squares means and standard error of least squares means (SEM). Cows were fed a basal diet (Control) without oils or basal diet supplemented with pure oils or blends at 4% of total DM intake as follows: 0.8 kg SO, 0.6 kg SO and 0.2 kg LO (SO75 - LO25), 0.4 kg SO and 0.4 kg LO (SO50 - LO50), 0.2 kg SO and 0.6 kg LO (SO25 - LO75) and 0.8 kg LO. [2]Treatment effect. [3]Consociated pasture containing *Bromus unioloides*, *Festuca arundinacea*, *Trifolium pratense* and *trifolium repens*. [a,d]Means in the same row with different superscripts differ significantly for treatment effect with *P*-value as mentioned in column for significance at $p < 0.05$ (Test Tukey-Kramer).

Table 6. Milk fatty acid (FA) composition from grazing dairy cows supplemented or not (Control) with combinations of soybean (SO) and linseed (LO) oils at different percentages (w/w).

FA	Treatment[1]							*P*[2]
g/100 g of FA reported	Control	SO100	SO75 - LO25	SO50 - LO50	SO25 - LO75	LO100	SEM	T
C4:0	2.38^{a}	2.41^{a}	2.47^{a}	2.18^{a}	2.60^{a}	2.46^{a}	0.15	<0.0001
C6:0	1.50^{a}	1.25^{ab}	1.35^{ab}	1.11^{b}	1.30^{ab}	1.34^{ab}	0.10	0.02
C8:0	0.93^{a}	0.66^{b}	0.74^{b}	0.60^{b}	0.72^{b}	0.74^{b}	0.06	0.0002
C10:0	2.20^{a}	1.39^{b}	1.55^{b}	1.27^{b}	1.54^{b}	1.55^{b}	0.13	<0.0001
C12:0	2.58^{a}	1.60^{b}	1.79^{b}	1.52^{b}	1.73^{b}	1.73^{b}	0.12	<0.0001
C14:0	10.21^{a}	6.78^{b}	7.27^{b}	6.42^{b}	6.75^{b}	6.95^{b}	0.32	<0.0001
cis-9 C14:1	0.88^{a}	0.43^{b}	0.48^{b}	0.37^{b}	0.42^{b}	0.34^{b}	0.08	<0.0001
C15:0	0.98^{a}	0.68^{b}	0.72^{b}	0.73^{b}	0.69^{b}	0.68^{b}	0.03	<0.0001
C16:0	25.69^{a}	19.49^{b}	20.07^{b}	19.53^{b}	19.52^{b}	18.96^{b}	0.66	<0.0001
C16:1	0.88^{a}	0.58bc	0.62^{b}	0.40^{bc}	0.54^{bc}	0.38^{c}	0.07	<0.0001
C17:0	0.52^{a}	0.32^{b}	0.31^{b}	0.34^{b}	0.34^{b}	0.33^{b}	0.03	<0.0001
C18:0	12.78^{c}	14.11^{bc}	13.86^{bc}	14.10^{bc}	16.15^{a}	15.33^{ab}	0.67	0.004
C18:1 Isomers								
Trans-6 - 8	0.16^{c}	0.38^{ab}	0.32^{b}	0.41^{a}	0.36^{ab}	0.36^{ab}	0.03	<0.0001
Trans-9	0.23^{b}	0.53^{a}	0.48^{a}	0.52^{a}	0.47^{a}	0.50^{a}	0.02	<0.0001
Trans-10	0.46^{c}	1.48^{ab}	0.95^{bc}	1.80^{a}	1.13^{abc}	0.91^{bc}	0.29	0.006
Trans-11 (VA)	3.49^{c}	8.17^{ab}	7.82^{b}	9.20^{a}	7.67b	8.15^{ab}	0.38	<0.0001
Total *trans*	4.34^{c}	10.56^{ab}	9.57^{b}	11.93^{a}	9.63b	9.91^{b}	0.52	<0.0001
cis-9 C18:1	26.14^{b}	27.80^{ab}	27.50^{ab}	27.45^{ab}	27.76^{ab}	28.10^{a}	0.68	0.02
cis-11C18:1	2.15^{a}	2.10^{a}	1.92^{ab}	1.95^{ab}	1.92^{ab}	1.78^{b}	0.09	0.01
C18:2 (n − 6)	1.96^{b}	3.36^{a}	3.50^{a}	3.44^{a}	2.87^{a}	2.74^{a}	0.29	<0.0001
C18:3 (n − 3)	0.35^{d}	0.40^{d}	0.64^{c}	0.73^{bc}	0.85^{ab}	1.05^{a}	0.07	<0.0001
cis-9*trans*-11 C18:2 (CLA)	1.56^{c}	3.03^{a}	3.21^{a}	3.24^{a}	2.33^{b}	2.96^{a}	0.22	<0.0001
Short chain FA[3]	7.02^{a}	5.70^{b}	6.11^{ab}	5.16^{b}	6.17^{ab}	6.08^{ab}	0.42	0.005
Medium chain FA[4]	41.69^{a}	29.30^{b}	31.21^{b}	29.18^{b}	29.70^{b}	29.15^{b}	0.98	
Long chain FA[5]	49.27^{b}	61.32^{a}	60.21^{a}	62.70^{a}	61.52^{a}	61.86^{a}	1.08	<0.0001
Saturated FA (SFA)	59.76^{a}	48.24^{b}	50.10^{b}	47.74^{b}	51.28^{b}	49.95^{b}	1.24	<0.0001
Unsaturated FA (UFA)	38.21^{b}	48.07^{a}	47.43^{a}	49.30^{a}	46.12^{a}	47.14^{a}	1.17	<0.0001
SFA/UFA	1.58^{a}	1.01^{b}	1.06^{b}	0.97^{b}	1.12^{b}	1.06^{b}	0.06	<0.0001
AI[6]	1.85^{a}	1.05^{b}	1.12^{b}	1.00^{b}	1.09^{b}	1.07^{b}	0.07	<0.0001
ΔD products	35.77^{b}	43.93^{a}	42.97^{a}	44.72^{a}	42.04^{a}	43.00^{a}	0.99	<0.0001
Substrates[8]	54.51^{a}	51.50^{b}	51.76^{b}	52.99^{ab}	53.01^{ab}	52.01^{b}	0.85	<0.0001
Índex[7]	0.40^{b}	0.46^{a}	0.45^{a}	0.46^{a}	0.44^{a}	0.45^{a}	0.008	<0.0001
De novo FA (C4:0-C15:1)	21.07^{a}	15.60b	16.35^{b}	14.17^{b}	15.59^{b}	15.70^{b}	0.81	<0.0001
Preformed FA (>17:0)	50.70^{b}	61.26^{a}	60.16^{a}	62.57^{a}	61.42^{a}	61.72^{a}	1.06	<.0001
n − 6/n − 3 FA	5.94^{b}	8.53^{a}	5.66^{b}	4.86^{c}	3.47^{d}	2.76^{d}	0.26	0.0008
CLA/AV	0.44^{a}	0.37^{abc}	0.42a^{b}	0.33b^{c}	0.31^{c}	0.37^{bc}	0.03	<0.0001
∑(C12:0 - C16:0)	38.48^{a}	27.43^{b}	29.13^{b}	27.47^{b}	28.00^{b}	27.64^{b}	0.96	<0.0001

