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A meta-analysis to determine the optimal values of precursors in the diet of dairy cows that increase the concentration of conjugated linoleic acid (CLA) in milk

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ABSTRACT. The concentration of conjugated linoleic acid (CLA) in dairy products derived from ruminants, such as cow's milk, is mainly influenced by the dietary lipid profile. The current study aimed to establish the relationship between fatty acid (FA) profiles in various diets and the concentration of CLA in bovine milk using a meta-analysis. A total of 63 articles were selected, comprising 35 studies involving oilseeds, 13 – fish oil, and 15 – grass as the sole food source. A general linear model was constructed for each ingredient and CLA concentration was optimised with respect to the FA that showed the greatest effect (C18:0, C18:2 and C18:3) in the model. The relationship between FA concentrations in the diets, presentation method (seeds and pasture), type (seeds), and basal diet (fish oil), and the CLA content in milk was assessed using analysis of covariance, followed by Tukey's test (P = 0.05). It has been demonstrated that an increase in the total concentration of C18:0, C18:2, and C18:3 in the diets does not necessarily guarantee a higher CLA level in milk. This phenomenon is attributed to ruminal FA metabolism and metabolic processes within the animal itself. Consequently, it can be concluded that a quadratic relationship exists between the concentration of dietary FAs and the concentration of CLA in milk.

Introduction

Bovine milk's lipid fraction contains conjugated linoleic acid (CLA), a fatty acid (FA) known for its protective effects against the development of certain chronic diseases (Castro et al., 2019). Likewise, the consumption of bovine milk and its derivatives contributes to a reduction in the incidence of cardiovascular diseases, with a 13% decrease in the risk of overweight or obesity (Gagliostro et al., 2018; Sánchez et al., 2020). CLA is formed in the process of biohydrogenation that occurs in the rumen. During this process, the bacterium *Butyrivibrio fibrisolvens* transforms linoleic acid (C18:2) and α-linolenic acid (C18:3) into C18:2 cis-9 trans-11 isomers. Simultaneously, the enzyme Δ-9 desaturase eliminates hydrogen atoms via desaturation (Purba et al., 2020), primarily utilising stearic acid (C18:0) as its substrate to convert it into oleic acid (C18:1) (Gómez-Cortés et al., 2019). As a result, this process accounts for the production of 70–95% of C18:2 cis-9 trans-11 present in milk (Purba et al., 2020).

The CLA content in milk is influenced by intrinsic factors related to the animal, such as breed, number of lactations and lactation period (Flores et al., 2011; Roca-Fernández et al., 2014; Acosta-Balcazar et al., 2022). According to Prieto-Manrique et al. (2016), the quantity of CLA in milk tend to increase as the days in lactation progress. In contrast, Kliem and Shingfield (2016) have argued that the highest production of CLA occurs in the first third of the lactation period. However, Mojica-Rodríguez et al. (2019) have reported that CLA production in milk is not influenced by the lactation status, contradicting the findings of Acosta-Balcazar et al. (2022).

Furthermore, CLA levels can be affected by external factors associated with the animal. In this regard, feed is the primary factor influencing milk FA composition (Granados-Rivera et al., 2017). The source of nutrition (forage species, preserved forages, concentrate-based diets, oilseeds, vegetable oils and fish oil) provides the dietary supply of polyunsaturated FAs, which modify the rate and completeness of the biohydrogenation process (Castro-Hernández et al., 2014; Muruz and Çetinkaya, 2019).

Due to the protective effects of CLA, extensive research efforts have been dedicated over the years to increase its content in milk. This has been attempted through the incorporation of lipid supplements like oilseeds, vegetable oils and marine-derived fats such as fish oil into the diets of dairy animals, thereby elevating the intake of CLA precursors (Prieto-Manrique et al., 2016). Oilseeds and vegetable oils are added to the cows' diets not only to increase their energy intake but also to improve the efficiency of milk fat synthesis. The extent to which these supplements increase CLA levels in milk depends on factors such as the species, form of treatment, amount and interaction with the type of basal diet (Sterk et al., 2012; Prieto-Manrique et al., 2016).

On the other hand, the use of fish oil enhances reproductive performance and can improve the nutritional value of milk fat (Juchem et al., 2008). Fish oil has demonstrated the ability to increase trans vaccenic acid (TVA) production by more than 100%, despite its low linoleic and α-linolenic contents (<3%). Simultaneously, the oil has been shown to stimulate CLA production from linoleic acid and α-linolenic acid contained in other ingredients incorporated into various diets used in milk production (AbuGhazaleh et al., 2002).

Similarly, the consumption of fresh forage can increase CLA levels in milk, because it provides a higher quantity of linoleic and α-linolenic acid to the cows' diet (Walker et al., 2004; Aguilar et al., 2009; Ortega-Pérez et al., 2013). In fact, these fatty acids make up approximately 95% of the lipid fraction found in pasture forage (Acosta-Balcazar et al., 2022). The content of these precursors is affected by various factors, such as plant species and variety, climate, light intensity, precipitation, fertilisation or growth stage (Kalač and Samková, 2010).

Nevertheless, the extent to which the FA profile, especially the concentration of precursors, in the diet affects CLA concentration in milk remains uncertain. Therefore, the objective of the present work was to analyse the results of different studies that have employed supplements in dairy cow diets, including oilseeds, fish oil and grazing to ascertain the relationship between the FA profile of these diets and the concentration of CLA in milk. Additionally, this study aimed to determine the requisite amount of precursors needed to enhance CLA levels, employing a meta-analysis approach.

Material and methods

Search strategy

Scientific articles were searched, focusing on evaluating the effects of oilseeds, fish oil, and grass consumption as a strategy to improve the fatty acid profile of milk and increase its CLA content.

The platforms Google Scholar, Web of Science and ScienceDirect were used for this search. Keywords used for article retrieval included: 'oilseeds', 'fish oil'. 'grazing', 'diet', 'CLA', 'fatty acids', and 'cow milk'.

Selection criteria

The selection criteria for articles were as follows: (i) the study had to exclusively concern bovine milk; (ii) the work had to report the lipid profile of experimental diets (treatments) and milk; (iii) the reported FAs in the diets had to include C18:0, C18:2 and C18:3 (stearic acid, linoleic acid and α-linolenic acid); (iv) the unit of measurement for the lipid profiles of the diet and milk had to be in g/100 g FAs; and (v) in the milk lipid profile, the reported CLA content had to include C18:2 cis-9 trans-11 isomer. If the article reported total CLA instead of the C18:2 cis-9 trans-11 isomer, it was determined that 92% of the total CLA was the C18:2 cis-9 trans-11 isomer, following the procedure of Siurana and Calsamiglia (2016).

Database

A total of 63 articles published from 2000 to 2020 were selected, covering 139 experiments. Among these studies experiments, 35 articles involved the use of oilseeds, 13 – utilised fish oil, and 15 – mentioned grazing as the exclusive feeding source. The collected experimental data included the number of animals, days in lactation, experiment duration (days), basal diet (for oilseeds and fish oil), the content of C18:0, C18:2 and C18:3 (stearic acid, linoleic acid and α-linolenic acid) in the diet or pasture, and C18:2 cis-9 trans-11 isomer (CLA) content in milk. For studies involving oilseeds, their type and form were also considered, and the type of pasture for grazing experiments. The selection process is presented in PRISMA in Figure 1, and the studies included in this meta-analysis are listed in Table 1.

Data encoding

The 35 articles that focused on oilseeds comprised a total of 90 experiments involving 605 animals, which were categorised in two ways: A) type of oilseed (1. flaxseed n = 161, 2. soybean n = 178, 3. sunflower n = 106, 4. canola n = 44, 5. rapeseed n = 45, 6. cotton n = 31, and 7. peanut n = 40); B) presentation (1. oil n = 397, 2. pressed seed n = 57, 3. raw or whole seed n = 49, and 4. processed seed n = 102; including ground, roasted, paste and flour forms). For fish oil, the 13 studies included 23 experiments with a total of 123 animals, categorised based on the basal diet (1. maize silage or alfalfa hay n = 64, 2. processed maize n = 33, and 3. oat, soy, and barley n = 26). The 15 articles related to grazing included 26 experiments with a total of 312 animals and were categorised solely by pasture type (1. grasses n = 194, and 2. combination of grasses and legumes n = 118).

