SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2024v45n2p329

# Sunflower oil supplementation in the diets of lactating cows: productive and nutritional performance

# Suplementação com óleo de girassol em dietas de vacas em lactação: parâmetros zootécnicos

Fábio Antunes Rizzo[1]; Ana Carolina Fluck[2]*; Jorge Schafhäuser Junior[3]; Rudolf Brand Scheibler[2]; José Laerte Nörnberg[4]; Diego Prado de Vargas[5]; Lívia Argoud Lourenço[2]; Ana Paula Binato de Souza[6]; Jamir Luís Silva da Silva[3]

## Highlights

Sunflower oil in its free form can be used as an energy source for lactating cows.
It is necessary to use feed-strategies to high up the energy level up to 110g/kg DM.
Chopped alfalfa hay absorbed part of sunflower oil reducing its negative effects.

## Abstract

Eight Jersey cows (2nd-4th lactation; 483 ± 43 kg body weight; milk yield 21±2.2 kg day$^{-1}$) were used in a double 4x4 latin square design to evaluate whether the inclusion of increasing levels of sunflower oil in the concentrate up to the limit of 110 g kg$^{-1}$ DM of EE, in replacement to corn grain, has an impact on milk production and composition, feed efficiency, energy balance, intake and digestibility of diets from Jersey cows. The treatments consisted in lipid supplementation with increasing levels of sunflower oil replacing the corn grain and wheat bran of concentrate, including: CD (control diet), without sunflower oil and with 38 g kg$^{-1}$ of EE dry matter (DM); and three treatmens with sunflower oil concentrate-included: SF65= 65 g kg$^{-1}$ DM of EE; SF86=86 g kg$^{-1}$ DM of EE and SF110=110 g kg$^{-1}$ DM of EE. The increase of EE in diets did not affect the dry matter intake, neutral detergent fiber or crude protein. There was a linear increase in the digestibility coefficients for organic matter, crude protein, EE, neutral detergent fiber

[1] PhD in Animal Science, Center of Veterinary Medicine, Universidade de Caxias do Sul, UCS, Caxias do Sul, RS, Brazil. E-mail: rizzo.fabioantunes@gmail.com
[2] PhD. in Animal Science, Postgraduate Program in Animal Science, Faculdade Eliseu Maciel, Universidade Federal de Pelotas, UFPel, Capão do Leão, RS, Brazil. E-mail: anacarolinafluck@yahoo.com.br; rudolf_brand@hotmail.com; liviargoud@gmail.com
[3] PhD. in Animal Science, Researcher at Empresa Brasileira de Pesquisa Agropecuária, EMBRAPA, Pelotas, RS, Brazil. E-mail: jorge.junior@embrapa.br; jamir.silvadasilva@gmail.com
[4] PhD. in Animal Science, Food Science and Technology Department, Universidade Federal de Santa Maria, UFSM, Santa Maria, RS, Brazil. E-mail: jlnornberg@gmail.com
[5] PhD. in Food Science and Technology, Center of Veterinary Medicine, Universidade de Santa Cruz do Sul, UNISC, Santa Cruz do Sul, RS, Brazil. E-mail: diegodevargas@hotmail.com
[6] PhD. in Biotechnology, Postgraduate Program in Biotechnology, Universidade do Vale do Taquari, UNIVATES, Lajeado, RS, Brazil. E-mail anapaulabinato@gmail.com
* Author for correspondence

Received: June 27, 2023 Approved: Feb. 23, 2024

and blood levels of cholesterol. Treatments did not affect milk production, fat content, lactose or total solids. It is possible to use up to a limit of 110 g $kg^{-1}$ DM of EE in the diet, decreasing the concentrate total amount.
**Key words:** Blood metabolic profile. Digestibility. *Helianthus annus.* High-fat diet. Intake.

## Resumo

Oito vacas Jersey (2ª-4ª lactação; peso corporal 443 ± 63 kg; produção de leite, 21±2.2 kg dia/dia) foram utilizadas em delineamento duplo quadrado latino 4x4 para avaliar se a inclusão de níveis crescentes de óleo de girassol no concentrado até o limite de 110 g $kg^{-1}$ DM of EE, em substituição ao milho, tem impacto sobre a produção e composição do leite, eficiência alimentar, balanço energético, consumo e digestibilidade das dietas, e o perfil de ácidos graxos da gordura do leite de vacas da raça Jersey. Para a dieta controle não houve inclusão de óleo de girassol, com concentração de extrato etéreo (EE) de 37.6 g $kg^{-1}$ MS entre a mistura de concentrado e volumoso, os demais tratamentos consistiram na inclusão de níveis crescentes de óleo de girassol nas concentrações 65.3; 85.6 ou 110 g $kg^{-1}$ de EE na MS. O aumento do EE nas dietas não afetou o consumo de matéria seca, fibra em detergente neutro ou proteína bruta. Houve aumento linear nos coeficientes de digestibilidade para matéria orgânica, proteína bruta, EE, fibra em detergente neutro e níveis sanguíneos de colesterol. Os tratamentos não afetaram a produção de leite, teor de gordura, lactose ou sólidos totais. É possível utilizar até o limite de 110 g $kg^{-1}$ de EE na dieta em substituição ao grão de milho, diminuindo a quantidade de concentrado ingerido.
**Palavras-chave:** Perfil metabólico sanguíneo. Digestibilidade. *Helianthus annus.* Dieta rica em gordura. Consumo.

## Introduction

The increased genetic merit of dairy cows has been associated with high nutritional requirements, necessitating an increased inclusion of feeds rich in non-fibrous carbohydrates. It is known that high levels of lipids in concentrate in the diet (particularly with the fiber diet) can modify the ruminal pH, which affects the rumen microflora, the fermentation process, and decreases degradability, leading to metabolic disorders, like acidosis (Elmhadi et al., 2022; Ramos et al., 2021). Alternatively, additional lipids can be supplemented as a higher-energy diet concentrate, replacing carbohydrate sources, increasing the energy density of the diet.

Due to the use of lipid sources of animal origin being prohibited in Brazil and the high cost of purchasing commercial inputs intended for this purpose (such as calcium salts of fatty acids), alternative sources of lipid supplementation are constantly researched, such as oils vegetables (Souza et al., 2023; Kliem et al., 2017; Meignan et al., 2017), however, much of this research has focused on evaluating the modification of the fatty acid profile in milk, not paying attention to the actual performance of the animal when the lipid levels appropriate for lactating cow diets are exceeded. The vegetable oils most used today in ruminant feeding due to their greater availability and mainly due to the costs of their use are soybean, linseed, sunflower, and canola oils, whose usage

costs are favorable when compared to the cost of commercial protected fats available on the market.