[1]Values are expressed as least squares means and standard error of least squares means (SEM). Cows were fed a basal diet (Control) without oils or basal diet supplemented with pure oils or blends at 4% of total DM intake as follows: 0.8 kg SO, 0.6 kg SO and 0.2 kg LO (SO75 - LO25), 0.4 kg SO and 0.4 kg LO (SO50 - LO50), 0.2 kg SO and 0.6 kg LO (SO25 - LO75) and 0.8 kg LO. [2]Treatment effect. [3]Short chain FA (C6:0 to C10:0). [4]Medium chain FA: (C12:0 to C17:1). [5]Long chain FA: (C18:0 to C22:6). [6]Atherogenicity index: (C12 + 4 * C14 + C16)/(∑UFA). UFA: *cis*-9 C14:1, C16:1, *cis*-9 C18:1, *cis*-11 C18:1, *trans*-11 C18:1, C18:3, C18:2, C18:2 *cis*-9 *trans*11 CLA. The detrimental FA *trans*-6-8, 9, 10 C18:1 were excluded. [7]Index: ([∑Δ9Dproducts]/[∑Δ9D products + Susbstrates]). [8]Substrates:C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + *Trans*11 C18:1. [a,d]Means in the same row with different superscripts differ significantly for treatment effect with *P*-value as mentioned in column for significance at $p < 0.05$ (Test Tukey-Kramer).