Table 1. Studies selected for meta-analysis

No.	Ingredient and presentation	Study	No.	Ingredient and presentation	Study
1	Extruded soybeans	AbuGhazaleh et al., 2002	33	Roasted sunflower seed	Sarrazin et al., 2004
2	Extruded soybeans, soybean oil	Allred et al., 2006	34	Canola oil	Vafa et al., 2012
3	Extruded soybeans	Bailoni et al., 2004	35	Canola oil	Welter et al., 2016
4	Soybean oil, whole raw soybean	Barletta et al., 2016	36	Fish oil	AbuGhazaleh et al., 2002
5	Linseed oil	Benchaar et al., 2012	37	Fish oil	AbuGhazaleh et al., 2007
6	Whole flaxseed	Caroprese et al., 2010	38	Fish oil	Allred et al., 2006
7	Whole cottonseed	Castaño et al., 2014	39	Fish oil	Bharathan et al., 2008
8	Linseed oil, soybean oil	Castro et al., 2019	40	Fish oil	Brown et al., 2008
9	Extruded soybeans, canola seed, whole cottonseed	Chen et al., 2008	41	Fish oil	Caroprese et al., 2010
10	Canola seed	Chichlowski et al., 2005	42	Fish oil	Donovan et al., 2000
11	Extruded linseed, linseed oil, whole crude linseed	Chilliard et al., 2009	43	Fish oil	Gulatti et al., 2003
12	Sunflower oil	Cruz-Hernández et al., 2007	44	Fish oil	Pirondini et al., 2015
13	Raw soybeans, roasted soybeans, soybean oil, linseed oil	Dhiman et al., 2000	45	Fish oil	Rego et al., 2005b
14	Ground rapeseed, extruded linseed	Egger et al., 2007	46	Fish oil	Toth et al., 2019
15	Roasted soybean, extruded soybean	Fatahnia et al., 2018	47	Fish oil	Vafa et al., 2012
16	Linseed oil	Flowers et al., 2008	48	Fish oil	Whitlock et al., 2006
17	Whole roasted flaxseed, cracked roasted soybean	Gao et al., 2009	49	Grasses	Bargo et al., 2006
18	Peanut cake	Giacomazza-Cerutti et al., 2016	50	Grasses	Corazzin et al., 2019
19	Rapeseed oil, sunflower-seed oil, camelina-seed oil, camelina expeller	Halmemies-Beauchet-Filleau et al., 2011	51	Grasses and legumes	Flores et al., 2011
20	Rapeseed meal, rapeseed crushed, rapeseed oil	Hoffmann et al., 2016	52	Grasses and legumes	Kay et al., 2004
21	Rapeseed oil, soybean oil, linseed oil	Jacobs et al., 2011	53	Grasses and legumes	Kay et al., 2005
22	Sunflower oil	Kay et al., 2004	54	Grasses and legumes	Kay et al., 2006
23	Rapeseed cake, extruded soyabean	Kudrna and Marounek, 2006	55	Grasses and legumes	Kay et al., 2007
24	Whole sunflower seed, extruded linseed	Kudrna and Marounek, 2008	56	Grasses and legumes	Khanal et al., 2007
25	Canola oil and soybean oil	Loor and Herbein, 2003	57	Grasses and legumes	Mackle et al., 2003
26	Whole rapeseed, whole cottonseed, whole linseed	Muñoz et al., 2019	58	Grasses and legumes	Nantapo et al., 2014
27	Extruded canola	Neves et al., 2009	59	Grasses	Plata-Reyes et al., 2018
28	Rubber seed oil, flaxseed oil	Pi et al., 2016	60	Grasses	Prieto-Manrique et al., 2016
29	Sunflower oil, soybean oil	Rego et al., 2005a	61	Grasses and legumes	Rego et al., 2004
30	Rapeseed oil, sunflower oil, linseed oil	Rego et al., 2009	62	Grasses	Vibart et al., 2017
31	Whole cottonseed	Reveneau et al., 2005	63	Grasses	Ward et al., 2003
32	Sunflower oil, linseed oil	Roy et al., 2006			

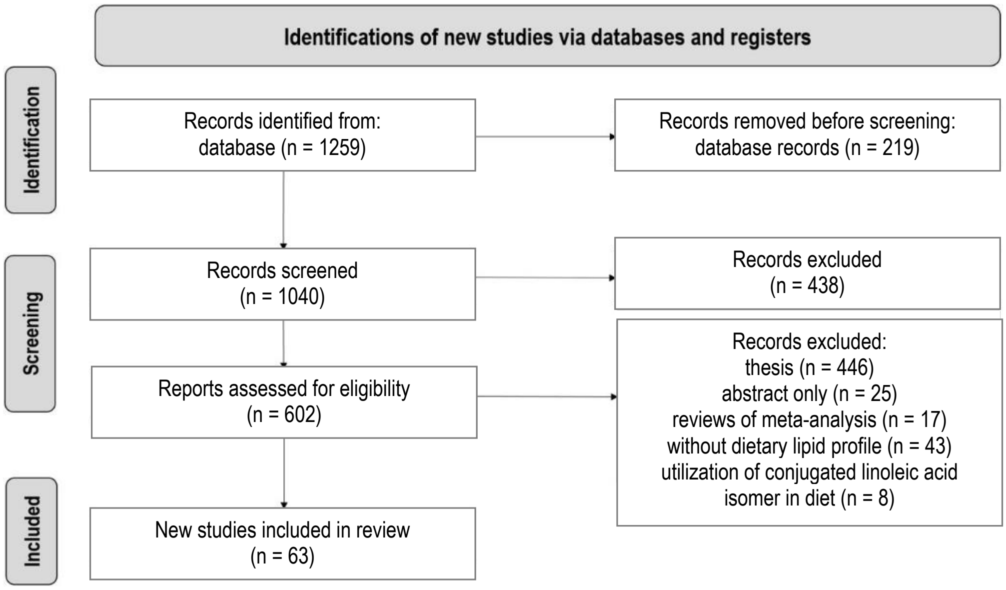

Figure 1. Flowchart of systematic review (PRISMA) from initial search to selection of articles included in this meta-analysis

$$\begin{aligned} CLA_{ijkl} = {} & \beta_{0jkl} + \beta_1 d_i + \beta_2 t_i + \beta_3 t_i^2 + \beta_4 C18{:}0_i + \beta_5 C18{:}2_i + \beta_6 C18{:}3_i + \beta_7 C18{:}0_i^2 + \beta_8 C18{:}2_i^2 + \\ & \beta_9 C18{:}3_i^2 + \beta_{10} t_i C18{:}0_i + \beta_{11} t_i C18{:}2_i + \beta_{12} t_i C18{:}3_i + \beta_{13} t_i^2 C18{:}0_i + \beta_{14} t_i^2 C18{:}2_i + \beta_{15} t_i^2 C18{:}3_i + \\ & \beta_{16} t_i C18{:}0_i^2 + \beta_{17} t_i C18{:}2_i^2 + \beta_{18} t_i C18{:}3_i^2 + \beta_{19} t_i^2 C18{:}0_i^2 + \beta_{20} t_i^2 C18{:}2_i^2 + \beta_{21} t_i^2 C18{:}3_i^2 + \varepsilon_{ijkl} \\ & i = 1, 2, \ldots, n;\ j = 1, 2, 3;\ k = 1, 2, \ldots, 4;\ l = 1, 2, \ldots, 7 \end{aligned} \tag{1}$$

Data processing

A general linear model was generated to explore the relationships between milk CLA level and variables of interest collected from the experiments. This model includes several parameters (β_{0-21}), which represent the effects of the following variables: case study (i), days in lactation (d), time of diet inclusion (t), stearic acid ($C18{:}0$), linoleic acid ($C18{:}2$), α-linolenic acid ($C18{:}3$), interactions between them and random error (ε). Additionally, presentation (j), type (k) and diet (l) were used as factors.

For each component (fish oil, oilseeds, and pasture), less significant effects were removed from the general linear model (Equation 1). Subsequently, the CLA function with respect to each fatty acid (C18:0, C18:2 and C18:3) was derived. The derivatives were set to zero, and each equation was solved by defining the fatty acids as variables (Equation 2). This procedure was carried out for each of the ingredients.

$$\frac{\partial CLA}{\partial C18{:}\underline{i}} = 0,\ \underline{i} = 0, 2, 3 \tag{2}$$

Using Equation 2, the optimum value of each fatty acid ($C18{:}\underline{i}$, $\underline{i} = 0, 2, 3$) was obtained and the second derivative criterion (Larsson and Edwards, 2014) was applied to determine whether optimum value corresponded to a minimum value (Equation 3) or a maximum value (Equation 4).

$$\frac{\partial'' CLA}{\partial'' C18{:}\overset{*}{\underline{i}}} > 0,\ \underline{i} = 0, 2, 3 \tag{3}$$

When the second derivative was greater than zero, a minimum CLA concentration was obtained by setting the concentration of $C18{:}\underline{i}$, to $C18{:}\overset{*}{\underline{i}}$, $\underline{i} = 0, 2, 3$, of FA.

$$\frac{\partial'' CLA}{\partial'' C18{:}\overset{*}{\underline{i}}} < 0,\ \underline{i} = 0, 2, 3 \tag{4}$$

Conversely, when the second derivative was lower than zero, a maximum CLA concentration was obtained by setting the concentration of $C18{:}\underline{i}$, to $C18{:}\overset{*}{\underline{i}}$, $\underline{i} = 0, 2, 3$ of the FA.

If the optimal value of the precursor $C18{:}\underline{i}$, to $C18{:}\overset{*}{\underline{i}}$, $\underline{i} = 0, 2, 3$ corresponded to a minimum value (Equation 3), then the concentration of the precursor that increased CLA level in milk was the one that deviated the farthest from $C18{:}\overset{*}{\underline{i}}$ (Figure 2). Three different scenarios could be presented: $C18{:}\overset{*}{\underline{i}}$ fell within the range of the sample data ($x_1, ..., x_n$); in this case, Equation 1 was evaluated using the lowest ($x_{(1)}$) and highest ($x_{(n)}$) concentration values of the precursor in the sample, and the one that favours the higher concentration of CLA

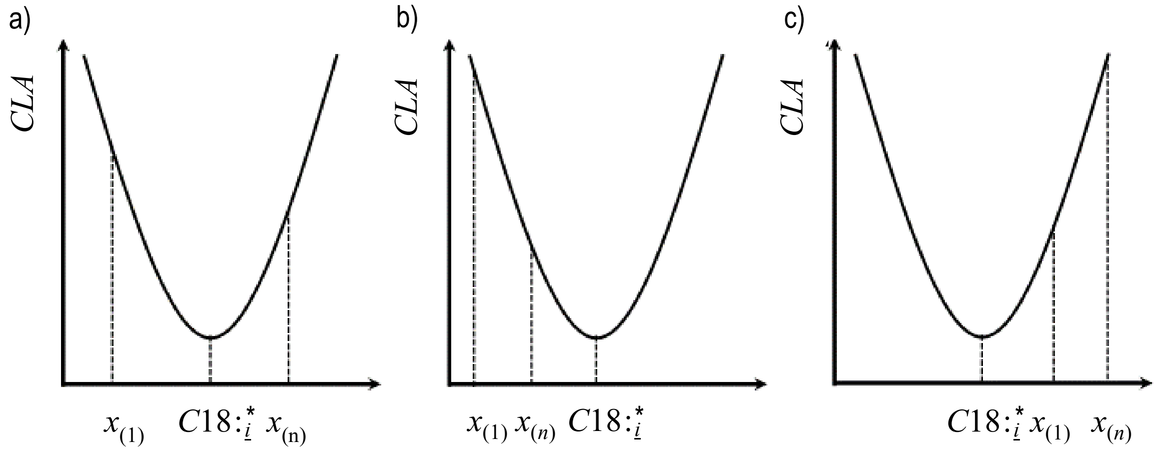

Figure 2. Scenarios for identifying the precursor concentration that favours the highest conjugated linoleic acid (CLA) concentration in milk, when the optimal value of the precursor ($C18{:}\underline{i}^*$) is associated with the minimum value $x_{(1)}$. Data for the lowest precursor concentration in the sample $x_{(n)}$; data for the highest precursor concentration in the sample.

was selected. For Figure 2a, this was ($x_{(1)}$). If $C18{:}\underline{i}^*$ was located to the right or left of the sample data (x_1, ..., x_n), the precursor concentration contributing to the highest CLA concentration was furthest from $C18{:}\underline{i}^*$, which in the case of Figures 2b and 2c was ($x_{(1)}$) and ($x_{(n)}$), respectively.

