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Effect of postruminal supply of linseed oil in dairy cows: 1. Production performance and fate of postruminally available α-linolenic acid

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Research Article

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Abstract

Triacylglycerols (TAG) are the primary sources of preformed fatty acids (FA) for lipid synthesis in the mammary gland. However, polyunsaturated FA escaping ruminal biohydrogenation are selectively incorporated into cholesterol esters (CE) and phospholipids (PL). The aim of the current experiment was to study the effects of abomasal infusion of increasing amount of linseed oil (L-oil) on plasma distribution of α-linolenic acid (α-LA) and its transfer efficiency into milk fat. Five rumen-fistulated Holstein cows were randomly distributed in a 5 × 5 Latin square design. Abomasal infusion of L-oil (55.9% α-LA) was performed at the rate of 0, 75, 150, 300, and 600 ml/d. Concentrations of α-LA increased quadratically in TAG, PL, and CE; a less steep slope was observed with an inflexion at an infusion rate of 300 ml L-oil per day. The increase in plasma concentration of α-LA was of a lower magnitude in CE as compared with the other two fractions, resulting in a quadratic decrease in relative proportion of this FA circulating as CE. The transfer efficiency into milk fat increased from 0 to 150 ml L-oil infused, and a plateau was maintained thereafter with greater levels of infusion (quadratic response). This pattern resembles the quadratic response of the relative proportion of α-LA circulating as TAG, and the relative concentration of this FA in TAG. Increasing the postruminal supply of α-LA partly overcame the segregation mechanism of absorbed polyunsaturated FA in different plasma lipid classes. Proportionately more α-LA was then esterified as TAG, at the expense of CE, increasing its efficiency of transfer into milk fat. This mechanism appears to be surpassed in its turn when L-oil infusion was increased over 150 ml/d. Nevertheless, the yield of α-LA in milk fat continued to increase, but at a slower rate at the highest levels of infusion.

Bovine milk fat contains a low concentration of α-linolenic acid (*cis*-9, *cis*-12, *cis*-15 18:3), ranging from 0.5 to 2.0% of total fatty acids (FA; Kaylegian and Lindsay, 1995; Lindmark Månsson, 2008). The main reason for the low secretion of this n-3 FA in milk, despite its abundance in several feed ingredients, is the extensive biohydrogenation taking place in the rumen. Reviewing the literature, Doreau and Ferlay (1994) reported a rate of hydrogenation of *cis*-9, *cis*-12, *cis*-15 18:3 ranging between 85 and 100%, with an average of 92%.

Under conditions of low FA intake, dietary *cis*-9, *cis*-12, *cis*-15 18:3 escaping ruminal biohydrogenation is selectively incorporated, after its absorption in the small intestine, into the plasma cholesterol esters (CE) and phospholipids (PL; Tyburczy *et al.*, 2008). A limited portion is esterified to triacylglycerols (TAG) or remains in its free form. Less *cis*-9, *cis*-12, *cis*-15 18:3 is thus available for milk fat synthesis, as TAG are the primary sources of preformed FA for lipid synthesis in the mammary gland (Christie, 1981). Such segregation process represents another obstacle in the transfer of dietary *cis*-9, *cis*-12, *cis*-15 18:3 to milk fat. However, as suggested by Christie (1981), when large amounts of polyunsaturated FA reach the intestines, the mechanism for segregating these components is exceeded and the surplus could be incorporated into TAG. In line with this premise, postruminal supply of *cis*-9, *cis*-12, *cis*-15 18:3, *via* abomasal infusion of linseed oil (L-oil), increased milk fat concentration of this n-3 FA up to 14.3% of milk fat (Fauteux *et al.*, 2016). This represents an apparent recovery of 27.7%, which is much greater than the efficiency of transfer of 1.95% reported in a meta-analysis (of 16 treatment means) by Leduc *et al.* (2017) for dietary L-oil.