(Table 6). Content of oleic acid resulted higher (+7%, $p < 0.05$) only in LO100. Compared to Control, the increase of the linoleic acid content in milk resulted high (62%, $p < 0.05$) in cows receiving supplementary oils without differences between blends. Linolenic acid gradually increased when LO replaced SO. The basal milk n − 6/n − 3 ratio (5.94) was increased ($p < 0.05$) up to 8.53 in SO alone and the inclusion of 25% LO in the blend allowed to maintain the ratio in values near to 5.66 and close to Control records. Concomitant increases in LO at 50%, 75% and 100% of the blend significantly reduced the n − 6/n − 3 ratio to 4.86; 3.47 and 2.76 respectively. Basal content (g/100g FA) of *trans*-9 C18:1 (0.23) and *trans*-10 C18:1 (0.46) were increased by oil intake (Table 6) reaching maximal values of 0.53 (*trans*-9) and 1.80 (*trans*-10) in SO100 and SO50-LO50 (Table 6). No differences ($p > 0.05$) between blends were detected for milk content of *trans*-9 C18:1 and a defined response-pattern in the case of *trans*-10 C18:1 was not observed.

In mik from Control cows, VA content represented 80.41% of the total *trans*-C18:1 remaining high (77% to 82%) after oil intake (Table 6). In Control treatment, *trans*-9 and *trans*-10 C18:1 represented 5.30% and 10.60% of the total *trans*-C18: 1 remaining low after oil intake (11.5% and 28.9%, respectively). VA and RA were highly correlated ($r^2 = 0.80$) with an estimated rate of conversion of 32.8% (Figure 1) or 37.3% when the RA/VA ratio was used as an estimator (Table 6).

Content of VA in Control milk averaged 3.49 g/100g FA (Table 6) and increased ($p < 0.05$) after oil intake reaching maximal values in SO50 - LO50 (9.20 g/100 g FA) and SO100 (8.17 g/100 g FA). Basal RA content in milk (1.56 g/100g FA, Table 6) increased ($p < 0.05$) after oil intake showing the highest numerical value in SO50 - LO50 (3.24 g/100g FA) and the lower in SO25 - LO75 (2.33 g/100g FA). The SO50 - LO50 treatment also yielded the highest *trans*-9 (0.52) and *trans*-10 C18:1 (1.80 g/100g FA) contents. Milk VA and RA showed a high variable response to oil intake within treatments (Figure 1) without a well-defined response-pattern (Table 6). Total unsaturated FA content and the unsatu-

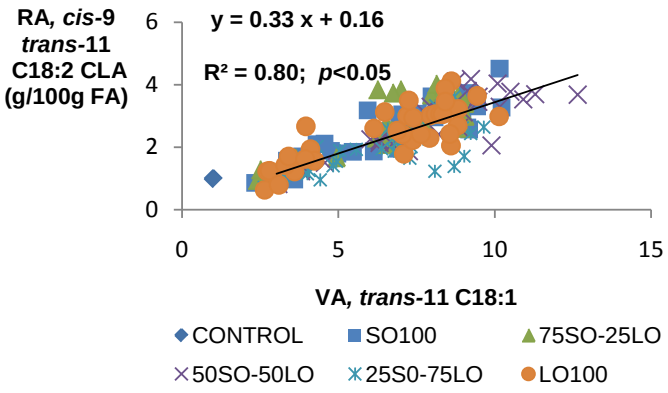

Figure 1. Relationship between rumenic (RA, *cis*-9, *trans*-11 C18:2) and vaccenic (VA, *trans*-11 C18:1) acids in milk from cows supplemented or not (Control) with combinations of soybean (SO) and linseed (LO) oils at different percentages (w/w).

rated/saturated/ratio in milk were higher in oil compared to Control ($p < 0.05$) treatment without differences between oil blends.

Plasma metabolite concentration (glucose, non-esterified fatty acids, triglyceride and urea) were not affected (data not shown). Compared to Control (199.4 mg/dl), circulating levels of plasma cholesterol increased ($p < 0.05$) in SO50 (229.3 mg/dl), SO25 (231.9 mg/dl) and LO 100 (236.4 mg/dl).

