Lipid Sources To Jersey Cows: Productive, Nutritional Effects and Milk Fatty Acid Compounds

Ana Paula Binato de Souza

Universidade Federal da Pelotas

Jorge Schafhauser Junior

Empresa Brasileira de Pesquisa Agropecuária: Embrapa Agroenergia

Rudolf Brand Scheibler

UFPEL: Universidade Federal de Pelotas

Ana Carolina Fluck (✉ anacarolinafluck@yahoo.com.br)

UTFPR: Universidade Tecnológica Federal do Parana <https://orcid.org/0000-0001-9133-2446>

Fábio Antunes Rizzo

UFPEL: Universidade Federal de Pelotas

José Laerte Nörnberg

UFSM: Universidade Federal de Santa Maria

Diego Prado de Vargas

Unoesc Campus de Xanxerê: Universidade do Oeste de Santa Catarina - Campus Xanxere

Research Article

Keywords: bypass fat, energy balance, intake, Jersey cows, lipid source.

Posted Date: June 30th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-588948/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

The aim was to assess if the replacement of fatty acid salts by oil seeds in diet of dairy cows and their effects about intake and digestibility, milk production and composition, feed efficiency, metabolic profile and milk fatty acid profile. Lipid sources evaluated were calcium salts of fatty acids (CS), linseed (LI), sunflower (SF) and soybean (SY). Diets were iso in protein, fiber and energy, with 58 g/kg (mean) of crude fat in dry matter. There were no differences ($P > 0.05$) in the intake of nutrient fractions. The protein digestibility was lower for SY ($P < 0.001$) due the fraction derived from the grain in that treatment. The ether extract digestibility was higher for CS treatment ($P < 0.001$), and no difference was found between the grains sources. Milk production was higher in CS, when milk production was corrected for energy, these differences disappeared. Little effect on milk composition (g/kg) was observed, only for lactose. The sources used do not affect feed efficiency, energy balance and blood metabolic profile. in CS. When observed milk fatty acid profile, there was an increase influence by CS There was no difference for saturated and polyunsaturated fatty acids (PUFA), however, monounsaturated fatty acids (MUFA) were higher for SF ($P = 0.0172$). The lipid sources evaluated can be used in early lactation without negative effects being able to replace calcium salts of fatty acids in diets.

Introduction

The progress in management practices for dairy herds has provided better conditions for the animals expressing high yields. However, the increase in the individual milk production in most dairy herds in early lactation promote a negative energy balance period (BEN), even being offered high-energy diets. This fact is responsible for reducing the productive and reproductive performance in postpartum, increase the incidence of metabolic disorders and diseases in dairy cows (Wildman et al, 1982; Ruegg & Milton, 1995). Bonded it, the growing concern of the population to consume animal products with reduced levels of saturated fatty acids makes research aimed at the nutrition of dairy cows to aim to reduce this content in dairy products.

Than, to minimize the effects of metabolic challenge and improve the quality of dairy products, fat sources have been used in animal feeding, to raise the energy density of diets without negative effects (Onetti & Grummer, 2004). There are numerous fat sources available into this purpose like free fat, particularly in the form of vegetable oils readily available to the rumen, or non-degradable rumen sources, which can be artificially protected, mainly in the form of salts calcium fatty acids, or naturally protected sources, as the oilseeds (Jenkins, 1998; Naik, 2013). Worldwide, there are many commercial products presented like fatty acid calcium salts available for cattle nutrition, some individual characteristics, but all with high costs that can limit its use for dairy farms, by the high cost of diets when included.

The hypothesis was that it is possible to substitute calcium salts of fatty acids by whole oil seeds, assuming that the fat in the seeds is naturally protected by the integument of the shell and/or associated with the protein matrix and hence with slow release in rumen, generating no harmful effects on ruminal fermentation. The commercial protected fat replacement was tested by oilseed sunflower, linseed and

soybean in the diet of lactating Jersey cows, and its effects on the intake and digestibility of nutrients, metabolic and productive parameters.

Materials And Methods

Animals and location

The experiment was from September to December, 2013, at the Empresa Brasileira de Pesquisa Agropecuária (Embrapa), research field located in Southern Brazil (geographical coordinates 31° 52" S and 52° 29" W, altitude 13.24 m). The study was approved by following the norms of the Committee on Animal Research and Experimentation from Universidade Federal de Pelotas (case no.6850).

Eight 40 days milking multiparous Jersey cows, in 407 kg average weight and milk production of 20±2 kg between second and fourth lactation, were housed into a free stall and adapted to the experimental diets for 15 days before the start of the experiment.