Despite this information, it appears that the quantitative aspect of the distribution mechanism of dietary polyunsaturated FA between plasma lipid classes and the resulting impact on their transfer efficiency into milk fat are not fully understood. The objective of our research was to determine the impact of postruminal supply of increasing amounts of L-oil, as a source of *cis*-9, *cis*-12, *cis*-15 18:3, on lactation performance, FA profile of plasma lipid fractions, and yield of milk FA. We hypothesised that a threshold value exists beyond which the postruminal supply of alpha-linolenic acid overcome the segregation mechanism generally observed during

intestinal absorption and plasma transport, leading to a more even distribution of this fatty acid in varying lipid classes, and to an increased efficiency of its transfer to milk fat.

Materials and methods

Animals, feeding, and treatments

The experimental procedures involving dairy cows followed the guidelines of the Canadian Council on Animal Care (2009) and were approved by the Université Laval Animal Care Committee (Protocol # 2015001). The trial was conducted in an air-conditioned tie-stall facility at the Centre de Recherche en Sciences Animales de Deschambault, QC, Canada.

Five Holstein cows in early lactation (36 ± 2 d in milk; mean ± SD), weighing 732 ± 66 kg, and fitted with a rumen cannula (Ankom Technology, Macedon, NY, USA) were fed a total mixed ration based on corn and grass silages (online Supplemental Table S1) and formulated to meet or exceed energy and nutrient requirements (National Research Council, 2001). This total mixed ration was offered once daily at 10:00 h and the amount served was adjusted to obtain approximately 10% of refusals, ensuring *ad libitum* feeding conditions. Silages were sampled once a week and dried at 55°C for 72 h to determine their dry matter content and to adjust the proportions of feed ingredients in the total mixed ration on an as-fed basis. Free access to drinking water was available throughout the experiment.

Cows were randomly distributed in a 5 × 5 Latin square design with experimental periods of 21 d. During the first 14 d of each period, cows received an abomasal infusion of L-oil (Pokonobe Industries Inc., Westmount, QC; containing 5.6% 16:0, 3.4% 18:0, 18.4% *cis*-9 18:1, 0.7% *cis*-11 18:1, 14.9% *cis*-9, *cis*-12 18:2, 55.9% *cis*-9, *cis*-12, *cis*-15 18:3, and 0.2% 20:0) at the rate of 0, 75, 150, 300, and 600 ml/d. Oil was continuously delivered to the abomasum using peristaltic pumps (Flexiflo Patrol Pump; Abbott Nutrition Canada, Saint-Laurent, QC, Canada) connected to an infusion apparatus as described by Gressley *et al.* (2006). Each treatment period was followed by a 7-d washout interval.

Experimental measurements, samplings and analyses

Feed offered and refused was weighed and samples of the total mixed ration were collected from d 12 to 14 of each infusion period. Samples were dried at 55°C for 48 h to determine dry matter content and ground to 1 mm using a Wiley mill (model 4, Arthur H. Thomas Co., Philadelphia, PA). After being pooled by period, samples were analysed for residual moisture, neutral detergent fibre, acid detergent fibre, crude protein, and ash as described in the online Supplementary File, materials and methods. Determination of dietary FA composition was carried out by gas chromatography as described by Jenkins (2010) with modifications (Villeneuve *et al.*, 2013).

Cows were milked twice daily at 7:00 and 17:00 h. Milk yield was measured using calibrated milk meters (Flomaster Pro, DeLaval, Tumba, Sweden), and samples were collected at each milking from d 12 to 14 on each infusion period. These samples were preserved with bronopol and stored at 4°C. At the end of each period, these samples were sent to a commercial laboratory (Lactanet, Ste-Anne-de-Bellevue, QC, Canada) where they were analysed for fat, protein, lactose, and urea-N concentrations by infrared absorption spectroscopy using a Foss MilkoScan FT 6000 (Foss, Hillerød, Denmark), and for somatic cell count determination using a Fossomatic FC (Foss). Yield of energy-corrected milk was calculated as described by Madsen *et al.* (2008). An additional set of milk samples without preservative were harvested during the last 3 d of each infusion and stored at −20°C for later determination of the FA profile as described in a companion paper (Rico *et al.*, 2023 in press).