4. Discussion

4.1. Pasture and Oil Characteristics

The daily strip-grazing system allowed to provide 29 kg DM/cow.day considered adequate to maximize pasture intake [25]. In grazing conditions, pasture intake should be maximal when herbage is offered at a rate of 45 g pasture OM/kg BW [25]. From the average BW of cows (563 kg) and the average OM content of pastures (90 g OM/100g DM, **Table 1**) it can be calculated that a non-limitant herbage allowance should be around 22.8 kg DM which resulted lower to that obtained in the present experiment. Maximal DM intake should be obtained when pasture allowance was 45 to 55 g DM/kg BW per day [26]. The average BW of cows (563 kg, **Table 4**) suggests an optimal range in pasture allowance of 25 to 31 kg DM. Thus, the herbage allowance obtained (29 kg DM/cow.day) was within the optimal range. Pasture DM content (21.85%) was over the critical range of 15% - 18% proposed to decrease voluntary intake [27]. In turn, NDF (46.23%) and CP (15.1%) contents were in the range of 40% - 50% (NDF) and 15% - 25% (CP) considered as adequate for well managed pastures [25]. In our experiment, pasture quality and quantity were sufficiently enough to maintain or increase total DM and energy intake of cows (**Table 5**).

SO represented a good source of oleic (21.55%) and essentially linoleic (53.55%) acids as reported in [28] [29]. In LO, linolenic acid content (41.9%, **Table 2**) resulted lower to the 55% value reported by others [7] [28] [30] but near to values informed in [31] [32]. Linolenic acid in pasture was nevertheless higher than reported by [33] and [30].

4.2. Milk Yield and Composition and Changes in Body Weight

In [34], supplementing SO or LO alone or in combination at 4% of DM intake increased milk yield (16.7%) compared to Control without differences between oils. In our trial, the average increase in oil-supplemented cows over Control was somewhat moderate (9.4%) and mainly explained by both oil blends at a ratio of 75:25 (**Table 3**). Since milk production at SO100 and LO100 did not differ from Control, a synergic effect on milk output of both 75:25 combinations can be expected. Comparison between oil blends did not reveal a specific effect on milk production. A high frequency of favorable effects on milk production after the inclusion of unprotected vegetable oils in the diet was reported by [35]. The lack of differences between SO100 and LO100 respect to Control (**Table 3**) was also observed in the meta-analysis by [28] suggesting the absence of any net advan-

tage of one or another oil over milk production. Feeding LO at 3% or 4% DM intake increased milk production in [34] a result not observed in other trials [7] [33]. Supplementary SO at 2.9% ± 1.2% of DMI (533 ± 228 g/day) did not affect milk production in the experiments reviewed by [28] or when SO was fed at 3.5% to 5% of DM intake [36] [37] [38]. In addition, LO supply (1% to 7% of DM intake) did not affect milk production in [28] [30] [33] [36]. In our experiment, the higher yield of FCM from cows in SO75 - LO25 was explained by the higher volume of milk produced since milk fat content did not change (**Table 3**). These results suggest that energy excreted in milk was the same across treatments as reported by [34]. Unsaturated lipid supply generally has neutral effects on yield of FCM both in non-grazing [39] as in grazing experiments [40]. In a wide dose-range of lipid supplementation (0.2 to 1.0 kg/day) it has been observed that unsaturated lipids decrease milk fat content and fat yield by 8% in grazing dairy cows [40]. The lowest milk fat content observed in the 50 - 50 treatment (**Table 3**) was consistent with the higher levels of *trans*-10 C18:1 (**Table 6**) because both parameters were negative correlated (**Figure 2**). A direct relationship between increasing milk levels of *trans*-10 C18:1 and the reduction of *de novo* mammary synthesis has been previously reported [41] a fact that contributes to explain the lower milk fat content observed.

The lack of negative effects of oil supply on milk protein content (**Table 3**) is a relevant result as this parameter not only affects milk price but also determines the speed and quality of coagulation in cheese production. In confined production systems, supplementation with unprotected lipids often decrease milk protein content [35] [39] [42] while in pasture based diets this parameter is often not affected [40] [43]. Feeding LO does not appear to affect either milk fat [28] [34] nor milk protein contents or production [7] [33] [34].