Treatments and experimental diets

The treatments evaluated were calcium salts of fatty acids (CS) (Megalac-E®) and grain of Linseed (LI), Sunflower (SF) and Soybean (SY), to natural protection of lipids of the diet (Table 1) formulated to reach the same levels of energy, crude protein and neutral detergent fiber (Table 2) tested in performance simulator, NRC (2001). The forage used was corn silage, provided twice daily and adjusted to 100 g/kg of orts. The concentrate was provided three times of day, individually, separated of forage, to ensure total intake.

Experimental management

Each experimental period was conducted over 21 days, with a 17-day adaptation and a 4-day measurement period and cows were milked twice a day. Feed refusals were removed and weighed daily prior to delivery of the next meal. To estimate the fecal excretion was administered 10 g of chromium oxide (Cr₂O₃) shared into two daily doses, after milking. The faeces samples were collected out in the morning and afternoon, during the four-day collection periods, with the individual samples stored in a freezer (-18 °C), forming a sampling-period per cow.

Blood samples were taken at 15th and 17th days of each period, immediately after the morning milking, by jugular puncture. These were kept at rest for 10 minutes and was then centrifuged at 8000 rpm for 10 minutes, then placed in an isothermal box chilled and sent for analysis in a commercial laboratory.

Evaluations

Milk production was obtained by the average milk produced in the last four days for each period. Milk adjusted for energy was obtained by the equation described by Sjaunja et al. (1990). Milk analysis consisted in protein, fat, total solids, lactose and urea infrared spectroscopy according to AOAC 1996

(method 972.16), somatic cell count (SCC) by flow cytometry and the values processed according to the methodology described by Gerge and Shook (1993), in which somatic cell score (SCS) = $[\log_2 (\text{SCC}/100)] + 3$.

Diet ingredients composition (Table 1), refusal feed and faeces were analyzed to dry matter (DM), organic matter (OM) and crude protein (CP) according to AOAC (1996, 967.03 methods, 942.05 and 954.05, respectively), ether extract (EE) in filter bags system, developed by Ankom® Technology Inc. (Macedon, NY) (LIU, 2011), insoluble acid detergent fiber (ADF) and insoluble neutral detergent fiber (aNDF), with the addition of α -amylase, but without sodium sulfite, and acid detergent lignin (ADL) as described by Senger, et. al. (2008). The non-fiber carbohydrates (NFC) were estimated by the equation of Hall (2003).

The energy content of the diet was obtained from equations of NRC (2001) for digestible energy, metabolizable energy and net energy of lactation (NEL); the apparent digestibility was considered for energy calculations. The energy balance (EB) was estimated by NRC equations (2001).

Serum concentrations of glucose, total cholesterol and triglycerides were made by enzymatic colorimetric method, non-esterified fatty acids (NEFA) by enzymatic spectrophotometry, urea and the concentrations of the enzymes aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT) by kinetic enzymatic at a commercial laboratory.

For milk fatty acids profile, the crude fat was extract from milk samples by Bligh & Dyer method (1959) and the transesterification and methylation by Christie (1982). The fatty acid profile was determined according to Simionato et al. (2010). The identification of fatty acid methyl esters (Emag) was performed by comparing the retention times of the sample constituents, having used a mixture composed by 37Emag (24056, Supelco, 37 Component Fame Mix) and transvacenic acid methyl ester standard (18:1n7- t11) (46905-U, trans-11-octadecenoic methyl ester), with mixture of isomers of rumenic acid methyl ester (CLA, 18:2n7 - c9, t11) and t10,c12-octadecadienoico acid (CLA, 18:2n6 - t10,c12) (O5632, Linoleic acid, conjugated methyl ester).

Experimental design and statistical analysis

The experimental design was a double Latin square (4 x 4) with four treatments and four periods. Each animal was an experimental unit and the square was the repeated measure. It was used the SAS *Glimmix* procedure (SAS Institute Inc, 2013) followed the general linear mixed models methodology, with the choice of the distribution that would best fit the data. This decision was made using the corrected Akaike value. The analysis of variance followed the mathematical model:

$$Y_{ijkl} = \mu + \alpha_i + \alpha_j + p_k + s_l (\alpha \cdot p)_{ik} + \varepsilon_{ijkl}$$

Where: Y_{ijk} is the observation concerning the i-th treatment (α_i) in the j-th animal (α_j) of the k-th period (p_k) in the l-th square (s_l). Treatments were considered as fixed effect. The periods and the interaction treatments*periods as random effects. Data were submitted to analysis of variance and the treatment

effect was evaluated by F test with 5% of error probability. When significant, means were compared by the Tukey-Kramer test ($P = 0.05$).

Results

Apparent digestibility

The digestibility (Table 4) of DM, OM, and aNDF were not affected by treatments ($P > 0.05$). The CP digestibility was lower for SY treatment ($P = 0.0014$). Fat content digestibility was higher to CS treatment ($P = 0.0053$). The NFC was superior for SW ($P = 0.0435$) and lower in SW.