According to National Research Council [NRC] (2001), the supplementation of lipids in the diets of dairy cows should not exceed 60 to 70 g $kg^{-1}$ of dry matter intake (DMI), since larger amounts can result in a reduction in total consumption (Bionaz et al., 2020) and therefore may reduce or even eliminate the advantages of lipid addition. Among the available lipid sources, the use of supplemental vegetable oil should not exceed 20 to 30 g $kg^{-1}$ of diet dry matter (DM) to prevent the negative effects on dry matter intake, ruminal fermentation and digestibility, milk production, and net energy intake (Panahiha et al., 2022). The results obtained using oil as a free form supplementation have been controversial, reflecting variations in the roughage source, shape and daily frequency of feeding, concentrate composition, and mineral levels in the diet, particularly calcium.

Despite presenting negative impacts, the use of energy sources with high lipid density is considered an alternative source of replacing carbohydrate sources in the ruminant diet, increasing the energy concentration of the diet (C. S. Silva et al., 2023). In tropical countries, such as Brazil, high temperatures, linked with the reduction in dry matter intake of lactating cows, directly impacts the milk production of these animals. In this way, to replace a traditional energy source of carbohydrates, such as corn, to vegetable oils, ensures that the animal intake a greater energy supply, even when there is a reduction in intake.

However, the lipid supplementation not always leads to a greater milk production. There are situations in which the DM intake is reduced by the addition of free fat and the increase in energy intake from a lipid source does not prove to be compensatory. In this sense, we evaluated if the inclusion of increasing levels of free-form sunflower oil in the concentrate up to the limit of 110 g $kg^{-1}$ DM of EE, in replacement of corn, has an impact on milk production and composition, feed efficiency, energy balance, intake and digestibility of diets, and the fatty acid profile of milk fat from Jersey cows

## Materials and Methods

### *Location, animals, treatments and experimental design*

The experiment was carried out at the Temperate Climate Agricultural and Livestock Research Center of the Brazilian Agricultural Research Corporation (EMBRAPA), in South of Brazil (31º 52' 20" south latitude and 52º 21' 24" west longitude, Rio Grande do Sul state (RS), Brazil). The study was carried out upon approval by the Ethics Committee on Animal Use of the Federal University of Pelotas, Brazil, (case n° 4847).

Eight lactating multiparous Jersey cows ($2^{nd}$-$4^{th}$ lactation, 90± 5 days in milk, 483 ± 43 kg body weight; milk yield, 21±2.2 kg $day^{-1}$) were used in a latin square design. The animals were individually housed in a free-stall barn. The treatments consisted of increasing the levels of dietary supplementation with sunflower oil. The diets (Table 1) were formulated considering the chemical composition of the ingredients (Table 2), providing similar daily quantities (kg) of crude protein (CP), net energy for lactation (NEl) and neutral detergent insoluble fiber

(NDF), and were tested in a performance simulator (NRC, 2001). Treatments consisted in a supplementation with increasing levels of sunflower oil replacing the corn grain and wheat bran of concentrate, including: CD (control diet), without sunflower oil and with 38 g ether extract (EE)/kg dry matter (DM); and three treatments with sunflower oil included in the concentrate meal as SF65= 65 g EE/kg DM; SF86=86 g EE/kg DM; and SF110=110 g EE/kg DM. A supplementary calcium source, dicalcium phosphate was added to the SF65, SF86 and SF110 diets supplemented with sunflower oil at a daily ratio of 28, 56 and 84 g/day, on a DM basis, respectively.

**Table 1**
**Ingredients and chemical composition of the experimental diets (g kg$^{-1}$ DM)**

| Ingredients | Treatments | | | |
|---|---|---|---|---|
| | CD | SF65 | SF86 | SF110 |
| **Forage**[A] | 499.8 | 541.7 | 584.4 | 601.7 |
| **Concentrate** | 500.2 | 458.3 | 415.6 | 398.3 |
| Corn, grain | 298.3 | 245.0 | 170.4 | 132.1 |
| Wheat, bran | 99.9 | 81.9 | 67.8 | 37.3 |
| Soybean, meal | 87.4 | 89.9 | 110.6 | 132.9 |
| Mineral-vitamin[B] | 17.0 | 17.0 | 17.0 | 17.0 |
| Dicalcium phosphate | 5.6 | 5.6 | 5.6 | 5.6 |
| Sunflower oil | - | 25.0 | 48.5 | 73.3 |
| Experimental diets chemical composition | | | | |
| Item* | Treatments | | | |
| | CD | SF65 | SF86 | SF110 |
| EDMI | 18.00 | 16.70 | 15.91 | 15.30 |
| NDF | 6.5 | 6.5 | 6.3 | 6.2 |
| CP | 3.0 | 2.8 | 2.9 | 2.8 |
| EE | 0.5 | 0.9 | 1.2 | 1.5 |
| NFC | 7.4 | 6.6 | 5.7 | 5.0 |
| Ca | 0.18 | 0.18 | 0.18 | 0.18 |
| NEL (MJ day$^{-1}$) | 118.2 | 120.8 | 119.0 | 117.9 |

CD= 38 g EE kg$^{-1}$ DM, without sunflower oil, SF65= 6G g EE kg$^{-1}$ DM, SF86=86 g EE kg$^{-1}$ DM, SF110 = 110 g EE kg$^{-1}$ DM, with inclusion of sunflower oil for energy foods replacement, A: Mixture of corn silage and chopped alfalfa hay, at a ratio of 1/1 in dry matter basis, B Minimum Composition per kg: Ca-230 g, P-95 g, Mg-1.1 g, Na-60 g, S-12 g, Vit. A-120.000 UI, Vit. D3-30.000 UI, Vit. E-1200 UI, Se-20 g, Zn-3 g, Lasalocid-1000
*EDMI, estimated dry matter intake, aNDF, neutral detergent fibre, using a heat stable amylase and corrected for ash, CP, crude protein, EE, ether extract, NFC, carbohydrates non - fibre, Ca, calcium, NEl, net energy of lactation.