On d 13 of each infusion period, blood was withdrawn from the coccygeal vessel into evacuated plasma separation tubes containing EDTA (Vacutainer 366430, Becton Dickinson, Franklin Lakes, NJ, USA) at 09:30 (preprandial) and 15:30 (postprandial) h. Blood was placed on ice until centrifuged at 956 × ***g*** for 15 min and 4°C. The supernatant was transferred to microtubes and frozen at −20°C for further FA analysis of lipid classes (TAG, PL, CE, and FFA) as described in the online Supplementary File, materials and methods.

Statistical analysis

Data were analysed with the MIXED procedure of SAS (SAS Institute Inc., Cary, NC) as a 5 × 5 Latin square design according to the following model:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + C_l(S_k) + \varepsilon_{ijkl}$$

where Y_{ijkl} is the individual observation, μ the overall mean, T_i the fixed effect of treatment ($i = 1$ to 5), P_j the random effect of period ($j = 1$ to 5), S_k the random effect of sequence ($k = 1$ to 5), $C_l(S_k)$ the random effect of cow ($l = 1$–5) nested in sequence, and ϵ_{ijkl} the residual error terms. Linear and quadratic contrasts for treatment effect were performed. Differences between treatments were declared at $P \leq 0.05$.

Results

The online Supplementary File provides detailed data for dry matter intake, milk yield and composition (Table S2), intake, secretion and transfer efficiencies of individual FA (Table S3), FA yields (Table S4) and fatty acid profiles in TAG (Table S5), PL (Table S6), CE (Table S7) and FFA (Table S8) as well as the distribution of FA among plasma lipid classes (Table S9). Intake of the total mixed ration decreased linearly with increasing levels of L-oil infused into the abomasum (Fig. 1a). However, the estimated energy intake was not affected by treatments (Fig. 1b). Increasing levels of infusion linearly decreased the yield of energy-corrected milk (Fig. 1d). Milk fat concentration decreased quadratically, reaching a plateau at 300 ml of L-oil/d, whereas fat yield decreased linearly with increasing doses of L-oil (Fig. 1e and 1f). Milk protein and lactose concentrations remained stable, but protein yield decreased linearly with the level of oil infusion whereas lactose yield remained unchanged (Fig. 1g to 1j). Intake of *cis*-9, *cis*-12, *cis*-15 18:3 increased linearly with the level of infusion (Fig. 2a).

Plasma concentrations of *cis*-9, *cis*-12, *cis*-15 18:3 circulating as TAG, PL and CE increased linearly and quadratically, whereas the concentration of this FA in its free form increased linearly with the amount of L-oil infused (Fig. 3, left panels). Similar variations were observed when concentrations of *cis*-9, *cis*-12, *cis*-15 18:3 were expressed in relative proportions of total FA in each of these plasma fractions (Fig. 3, right panels). Finally, when expressed as proportions of total circulating *cis*-9, *cis*-12, *cis*-15 18:3 (Fig. 4), concentrations of this FA increased linearly and