Production, milk composition and milk fatty acid profile, feed efficiency and energy balance

Milk yield (kg/d) was higher in CS in relation of SF and SY and the LI did not differ from the others (Table 5), however, milk yield corrected to energy (ECM), feed efficiency (FE) and energy balance were not affected by treatments ($P > 0.05$). The milk composition (g/kg) as well as individual and total solids was not affected by treatments, except for lactose production that was higher in CS treatment compared to SY, but not of the LI and SF.

In ascending order, the main fatty acids found in milk were C16:0 (palmitic acid), C18:1n9c (oleic acid) and C18:0 (stearic acid). Linoleic conjugate acid (CLA; C18:2c9t11) was superior ($P < 0.001$) in CS. When observed milk fatty acid profile, there was an increase influence by CS on C18:1n9t (elaidic acid), C18:2c9t11 (CLA-conjugate linoleic acid), C18:2t10c12, C18:1n11t (vacenic acid), C:182n6c (linoleic acid). For C16:0 (palmitic acid), milk fatty acid profile was lower ($P < 0.001$) in SF treatment and C:20 LI treatment ($P = 0.0017$). C18:0 (stearic acid) and C18:1n9c has superior values for SF treatment ($P = 0.003$ and $P = 0.0084$ respectively). There was no difference for saturated and polyunsaturated fatty acids (PUFA), however, monounsaturated fatty acids (MUFA) were higher for SF ($P = 0.0172$).

Biochemical blood profile

The use of different lipid sources in diets did not affect ($P > 0.05$) blood metabolic profile of animals (Table 6).

Discussion

Feed intake

There was no effect of treatment on DM, OM, CP, EE, aNDF and energy intake, because the diets were formulated to be similar in protein, energy and fiber. When added fat in the form of calcium salts in the diet of lactating cows, depressive effects on consumption can be observed (NRC, 2001), and are associated with low palatability, unsaturation and chain length of acids fatty, as well as metabolic effects to the release of cholecystokinin (Allen, 2000). None of these possible effects was observed in our study. Results about nutrient intake are varied, but Kennelly (1996) suggested that fat addition in ruminants

diets like whole oil seeds, proved to have less detrimental effect on dry matter intake, due to the slower release seed oil than the same amount, fed as free oil, resulting in no effect on dry matter intake. Petit et al. (2009) also observed that cows in early lactation fed with the highest level of linseed (150 g/kg of dry matter) did not reduce dry matter intake.

In studies with calcium salts of fatty acids, although there was a lower dry matter intake, Jenkins (1993) report that the energy intake did not fall because it increase in energy density or metabolic efficiency, without affecting milk production. Onetti & Grummer (2004) confirmed this hypothesis in a review at that the author compiled data from 41 trials that assessed supplementation from different sources and levels of fat to lactating cows. From 23 experiments evaluated, using calcium salts of fatty acids as the fat source, it observed a reduction in dry matter intake of 0.97 kg/d. However, when analyzing the milk production of 21 of these studies, the average milk production was increased by 1.29 kg/d. Thus, it is assumed that the net energy intake was maintained or increased with lipids supplementation. That is one of the main objectives of the use of fat in diets of lactating cows, to increase the energy density, particularly avoiding non-fiber carbohydrates, in order to avoid acidosis risks in high producing animals.

The lack of effects on feed intake confirms our hypothesis, that oilseeds can be used as lipid sources to replace commercial protected fat, probably because they are processed at different parts of digestive tract. Besides that, there was no effect on net energy intake, that depends how and where the diet is metabolized. Probably, the slow release of oil in the rumen, leads to a partial protection, as suggested by Kennelly (1996).

The use of oil like whole grains, especially LI and SY provided a significant reduction in soybean meal use at diet, major proteic ingredient of livestock feeding, which has a high cost when compared to grain used.

The lower intake of NFC in SF and SY was not enough to reduce milk yield corrected to energy and milk solids production, therefore the diets were appropriate.

Apparent Digestibility

The absence of differences in DM, OM and aNDF ensures that lipid sources LI, SF and SY not causes negative effects on the digestive process.

Other results about digestibility of nutrients in dairy cows are quite heterogeneous. Martin et al., (2008) observed reductions, while Gonthier et al. (2004) found increase and Doreau et al. (2009) not found effect.

The main determinants of these effects are the amount of lipids and how they are fed (free oil or seed). In our study, these two factors contributed to the results and the few differences observed, since the inclusion of fat from LI sources, SF and SY occurred at an intermediate level, less than 35 g/kg of DM intake, maintaining low ruminal availability.

The digestibility of protein was less in SY. This is associated with a higher protein inclusion coming from soybean grain. In the other treatments was used soybean meal, with higher protein digestibility. Another associated factor may be related to reduced digestibility of soybean seed, occurring loss in feces.