**Table 2**
**Effects of increasing levels of sunflower oil on the voluntary intake and apparent digestibility**

| Item | Treatment | | | | SEM | *P*-value | |
|---|---|---|---|---|---|---|---|
| | CD | SF65 | SF86 | SF110 | | Linear | Quadratic |
| Intake | | | | | | | |
| DM (kg day$^{-1}$) | 17.4 | 17.2 | 17.1 | 16.1 | 0.332 | 0.253 | 0.606 |
| DM (g kg$^{-1}$ of BW) | 42.4$^{a}$ | 42.0$^{ab}$ | 41.2$^{ab}$ | 39.1$^{b}$ | 0.078 | 0.053 | 0.459 |
| DMConc (kg day$^{-1}$) | 8.71 | 7.89 | 7.11 | 6.41 | - | *** | - |
| DMFor (kg day$^{-1}$) | 8.70 | 9.32 | 10.0 | 9.69 | 0.332 | 0.299 | 0.543 |
| For:Conc (g kg$^{-1}$ of DM) | 494 | 543 | 586 | 593 | 0.784 | <0.001 | 0.105 |
| OM (kg day$^{-1}$) | 16.2$^{a}$ | 16.0$^{a}$ | 15.8$^{ab}$ | 14.8$^{b}$ | 0.296 | 0.164 | 0.593 |
| aNDFom (kg day$^{-1}$) | 5.96 | 6.03 | 6.06 | 5.59 | 0.233 | 0.564 | 0.518 |
| aNDFom (g kg$^{-1}$ of BW) | 14.9$^{a}$ | 15.2$^{ab}$ | 14.4$^{ab}$ | 13.5$^{b}$ | 0.049 | 0.104 | 0.101 |
| CP (kg day$^{-1}$) | 3.04 | 2.97 | 3.12 | 2.88 | 0.079 | 0.512 | 0.459 |
| EE(kg day$^{-1}$) | 0.66 | 1.05 | 1.45 | 1.74 | 0.023 | <0.001 | 0.179 |
| NFC (kg day$^{-1}$) | 6.63 | 5.98 | 5.31 | 4.44 | 0.148 | <0.001 | 0.510 |
| Ca (kg day$^{-1}$) | 0.16 | 0.17 | 0.17 | 0.17 | 0.373 | 0.521 | 0.428 |
| NEl (MJ day$^{-1}$) | 133.1 | 139.7 | 152.7 | 146.9 | 0.902 | 0.003 | 0.141 |
| Digestibility | | | | | | | |
| DM (kg day$^{-1}$) | 69.1 | 70.4 | 71.8 | 71.3 | 1.557 | 0.255 | 0.569 |
| OM (kg day$^{-1}$) | 71.5 | 72.7 | 74.4 | 76.1 | 1.383 | 0.018 | 0.939 |
| CP (kg day$^{-1}$) | 70.2 | 72.3 | 75.4 | 76.9 | 1.486 | <0.001 | 0.699 |
| EE (kg day$^{-1}$) | 68.1 | 82.9 | 87.5 | 89.9 | 2.132 | <0.001 | 0.011 |
| aNDFom (kg day$^{-1}$) | 54.4 | 57.8 | 59.8 | 62.2 | 1.792 | 0.003 | 0.842 |
| NFC (kg day$^{-1}$) | 88.7 | 89.6 | 87.3 | 84.8 | 0.847 | <0.001 | 0.046 |

CD= 38 g EE kg$^{-1}$ DM, without sunflower oil, SF65= 65 g EE kg$^{-1}$ DM, SF82=86 g EE kg$^{-1}$ DM, SF110 = 110 g EE kg$^{-1}$ DM, with inclusion of sunflower oil for energy foods replacement. DMConc, dry matter intake of concentrate, DMFor, dry matter intake of forage, For:Conc, roughage:concentrate ratio, OM, organic matter, aNDFon, neutral detergent fibre corrected for ash, CP, crude protein, EE, ether extract, NFC, carbohydrates non fibre, Ca, calcium, NEl, net energy of lactation, SEM, standard error of means. $^{a,b}$ Values within a row with different superscripts differ significantly at $p<0.05$ in ANOVA.
DM Conc. (kg/day) = 9.8691 - 0.0317x, $R^2$=0.9982
NFC (kg/day) = 7.7935-0.0298x, $R^2$= 0.9916
For:Conc (g/kg of DM) = 45.565 + 0.1388x, $R^2$=0.8549
EE (kg/day) = 0.1086+0.0151x, $R^2$=0.9969
NEl (MJ/day) = 126.32+0.2213x, $R^2$=0.7300
DMI, with inclusion of sunflower oil for energy foods replacement.
OM = 69.0860+0.0616x, $R^2$=0.9975
CP = 66.5327+0.0975x, $R^2$=0.9825
EE = 59.7492+0.2969x, $R^2$=0.8891
aNDFom = 50.6664+0.1077x, $R^2$=0.9893
NFC = 91.8631-0.0582x, $R^2$=0.7370.

The roughage portion consisted of corn silage and chopped alfalfa hay with a particle size of approximately 3-4 cm, at a 1/1 ratio, provided twice daily, enabling *ad libitum* intake and 10% leftovers, which were collected and sampled daily. The cows were pre-adapted to the management for 20 days before the experiment and each experimental period was conducted over 15 days, with a 10-day adaptation and a 5-day measurement period (Scheibler et al., 2015). Milk samples of two consecutive milkings (morning and afternoon) of all experimental animals were collected on days 11$^{th}$ to 15$^{th}$ of each period and mixed according to milk production.

The concentrate ration containing sunflower oil was provided 3 times a day (at 08:00; 14:00 and 20:00 h). All diets were supplemented with an additional source of calcium (dicalcium phosphate) to provide concentrations higher in relation to the estimated calcium need. The roughage portion of the diets was provided separately at a 1/1 ratio of dry matter composed of corn silage and chopped alfalfa hay, with a 3-5 cm particle size, offered *ad libitum* twice a day, with 10% of DM leftover.