The lack of differences in BW gain (**Table 4**) was consistent with [30] [44] [45] [46] and the similar plasma NEFA concentrations (data not shown). In fact, supplementation with unsaturated lipids does not appear to reduce BW loss in lactating cows or favor the reconstitution of body reserves in lactating cows [47].

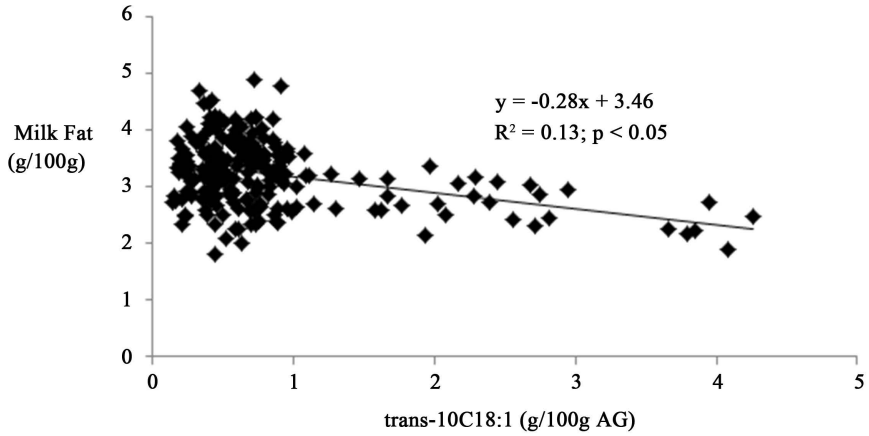

Figure 2. Relationship between milk fat content and *trans*-10 C18:1 in milk.

4.3. Dry Matter Intake

In our trial, the effect of supplemental fat on DM intake showed different responses (**Table 5**) depending on the specific oil blend consumed. Pasture intake slightly increased in SO100 and SO75 while it was decreased in others treatments (SO50 - LO50; SO25 - LO75 and AL100) while total DM intake resulted higher in the SO100 and SO75 (**Table 5**). The inclusion of LO (3.2% ± 1.7% DM intake) or SO (2.9% ± 1.2% DM intake) in the ration did not affect DM intake of cows in the meta-analysis by [28] nor in [33] [34]. Feeding unsaturated FA is more likely to reduce feed intake than saturated FA owing to their potential negative effects on ruminal digestion. However the results are variable including negative [48], neutral [49] or even positive [50] [51] effects on rumen function. The forage concentrate ratio **(F/C)** is relevant because when LO was included at 3% of DM intake in a 65/35 F/C diet, positive effects were reported on FDN digestion with an opposite result when the F/C was 35:65 [51]. In the present trial, the F/C averaged 54:46 (**Table 5**).

4.4. Milk Fatty Acid Profile

The increase in mammary uptake of circulating FA after oil supply [52] may explain the changes in milk FA composition compared to Control treatment (**Table 6**) confirming ruminant milk fat plasticity [3] [28]. The consistency in content of butyric acid after oil intake (**Table 6**) is a frequently reported result [3] [28] which is of interest for its potential beneficial role in human health [3]. Butyric acid can be synthesized by an independent malonyl-CoA pathway and therefore not dependent on the activity of the acetyl CoA carboxylase that is inhibited by the uptake of the exogenous FA supplied by oils [3] [6].

The decrease in the total content of *de novo* synthesized FA (C4:0 to C15:1) was similar between the pure oils and their mixtures (**Table 6**) as reported in [34]. The effect is explained by the inhibition in the activity of lipogenic mammary enzymes such as Acetyl-CoA carboxylase [53] [54]. Antonacci *et al.* [55] also reported a reduction (−17.8%) in the total *de novo* synthesized FA content (from 22.49 to 18.48 g/100g FA) after feeding 0.7 kg of an SO70-LO30 blend to grazing dairy cows. In our study, the decrease in milk fat content (**Table 3**) was negatively correlated to *trans*-10 C18:1 content (**Figure 2**) in agreement with [41]. A high content of *trans*-10 C18: 1 or related metabolites like *trans*-10, *cis*-12 C18:2 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 [56]. In our study, the reduction in milk fat content (**Table 3**) occurred in part at the expense of the amount of hypercholesterolemic FA (**Table 6**) which improves the healthy value of milk and contributes to decrease the atherogenic potential of milk fat. In grazing dairy cows, supplementation with 0.7 kg/cow/day of an SO70 - LO30 mixture reduced the atherogenicity index of milk from 1.6 to 1.25 [55].