The CS showed higher digestibility of EE, probably because that is composed of fat free saponified that solubilizes widely in the abomasum (Naik, 2013). Chouinard et al. (1998) observed an increase in digestibility of DM, CP, and aNDF in diets with AG calcium salts when compared to a control diet. In LI, SF and SY, the way the fat is arranged in the grains, there may be some limitations, due to the presence of fibrous exospermas in grain, or the association of that fat to protein grain matrix (Ekeren et al., 1992), that can affect negatively both the access of ruminal microflora, as digestive enzymes in the small intestine. Supplementation with vegetable oil in free form (rich in unsaturated fatty acids) could be a trouble to ruminal fermentation, however when this is from oilseeds these disorders be reduced by slower fat release, without losses on nutrient digestibility (Coppock & Wilks, 1991), that seems to be confirmed in this study.

Production, milk composition and milk fatty acid profile, feed efficiency and energy balance

The higher milk production observed in CS may be related to the specific characteristics of this diet, because although it was not found effects of treatments on the digestibility of OM, the higher digestibility of EE and the highest intake of NFC may be responsible for increased lactose production, that has high correlation (0.9742; $r < 0.0001$) with milk production. However, it did not affect the energy corrected milk production and feed efficiency, that qualifies all diets as effective to input high energy densities and should be considered the availability. The costs of different oil sources may be important to decision about which will be used.

The absence of changes in milk fat and protein reinforces the theory of natural protection in whole grains as well as the uniformity of the diets because these milk components are variable, both as a result of dietary lipids, or microbial growth, either for lack of fermentable carbohydrates or deficiency in protein intake. The lower production of lactose observed in SY treatment may be related to reduced intake of NFC, however, is not considered a limiting factor, because no effects were observed in the ECM nor in solids.

The milk urea nitrogen (MUN) was above to the standards (10 - 16 mg/dL) proposed by Jonker et al. (1998) in LI and SF treatments, however, acceptable to the high protein levels of these diets (209 g/kg OM). So we can infer that the inclusion of lipids not depressed energy from NFC into rumen, with mean concentration of 368 g/kg OM.

Blood profile

No effects on blood profile ($P > 0.05$) confirmed the similarity of diets and the potential of lipid sources. Blood glucose (62.1 mg/dL), triglycerides (3.34 mg/dL) and NEFA (0.30 mmol/L) are consistent with the physiological values described by Kaneko et al. (2008).

The concentration of blood urea was 39.3 mg/dL, within the range 15-42 mg/dL described by Wittwer (2000), but lower than those described by Kaneko et al. (2008), that accept values between 42.8 and 64.3 mg/dL.

Serum cholesterol, although not reflected treatment differences, was higher than 120 mg/dL, described by Kaneko et al. (2008). The increase occurred in response to intake of high levels of lipids (Wittwer, 2000). Elliott et al. (1993), showed that the elevated cholesterol levels in diets with oil may be related to greater need to transportation of long chain fatty acids.

The NEFA means were 0.30 mmol/L (Table 6), that combined with energy balance data (Table 5) show that no BEN during the trial, due the effectiveness of the diets. The absence of effects on AST and GGT concentrations confirms that did not liver damage by the fat sources used.

It was observed that higher levels of CLA cis 9 trans 11 for CS, however, in the same way, also found, despite low, values of CLA cis 12 trans 10, its isomerized form. Several factors influence biohydrogenation in the rumen and can change the amount and composition of unsaturated fatty acids, both those destined for deposition in adipose tissue and those secreted in milk. Biohydrogenation can happen completely or with the formation of intermediate products, such as C18: 2c9t11, these compounds being absorbed in the ruminal walls and, in blood flow, can be absorbed by the mammary gland and incorporated into milk. Into the bloodstream, they can be absorbed by the mammary gland and incorporated into milk. However, when we observed the MUFA values, SY presented higher values than the other treatments. Unlike the other diets evaluated, where MUFA content was around 20% of the total fatty acids, SY presented values of 83.52%. This high content of MUFA in the composition of SY and its higher values in the final composition of milk compared to other diets evaluated, demonstrates that for total efficiency of biohydrogenation, a balance is needed between the fatty acid compounds present in the feed.

Conclusion

The replacement of fatty acid calcium salts for oilseeds such as soybeans, sunflower and linseed is possible within the levels used in this study, without negative effects on the production or animal health. The decision about the use these sources should reflect the market prices and availability. Sunflower showed higher levels of monounsaturated fatty acids in the final composition of the milk. Higher levels of CLA cis 9 trans 11 in milk were found when supplied with fatty acid calcium salts.