### *Measurements and analytical methods*

The cows were adapted to the trial diet for 20 days prior to the beginning of the experiment. During the five days of data collection, immediately after the morning milking, the cows were weighed within a balance precision of 100 g, with an average used as the animal weight for that period. The roughage orts were removed and weighed daily during milking in the morning, prior to the new meal and during each collection period, and the samples were stored in a freezer (-20 °C). Silage and hay samples were collected at each sampling period for each cow. To estimate fecal excretion, 10 g of chromium oxide ($Cr2O_3$) was provided in a tablet twice a day after milking and before the new diet supply (5 g in the morning and 5 g in the afternoon) with forced intake, starting seven days before the start of fecal sampling until the morning of the fifth day of each experimental period. Fecal samples were collected during five days from each experimental period, twice a day before milking, through voluntary evacuation or directly from the rectum using gloves. The samples were placed in plastic bags, identified, and stored in the freezer. At the end of each sampling period, the samples were mixed in equal proportions, forming a composite sample per period for each cow.

The dry matter (DM) by Method 967.03, and mineral matter by method 942.05 (Association of Official Analytical Chemistry [AOAC], 2019). Organic matter (OM) was estimated by the equation 1000-MM., crude protein (CP) content was assayed indirectly by N content according to methods 984.13 and 2001.11 (AOAC, 2019). Acid-insoluble detergent fiber corrected for ash (ADFom), insoluble neutral detergent fiber corrected for ash (aNDFom) with added α-amylase but without the use of sulfite, and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991). The ether extract content (EE) was evaluated in filter bags system, developed by Ankom Technology Inc. (Macedon, NY). The calcium (Ca) and chromium (Cr) contents were determined by atomic absorption spectrophotometry (D. J. Silva & Queiroz, 2002).

Dry matter intake and the dry matter constituents OM, CP, aNDFom, EE and NFC (kg/day) were determined as the difference between the roughage offer and orts, assuming concentrate afforded total intake. Fecal production estimates (PFT) were obtained with respect to the fecal concentrations of chromium using the equation (Silva & Queiroz, 2002):

PFT = (g Cr supplied / day) / (g Cr / g fecal DM)

The digestibility coefficients for dry matter, organic matter, ether extract, non-fibrous carbohydrates and true insoluble neutral detergent fiber corrected for ash were determined for the experimental diets.

The energy content of the diet was obtained using NRC (2001) equations for digestible energy (DE), metabolizable energy (ME), and net energy of lactation (NEL):

DE (Mcal/kg) = 0,04409*NDT (%)

ME (Mcal/kg) = 1,01*ED (mcal/kg) - 0,45

NEL 3x (Mcal/kg) = 0,0245*NDT (%) - 0,12

The apparent digestibility coefficients were considered for energy calculations. The energy balance (EB) was determined using the equations proposed by NRC (2001).

### *Milk production and composition*

The cows were milked twice a day at 7:00 and 19:00 h in a double 4×4 piped milking machine equipped with an automatic milk meter, with results expressed in kilogram of milk per milking. Milk samples of two consecutive milking (morning and afternoon) of all experimental animals were collected on the sixteenth and seventeenth experimental days and mixed according to milk production. Individual milk samples were collected during the morning and afternoon milking period, mixed in proportion to production, cooled to 5° C, and sent to the laboratory for the estimation of milk content variables: fat, protein, lactose, and total solids were determined by infrared spectroscopy using method 972.16 of AOAC (Cunniff, 1996).

To estimate the energy-corrected milk, milk production was adjusted for energy using the equation described by Sjaunja et al. (1990):

Milk yield = (kg milk × (((383×fat% + (242×protein%) + (165.4×lactose%) + 20.7)/3,14)

### *Biochemical blood profile*

Blood samples were taken at 14$^{th}$ to 15$^{th}$ days of each experimental period, immediately after the morning milking, by venipuncture of the jugular vein. The samples were kept at rest for 10 min, centrifuged at 7871 RCF for 10 min immediately after and placed in isothermal box, and sent for analysis at commercial laboratory. The blood parameters evaluated were glucose, triglycerides, blood urea, cholesterol, non-esterified free fatty acid (NEFA), gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST). The analysis of blood glucose, triglycerides, and total cholesterol was performed using an automated colorimetric enzymatic method an NEFA was determined through enzymatic spectrophotometric, blood urea was determined using automated kinetic methods, and AST and GGT was determined using an automated kinetic enzymatic method.

### *Statistical procedures*

The statistical analysis was performed using the SAS® application - Statistical Analysis System version 9.0 (Statistical Analysis System Institute [SAS Institute], 2013). The data were subjected to ANOVA based on the GLM procedure in a double latin square design (4 × 4), with four treatments and four periods, where each animal was considered an experimental unit, distributed by milk production and parity date, considering a cow as a random effect, with the following statistical model:

$$Yijkl = \mu + Qi + Tj + Pk + QTij + A(i)l + eijk,$$

where Yijkl = average values for each observation; μ = general average of the variable in the experiment; Qi = square effect, where i = 1 and 2; Tj = j effect of treatment, with j = 1, 2, 3 and 4; Pk = effect of period, with k = l, 2, 3 and 4; = QTij interaction between treatment square ie j; A () = l i cow effect within the i square; and eijk. = Experimental error.

The treatment means were adjusted by ordinary least squares with the LSMEANS command. After identifying significant treatments by ANOVA in the fixed effects model, regression analysis was performed. When no effect is shown in the regression analysis, means were compared by Tukey's test at 95% significance.

## Results and Discussion

No effects from the diets were observed for intake of DM, aNDFom, Ca and CP. However, a smaller DM, aNDn and OM were observed when sunflower oil was added in higher amounts (Table 2). Linear reductions were observed for the NFC and concentrate intake but increased linearly with roughage:concentrate ratios and energy as well as the EE intake. Differences in DMI with respect to body weight reflects the higher energy concentration in these diets, as roughage intake did not differ (Table 2). The concentrate volume after oil addition showed linear changes in the roughage:concentrate ratio, reaching 60:40 in treatments with greater oil inclusion. Similar results were obtained for the OM intake and the replacement of corn and wheat bran with sunflower oil, at the same level of energy input, did not show the same volumetric ratio.

Higher levels of EE may generate negative effects on rumen fermentation (Oliveira et al., 2021) and the digestibility of nutrients, particularly fiber (Kaltenegger et al., 2021). Changes in cellulolytic population and protozoa in the rumen microflora were observed (Matthews et al., 2019; Newbold et al., 2015); however, we propose that this effect was negligible since the forage intake did not differ. Neutral detergent fiber intake was similar, but the aNDFom with respect to body weight was reduced with increased oil inclusion following DM and OM intake, even in similar diets that had the same levels of fiber. The observation that fat supplementation decreased NDF intake because of lower fiber digestibility and slower adherence and attachment of rumen microorganisms was not consistent with the results of other studies using sunflower oil as lipid supplementation (Salles et al., 2019) or even other vegetables oil-source such as soybean oil (Barletta et al., 2016), linseed oil (Benchaar et al., 2015; Suksombat et al., 2014).