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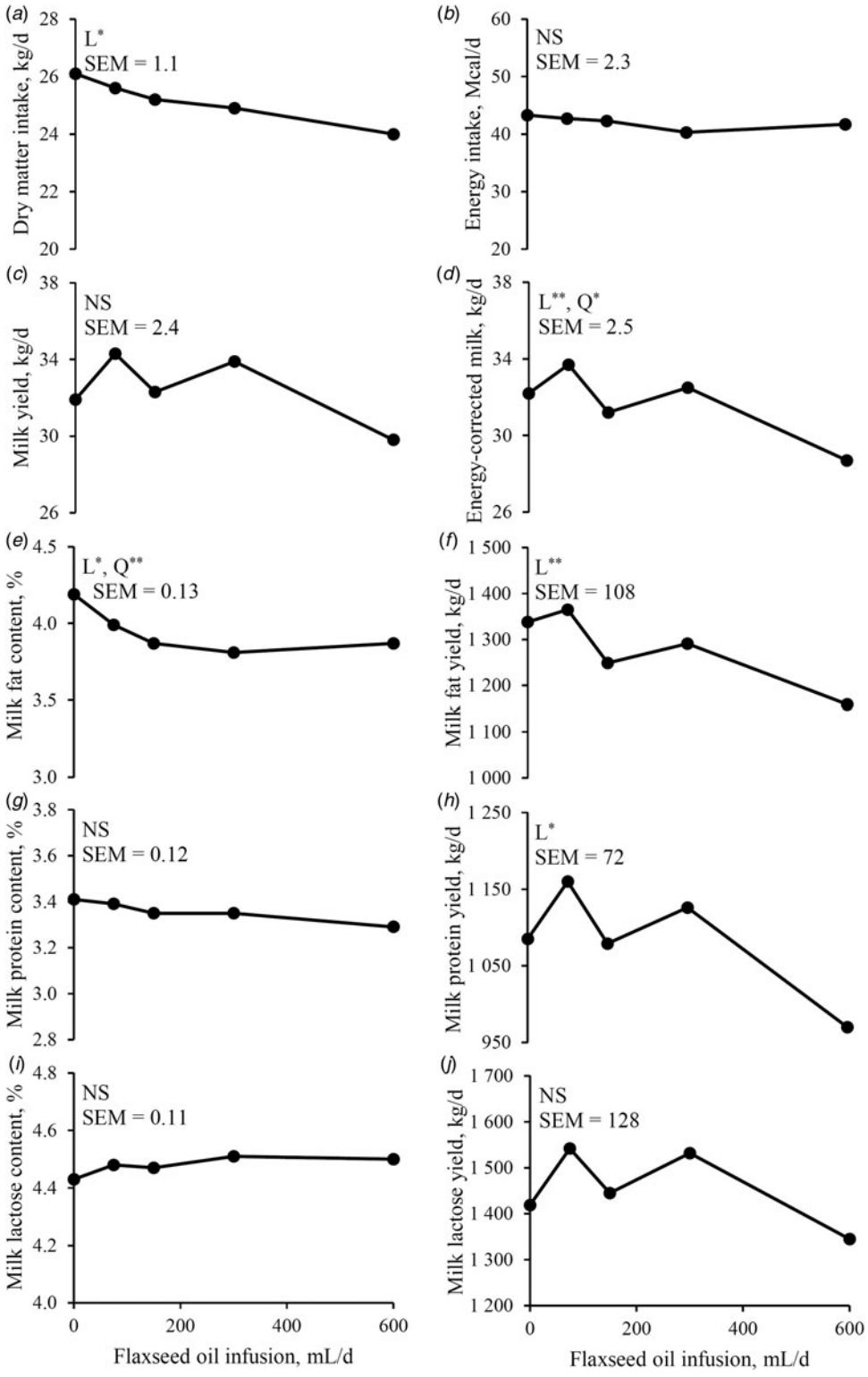

Figure. 1. Dry matter (a) and energy (b) intake, actual (c) and energy corrected (d) milk yield, and concentrations and yields of milk fat (e and f), protein (g and h) and lactose (i and j) in dairy cows abomasally infused with increasing levels of linseed oil. SEM, standard error of the mean; L = linear and Q, quadratic effects of the level of linseed oil infusion. *$P \leq 0.05$ and **$P \leq 0.01$. NS, not significantly affected ($P > 0.05$). Table values can be found in online Supplementary File, Table S2.

quadratically in PL and TAG, increased linearly in FFA and decreased linearly and quadratically in CE.