If the optimal value of the precursor ($C18{:}\underline{i}^*$, $\underline{i} = 0, 2, 3$ was the maximum value (Equation 4), then precursor concentration that increased CLA concentration in milk was the closest to $C18{:}\underline{i}^*$ (Figure 3). Three distinct scenarios could be outlined: $C18{:}\underline{i}^*$ was present within the sample data (x_1, ..., x_n); in this case Equation 1 was evaluated at the precursor concentration closest to the left (x_1^*) and to the right (x_2^*) of $C18{:}\underline{i}^*$. The concentration that resulted in the highest CLA concentration was selected, which in Figure 3a was (x_2^*). If $C18{:}\underline{i}$ was to the right or left of the sample data (x_1, ..., x_n), the precursor concentration favouring the highest CLA level was closest to $C18{:}\underline{i}^*$, which in the case of Figures 3b and 3c was ($x_{(n)}$) and ($x_{(1)}$), respectively.

Finally, Equation 1 was assessed using the precursor concentrations that led to the highest CLA concentration in each ingredient.

Statistical analysis

For each of the components (oilseeds, fish oil and pasture), the relationship between precursor concentrations in the diets, presentation effect (in oilseeds and pasture), type (in oilseeds), and basal diet (in fish oil) on the CLA content in milk was evaluated using analysis of covariance. Subsequently, the Tukey test ($P = 0.05$) was conducted to compare means between effects that showed statistical significance. Additionally, the linear relationship between total daily precursor concentration in

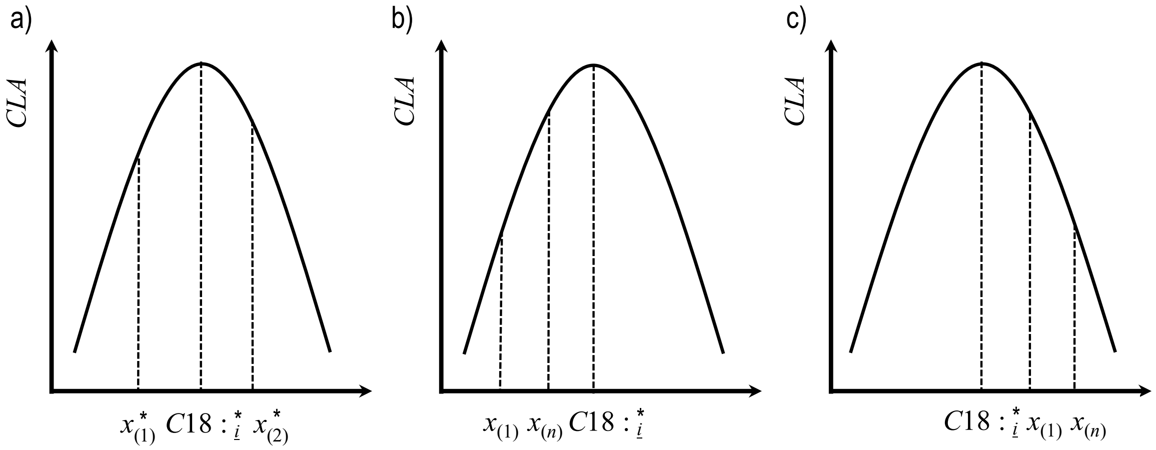

Figure 3. Scenarios for identifying the precursor concentration that favours the highest conjugated linoleic acid (CLA) concentration in milk when the optimal value of the precursor ($C18{:}\underline{i}^*$) is associated with the maximum value x_1^*; nearest precursor concentration to the left of the optimum value; x_2^*; nearest precursor concentration to the right of the optimum value $x_{(1)}$. Data for the lowest precursor concentration in the sample $x_{(n)}$; data for the highest precursor concentration in the sample

the diet and CLA levels in milk was assessed. The analyses were carried out using the GLM procedure implemented in SAS 9.0 (2002) statistical software. Finally, Equation 1 was evaluated using precursor concentrations that resulted in the highest CLA concentration in each component.

Results

The model for each ingredient demonstrated that CLA concentration in milk depended on the quadratic effects of the three precursors, *C*18:0, *C*18:2, and *C*18:3 (Table 2). For oilseeds, the quadratic effects, except for *C*18:2, were dependent on the quadratic effect of days of dietary inclusion (t^2). As regards fish oil, the quadratic effect of the precursor *C*18:3 was influenced by the linear effect of days of dietary inclusion (t). In the grazing model, the quadratic effects of all three precursors depended on the quadratic effect of days of dietary inclusion, t^2 (Table 2).

With respect to the optimal value *C*18:2*, based on the evaluated days of dietary inclusion, the maximum values were obtained for oilseeds and fish oil. For pasture, maximum values were obtained between days 0 and 61.1, while the minimum values occurred from day 61.1 to 65.

For the optimal value *C*18:3*, the maximum values were obtained from day 73.4 to 98 for oilseeds, from day 45.3 to 84 for fish oil, and from day 0 to 57.5 for pasture. The minimum values were obtained from day 0 to 73.4, from day 0 to 45.3, and from day 57.1 to 65, for oilseeds, fish oil, and grazing, respectively.

Oilseeds

In diets based on oilseeds, among the three evaluated precursors, linoleic acid (C18:2) required the highest daily supplementation (37.45 g/100 g FA) in cow feeding to maximise CLA concentration in milk (Table 4). Furthermore, there was no linear relationship ($P > 0.05$) between the total precursor concentration in the diet and milk CLA levels.

Table 2. Models with significant effects associated with conjugated linoleic acid (CLA) concentration using three types of dietary ingredients for dairy cows

Ingredient	Model
Oilseeds	$CLA_{ikl} = \beta_{0jkl} + \beta_1 d_i + \beta_2 t_i + \beta_3 t_i^2 + \beta_4 C18{:}0_i + \beta_5 C18{:}2_i + \beta_6 C18{:}3_i + \beta_7 C18{:}0_i^2 + \beta_8 C18{:}2_i^2 + \beta_9 C18{:}3_i^2 + \beta_{10} t_i C18{:}0_i + \beta_{11} t_i C18{:}2_i + \beta_{13} t_i^2 C18{:}0_i + \beta_{14} t_i^2 C18{:}2_i + \beta_{15} t_i^2 C18{:}3_i + \beta_{16} t_i C18{:}0_i^2 + \beta_{18} t_i C18{:}3_i^2 + \beta_{19} t_i^2 C18{:}0_i^2 + \beta_{21} t_i^2 C18{:}3_i^2 + \varepsilon_{ikl};$ $i = 1, 2, \ldots, n, k = 1, 2, \ldots, 7, l = 1, 2, \ldots, 4$
Fish oil	$CLA_{ij} = \beta_{0j} + \beta_1 d_i + \beta_2 t_i + \beta_3 t_i^2 + \beta_4 C18{:}0_i + \beta_5 C18{:}2_i + \beta_6 C18{:}3_i + \beta_7 C18{:}0_i^2 + \beta_8 C18{:}2_i^2 + \beta_9 C18{:}3_i^2 + \beta_{10} t_i C18{:}0_i + \beta_{11} t_i C18{:}2_i + \beta_{12} t_i C18{:}3_i + \beta_{13} t_i^2 C18{:}0_i + \beta_{14} t_i^2 C18{:}2_i + \beta_{15} t_i^2 C18{:}3_i + \beta_{18} t_i C18{:}3_i^2 + \varepsilon_{ij};$ $i = 1, 2, \ldots, n, j = 1, 2, 3$
Grazing	$CLA_{il} = \beta_{0l} + \beta_1 d_i + \beta_2 t_i + \beta_3 t_i^2 + \beta_4 C18{:}0_i + \beta_5 C18{:}2_i + \beta_6 C18{:}3_i + \beta_7 C18{:}0_i^2 + \beta_8 C18{:}2_i^2 + \beta_9 C18{:}3_i^2 + \beta_{13} t_i^2 C18{:}0_i + \beta_{14} t_i^2 C18{:}2_i + \beta_{15} t_i^2 C18{:}3_i + \beta_{16} t_i C18{:}0_i^2 + \beta_{17} t_i C18{:}2_i^2 + \beta_{18} t_i C18{:}3_i^2 + \beta_{19} t_i^2 C18{:}0_i^2 + \beta_{20} t_i^2 C18{:}2_i^2 + \beta_{21} t_i^2 C18{:}3_i^2 + \varepsilon_{il};$ $i = 1, 2, \ldots, n, l = 1, 2$

i – case study, *j* – base diet, *k* – type of seed, *l* – presentation of seed or grass, β_p, *p* = 0, 1, 2, ..., 21 – model parameters, *d* – days in lactation, *t* – days of ingredient inclusion, *C*18:0 – stearic acid, *C*18:2 – linoleic acid, *C*18:3 – α-linolenic acid, (t^2, $C18{:}0^2$, $C18{:}2^2$, $C18{:}3^2$) – covariates squared, *ε* – random error

In each of the three ingredients, the optimal values of the three precursors (*C*18:0*, *C*18:2*, *C*18:3*) are functions that depend on days of dietary inclusion (Table 3). In oilseeds, with the optimal value *C*18:0*, the maximum CLA values were obtained between days 56.3 and 79.6, while the minimum values were obtained for days 0 to 56.3 and 79.6 to 98 days. For fish oil, the optimum values were minimum values, while for grazing, the maximum value occurred from day 21.3 to 65, while the minimum values occurred for the period from day 0 to 21.3.

Moreover, increasing the quantity of the precursor in the diet did not guarantee a higher CLA concentration in milk. This was evident from the fact that the highest precursor amount (88.54 g/100 g FA) was provided on day 70, yet the highest CLA concentration was recorded on day 42 (2.78 g/100 g FA, Table 4).