Although the NFC intake differed, this effect did not influence energy intake. Similarly, this effect did not influence bacterial

growth and fiber fermentation, most likely because NFC levels were adequate. Even though the diets were similar in energy intake, it is likely that the DM, CP and aNDFom intake as well as increasing EE intake contributed to the overall increase in the net energy intake (MJ $d^{-1}$). According to Williams et al. (2021), fat supplementation either maintains or increases the energy intake if the total DM intake is not significantly reduced, as observed in the present study.

Formulation factors mitigated the adverse effects of high levels of oil in free form, like the use of chopped alfalfa hay can absorbed part of the vegetable oil, minimizing its negative effects (Panahiha et al., 2022). Mohammadabadi et al. (2021), in an *in vitro* assay, reported that diets where the only source of roughage was corn silage showed a larger decrease in intake compared with diets containing 25 and 50% alfalfa hay. Another factor is particle size. Chopped forage increased the flow, thereby contributing to the maintenance of forage intake levels. Coppock and Wilks (1991) proposed that the DM intake level influences the reactivity of free fat in the rumen, and animals consuming 40 g DM $kg^{-1}$ BW or more have higher digestion flow rates, thereby reducing the negative effects of free fat over the ruminal activity.

The high availability of Ca in the diet has promoted saponification in part of the oil in free form, aiding in rumen fermentation. This is particularly true for fibers since its limiting for fixing microbial colonization onto food particles. The analysis of the intake profiles suggested that sunflower oil supplementation promotes the intake of less acidogenic diets and increases daily energy intake (MJ $day^{-1}$), despite slight reductions in the DMI with respect to body weight.

Diets with sunflower oil inclusion resulted in a linear increase in digestibility of OM, CP, EE and aNDFom but reduced linearly in the digestibility of NFC (Table 2). The linear increase in CP digestibility (Table 2) may be associated with differences in the digestibility of concentrate constituents (NRC, 2001), as the use of soybean meal increased, and the use of wheat bran decreased with the increasing use of sunflower oil. Newbold et al. (2015) reported that diets when using vegetable oils, mainly soybean and sunflower, decrease the protozoa population and this can be linked to the protozoa through lower bacterial predation. Also, Vargas et al. (2020) cited that diets using vegetable oil as lipid supplement manipulate the microbial community and the rumen fermentation, which will directly impact digestibility.

The increase in EE digestibility likely reflects the complete lipid availability of vegetable oil in the small intestine and intestinal absorption and the increased digestibility of unsaturated fatty acids, such as sunflower fat (Bionaz et al., 2020). Another studies (Vargas et al., 2020; Freitas et al., 2018; Vargas-Bello-Pérez et al., 2016) describe that large amounts of vegetable oil in the ruminant diet can impair rumen fermentation. However, it is thought that the synergistic effects and diet associations promote an improvement in rumen fermentation through a higher share of roughage in DMI. In this less acidogenic environment, combined with increased calcium availability, aNDFom digestibility is improved with increased level of ether extract by sunflower oil inclusion.

Lima et al. (2014) reported no negative effects on DM, OM and NDF digestibility when using dietary lipid supplements as opposed to Chamberlain and Peters (2016)

that observed a decreased in the OM intake and digestibility. However, Frank et al. (2022), evaluating the relationship between different levels of calcium salts of palm oil and supplementation and oil levels, described that increasing calcium levels decreased depression in DM digestibility through increased oil levels in the diet.

Milk yield, milk yield corrected for energy, fat content and daily fat production, lactose, and total solids were not affected by the treatments. The milk protein content showed a linear decrease, while daily production was lower only in the treatment with the largest oil inclusion. The lactose concentration was lower in the treatment with more oil inclusion. Changes in protein and lactose concentrations reduced the significant effects on total solids content. Higher levels of sunflower oil did not affect milk yield; however, oil supplementation positively influenced the feed efficiency (Table 3). These highlight the importance of proper nutritional arrangement in the diet and suggest the use of alternative foods to reduce the adverse effects of oil in free form supplementation. The use of high levels of oil to replace carbohydrates induced a linear increase in the daily energy intake and energy balance (Table 2).

**Table 3**
**Effect of diets containing increasing levels of sunflower oil on milk yield (MY), energy corrected milk (ECM) , milk composition, feed efficiency (FE) and energy balance (EB)**

| Item | Treatment | | | | SEM | *P*-value | |
|---|---|---|---|---|---|---|---|
| | CD | SF65 | SF86 | SF110 | | Linear | Quadratic |
| MY (kg day$^{-1}$) | 23.4 | 24.2 | 24.7 | 23.3 | 0.378 | 0.981 | 0.355 |
| ECM (kg day$^{-1}$) | 22.82 | 22.98 | 23.50 | 22.40 | 0.678 | 0,865 | 0,542 |
| Milk Fat (g kg$^{-1}$) | 3.68 | 3.60 | 3.51 | 3.42 | 0.162 | 0,274 | 0,961 |
| Milk Fat (kg day$^{-1}$) | 0.86 | 0.86 | 0.89 | 0.86 | 0.052 | 0,908 | 0,695 |
| Milk Protein (g/kg) | 3.48 | 3.34 | 3.34 | 3.26 | 0.051 | 0,007 | 0,666 |
| Milk Protein (kg day$^{-1}$) | 0.81$^{a}$ | 0.81$^{a}$ | 0.81$^{a}$ | 0.77b | 0.010 | 0,306 | 0,351 |
| Lactose (g kg$^{-1}$) | 4.76$^{a}$ | 4.70$^{a}$ | 4.73$^{a}$ | 4.66b | 0.019 | 0,063 | 0,885 |
| Lactose (kg day$^{-1}$) | 1.11 | 1.14 | 1.16 | 1.10 | 0.017 | 0,980 | 0,458 |
| Total solids (g kg$^{-1}$) | 12.94 | 12.61 | 12.51 | 12.22 | 0.189 | 0,035 | 0,969 |
| Total solids (kg day$^{-1}$) | 3.03 | 3.06 | 3.07 | 2.99 | 0.065 | 0,708 | 0,417 |
| FE (kg milk kg$^{-1}$ DM) | 1.35$^{b}$ | 1.43$^{a}$ | 1.45$^{a}$ | 1.45$^{a}$ | 0.025 | 0.108 | 0.259 |
| EB (MJ day$^{-1}$) | 29.29 | 38.07 | 46.44 | 48.11 | 0.913 | 0.002 | 0.398 |