Declarations

Acknowledgment

The authors would like to thanks the Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

Funding

Not applicable

Conflicts of interest/Competing interests

Not applicable

Availability of data and material

Not applicable

Code availability

Not applicable

Authors' contributions

Conception and design of study: Schafhauser Junior J, Scheibler RB, Souza APB, Rizzo FA.

Acquisition of data: Souza APB, Rizzo FA, Scheibler, RB, Fluck AC, Vargas DP.

Analysis and/or interpretation of data: Souza APB, Schafhauser Junior J, Scheibler RB, Fluck AC.

Drafting the manuscript: Souza APB, Fluck AC.

Critical review/revision: Schafhauser Junior J, Nörnberg JL, Fluck AC.

Ethics approval

This study was approved by Ethics Committee on Animal Use of the Technologic Federal University of Paraná – Brazil, (number 09/2018)

Consent to participate

Not applicable

Consent for publication

Not applicable

References

1. Allen, MS 2000. Effects of diet on short-term regulation of feed intake by lactating dairy cattle. *Journal of Dairy Science*, 83:7, 1598–1624. DOI: 10.3168/jds.S0022-0302(00)75030-2.

2. Association of Official Analytical Chemists (AOAC), Official Methods of Analysis, 16th ed. AOAC, Washington, DC, USA, 1996.
3. Bligh EG, Dyer WJ 1959. A rapid method of total lipid extraction and purification. *Can Journal of Biochemistry and Physiology*, 37:8, 911–917. DOI: 10.1139/o59-099.
4. Chouinard, PY.; Girard, V.; Brisson, GJ. 1998. Fatty acid profile and physical properties of milk fat from cows fed calcium salts of fatty acids with varying unsaturation. *Journal of Dairy Science*, 81:2, 471–481. DOI: 10.3168/jds.S0022-0302(98)75599-7.
5. Coppock, CE; Wilks, DL. 1991. Supplemental fat in high energy rations for lactating cows: Effects on intake, digestion, milk yield, and composition. *Journal Animal Science*, 69:9, 3826–3837. DOI: 10.2527/1991.6993826x.
6. Doreau, M; Laverroux, S; Normand, J; Chesneau, G; Glasser, F. 2009. Effect of linseed fed as rolled seeds, extruded seeds or oil on fatty acid rumen metabolism and intestinal digestibility in cows. *Lipids*, 44:1, 53–62. DOI: 10.1007/s11745-008-3250-x.
7. Elliott, JP; Drackley, JK; Schauff, DJ; Jaster, EH. 1993. Diets containing high oil corn and tallow for dairy cows during early lactation. *Journal of Dairy Science*, 76:3, 775–789. DOI: 10.3168/jds.S0022-0302(93)77401-9.
8. Ekeren, PA; Smith, DR; Lunt, DK; Smith, SB. 1992. Ruminal biohydrogenation of fatty acids from high-oleate sunflower seeds. *Journal of Animal Science*, 70:8, 2574–2580. DOI: 10.2527/1992.7082574x.
9. George, E.; Shook, BS. 1993. Genetic improvement of mastitis through selection on somatic cell count. *Veterinary Clinics of North America: Food Animal Practice*, 9:3, 563–577. DOI: 10.1016/S0749-0720(15)30622-8.
10. Gonthier, C; Mustafa, AF; Berthiaume, R; Petit, HV; Martineau, R; Ouellet, DR. 2004. Effects of feeding micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. *Journal of Dairy Science*, 87:6, 1854–1863. DOI: 10.3168/jds.S0022-0302(04)73343-3.
11. Hall, MB. 2003. Challenges with non-fiber carbohydrate methods. *Journal of Animal Science*, 81:12, 3226–3232. DOI: 10.2527/2003.81123226x.
12. Jenkins, TC. 1993. Lipid metabolism in the rumen. *Journal of Dairy Science*, 76:12, p.3851–3863. DOI: 10.3168/jds.S0022-0302(93)77727-9.
13. Jenkins, TC. The benefits and limitations of fat in dairy rations. In: *Proceedings of the Mid-South Ruminant Nutrition Conference*. Texas, USA.
14. Jonker, JS; Kohn, RA; Erdman, RA. 1998. Using milk urea nitrogen to predict nitrogen excretion and utilization efficiency in lactating dairy cows. *Journal of Dairy Science*, 81:10, 2681–2692. DOI: 10.3168/jds.S0022-0302(98)75825-4.
15. Kaneko, JJ; Harvey, J; Bruss, M. *Clinical biochemistry of domestic animals*. Academic press, 6th ed. 2008.
16. Kennelly, JJ. 1996. The fatty acid composition of milk fat as influenced by feeding oilseeds. *Animal Feed Science and Technology*, 60:3, 137–152. DOI: 10.1016/0377-8401(96)00973-X.