Regarding the type of oilseed, the best CLA production in milk was achieved when the basal diet contained cottonseed (2 g/100 g FA, Table 4). In terms of presentation, the highest milk CLA levels occurred when vegetable oil was added to the basal diet (1.95 g/100 g FA, Table 4).

Table 3. Equations of the optimal values of the precursors (*C*18:0, *C*18:2 and *C*18:3) and the second derivative of the conjugated linoleic acid (CLA) model with respect to the precursors in each ingredie

Ingredient	*C*18:0	*C*18:2	*C*18:3
Optimum			
oilseeds	$\frac{-(\beta_4+\beta_{10}t+\beta_{13}t^2)}{2\beta_7+2\beta_{16}t+2\beta_{19}t^2}$	$\frac{-(\beta_5+\beta_{11}t+\beta_{14}t^2)}{2\beta_8}$	$\frac{-(\beta_6+\beta_{15}t^2)}{2\beta_9+2\beta_{18}t+2\beta_{21}t^2}$
fish oil	$\frac{-(\beta_4+\beta_{10}t+\beta_{13}t^2)}{2\beta_7}$	$\frac{-(\beta_5+\beta_{11}t+\beta_{14}t^2)}{2\beta_8}$	$\frac{-(\beta_6+\beta_{12}t+\beta_{15}t^2)}{2\beta_9+2\beta_{18}t}$
grazing	$\frac{-(\beta_4+\beta_{13}t^2)}{2\beta_7+2\beta_{16}t+2\beta_{19}t^2}$	$\frac{-(\beta_5+\beta_{14}t^2)}{2\beta_8+2\beta_{17}t+2\beta_{20}t^2}$	$\frac{-(\beta_6+\beta_{15}t^2)}{2\beta_9+2\beta_{18}t+2\beta_{21}t^2}$
Second derivative			
oilseeds	$\begin{cases}2\beta_7+2\beta_{16}t+2\beta_{19}t^2>0, \text{ if } 0<t<56.3 \text{ or } 79.6<t<98\\2\beta_7+2\beta_{16}t+2\beta_{19}t^2<0, \text{ if } 56.3<t<79.6\end{cases}$	$2\beta_8 = -2.18 \times 10^{-4}$	$\begin{cases}2\beta_9+2\beta_{18}t_i+2\beta_{21}t^2>0, \text{ if } 0<t<73.4\\2\beta_9+2\beta_{18}t_i+2\beta_{21}t^2<0, \text{ if } 73.4<t<98\end{cases}$
fish oil	$2\beta_7 = 0.698$	$2\beta_8 = -0.030$	$\begin{cases}2\beta_9+2\beta_{18}t_i>0, \text{ if } 0<t<45.3\\2\beta_9+2\beta_{18}t_i<0, \text{ if } 45.3<t<84\end{cases}$
grazing	$\begin{cases}2\beta_7+2\beta_{16}+2\beta_{19}t^2>0, \text{ if } 0<t<21.3\\2\beta_7+2\beta_{16}t+2\beta_{19}t^2<0, \text{ if } 21.3<t<65\end{cases}$	$\begin{cases}2\beta_8+2\beta_{17}t+2\beta_{20}t^2>0, \text{ if } 61.1<t<65\\2\beta_8+2\beta_{17}t+2\beta_{20}t^2<0, \text{ if } 0<t<61.1\end{cases}$	$\begin{cases}2\beta_9+2\beta_{18}t+2\beta_{21}t^2>0, \text{ if } 57.1<t<65\\2\beta_9+2\beta_{18}t+2\beta_{21}t^2<0, \text{ if } 0<t<57.1\end{cases}$

β_{4-21} – model parameters, t – days of ingredient inclusion, t^2 – squared days of inclusion of the ingredient, *C*18:0 – stearic acid, *C*18:2 – linoleic acid, *C*18:3 – α-linolenic acid

Table 4. Maximum conjugated linoleic acid (CLA) concentration in milk (g/100 g fatty acid (FA)) associated with the combination of optimal precursor values (g/100 g FA) as a function of time (days) when diets supplemented with seven types of seeds were offered in four presentations

Days of inclusion	Optimum values			Sum of precursors	Maximum CLA values																	
					Flax				Soybean				Sunflower				Canola	
	*C*18:0*	*C*18:2*	*C*18:3*		P1	P2	P3	P4	P1	P2	P3	P4	P1	P2	P3	P4	P1	P2
10	5.25	28.65	3.50	37.40	2.63	2.36	1.93	2.18	2.93	2.66	2.23	2.48	3.13	2.86	2.43	2.68	2.64	2.37
14	4.10	54.50	5.40	64.00	1.53	1.26	0.83	1.08	1.83	1.56	1.13	1.38	2.03	1.75	1.33	1.58	1.54	1.26
21	2.10	51.87	1.53	55.50	0.76	0.49	0.06	0.31	1.06	0.79	0.36	0.61	1.26	0.99	0.56	0.81	0.77	0.50
23	3.90	46.40	30.20	80.50	1.05	0.78	0.35	0.60	1.35	1.08	0.65	0.90	1.55	1.28	0.85	1.10	1.06	0.79
30	2.40	31.40	36.90	70.70	1.11	0.84	0.41	0.66	1.41	1.14	0.71	0.96	1.61	1.33	0.91	1.16	1.12	0.84
35	4.20	38.50	24.10	66.80	1.27	1.00	0.57	0.82	1.57	1.30	0.87	1.12	1.77	1.50	1.07	1.32	1.28	1.01
42	3.80	18.40	49.00	71.20	2.76	2.49	2.06	2.31	3.06	2.79	2.36	2.61	3.26	2.98	2.56	2.81	2.77	2.50
49	3.20	26.20	40.60	70.00	2.34	2.06	1.64	1.89	2.64	2.36	1.94	2.19	2.83	2.56	2.13	2.38	2.35	2.07
57	3.90	52.43	8.58	64.91	0.94	0.67	0.24	0.49	1.24	0.97	0.54	0.79	1.44	1.17	0.74	0.99	0.95	0.68
60	5.47	14.62	1.07	21.16	1.11	0.84	0.41	0.66	1.41	1.14	0.71	0.96	1.61	1.34	0.91	1.16	1.12	0.85
63	5.23	27.12	38.59	70.94	2.57	2.30	1.87	2.12	2.87	2.60	2.17	2.42	3.07	2.80	2.37	2.62	2.58	2.31
70	14.60	48.95	24.99	88.54	1.00	0.72	0.30	0.55	1.30	1.03	0.60	0.85	1.49	1.22	0.79	1.04	1.01	0.73
84	6.65	49.69	11.38	67.72	1.93	1.66	1.23	1.48	2.23	1.96	1.53	1.78	2.43	2.15	1.72	1.97	1.94	1.66
98	4.40	35.50	5.71	45.61	0.99	0.72	0.29	0.54	1.29	1.02	0.59	0.84	1.48	1.21	0.78	1.03	1.00	0.72
Mean (precursors)	4.94	37.45	20.11															
Mean (oilseeds)					1.21^{g}				1.51^{e}				1.71^{c}				1.22^{f}	
Mean (presentation)					P1 = 1.95^{1}				P2 = 1.67^{2}				P3 = 1.25^{4}				P4 = 1.50^{3}	

Continuation

Days of inclusion	Optimum values			Maximum CLA values														CLA per day of inclusion
				Canola		Rapeseed				Cotton				Peanut				
	*C*18:0*	*C*18:2*	*C*18:3*	P3	P4	P1	P2	P3	P4	P1	P2	P3	P4	P1	P2	P3	P4	
10	5.25	28.65	3.50	1.94	2.19	2.97	2.69	2.27	2.52	3.42	3.15	2.72	2.97	3.36	3.09	2.66	2.91	2.65^{B}
14	4.10	54.50	5.40	0.84	1.09	1.86	1.59	1.16	1.41	2.32	2.04	1.62	1.87	2.26	1.99	1.56	1.81	1.55^{F}
21	2.10	51.87	1.53	0.07	0.32	1.10	0.82	0.40	0.65	1.55	1.28	0.85	1.10	1.49	1.22	0.79	1.04	0.78^{M}
23	3.90	46.40	30.20	0.36	0.61	1.39	1.11	0.68	0.93	1.84	1.57	1.14	1.39	1.78	1.51	1.08	1.33	1.07^{I}
30	2.40	31.40	36.90	0.42	0.67	1.44	1.17	0.74	0.99	1.90	1.63	1.20	1.45	1.84	1.57	1.14	1.39	1.13^{H}
35	4.20	38.50	24.10	0.58	0.83	1.61	1.33	0.90	1.16	2.06	1.79	1.36	1.61	2.00	1.73	1.30	1.55	1.29^{G}
42	3.80	18.40	49.00	2.07	2.32	3.09	2.82	2.39	2.64	3.55	3.28	2.85	3.10	3.49	3.22	2.79	3.04	2.78^{A}
49	3.20	26.20	40.60	1.64	1.89	2.67	2.40	1.97	2.22	3.13	2.85	2.42	2.68	3.07	2.80	2.37	2.62	2.36^{D}
57	3.90	52.43	8.58	0.25	0.50	1.28	1.00	0.57	0.83	1.73	1.46	1.03	1.28	1.67	1.40	0.97	1.22	0.96^{L}
60	5.47	14.62	1.07	0.42	0.67	1.45	1.17	0.75	1.00	1.90	1.63	1.20	1.45	1.85	1.57	1.14	1.39	1.13^{H}
63	5.23	27.12	38.59	1.88	2.13	2.91	2.63	2.21	2.46	3.36	3.09	2.66	2.91	3.31	3.03	2.60	2.85	2.59^{C}
70	14.60	48.95	24.99	0.31	0.56	1.33	1.06	0.63	0.88	1.79	1.51	1.09	1.34	1.73	1.46	1.03	1.28	1.02^{J}
84	6.65	49.69	11.38	1.24	1.49	2.26	1.99	1.56	1.81	2.72	2.44	2.02	2.27	2.66	2.39	1.96	2.21	1.95^{E}
98	4.40	35.50	5.71	0.30	0.55	1.32	1.05	0.62	0.87	1.78	1.50	1.08	1.33	1.72	1.45	1.02	1.27	1.01^{K}
Mean (precursors)	4.94	37.45	20.11															
Mean (oilseeds)				1.22^{f}		1.54^{d}				2.00^{a}				2.94^{b}				
Mean (presentation)				P1 = 1.95^{1}		P2 = 1.67^{2}				P3 = 1.25^{4}				P4 = 1.50^{3}				
β																		0.0008^{ns}

The optimum precursor values and maximum CLA values for oilseeds were obtained by evaluating the equation in Tables 2 and 1, respectively; P1 – oil, P2 – pressed seed, P3 – raw or whole seed, P4 – processed seed; different letters between: $^{A–M}$ – inclusion days, $^{a–f}$ – seed types, and ^{1,2,3} – presentations indicate significant statistical difference at $P = 0.05$; β – estimator of the linear relationship between the sum of precursors and the average CLA concentration per day of inclusion; ns – not significant ($P > 0.05$)

Fish oil

In diets supplemented with fish oil, similarly to oilseeds, linoleic acid (C18:2) had to be added at the highest dose per day (26.62 g/100 g FA) in order to achieve CLA concentration increments in milk (Table 5).