Milk fat concentration of *cis*-9, *cis*-12, *cis*-15 18:3 increased linearly as the level of infusion of L-oil increased (Fig. 2b). Milk secretion and the transfer efficiency from diet + infusion to milk fat of *cis*-9, *cis*-12, *cis*-15 18:3 increased linearly and quadratically as the level of infusion of L-oil increased (Fig. 2c and 2d). Regarding the quadratic response of

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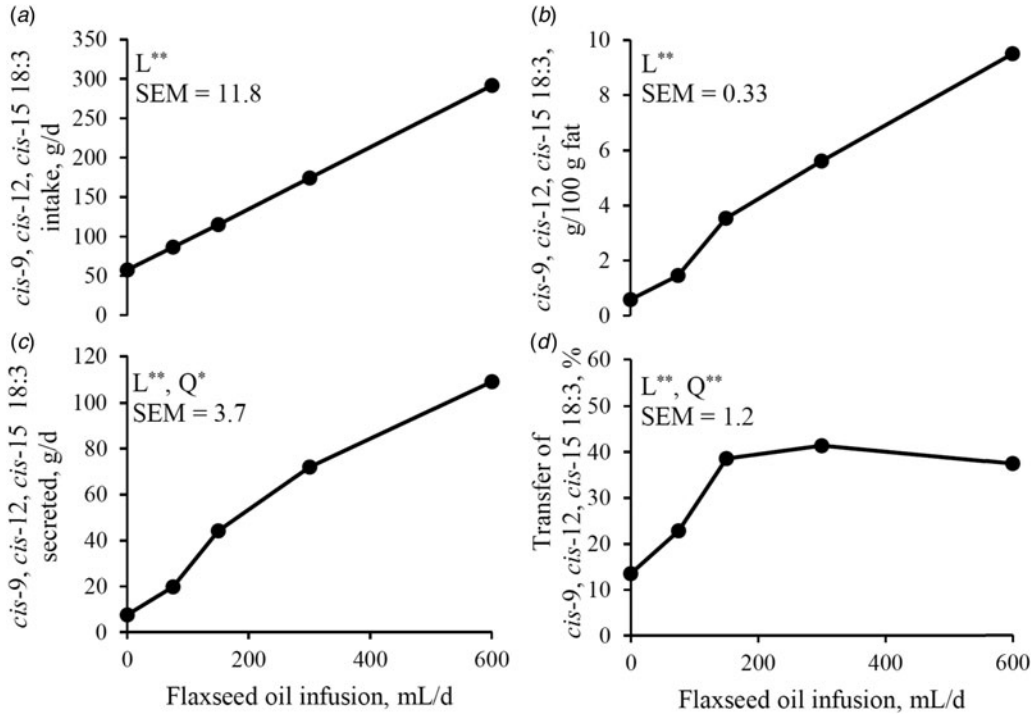

Figure. 2. Intake (A), milk concentration (B) and secretion (C), and apparent transfer efficiency from diet to milk fat (D) of *cis*-9, *cis*-12, *cis*-15 18:3 in dairy cows abomasally infused with increasing levels of linseed oil. SEM, standard error of the mean; L, linear and Q, quadratic effects of the level of linseed oil infusion. $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. Table values can be found in online Supplementary File, Tables S3 and S4.

transfer efficiency, a plateau was reached at a daily infusion rate of 150 ml L-oil.

Discussion

Dairy cows were abomasally infused with L-oil in the current trial as a convenient method to avoid ruminal biohydrogenation of its constituent polyunsaturated FA. In a review, Benson *et al.* (2001) reported that abomasal infusion of L-oil decreased the intake of the total mixed ration. However, as the energy density of FA from L-oil is greater than that of the ingredients of the diet (National Research Council, 2001), lower dry matter intake was presumably a mechanism to maintain a constant ingestion of energy (Bull *et al.*, 1976). This satiety effect of abomasally infused polyunsaturated FA appears to be mediated through glucagon-like peptide-1 (7–36) amide (Litherland *et al.*, 2005), products of proglucagon processing (pancreatic glucagon, gut glucagon, and glucagon-like peptide-1; Benson and Reynolds, 2001) and cholecystokinin (Chelikani *et al.*, 2004), whereas leptin does not seem to be involved (Chelikani *et al.*, 2004).