CD= 38 g EE kg$^{-1}$ DM, without sunflower oil, SF65= 65 g EE kg$^{-1}$ DM, SF82=86 g EE kg$^{-1}$ DM, SF110 = 110 g EE kg$^{-1}$ DM, with inclusion of sunflower oil for energy foods replacement, $^{a,b}$ Values within a row with different superscripts differ significantly at $p<0.05$ in ANOVA, MY, milk ield, ECM - energy corrected milk = kg milk × ((383 fat% + 242 protein% + 165.4 lactose% + 20.7)/3140) (Sjaunja et al., 1990).
Milk Protein (g/kg) = 3,55383-0,026282x, $R^2$= 0,8200
Total solids (g/kg) = 13,28348-0,097669x, $R^2$= 0,9567
Energy Balance (MJ / day) = 19.8125+0.2719x, $R^2$=0.9418.

Distinct authors cited a concerning and divergent results about milk production using unsaturated oils. There are reports of increased milk production (Rodrigues et al., 2019; Ye et al., 2009), no effects on milk production (Benchaar et al., 2015) or decreased milk production (Rodrigues et al., 2019). These variations are associated with the effects of dietary Ca content, roughage source, lactation age and animal's production.

The absence of effects on fat production and content are probably due to incorporation of dietary fatty acids directly into milk fat (Ungerfeld et al., 2019) and the shorter metabolic route to fat synthesis at the mammary gland may be associated with the absence of effects on the milk yield. In this sense, the improvement in forage to concentrate ratio reached by the inclusion of oil and reduction of non-fibrous carbohydrates could improve the rumen environment, avoiding depression in milk fat synthesis.

The reduction in NFC content with a higher proportion of forage consumed, especially in treatments with greater inclusion of oil, could improve the rumen environment and growth of cellulolytic bacteria, keeping milk fat content and milk fat production stable. Moreover, the reduction in intake and digestibility of NFC by removal of corn and wheat bran resulted in less energy available to the rumen bacteria. In this situation, dietary protein can be used as energy source by microorganisms. These results explain the increase in serum levels of plasma urea (Table 4) and the reduction in the microbial source of amino acids for milk protein synthesis.

**Table 4**
**Effect of increasing levels of sunflower oil on biochemical blood profile**

| Item | Treatment | | | | SEM | *P*-value | |
|---|---|---|---|---|---|---|---|
| | CD | SF65 | SF86 | SF110 | | Linear | Quadratic |
| Glucose (mg $dL^{-1}$) | 63.6 | 62.7 | 63.3 | 63.5 | 1.192 | 0.785 | 0,408 |
| Triglycerides (mg $dL^{-1}$) | 3.56 | 3.33 | 3.06 | 3.63 | 0.360 | 0,888 | 0,390 |
| Blood urea (mg $dL^{-1}$) | 42.3 | 43.1 | 46.1 | 48.8 | 1.959 | 0.037 | 0,751 |
| Total cholesterol (mg $dL^{-1}$) | 167.5 | 203.9 | 224.3 | 227.9 | 6.054 | <0.001 | 0,120 |
| NEFA (mmol $L^{-1}$) | 0.271 | 0.291 | 0.301 | 0.351 | 0.019 | 0,090 | 0,791 |
| AST (U $L^{-1}$) | 100.1 | 101.1 | 114.6 | 108.0 | 5.889 | 0,267 | 0,398 |
| GGT (U $L^{-1}$) | 38.7 | 39.4 | 42.6 | 41.0 | 2.325 | 0,990 | 0,657 |

CD= 38 g EE $kg^{-1}$ DM, without sunflower oil, SF65= 65 g EE $kg^{-1}$ DM, SF82=86 g EE $kg^{-1}$ DM, SF110 = 110 g EE $kg^{-1}$ DM, with inclusion of sunflower oil for energy foods replacement. NEFA = non-esterified fatty acids, AST = aspartate aminotransferase, GGT = gamma-glutamyl transferase, EE = ether extract, DMI = dry matter intake.
Total cholesterol = 140,0179+0,9351x, $R^2$=0,8889
Blood urea = 38.5122+0.0905x, $R^2$=0.9773.

Increasing linear effects for blood concentrations of urea and total cholesterol were observed. The other evaluated parameters were not affected (Table 4). The levels of serum glucose, triglycerides and NEFA showed no differences, which was consistent with previous results examining physiological profiles in dairy cows (Kaneko et al., 2008). As describe by Hubner et al. (2022), the hypoglycemic conditions of dairy cows are associated with the occurrence of ketosis and energy deficiencies, but this was not observed in the present study.

The blood urea concentration increased because the digestibility of crude protein reduced availability and digestibility of NFC, which increased the concentration of ruminal ammonia. Total cholesterol increased linearly with increasing ether extract in the diet, reflecting the increased synthesis and cholesterol demand for the digestion, absorption, and transport of lipids (Bionaz et al., 2020). These results are consistent with previous study involving lipid supplementation with vegetable oil carried out by Oliveira et al. (2021), which included soybean or linseed oil in Holstein diets and describe cholesterol near to 200mg $dL^{-1}$ for both treatments, exceeding the upper limit of 120 mg $dL^{-1}$ suggested by Kaneko et al. (2008).

The serum concentrations of AST did not differ between treatments and was within the physiological profile previously described by Kaneko et al. (2008). Similarly, the serum concentrations of gamma glutamyl transferase GGT did not differ between treatments (Table 4); however, the average of 40.41 U $L^{-1}$ was higher than the values reported by Oliveira et al. (2021) under similar conditions. Despite this finding, the absence of differences between the treatments tested in the present study assumes that the animals supplemented with fat during lactation did not experience significant changes in the liver tissue.

## Conclusions

The increased energy density reduced the daily amount of concentrate to be supplied and improved feed efficiency without negatively affecting intake, digestibility, or performance, being possible to use up to a limit of 110 g $kg^{-1}$ of EE in total dry matter in the diet of Jersey dairy cows from supplementation with sunflower oil, in replacement to corn grain.