It is worth noting that, as with oilseeds, the CLA content in milk per inclusion day did not show a linear relationship ($P > 0.05$) with total precursor concentrations in the diets. The highest CLA concentrations were recorded on days 21 (10.44 g/100 g FA) and 12 (9.55 g/100 g FA), with total precursor concentrations amounting to 69.13 and 24.70 g/100 g FA, respectively. Thus, one would expect that on day 28, the third day with a higher CLA concentration (7.93 g/100 g FA), the sum of its precursors would be lower than on day 12. However, the total precursor concentration was higher than expected (31.94 g/100 g FA, Table 5). Of the three evaluated diets, the highest CLA concentration in milk was recorded when fish oil was added to the maize silage-based diet (5.814 g/100 g FA, Table 5).

Table 5. Maximum conjugated linoleic acid (CLA) concentration values (g/100 g fatty acid (FA)) associated with the combination of optimal precursor values (g/100 g FA) as a function of time (days) when diets supplemented with fish oil were offered

Days of inclusion	Optimum values			Sum of precursors	Maximum values (diets)			CLA per day of inclusion
	*C*18:0	*C*18:2	*C*18:3		Maize silage	Processed maize	Oats, soybeans or barley	
12	3.55	18.41	2.74	24.70	11.74	8.53	8.39	9.55^{A}
21	2.94	31.81	34.38	69.13	12.63	9.42	9.28	10.44^{A}
26	2.70	39.80	6.50	49.00	4.24	1.04	0.89	2.05^{CD}
28	2.32	26.75	2.87	31.94	10.12	6.91	6.76	7.93^{B}
35	4.08	25.67	3.58	33.33	2.70	0.00	0.00	0.42^{E}
42	3.53	31.06	4.25	38.84	0.65	0.00	0.00	0.90^{DE}
60	5.44	34.48	1.46	41.38	4.82	1.61	1.46	2.63^{C}
70	6.40	33.34	16.13	55.87	1.59	0.00	0.00	0.53^{DE}
84	6.68	25.30	3.87	35.85	3.84	0.63	0.48	1.65^{CDE}
Mean (precursors)	4.18	26.62	8.42					
Mean (diet)					5.81^{a}	3.12^{b}	3.02^{b}	
β								0.022^{ns}

The optimum precursor values and maximum CLA values for fish oil were obtained by evaluating the equation in Tables 2 and 1, respectively; different letters between: $^{A–E}$ – inclusion days, and ^{ab} – diets indicate significant statistical difference at $P = 0.05$; β – estimator of the linear relation-

Table 6. Maximum conjugated linoleic acid (CLA) concentration (g/100 g fatty acid (FA)) associated with the combination of optimal precursor values (g/100 g FA) depending on the time (days) of animal grazing

Days grazing	Optimum value			Sum of precursors	Maximum value		CLA per day grazing
	*C*18:0	*C*18:2	*C*18:3		Grasses	Grasses with legumes	
10	6.70	12.50	48.90	68.10	2.62	2.00	2.31^{D}
11	8.20	13.00	46.50	67.70	3.15	2.52	2.83^{B}
14	2.20	13.30	48.70	64.20	2.25	1.62	1.93^{E}
21	1.95	15.19	34.14	51.28	1.82	1.19	1.50^{I}
22	1.40	6.70	48.90	57.00	2.68	2.06	2.37^{C}
24	2.93	19.77	44.06	66.76	1.71	1.09	1.40^{J}
28	1.58	10.54	44.96	57.08	3.56	2.94	3.25^{A}
36	2.00	9.20	57.30	68.50	1.88	1.26	1.57^{H}
42	1.05	19.80	55.60	76.45	1.55	0.93	1.24^{K}
48	1.26	15.18	55.93	72.37	2.23	1.61	1.92^{F}
60	2.00	9.20	57.30	68.50	2.17	1.55	1.86^{G}
64	2.34	10.54	55.59	68.47	2.25	1.63	1.94^{E}
Mean (precursors)	2.80	12.91	49.82				
Mean (presentation)					2.32^{a}	1.70^{b}	
β							-0.027^{ns}

The optimum precursor values and maximum CLA values for pasture grazing were obtained by evaluating the equation in Tables 2 and 1, respectively; different letters between: $^{A–K}$ – mean days grazing, and ^{ab} – presentations indicate significant statistical difference at $P = 0.05$; β – estimator of the linear relationship between the sum of precursors and the average CLA concentration per day of inclusion; ^{ns} – not significant ($P > 0.05$)

Pasture

In diets based on fresh pasture, α-linolenic acid (C18:3) required the highest supplementation (49.82 g/100 g FA) to increase CLA concentration in milk (Table 6). Considering inclusion days, the highest CLA concentration (3.25 g/100 g FA) was observed on day 28, with a total precursor level of 57.08 g/100 g FA. However, there was no linear relationship ($P > 0.05$) with total precursor concentration, as their total concentration was higher on subsequent days (Table 6).

Regarding the type of pasture, it was evident that grasses contributed ($P = 0.05$) to higher CLA levels in milk (2.32 g/100 g FA) compared to the combination of grasses with legumes (1.70 g/100 g FA; Table 6).

Discussion

As the days of experimental evaluation progressed, the lactation period of the animals also advanced, potentially affecting CLA concentration. In this regard, Kliem and Shingfield (2016) have reported that milk contains a higher proportion of polyunsaturated fatty acids (PUFA) when the cow is in the first third of lactation. This is because longer-chain FA are mobilised from adipose tissue and contribute to a higher proportion of total FA secreted in milk due to the negative energy balance that typically occurs at this stage (Bilal et al., 2014). However, Prieto-Manrique et al. (2016) have argued that CLA concentrations increases with the days of lactation.

Oilseeds

Incorporating oilseeds or vegetable oils into dairy cow diets has been shown to lead to changes in the FA profile (Martínez-Marín et al., 2013) due to their high PUFA content (Kliem and Shingfield et al., 2016). Diets containing oilseeds such as soybean, sunflower, rapeseed and cottonseed predominantly contain linoleic acid (Roca-Fernandez et al., 2014); a PUFA that is transformed in the biohydrogenation process that occurs in the animal's rumen, resulting in CLA production (Purba et al., 2020).

The potential of vegetable oils and oilseeds to increase the CLA content in milk is limited, even when provided in large quantities or for extended periods (Kliem and Shingfield, 2016). CLA concentration in milk not only depends on dietary manipulation but also on seasonal factors and lactation numbers (Maria-Patiño, 2011). Glasser et al. (2008) have emphasized that the increase in CLA also relies on ruminal metabolism (hydrolysis, isomerisation and biohydrogenation of dietary F) and animal metabolism (mobilisation, uptake and synthesis of FA in the mammary gland).

Furthermore, changes in the milk FA profile primarily depend on the type or species of oilseed and its processing (pressed, ground, heated, whole or unprocessed) (Prieto-Manrique et al., 2016). In this study, the highest CLA concentrations in milk have been achieved by adding cottonseed to the diet. Cottonseed is known for its high linoleic acid concentration, ranging from 500 to 573 g/kg FA, depending on its form, whether whole or in oil (Besharati and Taghizadeh, 2014; Castaño et al., 2014).

When evaluating different presentations, the use of vegetable oil tend to result in a higher CLA content. This effect arises from vegetable oil's capacity to inhibit ruminal microbial activity, leading to a higher flow of linoleic acid and CLA from feedstuffs to the mammary gland through the bloodstream (He and Armentano, 2011). For this reason, some researchers caution against including vegetable oils in dairy cow diets due to their potential toxic effect on rumen microbial growth (Muruz and Çetinkaya, 2019). Furthermore, oils with higher amounts of PUFAs reduce the content of short- and medium-chain FAs, as supplementary oils increase FA transport from the intestine to the mammary glands and reduce *de novo* synthesis. Adding FAs in free form to the diets of dairy cows increases their levels in milk and inhibits the synthesis of short and mediumchain FAs in the mammary gland (Castro et al., 2019).

On the other hand, incorporating untreated seeds into the diet, such as raw or whole seeds, can also elevate the concentration of CLA in milk without significantly impacting the fat content. This occurs because there is limited interaction of PUFAs with rumen bacteria, thus they do not interfere with their function (Prieto-Manrique et al., 2016; Siurana and Calsamiglia, 2016).

Fish oil

Fish oil can be used in dairy cow diets to increase the concentration of PUFAs in milk, including eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and CLA (Acosta-Balcazar et al., 2022). The extent of the increase in milk CLA level depends on the source of marine lipids (fish, plankton or algae) and additions to the basal diet (Kairenius et al., 2015). Similar to oilseeds, an increase in CLA levels caused by fish oil is affected by ruminal biohydrogenation and the animal's metabolism (Prieto-Manrique et al., 2016; Siurana and Calsamiglia, 2016).

Kliem and Shingfield (2016) have suggested that diets based on maize silage can cause a decrease in ruminal pH, altering the microbial flora and leading to changes in PUFA biohydrogenation phases. Ortega-Pérez et al. (2013) have reported that a ruminal pH of 6.0 is conducive to CLA production, which can be achieved through an appropriate forage/concentrate ratio. Additionally, PUFAs present in fish oil inhibit the activity of Δ9-desaturase enzyme by up to 30.2%. This enzyme is responsible for converting trans-vaccenic acid (TVA) to stearic acid in the final phase of biohydrogenation (Alizadeh et al., 2012; Pirondini et al., 2015; Prieto-Manrique et al., 2016). The inhibition of Δ9-desaturase results in the accumulation of TVA in ruminal fluid, ultimately leading to higher CLA concentrations.