17. Liu, K. 2011. Selected topics in the analysis of lipids: modification of an AOCS official method for crude oil content in distillers grains. Urbana: The AOCS Lipid Library.
18. Martin, C; Rouel, J.; Jouany, JP; Doreau, M; Chilliard, Y. 2008. Methane output and diet digestibility in response to feeding dairy cows crude linseed, extruded linseed, or linseed oil. *Journal of Animal Science*, 86:10, 2642–2650. DOI: 10.2527/jas.2007-0774.
19. Naik, PK. 2013. Bypass fat in dairy ration-a review. *Animal Nutrition Feed Technology*, 13:1, 147 – 63.
20. NRC - National Research Council.2001. 7.ed. Washington, DC.: National Academy of Sciences, 81p.
21. Onetti, SG; Grummer, RR. 2004. Response of lactating cows to three supplemental fat sources as affected by forage in the diet and stage of lactation: a meta-analysis of literature. *Animal Feed Science and Technology*, 115:1, 65–82. DOI: 10.1016/j.anifeedsci.2004.02.009.
22. Petit, HV; Gagnon, N. 2009. Milk concentrations of the mammalian lignans enterolactone and enterodiol, milk production, and whole tract digestibility of dairy cows fed diets containing different concentrations of flaxseed meal. *Animal Feed Science and Technology*, 152:1, 103–111. DOI: 10.1016/j.anifeedsci.2009.04.004.
23. Ruegg, PL; Milton, RL. 1995. Body condition scores of Holstein cows on Prince Edward Island, Canada: relationships with yield, reproductive performance, and disease. *Journal of Dairy Science*, 78:3, 52–564. DOI: 10.3168/jds.S0022-0302(95)76666-8.
24. Senger, CCD; Kozloski, GV; Sanchez, LMB; Mesquita, FR; Alves, TP; Castagnino, DS. 2008. Evaluation of autoclave procedures for fiber analysis in forage and concentrate feedstuffs. *Animal Feed Science and Technology*, 146:1–2, 169–174. DOI: 10.1016/j.anifeedsci.2007.12.008.
25. Sjaunja, LO; Baevre, L; Junkkarinen, L; Pedersen, Jørn. 1990. A Nordic proposal for an energy corrected milk (ECM) formula. In: *Proceedings of the 27th Session of International Committee of Recording and Productivity of Milk Animal*, France, 156–157.
26. SAS Institute Inc. 2013. SAS/STAT® 13.1 User's Guide. SAS Institute Inc., Cary, NC.
27. Wildman, EE; Jones, GM; Wagner, PE; Boman, RL; Troutt Jr, H.F.; Lesch, TN. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. *Journal of Dairy Science*, 65:3, 495–501. DOI: 10.3168/jds.S0022-0302(82)82223-6.
28. Wittwer, F. 2000. Diagnosis of metabolic energy imbalances in cattle herds. In: *González FHD, Barcellos JO, Ospina H, Ribeiro L. A metabolic profile in ruminants: its use in nutrition and nutritional diseases*. Porto Alegre: Brasil, 9–22.

Tables

Table 1. Diets composition

§ g/kg of dry matter; Calcium salts (CS)= Calcium salts of fatty acids (Megalac-E®); Linseed (LI), Sunflower (SF) and Soybean (SY)= Whole grains; Mineral-vitamin, Minimum Composition per kg: Ca-229g; P-95g; Mg-1.1g; Na-60g; S-12g; Vit. A-120.000 UI; Vit. D3-30.000 UI; Vit. E-1200 UI; Se-20g; Zn-3g; Lasalocid-1000mg.

Ingredients [§]	Treatment			
	CS	LI	SF	SY
Corn, silage	533.2	538.1	526.3	503.8
Concentrate	466.8	461.9	473.7	496.2
Calcium salts	29.7	-	-	-
Linseed	-	74.7	-	-
Sunflower	-	-	127.4	-
Soy bean	-	-	-	145.3
Corn, grain	144.2	124.4	127.1	151.6
Soybean meal	173.8	155.4	177.8	85.4
Wheat bran	96.3	75.2	9.3	82.0
Mineral-vitamin	11.9	14.9	15.3	15.2
Limestone	1.8	8.0	7.5	7.5
Alcamix ®	9.1	9.1	9.3	9.3

Table 2. Chemical composition of the diets

Diets				
Compounds	CS	LI	SF	SY
Dry Matter [*]	400.1	398.6	402.4	416.3
Organic Matter ^{**}	931.8	929.1	932.1	931.3
Crude Protein [§]	204.0	209.6	212.7	211.6
Crude fat ^{**}	61.1	62.9	63.6	62.3
aNDF ^{**}	333.5	333.4	333.3	333.5
NFC ^{**}	377.8	365.8	363.2	365.3
NEL ^{§§}	8.89	8.91	8.81	8.78
SFA ⁺⁺	44.46	12.66	6.43	19.76
MUFA ⁺⁺	23.19	22.26	83.52	20.45
PUFA ⁺⁺	32.35	65.08	10.05	59.79