## References

Association of Official Analytical Chemistry (2019). *Official methods of analysis* (21nd ed.). AOAC.

Barletta, R. V., Gandra, J. R., Bettero, V. P., Araújo, C. E., Del Valle, T. A., Almeida, G. F., Jesus, E. F., Mingoti, R. D., Benevento, B. C., Freitas, J. E., Jr., & Rennó, F. P. (2016). Ruminal biohydrogenation and abomasal flow of fatty acids in lactating cows: Oilseed provides ruminal protection for fatty acids. *Animal Feed Science and Technology, 219*(2016), 111-121. doi: 10.1016/j.anifeedsci.2016.06.011

Benchaar, C., Hassanat, F., Martineau, R., & Gervais, R. (2015). Linseed oil supplementation to dairy cows fed diets based on red clover silage or corn silage: Effects on methane production, rumen fermentation, nutrient digestibility, N balance, and milk production. *Journal of*

*Dairy Science, 98*(11), 7993-8008. doi: 10.3168/jds.2015-9398

Bionaz, M., Vargas-Bello-Pérez, E., & Busato, S. (2020). Advances in fatty acids nutrition in dairy cows: from gut to cells and effects on performance. *Journal of Animal Science and Biotechnology, 11*(1), 110. doi: 10.1186/s40104-020-00512-8

Chamberlain, M. B., & Peters, E. J. de. (2016). Impacts of feeding lipid supplements high in palmitic acid or stearic acid on performance of lactating dairy cows. *Journal of Applied Animal Research, 45*(1), 126-135. doi: 10.1080/09712119.2015.1124327

Coppock, C. E., & Wilks, D. L. (1991). Supplemental fat in high-energy rations for lactating cows: effects on intake, digestion, milk yield, and composition. *Journal of Animal Science, 69*(9), 3826-3837. doi: 10.2527/1991.6993826x

Cunniff, P. (1996). *Official methods of analysis.* 15th ed. (2nd rev.). Association of Official Analytical Chemistry, Gaithersburg, Maryland.

Elmhadi, M. E., Ali, D. K., Khogali, M. K., & Wang, H. (2022). Subacute ruminal acidosis in dairy herds: Microbiological and nutritional causes, consequences, and prevention strategies. *Animal Nutrition, 10*(2022), 148-155. doi: 10.1016/j.aninu.2021.12.008

Frank, E., Livshitz, L., Portnick, Y., Kamer, H., Alon, T., & Moallem, U. (2022). The effects of high-fat diets from calcium salts of palm oil on milk yields, rumen environment, and digestibility of high-yielding dairy cows fed low-forage diet. *Animals, 12*(16), 2081. doi: 10.3390/ani12162081

Freitas, J. E., Jr., Takiya, C. S., Del Valle, T. A., Barletta, R. V., Venturelli, B. C., Vendramini, T. H. A., Mingoti, R. D., Calomeni, G. D., Gardinal, R., Gandra, J. R., Bettero, V. P., Ferreira de Jesus, E., Oliveira, M. D. S., & Rennó, F. P. (2018). Ruminal biohydrogenation and abomasal flow of fatty acids in lactating cows fed diets supplemented with soybean oil, whole soybeans, or calcium salts of fatty acids. *Journal of Dairy Science, 101*(9), 7881-7891. doi: 10.3168/jds.2017-13666

Hubner, A., Canisso, I. F., Peixoto, P. M., Coelho, W. M., Jr., Ribeiro, L., Aldridge, B. M., Menta, P., Machado, V. S., & Lima, F. S. (2022). Characterization of metabolic profile, health, milk production, and reproductive outcomes of dairy cows diagnosed with concurrent hyperketonemia and hypoglycemia. *Journal of Dairy Science, 105*(11), 9054-9069. doi: 10.3168/jds.2021-21327

Kaltenegger, A., Humer, E., Pacífico, C., & Zebeli, Q. (2021). Feeding dairy cows bakery by-products enhanced nutrient digestibility but affected fecal microbial composition and pH in a dose-dependent manner. *Journal of Dairy Science, 104*(7), 7781-7793. doi: 10.3168/jds.2020-19998

Kaneko, J. J., Harvey, J. W., & Bruss, M. L. (2008). *Clinical biochemistry of domestic animals* (6nd ed.). Academic Press.

Kliem, K. E., Humphries, D. J., Reynolds, C. K., Morgan, R., & Givens, D. I. (2017). Effect of oilseed type on milk fatty acid composition of individual cows, and also bulk tank milk fatty acid composition from commercial farms. *Animal, 11*(2), 354-364. doi: 10.1017/S1751731116001403

Lima, L. S., Santos, G. T., Schogor, A. B., Damasceno, J. C., Marchi, F. E., Santos, N. W., Santos, F. S., & Petit, H. V. (2014). Effect of abomasal or ruminal supplementation of citrus pulp and soybean oil on nutrient digestibility and ruminal fermentation of dairy cows. *Animal Feed Science and Technology, 189*(2014), 123-129. doi: 10.1016/j.anifeedsci.2013.12.008

Matthews, C., Crispie, F., Lewis, E., Reid, M., O'Toole, P. W., & Cotter, P. D. (2019). The rumen microbiome: a crucial consideration when optimizing milk and meat production and nitrogen utilization efficiency. *Gut Microbes, 10*(2), 1-18. doi: 10.1080/19490976.2018.1505176

Meignan, T., Lechartier, C., Chesneau, G., Bareille, N. (2017). Effects of feeding extruded linseed on production performance and milk fatty acid profile in dairy cows: a meta-analysis. *Journal of Dairy Science, 100*(6), 4394-4408. doi: 10.3168/jds.2016-11850

Mohammadabadi, T. T., Chaji, M., Direkvandi, E., & Alqaisi, O. (2021). O. Effect of replacing alfalfa hay with Leucaena leucocephala (*L. Leucocephala*) leaves on in vitro gas production, digestibility and *in situ* degradability in buffalo. *Acta Scientiarum: Animal Science, 43*, e52129. doi: 10.4025/actascianimsci.v43i1.52129

Newbold, C. J., De La Fuente, G., Belanche, A., Ramos-Morales, E., & McEwan, N. R. (2015). The role of ciliate protozoa in the rumen. *Frontiers in Microbiology, 6*, 1313. doi: 10.3389/fmicb.2015.01313

National Research Council (2001). *Nutrient requirements of dairy cattle* (7nd rev. ed.). National Academy of Science.