It should be emphasised that fish oil supplements (at concentrations not exceeding 7% dry matter) were shown to reduce FA synthesis in the mammary gland (Alizadeh et al., 2012) without altering the organoleptic properties of milk (Prieto-Manrique et al., 2016).

Grazing

Fresh pasture grazing is known to increase the proportion of PUFAs in milk, and their CLA content can be up to five times higher (Ortega-Pérez et al., 2013). Of the lipid content of pasture feed (8–10% dry matter), 95% corresponds to linoleic and α-linolenic acids, with α-linolenic acid being most abundant, ranging from 50 to 75% (Toyes-Vargas et al., 2013; Acosta-Balcazar et al., 2022). Of the total content of α-linolenic acid from pasture forage, 74% is converted to TVA in the rumen and later transformed into CLA by Δ9-desaturase (Prieto-Manrique et al., 2016).

The effect of pasture diet depends on factors such as availability, allocation, grazing time, botanical composition, growth stage, lipid concentration, type and level of supplementation, as well as concentrate provided to the animals (Castro-Hernández et al., 2014; Prieto-Manrique et al., 2016; Corazzin et al., 2019). However, milk CLA levels are also influenced by rumen PUFA metabolism and animal metabolism, as in the case of oilseeds and fish oil (Prieto-Manrique et al., 2016; Siurana and Calsamiglia, 2016).

Regarding the type of pasture, grasses can increase the CLA content in milk. This is because grasses contain α-linolenic acid in their leaf chloroplasts and produce high biomass, thereby providing more α-linolenic acid in the cows' diet and increasing milk CLA levels (Morales-Almaráz et al., 2010). It is important to mention that the inclusion of pasture feeding has been found to have no effect on fat content, as it stimulates higher acetic acid production utilised in fat synthesis (Osorio and Vinazco, 2010).

Conclusions

There was no linear relationship between the total concentration of dietary precursors and the concentration of conjugated linoleic acid (CLA) in milk. However, a quadratic relationship was found between each of the dietary precursors and the CLA content. Furthermore, the concentration of precursors that contributed to higher CLA concentrations was time-dependent.

For diets based on oilseeds and fish oil, linoleic acid was the precursor that required the highest supplementation dose to increase CLA concentration in milk, while in diets based on fresh pasture grazing, it was α-linolenic acid.

The highest CLA concentration in milk was recorded within the first 21 days when diets were supplemented with fish oil and on day 28 if fresh pasture forage was consumed.

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Conflict of interest

The Authors declare that there is no conflict of interest.

References

AbuGhazaleh A.A., Holmes L.D., 2007. Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. J. Dairy Sci. 90, 2897–2904, https://doi.org/10.3168/jds.2006-684

AbuGhazaleh A.A., Schingoethe D.J., Hippen A.R., Kalscheur K.F., Whitlock L.A., 2002. Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. J. Dairy Sci. 85, 2266–2276, https://doi.org/10.3168/jds.S0022-0302(02)74306-3

Acosta-Balcazar I.C., Granados-Rivera L.D., Salinas-Chavira J., Estrada-Drouaillet B., Ruiz-Albarrán M., Bautista-Martínez Y., 2022. Relationship between the composition of lipids in forages and the concentration of conjugated linoleic acid in cow's milk: A review. Animals 12, 1621, https://doi.org/10.3390/ani12131621

Aguilar G.O.X., Moreno M.B.M., Pabón R.M.L. Carulla F.J.E., 2009. Effect of consumption of kikuyu (*Pennisetum clandestinum*) or ryegrass (*Lolium hibridum*) on the concentration of conjugated linoleic acid and the fatty acid profile of milk fat (in Spanish). Livest. Res. Rural. Dev. 21, http://www.lrrd.org/lrrd21/4/agui21049.htm

Alizadeh A.R., Alikhani M., Ghorbani G.R., Rahmani H.R., Rashidi L., Loor J.J., 2012. Effects of feeding roasted safflower seeds (variety IL-111) and fish oil on dry matter intake, performance and milk fatty acid profiles in dairy cattle. J. Anim. Physiol. Anim, Nutr. 96, 466–473, https://doi.org/10.1111/j.1439-0396.2011.01165.x

Allred S.L., Dhiman T.R., Brennand C.P., Khanal R.C., McMahon D.J., Luchini N.D., 2006. Milk and cheese from cows fed calcium salts of palm and fish oil alone or in combination with soybean products. J. Dairy Sci. 89, 234–248, https://doi.org/10.3168/jds.S0022-0302(06)72088-4

Bailoni L., Bortolozzo A., Mantovani R., Simonetto A., Schiavon S., Bittante G., 2004. Feeding dairy cows with full fat extruded or toasted soybean seeds as replacement of soybean meal and effects on milk yield, fatty acid profile and CLA content. Ital. J. Anim. Sci. 3, 243–258, https://doi.org/10.4081/ijas.2004.243

Bargo F., Delahoy J.E., Schroeder G.F., Muller, L.D., 2006. Milk fatty acid composition of dairy cows grazing at two pasture allowances and supplemented with different levels and sources of concentrate. Anim. Feed Sci. Technol. 125, 17–31, https://doi.org/10.1016/j.anifeedsci.2005.05.010

Barletta R.V., Gandra J.R., Bettero V.P. et al., 2016. Ruminal biohydrogenation and abomasal flow of fatty acids in lactating cows: Oilseed provides ruminal protection for fatty acids. Anim. Feed Sci. Technol. 219, 111–121, https://doi.org/10.1016/j.anifeedsci.2016.06.011

Benchaar C., Romero-Pérez G.A., Chouinard P.Y., Hassanat F., Eugene M., Petit H.V., Côrtes C., 2012. Supplementation of increasing amounts of linseed oil to dairy cows fed total mixed rations: Effects on digestion, ruminal fermentation characteristics, protozoal populations, and milk fatty acid composition. J. Dairy Sci. 95, 4578–4590, https://doi.org/10.3168/jds.2012-5455

Besharati M., Taghizadeh, A., 2014. Effect of whole cottonseed, monensin and vitamin E on milk compositions and CLA content of milk fat of lactating dairy cows. Int. J. Biosci. 5, 420–432, http://dx.doi.org/10.12692/ijb/5.12.420-432

Bharathan M., Schingoethe D.J., Hippen A.R., Kalscheur K.F., Gibson M.L., Karges K., 2008. Conjugated linoleic acid increases in milk from cows fed condensed corn distillers solubles and fish oil. J. Dairy Sci. 91, 2796–2807, https://doi.org/10.3168/jds.2007-0938

Bilal G., Cue R.I., Mustafa A.F., Hayes J.F., 2014. Effects of parity, age at calving and stage of lactation on fatty acid composition of milk in Canadian Holsteins. Canad. J. Anim. Sci. 94, 401–410, https://doi.org/10.4141/cjas2013-172

Brown W., AbuGhazaleh A.A., Ibrahim S.A., 2008. Milk conjugated linoleic acid response to fish oil and linseed oil supplementation of grazing dairy cows. Asian-Aust. J. Anim. Sci. 21, 663–670, https://doi.org/10.5713/ajas.2008.70272

Caroprese M., Marzano A., Marino R., Gliatta G., Muscio A., Sevi, A., 2010. Flaxseed supplementation improves fatty acid profile of cow milk. J. Dairy Sci. 93, 2580–2588, https://doi.org/10.3168/jds.2008-2003

Castaño G.A., Pabón M.L., Carulla J.E., 2014. Concentration of *trans*-vaccenic and rumenic acids in the milk from grazing cows supplemented with palm oil, rice bran or whole cottonseed. Rev. Bras. Zootec. 43, 315–326, https://doi.org/10.1590/S1516-35982014000600006

Castro-Hernández H., González-Martínez F.F., Domínguez-Vara A., Pinos-Rodríguez J.M., Morales-Almaráz E., Vieyra-Alberto R., 2014. Effect of level of concentrate on milk fatty acid profile from grazing Holstein cows (in Spanish). Agrociencia 48, 765–775

Castro T., Martinez D., Isabel B., Cabezas A., Jimeno V., 2019. Vegetable oils rich in polyunsaturated fatty acids supplementation of dairy cows'diets: Effects on productive and reproductive performance. Animals 9, 205, https://doi.org/10.3390/ani9050205

Chen P., Ji P., Li S.L., 2008. Effects of feeding extruded soybean, ground canola seed and whole cottonseed on ruminal fermentation, performance and milk fatty acid profile in early lactation dairy cows. Asian-Aust. J. Anim. Sci. 21, 204–213, https://doi.org/10.5713/ajas.2008.70079

Chichlowski M.W., Schroeder J.W., Park C.S., Keller W.L., Schimek D.E., 2005. Altering the fatty acids in milk fat by including canola seed in dairy cattle diets. J. Dairy Sci. 88, 3084–3094, https://doi.org/10.3168/jds.S0022-0302(05)72990-8

Chilliard Y., Martin C., Rouel J., Doreau M., 2009. Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. J. Dairy Sci. 92, 5199–5211, https://doi.org/10.3168/jds.2009-2375

Corazzin M., Romanzin A., Sepulcri A., Pinosa M., Piasentier E., Bovolenta S., 2019. Fatty acid profiles of cow's milk and cheese as affected by mountain pasture type and concentrate supplementation. Animals 9, 68, https://doi.org/10.3390/ani9020068

Cruz-Hernández C., Kramer J.K.G., Kennelly J.J., Glimm D.R., Sorensen B.M., Okine E.K., Goonewardene L.A., Weselake, R.J., 2007. Evaluating the conjugated linoleic acid and trans 18:1 isomer in milk fat of dairy cows fed increasing amounts of sunflower oil and a constant level of fish oil. J. Dairy Sci. 90, 3786–3801, https://doi.org/10.3168/jds.2006-69

Dhiman T.R., Satter L.D., Pariza M.W., Galli M.P., Albright K., Tolosa M.X., 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci. 83, 1016–1027, https://doi.org/10.3168/jds.S0022-0302(00)74966-6