Despite a lack of impact on energy intake, yield of energy-corrected milk decreased linearly with the amount of L-oil infused. The decrease in dry matter intake may have led to a shortage of nutrients to support the synthesis of major milk constituents (fat, protein and lactose). In addition to the impact on yield, milk fat concentration also decreased with increasing levels of L-oil infusion. A quadratic response was apparent with a 9% decrease observed at 300 ml of L-oil/d.

When cows received the control treatment (0 ml/d L-oil), 83.5% of plasma *cis*-9, *cis*-12, *cis*-15 18:3 were circulating as CE, 14.9% as PL, 0.8% as TAG and 0.6% as FFA (Fig. 4). This distribution differs somewhat from data reported by Tyburczy *et al.* (2008; 66.3% as CE, 31.7% as PL, 1.7% as TG, and 0.3% as FFA) in cows in later lactation (259 ± 6 d post-partum) as compared to those used in the current trial (36 ± 2 d post-partum). Nevertheless, these results show that, at a low level of absorption, *cis*-9, *cis*-12, *cis*-15 18:3 is preferentially circulating as CE in blood plasma of dairy cows.

Concentrations of *cis*-9, *cis*-12, *cis*-15 18:3 increased quadratically in TAG, PL, and CE with level of infusion (Fig. 3). In these three fractions, a smaller slope was observed with an inflexion at an infusion rate of 300 ml of L-oil per day. The increase in plasma concentration of *cis*-9, *cis*-12, *cis*-15 18:3 was of a lower magnitude in CE as compared with the other two fractions. As a result, the relative proportion of *cis*-9, *cis*-12, *cis*-15 18:3 circulating as CE decreased quadratically as the level of L-oil infusion increased (Fig. 4). This observation is in line with the lower plasma proportion of *cis*-9, *cis*-12, *cis*-15 18:3 circulating as CE (66.3%) for a total concentration of 14.5 mg/dl in the experiment reported by Tyburczy *et al.* (2008), as compared with the current experiments where 83.5% of plasma *cis*-9, *cis*-12, *cis*-15 18:3 was circulating as CE, for a total concentration of 9.4 mg/100 g.

The efficiency of transfer from diet + infusion to milk fat initially increased from 0 to 150 ml of L-oil infused per day and a plateau was maintained thereafter (Fig. 2d). This pattern resembles the quadratic response of the relative proportion of *cis*-9, *cis*-12, *cis*-15 18:3 circulating as TAG (Fig. 4), and the relative concentration of this FA in TAG (Fig. 3).

These results partly support our hypothesis as, by increasing the postruminal supply of *cis*-9, *cis*-12, *cis*-15 18:3, we were able to partly overcome the segregation mechanism of absorbed polyunsaturated FA in different plasma lipid classes. Proportionately more *cis*-9, *cis*-12, *cis*-15 18:3 was then esterified as TAG, at the expense of CE, increasing the efficiency of transfer into milk fat. This