* g/kg of Wet matter; **g/kg of dry matter; §g/kg de organic matter; §§ MJ/kg organic matter; ++ In proportion of total fatty acids. Calcium salts (CS)= Calcium salts of fatty acids (Megalac-E®); Linseed (LI), Sunflower (SF) and Soybean (SY)= Whole grains; aNDF= insoluble fiber in neutral detergent; NFC= non-fibrous carbohydrates; NEL= net energy of lactation; SFA=saturated fatty acid, MUFA=monounsaturated fatty acid; PUFA= polyunsaturated fatty acid.

Table 3. Voluntary intake of dietary components.

Variables	Treatment				SEM	p-value	§ kg/day; §§ g/kg of Body Weight; §§§ MJ/day; Calcium salts (CS)= Calcium salts of fatty acids (Megalac-E®); Linseed (LI), Sunflower (SF) and Soybean (SY)= Whole grains; aNDF= insoluble fiber in neutral detergent; NFC= non-fibrous carbohydrates; NEL= net energy of lactation;
	CS	LI	SF	SY			
Dry Matter§	16.15	16.08	15.74	15.81	0.45	0.8974	Different letters represent significant differences (P=0.05) between the means by Tukey-Kramer test.
Dry Matter§§	40.20	40.09	39.09	39.36	1.48	0.9392	
Organic Matter§	15.05	14.94	14.67	14.73	0.31	0.9159	
Organic Matter§§	37.47	37.25	36.44	36.67	0.07	0.9488	
aNDF §	5.03	5.00	4.90	4.93	0.1875	0.9685	
aNDF §§	12.54	12.42	12.15	12.25	0.0461	0.9621	
Crude Protein §	3.06	3.12	3.11	3.11	0.0440	0.8709	
Crude Fat §	0.91	0.94	0.93	0.91	0.0166	0.6179	
NFC §	5.68	5.46	5.32	5.37	0.1012	0.4392	
NEL §§§	150.63	149.59	148.31	147.82	2.37	0.7373	

Tukey-Kramer test.

Table 4. Effect of treatments on nutrients digestibility

§ g/kg of compound; Calcium salts (CS)= Calcium salts of fatty acids (Megalac-E®); Linseed (LI)= Sunflower (SF) and Soybean (SY)= Whole grains; aNDF= insoluble neutral detergent fiber; NFC= non-fibrous carbohydrates. SEM¹= Standard Error; Different letters represent significant differences (P=0.05) between the means by Tukey-Kramer test.

Table 5. Milk production and energy balance.

Calcium salts (CS)= Calcium salts of fatty acids (Megalac-E®); Linseed (LI), Sunflower (SF) and Soybean (SY)= Whole grains; §kg/day; §§=kg milk × ((383 Fat% + 242 Protein% + 165.4 Lactose% + 20.7) / 3140)

Variables [§]	Treatment				SEM ¹	P-value	(Sjaunja et al., 1990); [†] kg milk/kg dry matter; *g/kg; [‡] Somatic cell count *1000/mL; ^{††} MJ/day; SEM ¹ = Standard Error; Different letters represent significant differences (P=0.05) between the means by Tukey-Kramer
	CS	LI	SF	SY			
Dry Matter	780.70	773.80	760.19	763.06	10.45	0.4185	
Organic Matter	793.38	785.84	770.43	774.32	10.05	0.3170	
Protein	842.70 ^a	837.74 ^a	852.70 ^a	806.98 ^b	9.08	0.0014	
Fat	791.64 ^a	702.42 ^{ab}	671.79 ^b	663.09 ^b	29.89	0.0053	
aNDF	611.51	623.01	593.38	599.06	20.30	0.7347	
NFC	914.21 ^a	900.31 ^{ab}	883.79 ^b	916.15 ^a	1.30	0.0435	

test.

Table 6. Effect of dietary treatments on blood parameters

Calcium salts (CS), Calcium salts of fatty acids (Megalac-E[®]); Linseed (LI), Sunflower (SF) and Soybean (SY), Whole grains; [§]mg/dL; ^{§§}mmol/L; [‡]U/L; NEFA= non-esterified fatty acids; GGT= gamma-glutamyl transferase; AST= aspartate aminotransferase. SEM¹= Standard Error; Different letters represent significant differences (P=0.05) between the means by Tukey-Kramer test.