Donovan D.C., Schingoethe D.J., Baer R.J., Ryali J., Hippen A.R., Franklin S.T., 2000. Influence of dietary fish oil on conjugated linoleic acid and other fatty acids in milk fat from lactating dairy cows. J. Dairy Sci. 83, 2620–2628, https://doi.org/10.3168/jds.S0022-0302(00)75155-1

Egger P., Holzer G., Segato S., Werth E., Schwienbacher F., Peratoner G., Andrighetto I., Kasal A., 2007. Effects of oilseed supplements on milk production and quality in dairy cows fed a hay-based diet (in Italian). Ital. J. Anim. Sci. 6, 395–405, https://doi.org/10.4081/ijas.2007.395

Fatahnia F., Azarfar A., Menatian S., Joz-Ghasemi Sh., Poormalekshahi A., Shokri A.N., 2018. Effect of diets containing roasted soybean, extruded soybean or their combination on performance and milk fatty acid profile of lactating Holstein cows. Iran. J. Vet. Res. 19, 276–282, PMCID: PMC6361601

Flowers G., Ibrahim S.A., AbuGhazaleh A.A., 2008. Milk fatty acid composition of grazing dairy cows when supplemented with linseed oil. J. Dairy Sci. 91, 722–730, https://doi.org/10.3168/jds.2007-0410

Flores G., Resch C., Fernández-Lorenzo B. et al., 2011. Effect of summer grazing of violet clover on the omega-3 content of bovine milk (in Spanish). Pastos 41, 79–99, http://polired.upm.es/index.php/pastos/article/view/1829/1830

Gagliostro G.A., Antonacci L.E., Carabajal A., López Pavlán J.M., Crujeira Y., 2018. Production of bovine milk reduced in saturated fat and naturally enriched in conjugated linoleic acid (in Spanish). INNOTEC 15, 37–43, https://doi.org/10.26461/15.01

Gao Y., Sun T., Li, J., 2009. Effect of oilseeds rich in linoleic and linolenic acids on milk production and milk fatty acid composition in dairy cows. Front. Agric. China. 3, 311–318, https://doi.org/10.1007/s11703-009-0022-1

Giacomazza-Cerutti W., Viegas J., Martins-Barbosa A. et al., 2016. Fatty acid profiles of milk and *Minas frescal* cheese from lactating grazed cows supplemented with peanut cake. J. Dairy Res. 83, 42–49, https://doi.org/10.1017/S0022029915000631

Glasser F., Ferlay A., Chilliard Y., 2008. Oilseed lipid supplements and fatty acid composition of cow milk: A meta-analysis. J. Dairy Sci. 91, 4687–4703, https://doi.org/10.3168/jds.2008-0987

Gómez-Cortés P., de la Fuente M. A., Juárez M., 2019. Trans fatty acids and conjugated linoleic acid in food: Origin and biological properties (in Spanish). Nutr. Hosp. 36, 479-486, https://dx.doi.org/10.20960/nh.2466

Granados-Rivera L.D., Hernández-Mendo O., Maldonado-Jaquez J.A., Bautista-Martínez Y., Granados-Zurita L., Quiroz-Valiente J., 2017. The trans-10, cis-12 isomer of conjugated linoleic acid in the nutrition of lactating cows. Rev. Chapingo Ser. Zonas Áridas, 16, 1–11, https://10.5154/r.rchsza.2017.10.008

Gulati S.K., McGrath S., Wynn P.C., Scott T.W., 2003. Preliminary results on the relative incorporation of docosahexaenoic and eicosapentaenoic acids into cow's milk from two types of rumen protected fish oil. Inter. Dairy J. 13, 339–343, https://doi.org/10.1016/S0958-6946(03)00004-9

Halmemies-Beauchet-Filleau A., Kokkonen T., Lampi A.M., Toivonen V., Shingfield, K.J., Vanhatalo A., 2011. Effect of plant oils and camelina expeller on milk fatty acid composition in lactating cows fed diets based on red clover silage. J. Dairy Sci. 94, 4413–4430, https://doi.org/10.3168/jds.2010-3885

He M., Armentano L.E., 2011. Effect of fatty acid profile in vegetable oils and antioxidant supplementation on dairy cattle performance and milk fat depression. J. Dairy Sci. 94, 2481–2491, https://doi.org/10.3168/jds.2010-3755

Hoffmann A., Görlich S., Steingass H., Terry H., Schollenberger M., Hartung K., Mosenthin, R., 2016. Milk production and milk fatty acids in dairy cows fed crushed rapeseed or rapeseed oil. Livest. Sci. 190, 31–34, https://doi.org/10.1016/j.livsci.2016.05.016

Jacobs A.A.A., van Baal J., Smits M.A., Taweel H.Z.H., Hendriks W.H., van Vuuren A.M., Dijkstra J., 2011. Effects of feeding rapeseed oil, soybean oil, or linseed oil on stearoyl-CoA desaturase expression in the mammary gland of dairy cows. J. Dairy Sci. 94, 874–887, https://doi.org/10.3168/jds.2010-3511

Juchem S.O., Santos J.E.P., Cerri R.L.A., Chebel R.C., Galvao K.N., Bruno R., DePeters E.J., Scott T., Thatcher W.W., Luchini D., 2008. Effect of calcium salts of fish and palm oils on lactational performance of Holstein cows. Anim. Feed Sci. Technol. 140, 18–38, https://doi.org/10.1016/j.anifeedsci.2007.01.018

Kairenius P., Ärölä A., Leskinen H., Toivonen V., Ahvenjärvi S., Vanhatalo A., Huhtanen P., Hurme T., Griinari J.M., Shingfield K.J., 2015. Dietary fish oil supplements depress milk fat yield and alter milk fatty acid composition in lactating cows fed grass silage-based diets. J. Dairy Sci. 98, 5653–5671, https://doi.org/10.3168/jds.2015-9548

Kalač P., Samková E., 2010. The effects of feeding various forages on fatty acid composition of bovine milk fat: A review. Czech J. Anim. Sci. 55, 521–537, https://doi.org/10.17221/2485-CJAS

Kay J.K., Mackle T.R., Auldist M.J., Thomson N.A., Bauman D.E., 2004. Endogenous synthesis of *cis*-9, *trans*-11 conjugated linoleic acid in dairy cows fed fresh pasture. J. Dairy Sci. 87, 369–378, https://doi.org/10.3168/jds.S0022-0302(04)73176-8

Kay J.K., Mackle T.R., Bauman D.E., Thomson N.A., Baumgard L.H., 2007. Effects of a supplement containing *trans*-10, *cis*-12 conjugated linoleic acid on bioenergetic and milk production parameters in grazing dairy cows offered ad libitum or restricted pasture. J. Dairy Sci. 90, 721–730, https://doi.org/10.3168/jds.S0022-0302(07)71556-4

Kay J.K., Roche J.R., Kolver E.S., Thomson N.A., Baumgard L.H., 2005. A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows. J. Dairy Res. 72, 322–332, https://doi.org/10.1017/S0022029905000944

Kay J.K., Roche J.R., Moore C.E., Baumgard L.H., 2006. Effects of dietary conjugated linoleic acid on production and metabolic parameters in transition dairy cows grazing fresh pasture. J. Dairy Res. 73, 367–377, https://doi.org/10.1017/S0022029906001944

Khanal R.C., Dhiman T.R., Boman R.L., McMahon D.J., 2007. Influence of supplementing dairy cows grazing on pasture with feeds rich in linoleic acid on milk fat conjugated linoleic acid (CLA) content. Asian-Australas. J. Anim. Sci. 20, 1374–1388, https://doi.org/10.5713/ajas.2007.1374

Kliem K.E., Shingfield K.J., 2016. Manipulation of milk fatty acid composition in lactating cows: Opportunities and challenges. Eur. J. Lipid Sci. Technol. 118, 1661–1683, https://doi.org/10.1002/ejlt.201400543

Kudrna V., Marounek M., 2006. The influence of feeding rapeseed cake and extruded soyabean on the performance of lactating cows and the fatty acid pattern of milk. J. Anim. Feed Sci. 15, 361, https://doi.org/10.22358/jafs/66907/2006

Kudrna V., Marounek M., 2008. Influence of feeding whole sunflower seed and extruded linseed on production of dairy cows, rumen and plasma constituents, and fatty acid composition of milk. Arch. Anim. Nutr. 62, 60–69, http://dx.doi.org/10.1080/17450390701780243

Larson R., Edwards B., 2014. Calculus, 10th Edition. Cengage Learning Publisher

Loor J.J., Herbein J.H., 2003. Dietary canola or soybean oil with two levels of conjugated linoleic acids (CLA) alter profiles of 18:1 and 18:2 isomers in blood plasma and milk fat from dairy cows. Anim. Feed Sci. Technol. 103, 63–83, https://doi.org/10.1016/S0377-8401(02)00259-6

Mackle T.R., Kay J.K., Auldist M.J., McGibbon A.K.H., Philpott B.A., Baumgard L.H., Bauman D.E., 2003. Effects of abomasal infusion of conjugated linoleic acid on milk fat concentration and yield from pasture-fed dairy cows. J. Dairy Sci. 86, 644–652, https://doi.org/10.3168/jds.S0022-0302(03)73642-X

Maria-Patiño E., 2011. Production and quality of buffalo milk (in Spanish). Tecnol. Marcha 24, 25–35

Martínez-Marín A.L., Pérez-Hernández M., Pérez-Alba L.M., Carrión-Pardo D., Gómez-Castro G., Garzón-Sigler A., 2013. Effect of oils and seeds in ruminant diets on milk fat fatty acid profile. Review (in Spanish). Rev. Mex. Cienc. Pecu. 4, 319–338

Mojica-Rodríguez J.E., Castro-Rincón E., Carulla-Fornaguera J.E., Lascano-Aguilar C.E., 2019. Grazing intensity on lipid profile in bovine milk in the Colombian dry tropics (in Spanish). Agron. Mesoam. 30, 783–802, https://doi.org/10.15517/am.v30i3.36312

Morales-Almaráz E., Soldado A., González A., Martínez-Fernández A., Domínguez Vara I., de la Roza Delgado B., Vicente F., 2010. Improving the fatty acid profile of dairy cow milk by combining grazing with feeding of total mixed ration. J. Dairy Res. 77, 225–230, https://doi.org/10.1017/S002202991000004X