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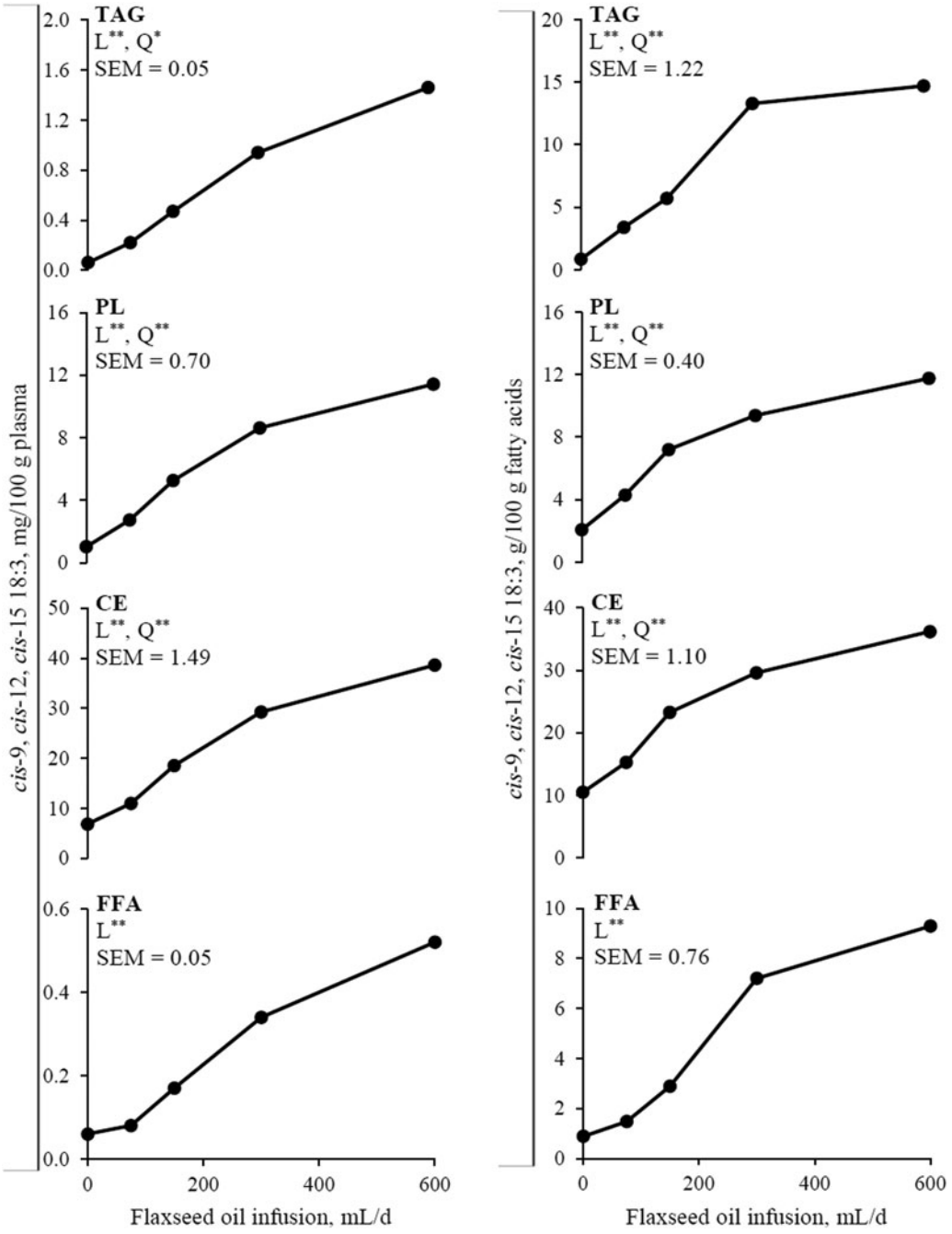

Figure. 3. Concentrations (left panels) and relative proportion (right panels) of *cis*-9, *cis*-12, *cis*-15 18:3 in plasma triacylglycerols (TAG), phospholipids (PL), cholesterol esters (CE), and free fatty acids (FFA) fractions in dairy cows abomasally infused with increasing levels of linseed oil. SEM, standard error of the mean; L, linear and Q, quadratic effects of the level of linseed oil infusion. $^{*}P \leq 0.05$ and $^{**}P \leq 0.01$. Table values can be found in online Supplementary File, Tables S5 to S8.

mechanism appeared to be surpassed in its turn when infusion increased over 150 ml/d, and transfer efficiency reached a plateau at greater supplies of L-oil. This observation is consistent with data reported by Baldwin *et al.* (1980) indicating that the relationship between plasma TAG concentration and mammary uptake is best described by Michaelis–Menten type kinetics. Nevertheless, the yield of *cis*-9, *cis*-12, *cis*-15 18:3 in milk fat continued to increase, but at a slower rate at the highest levels of infusion.