The reduction (33%) in myristic acid content (**Table 6**) is an important result because the pro-atherogenic role of C14:0 is considered to be very potent [1]. The reductions of 35% for C12:0 and 24% for C16:0 (**Table 6**) were comparable to those obtained in [55] and contribute to avoid an excessive consumption of unhealthy saturated fat. In the experiment of [34], the reductions of these three FA after supplementation at 4% DM intake with a SO50 - LO50 mixture or pure oils did not differ between treatments. In the present work, the reductions were within the range estimated from the meta-analysis performed by [28] for supplements with SO and LO with values of 42% - 37% (lauric), 23% - 24% (myristic) and 30% - 17% (palmitic).

Milk content of stearic acid increased only when LO was present at 75% and 100% of the blend without differences in treatments with a higher proportion of SO (**Table 6**). The results were consistent with that reported by [34] and could be linked to some possible inhibition of biohydrogenation from VA to C18:0 when high contents of linoleic acid are available [9] [10]. The effect of the oil blends on the content of C18:0 in milk was inconsistent, which agrees with other experiments that reported the lack of differences in milk stearic content in dairy cows supplemented with oils rich in C18:2n − 6 or C18:3n − 3 [55] [57].

In the meta-analysis by [28] all polyunsaturated FA supplements generate similar increases in the content of stearic and oleic acids in milk. In our trial, content of oleic acid numerically increased with oil supply but differed from Control only in LO100 (+7%). The increase in oleic acid after the addition of sunflower or soybean oils to the diet is a well-documented result [7] [28] [58] also observed when supplementing with LO [28] [34] [59] [60]. Oleic acid content in milk did not increase after the intake of an SO70 - LO30 mixture at 0.7 kg/cow/day in grazing dairy cows [55].

Linoleic acid content in milk from Control cows (2.96 g/100g FA, **Table 6**) was within the range (2% - 3%) suggested by [3]. In oil-enriched diets, linoleic acid content increased up to 2.74 - 3.50 g/100 g FA (**Table 6**) remaining below the 4% as reported by [3] and observed in [55] (3.25 to 3.92 g/100g FA) after supplying 0.7 kg/cow/day of an oil blend (SO70 - LO30).

The levels of RA achieved in treatments with pure oils (2.96 to 3.03 g/100g FA) were higher than those of 1.60 - 2.39 g/100g FA reported in [34] when rations with a high forage content (59%) were supplemented with oils at 4% of DM intake. These authors [34] obtained greater increases for both VA and RA using SO compared to LO with additive responses of the 50:50 blend but always lower to oils utilized in their pure form suggesting no synergistic effects. A higher and more complete ruminal biohydrogenation of PUFAs in animals that consumed LO would explain the response obtained [34]. In our trial, milk RA content in oil supplemented cows (2.33 to 3.24 g/100g) were higher than values reported in the meta-analysis by [28] when cows were suplemented with SO (1.02 ± 0.36 g/100g FA) or LO (1.75 ± 0.84 g/100g FA) and also to those obtained by [61] supplementing with 500 g/day of sunflower oil or SO (2.02

gRA/100g FA) to grazing dairy cows. They were also higher than observed in [62] using 0.9 kg/day of FA calcium salts (0.9 kg/cow/day) containing 30% linoleic acid but close to those reported in [55] (3.13 g/100 g for AR) using the mixture SO70:LO30 (0.7 kg/cow.day) in grazing dairy cows.

5. Conclusion

The results confirmed the existence of a broad plasticity in milk FA composition in response to PUFA feeding to grazing dairy cows which constitutes an effective tool to the farmer in order to improve the healthy and added value of milk with a potential benefit to the consumer's health. A net or well defined response over parameters linked to healthy value of milk was not detected after feeding soybean and linseed oils or blends at 4% of total DM intake. Taken together, the results suggest some advantage for the SO75:LO25 blend considering the relative costs of both oils, the positive effects on milk, fat and protein yields, the lower hypercholesterolemic FA content of milk and the increase in VA and RA content while maintaining a healthy n − 6/n − 3 ratio and very low levels of the detrimental *trans*-9 C18:1 and *trans*-10 C18:1 FA.

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