Oliveira, M. X. S., Palma, A. S. V., Reis, B. R., Franco, C. S. R., Marconi, A. P. S., Shiozaki, F. A., Reis, L. G., Salles, M. S. V., & Netto, A. S. (2021). Inclusion of soybean and linseed oils in the diet of lactating dairy cows makes the milk fatty acid profile nutritionally healthier for the human diet. *PLoS One, 16*(2), e0246357. doi: 10.1371/journal.pone.0246357

Panahiha, P., Mirzaei-Alamoutl, H., Kazemi-Bonchenari, M., & Aschenbach, J. R. (2022). Growth performance, nutrient digestibility, and ruminal fermentation of dairy calves fed starter diets with alfalfa hay versus corn silage as forage and soybean oil versus palm fatty acids as fat source. *Journal of Dairy Science, 105*(12), 9597-9609. doi: 10.3168/jds.2022-22165

Ramos, S. C., Jeong, C. D., Mamuad, L. L., Kim, S. H., Kang, S. H., Kim, E. T. D., Cho, Y. I., Lee, S. S., & Lee, S. S. (2021). Diet Transition from high-forage to high-concentrate alters rumen bacterial community composition, epithelial transcriptomes and ruminal fermentation parameters in dairy cows. *Animals, 11*(3), 838. doi: 10.3390/ani11030838

Rodrigues, J. P. P., Paula, R. M. de, Rennó, L. N., Costa, G. P., Hamade, V. C. E., Valadares, S. C., Fº., Rennó, F. P., & Marcondes, M. I. (2019). Effects of soybean oil supplementation on performance, digestion and metabolism of early lactation dairy cows fed sugarcane-based diets. *Animal, 13*(6), 1198-1207. doi: 10.1017/s1751731118002781

Salles, M., D'Abreu, L., Roma Júnior, L., Jr., César, M., Guimarães, J., Segura, J. G., Rodrigues, C., Zanetti, M. A., Pfrimer, K., &

Netto, A. (2019). Inclusion of sunflower oil in the bovine diet improves milk nutritional profile. *Nutrients, 11*(2), 481. doi: 10.3390/nu11020481

Scheibler, R.B., Schafhauser Junior, J., Rizzo, F.A., Nörnberg, J.L., Vargas, D.P., Silva, J.L.S., Fluck, A.C. & Fioreze, V.I. (2015). Replacement of corn grain by brown rice grain in dairy cow rations: Nutritional and productive effects, *Animal Feed Science and Technology, 208* (2015), 214-219. doi: 10.1016/j.anifeedsci.2015.07.013

Statistical Analysis System Institute (2013). *SAS/STAT® 13. 1 User's guide.* SAS Institute Inc.

Silva, C. S., Gama, M. A. S., Silva, E. A. M., Ribeiro, E. F., Souza, F. G., Monteiro, C. C. F., Mora-Luna, R. E., Oliveira, J. C. V., Santos, D. C., & Ferreira, M. D. A (2023). Nutritional quality of milk fat from cows fed full-fat corn germ in diets containing cactus opuntia and sugarcane bagasse as forage sources. *Animals, 13*(4), 568. doi: 10.3390/ani13040568

Silva, D. J., & Queiroz, A. C. de. (2002). *Análise de alimentos: métodos químicos e biológicos.* UFV.

Sjaunja, L. O., Baevre, L., Junkkarinen, L., & Pedersen, J. A. (1990). Nordic proposal for an energy corrected milk (ECM) formula. *Proceedings of the Session of International Committee of Recording and Productivity of Milk Animal,* Paris, France, 27.

Souza, A. P. B., Schafhauser, J., Jr., Scheibler, R. B., Fluck, A. C., Rizzo, F. A., Nörnberg, J. L., Vargas, D. P., & Lourenço, L. A. (2023). Supplementation with lipid sources in diets for Jersey cows in the first third of lactation. *Pesquisa Agropecuária Brasileira, 58*(1), e03149. doi: 10.1590/S1678-3921.pab2023.v58.03149

Suksombat, W., Than, L. P., Meeprom, C., & Mirattanaphrai, R. (2014). Effects of linseed oil or whole linseed supplementation on performance and milk Fatty Acid composition of lactating dairy cows. *Asian-Australasian Journal of Animal Science, 27*(7), 951-959. doi: 10.5713/ajas.2013.13665

Van Soest, P. J., Robertson, J. B., & Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *Journal of Dairy Science, 74*(10), 3583-3597. doi: 10.3168/jds.S0022-0302(91)78551-2

Vargas, J. E., Andrés, S., López-Ferreras, L., & López, S. (2020). Effects of supplemental plant oils on rumen bacterial community profile and digesta fatty acid composition in a continuous culture system (RUSITEC). *Anaerobe, 61*(2020), 102143. doi: 10.1016/j.anaerobe.2019.102143

Vargas-Bello-Pérez, E., Cancino-Padilla, N., Romero, J., & Garnsworthy, P. C. (2016). Quantitative analysis of ruminal bacterial populations involved in lipid metabolism in dairy cows fed different vegetable oils. *Animal, 10*(11), 1821-1828. doi: 10.1017/s1751731116000756

Ungerfeld, E. M., Urrutia, N. L., Vásconez-Montúfar, C., & Morales, R. (2019). Factors associated with the content of mammary-synthesized fatty acids in milk fat: a meta-analysis. *Journal of Dairy Science, 102*(5), 4105-4117. doi: 10.3168/jds.2018-15157

Williams, S. R. O., Milner, T. C., Garner, J. B., Moate, P. J., Jacobs, J. L., Hannah, M. C., Wales, W. J., & Marett, L. C. (2021). Dietary fat and betaine supplements offered to lactating cows affect dry matter intake, milk production and body temperature responses to an acute heat challenge. *Animals, 11*(11), 3110. doi: 10.3390/ani11113110

Ye, J. A., Wang, C., Wang, H. F., Ye, H. W., Wang, B. X., Liu, H. Y., Wang, Y. M., Yang, Z. Q., & Liu, J. X. (2009). Milk production and fatty acid profile of dairy cows supplemented with flaxseed oil, soybean oil, or extruded soybeans. *Acta Agriculturae Scandinavica - Section A, 59*(2), 121-129. doi: 10.1080/09064700903082252