In conclusion, whilst ruminal biohydrogenation and selective incorporation into plasma CE and PL are two obstacles in the transfer of polyunsaturated FA form diet to milk fat in lactating ruminants, we were able to study the fate of postruminally available *cis*-9, *cis*-12, *cis*-15 18:3 by using abomasal infusion of L-oil. At low doses the transfer efficiency of this FA into milk fat gradually increased as compared with control. This observation was consistent with the parallel increase in relative proportion of *cis*-9, *cis*-12, *cis*-15 18:3 circulating as plasma TAG known to be the main source of preformed FA acid taken up by the mammary gland for milk fat synthesis. However, the transfer into milk fat of FA circulating as TAG is a saturable process, and the efficiency plateaued at greater levels of L-oil infusion. Nonetheless, the yield of *cis*-9, *cis*-12, *cis*-15 18:3 in milk fat continued to increase, but at a slower rate at the highest infusion rates. The transport of FA in blood plasma and their subsequent

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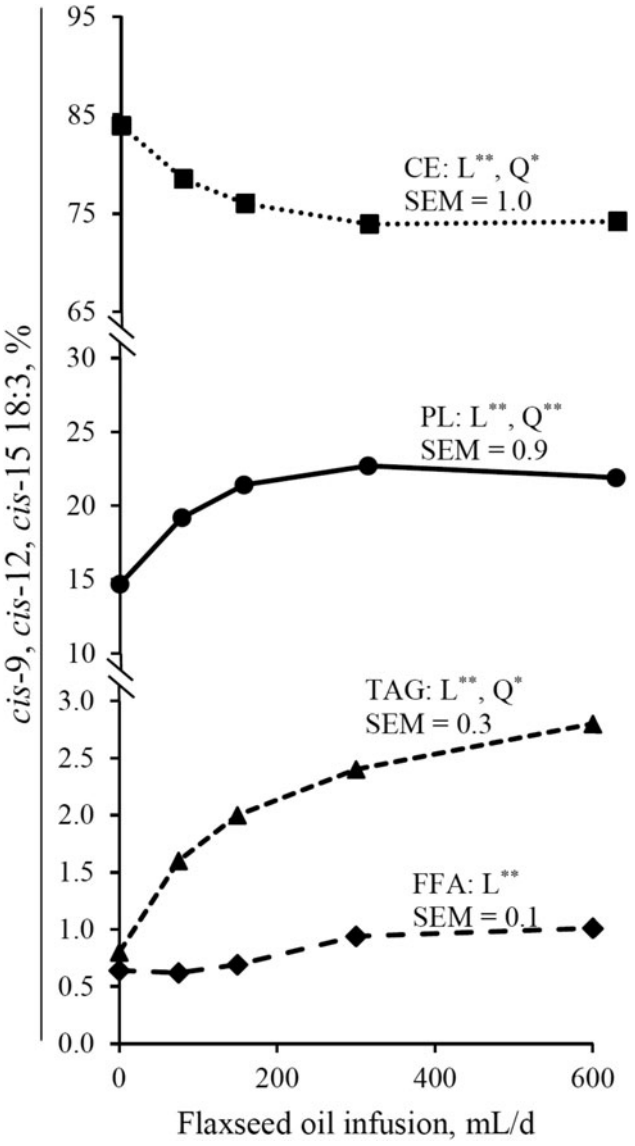

Figure. 4. Distribution of *cis*-9, *cis*-12, *cis*-15 18:3 among plasma lipid classes in dairy cows abomasally infused with increasing levels of linseed oil. SEM, standard error of the mean; TAG, triacylglycerols; PL, phospholipids; CE, cholesterol esters, and FFA, free fatty acids; L, linear and Q, quadratic effects of the level of linseed oil infusion. *$P \leq 0.05$ and **$P \leq 0.01$. Table values can be found in online Supplementary File, Table S9.

transfer to the mammary gland in dairy cows appear to be under a complex regulation which is not yet fully understood. A better comprehension of these mechanisms could help better predict the fate of essential FA in ruminants.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029923000250

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