Table 7. Effect of treatments on milk fatty acid profile¹

Variables	Treatment				SEM ¹	P-value
	CS	LI	SF	SY		
Milk Production [§]	26.51	25.57	24.99	24.66	0.96	0.5616
Milk Production II ^{§§}	26.93	26.39	25.55	25.67	0.63	0.5323
Feed Efficiency [†]	1.67	1.65	1.63	1.63	0.05	0.8858
Energy balance ^{††}	20.82	21.83	19.67	16.44	4.45	0.7849
milk urea nitrogen [*]	145.75	161.67	166.33	165.17	11.90	0.5612
Somatic Cell Count [‡]	101.75	58.81	149.62	36.31	32.54	0.0930
Milk fat						
g/kg	42.07	42.20	42.40	43.65	1.67	0.8962
kg/day	1.11	1.08	1.05	1.07	0.04	0.7023
Milk protein						
g/kg	32.35	33.81	32.88	32.72	0.09	0.5630
kg/day	0.86	0.86	0.82	0.81	0.03	0.5867
Lactose						
g/kg	47.72	47.86	47.67	48.10	0.03	0.7002
kg/day	1.26	1.224	1.19	1.18	0.05	0.5831
Total solids						
g/kg	131.44	134.01	132.53	134.20	1.87	0.6958
kg/day	3.47	3.42	3.30	3.30	0.11	0.5727

Item	Treatment				SEM	p-value
	CS	LI	SF	SY		
Glucose [§]	61.81	63.06	62.00	61.50	2.27	0.9658
Triglycerides [§]	3.57	2.85	3.51	2.63	1.322	0.6732
Blood urea [§]	36.17	38.987	43.06	38.87	2.371	0.3592
Cholesterol [§]	184.83	170.68	160.40	173.22	7.911	0.3460
NEFA ^{§§}	0.27	0.28	0.35	0.29	0.047	0.3066
GGT [‡]	35.06	37.62	35.06	37.56	1.75	0.5671
AST [‡]	89.25	85.68	89.81	84.44	3.69	0.2287

Fatty acids	Treatments				SEM ¹	P value
	CS	LI	SF	SY		
C16:0	296.22a	321.48a	262.66b	320.69a	12.7	0.0001
C16:1n7	9.18	10.36	10.71	10.28	0.65	0.3720
C18:0	171.46b	150.00b	200.61a	166.28b	7.19	0.0003
C18:1n9t	4.46a	2.50b	3.65a	2.59b	0.25	0.0001
C18:2c9t11	7.07a	3.43b	4.09b	3.15b	0.40	0.0001
C18:2t10c12	0.47a	0.33b	0.35b	0.30b	0.02	0.0022
C18:3n6	0.89	0.79	1.01	0.77	0.98	0.2515
C18:1n11t	33.46a	10.45b	11.87b	9.63b	2.13	0.0001
C18:1n9c	197.60b	209.85ab	258.78a	202.76b	14.55	0.0084
C18:2n6c	24.65a	20.57ab	15.27b	24.32a	2.16	0.0024
C18:3n3	2.42b	7.54a	1.99b	2.76b	0.87	0.0004
C20:4n6	1.27	1.28	1.17	1.18	0.13	0.9167
C20:0	2.03a	1.63b	1.94a	2.00a	0.08	0.0017
SFA	735.58	732.53	691.65	739.11	19.45	0.1297
MUFA	218.39b	228.79ab	277.42a	221.70b	16.54	0.0172
PUFA	44.69	42.13	29.84	39.62	4.47	0.0537
Unsaturated	263.02	271.13	308.35	261.46	20.92	0.2314
≤16 C [§]	519.55a	560.15a	468.21b	551.72a	22.52	0.0020
≤17C ^{§§}	215.50a	172.12b	223.18a	187.07b	7.50	0.0001

Calcium salts (CS), Calcium salts of fatty acids (Megalac-E[®]); Linseed (LI), Sunflower (SF) and Soybean (SY), Whole grains. C16:0 = Palmitic Acid; C16:1n7 = Palmitoleic Acid; C18:0 = Estearic Acid; C18:1n9t = Elaidic Acid; C18:2c9t11: CLA cis 9 trans 11; C18:2t10c12: CLA cis 12 trans 10; C18:3n6: Ácido γ-linolênico; C18:1n11t= Ácido Vacênico, C18:1n9c= Oleic Acid, C18:2n6c = Linoleic Acid; C18:3n3: Alfa-linolenic Acid; C20:4n6 = Araquidonic Acid; C20:0= Ecosanoic Acid.

Different letters represent significant differences (P=0.05) between the means by Tukey-Kramer test. SEM¹= Standard Error; ⁵SFA = saturated fatty acids; ⁶PUFA = polyunsaturated fatty acids; ⁷CLA = conjugated linolenic fatty acids. [§]Σ Fatty acids with less than 16 Carbons; ^{§§}Σ Fatty acids with more than 17 Carbons.

¹In proportion of total fatty acids.