Muñoz C., Sánchez R., Peralta A.M.T., Espíndola S., Yan T., Morales R., Ungerfeld E.M., 2019. Effects of feeding unprocessed oilseeds on methane emission, nitrogen utilization efficiency and milk fatty acid profile of lactating dairy cows. Anim. Feed Sci. Technol. 249, 18–30, https://doi.org/10.1016/j.anifeedsci.2019.01.015

Muruz H., Çetinkaya N., 2019. The effect of dairy cow feeding regime on functional milk production. Int. Adv. Res. Eng. J. 3, 1–6

Nantapo C.T.W., Muchenje V., Hugo A., 2014. Atherogenicity index and health-related fatty acids in different stages of lactation from Friesian, Jersey and Friesian x Jersey cross cow milk under a pasture-based dairy system. Food Chem. 146, 127–133, https://doi.org/10.1016/j.foodchem.2013.09.009

Neves C.A., dos Santos W.B.R., Santos G.T.D., da Silva D.C., Jobim C.C., Santos F.S., Visentainer J.V., Petit H.V., 2009. Production performance and milk composition of dairy cows fed extruded canola seeds treated with or without lignosulfonate. Anim. Feed Sci. Technol. 154, 83–92, https://doi.org/10.1016/j.anifeedsci.2009.08.002

Ortega-Pérez R., Espinoza-Villavicencio J.L., Palacios-Mechetnov E., Palacios-Espinosa A., Arjona-López O., Murillo-Amador B., Rivera-Acuña F., 2013. Fatty acid profile in milk from Chinampas (*Bos taurus*) cows fed with fresh forage from sarcocaulescent scrub or alfalfa hay (in Spanish). Arch. Med. Vet. 45, 45–51, http://dx.doi.org/10.4067/S0301-732X2013000100008

Osorio J.H., Vinazco, J., 2010. Bovine lipid metabolism and its relationship with diet, corporal condition, productive state and associated pathologies. Biosalud 9, 56–66

Pi Y., Gao S.T., Ma L., Zhu Y.X., Wang J.Q., Zhang J.M., Xu J.C., Bu D.P., 2016. Effectiveness of rubber seed oil and flaxseed oil to enhance the α-linolenic acid content in milk from dairy cows. J. Dairy Sci. 99, 5719–5730, https://doi.org/10.3168/jds.2015-9307

Pirondini M., Colombini S., Mele M., Malagutti L., Rapetti L., Galassi G., Crovetto G.M., 2015. Effect of dietary starch concentration and fish oil supplementation on milk yield and composition, diet digestibility, and methane emissions in lactating dairy cows. J. Dairy Sci. 98, 357–372, https://doi.org/10.3168/jds.2014-8092

Plata-Reyes D.A., Morales-Almaraz E., Martínez-García C.G., Flores-Calvete G., López-González F., Prospero-Bernal F., Valdez-Ruiz C.L., Zamora-Juárez Y.G., Arriaga-Jordán C.M., 2018. Milk production and fatty acid profile of dairy cows grazing four grass species pastures during the rainy season in small-scale dairy systems in the highlands of Mexico. Trop. Anim. Health Prod. 50, 1797–1805, https://doi.org/10.1007/s11250-018-1621-8

Prieto-Manrique E., Vargas-Sánchez J.E., Angulo-Arizala J., Mahecha-Ledesma L., 2016. Fat and fatty acids of cow milk grazing in four production systems (in Spanish). Agron. Mesoam. 27, 421–437, https://dx.doi.org/10.15517/am.v27i2.22022

Purba R.A.P., Paengkoum P., Paengkoum S., 2020. The links between supplementary tannin levels and conjugated linoleic acid (CLA) formation in ruminants: A systematic review and meta-analysis. PLoS One 15, e0216187, https://doi.org/10.1371/journal.pone.0216187

Rego O.A.D., Portugal P.V., Sousa M.B., Rosa H.J.D, Vouzela C.M., Borba A.E.S., Bessa R.J.B., 2004. Effect of diet on the fatty acid pattern of milk from dairy cows. Anim. Res. 53, 213–220, https://doi.org/10.1051/animres:2004010

Rego O.A., Alves S.P., Antunes L.M.S., Rosa H.J.D., Alfaia C.F.M., Prates J.A.M., Cabrita A.R.J., Fonseca A.J.M., Bessa R.J.B., 2009. Rumen biohydrogenation-derived fatty acids in milk fat from grazing dairy cows supplemented with rapeseed, sunflower, or linseed oils. J. Dairy Sci. 92, 4530–4540, https://doi.org/10.3168/jds.2009-2060

Rego O.A., Rosa, H.J.D., Portugal, P.V., Franco, T., Vouzela, C.M., Borba, A.E.S., Bessa, R.J.B. 2005a. The effects of supplementation with sunflower and soybean oils on the fatty acid profile of milk fat from grazing dairy cows. Anim. Res. 54, 17–24, https://doi.org/10.1051/animres:2005002

Rego O.A., Rosa H.J.D., Portugal P., Cordeiro R., Borba A.E.S., Vouzela C.M., Bessa R.J.B., 2005b. Influence of dietary fish oil on conjugated linoleic acid, omega-3 and other fatty acids in milk fat from grazing dairy cows. Liv. Prod. Sci. 95, 27–33, https://doi.org/10.1016/j.livprodsci.2004.11.040

Reveneau C., Ribeiro C.V.D.M., Eastridge M.L., St-Pierre N.R., Firkins J.L., 2005. Processing whole cottonseed moderates fatty acid metabolism and improves performance by dairy cows. J. Dairy Sci. 88, 4342–4355, https://doi.org/10.3168/jds.S0022-0302(05)73121-0

Roca-Fernández A.I., González-Rodríguez A., Vázquez-Yáñez O.P., López-Mosquera M.E., 2014. Effect of forage source and concentrate type on milk production and fatty acids profile of dairy cow (in Spanish). In: Pastos y PAC. Proceedings of the 53rd SEEP Scientific Meeting, La Coruña, Spain, pp. 453–460

Roy A., Ferlay A., Shingfield K.J., Chilliard Y., 2006. Examination of the persistency of milk fatty acid composition responses to plant oils in cows given different basal diets, with particular emphasis on *trans*-$C1_{8:1}$ fatty acids and isomers of conjugated linoleic acid. Anim. Sci. 82, 479–492, https://doi.org/10.1079/ASC200658

Sánchez M.A., Murray R.S., Montero J., Marchini M., Iglesias R., Saad G., 2020. Importance of milk and its potential effects on human health (in Spanish). Actual. Nutr. 21, 50–64, https://fi-admin.bvsalud.org/document/view/r8p94

SAS Institute Inc., 2002. Base SAS® 9.0 Procedures Guide. SAS Institute Inc., Cary, NC

Sarrazin P., Mustafa A.F., Chouinard P.Y., Raghavan G.S.V., Sotocinal S.A., 2004. Performance of dairy cows fed roasted sunflower seed. J. Sci. Food Agric. 84, 1179–1185, https://doi.org/10.1002/jsfa.1802

Siurana A., Calsamiglia S., 2016. A metaanalysis of feeding strategies to increase the content of conjugated linoleic acid (CLA) in dairy cattle milk and the impact on daily human consumption. Anim. Feed Sci. Technol. 217, 13–26, https://doi.org/10.1016/j.anifeedsci.2016.04.013

Sterk A., Van Vuuren A.M., Hendriks W.H., Dijkstra J., 2012. Effects of different fat sources, technological forms and characteristics of the basal diet on milk fatty acid profile in lactating dairy cows–A meta-analysis. J. Agric. Sci. 150, 495–517, https://doi.org/10.1017/S0021859611000979

Tóth T., Mwau P.J., Bázár G., Andrássy-Baka G., Hingyi H., Csavajda E., Varga L., 2019. Effect of feed supplementation based on extruded linseed meal and fish oil on composition and sensory properties of raw milk and ultra-high temperature treated milk. Inter. Dairy J. 99, 104552, https://doi.org/10.1016/j.idairyj.2019.104552

Toyes-Vargas E.A., Murillo-Amador B., Espinoza-Villavicencio J.L., Carrión-Palau L., Palacios-Espinosa, A., 2013. Chemical composition and precursors of vaccenic and rumenic acids in forage species in Baja California Sur, Mexico (in Spanish). Rev. Mex. Cienc. Pecu. 4, 373–386

Vafa T.S., Naserian A.A., Moussavi A.R.H., Valizadeh R., Mesgaran M.D., 2012. Effect of supplementation of fish and canola oil in the diet on milk fatty acid composition in early lactating Holstein cows. Asian-Aust. J. Anim. Sci. 25, 311–319, https://doi.org/10.5713/ajas.2010.10014

Vibart R.E., Tavendale M., Otter D., Schwendel B.H., Lowe K., Gregorini P., Pacheco D., 2017. Milk production and composition, nitrogen utilization, and grazing behavior of late-lactation dairy cows as affected by time of allocation of a fresh strip of pasture. J. Dairy Sci. 100, 5305–5318, https://doi.org/10.3168/jds.2016-12413

Walker G.P., Doyle P.T., Heard J.W., Francis S.A., 2004. Fatty acid composition of pastures. Anim. Produc. Austr. 1, 192–195

Ward A.T., Wittenberg K.M., Froebe H.M., Przybylski R., Malcolmson L., 2003. Fresh forage and solin supplementation on conjugated linoleic acid levels in plasma and milk. J. Dairy Sci. 86, 1742–1750, https://doi.org/10.3168/jds.S0022-0302(03)73760-6

Welter C., Martins C., de Palma S., Martins M., Dos Reis R., Schmidt L., Saran A., 2016. Canola oil in lactating dairy cow diets reduces milk saturated fatty acids and improves its omega-3 and oleic fatty acid content. PloS One 11, 1–16, https://doi.org/10.1371/journal.pone.0151876

Whitlock L.A., Schingoethe D.J., AbuGhazaleh A.A., Hippen A.R., Kalscheur K.F., 2006. Milk production and composition from cows fed small amounts of fish oil with extruded soybeans. J. Dairy Sci. 89, 3972–3980, https://doi.org/10.3168/jds.S0022-0302(06)72440-7