

Plasma Fatty Acid Profile of Gestating Ewes Supplemented with Fishmeal

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Abstract: Problem statement: The very long chain n-3 polyunsaturated fatty acids (>18C) cannot be adequately synthesized by ruminant tissues to meet their requirements; therefore, their concentration in body depends on the supply through feed. It may be possible to improve the essential fatty acid status of ruminant animals, during gestation by manipulating the maternal diet with fishmeal (FM). The objectives of this research were to (1) determine the effect of fishmeal supplementation on the plasma fatty acid profile of ewes during late gestation and (2) determine the status of the plasma docosahexaenoic acid (22:6n3) of lambs born to these ewes. **Approach:** Eight gestating ewes [Rideau-Arcott, 97±5 kg initial body weight, 100 days of gestation] were used in a completely randomized design. Ewes were individually-housed and fed either a control diet (supplemented with soybean meal) or a fishmeal supplemented diet. Blood samples were collected via jugular venipuncture for plasma fatty acids analysis on 100, 114, 128 and 142 days of gestation after morning feeding. Blood samples from the lambs were also collected via jugular venipuncture immediately after birth and before receiving their mothers' colostrum. Plasma fatty acids were analyzed by gas-liquid chromatography. **Results:** The ewes from both groups, i.e., control and fishmeal supplemented, had a similar fatty acid profile prior to supplementation (at 100 days, p>0.05). Thereafter, there was an increase in eicosapentaenoic acid (20:5n3), docosahexaenoic acid, total n3-PUFA and total very long chain n3-PUFA (>C18) contents in plasma for the fishmeal supplemented ewes compared to the control (p<0.03). There were no differences (p>0.05) in total saturated fatty acids, total monounsaturated fatty acids, total conjugated linoleic acid, total *trans*-18:1, total *cis*-18:1, or total n6-PUFA contents in ewe plasma between control and fishmeal supplemented groups. Lambs born to ewes fed the fishmeal supplemented diet had greater (p<0.05) plasma concentrations of eicosapentaenoic acid (0.7 Vs 0.4, g/100 g FA), docosahexaenoic acid (1.6 Vs 0.9, g/100 g FA) and total very long chain n3-PUFA (3.3 vs. 2.0, g/100 g FA) than lambs born to ewes fed the control diet. **Conclusion:** The ewes supplemented with fishmeal supplementation showed a positive response with the enrichment of docosahexaenoic acid, eicosapentaenoic acid and total very long chain n3-PUFA in plasma during gestation and these fatty acids were transferred to the fetus as well.

Kew words: Docosahexaenoic acid, Eicosapentaenoic acid, Fishmeal, Very long chain n3-PUFA, Ewe plasma, Gestation

INTRODUCTION

The very long chain n-3 polyunsaturated fatty acids (>18C, VL_n3-PUFA), such as, docosahexaenoic acid (DHA, 22:6n3), are essential for normal growth and development in mammals (Williams, 2000). A specific functional role of DHA had been shown in the fetus and early infant neural development, which might influence neonatal viability (Cattaneo *et al.*, 2006; Capper *et al.*, 2007). Docosahexaenoic acid is highly concentrated in brain tissues and selectively accumulated during later

stages of fetal development and infant brain growth (Brenna and Lapillonne, 2009; Hsieh and Brenna, 2009). Research suggests that DHA intake in pregnancy provides advantages to both the developing fetus and mother (Saldanha *et al.*, 2009). A major factor contributing to high lamb mortality rates in the sheep industry is hypothermia, due to delayed suckling and exhaustion of brown fat reserves (Singer, 1998). Studies in sheep have shown that supplementation of diets with fish oil, a source of VL_n3-PUFA, during late pregnancy reduced the latency of suckling in lambs (Capper *et al.*, 2006).

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It is well documented that the essential fatty acid (FA) status of ruminant animals at birth is less than satisfactory (Noble *et al.*, 1978). On the other hand, the VL_{n3}-PUFA cannot be adequately synthesized by ruminant tissues to meet their requirements; therefore, their concentration in body depends on the supply through feed. It may be possible to improve the essential fatty acid status of ruminant animals, during gestation by manipulating the maternal diet with Fishmeal (FM). The purposes of this study were to 1) determine the effect of FM-supplementation on the plasma FA profile of ewes during late gestation and 2) determine the status of plasma FA of lambs born to these ewes.

MATERIALS AND METHODS

All experimental procedures were done with the approval of the University of Guelph Animal Care Committee in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Animals and experimental treatments: Eight gestating ewes [Rideau-Arcott, 97±5 kg initial body weight, 100 days (d) of gestation] of similar nutritional and environmental background, were obtained from Ponsonby Sheep Research Station (University of Guelph, Ontario, Canada) and individually-housed in 4 by 6 foot indoor pens and randomly assigned to either a control diet (supplemented with soybean meal) or a FM-supplemented diet (2.64 kg/day as fed, comprising 0.312 kg protein-supplement, 0.441 kg mixed grain, 0.630 kg chopped hay, 1.261 kg alfalfa pellets). Ingredient composition of the diets is presented in Table 1. The nutrient requirements were based on both the body weight and gestational stage of the ewes determined by the Cornell Net Carbohydrate and Protein System for Sheep (Cornell University, Ithaca, NY). Feed was offered twice a day at 8:00 a.m. and 4:00 p.m., with orts being collected before the morning feeding. Animals were given ad libitum access to water throughout the duration of the study. The pooled samples of the experimental diets were analyzed for dry matter content by drying in an oven at 60°C for 48 h (AOAC, 1996). A subsample was ground using a Wiley Mill with a 1-mm screen (Thomas-Wiley, Philadelphia, PA) and stored at -20°C until analyzed. Samples were analyzed at a commercial laboratory (Agri-Food Laboratory,

Guelph, Ontario, Canada) for crude protein, crude fat, lignin, acid detergent fiber and neutral detergent fiber.

Blood sampling: Blood samples from the ewes were collected via jugular venipuncture on 100, 114, 128 and 142 day of gestation after morning feeding using heparin vacutainers (Becton, Dickinson and Company, Franklin Lks, NJ). Blood samples from the lambs were also collected via jugular venipuncture immediately after birth and before receiving their mothers' colostrum. Blood samples were centrifuged at 500×g for 15 min to separate the plasma. The plasma samples were kept at -20°C for subsequent fatty acids analysis.

Analysis of samples for fatty acids: Total lipid in plasma was extracted according to the method by Bligh and Dyer (1959) with minor modifications. A 0.20 mL of plasma was taken in a 15-mL screw-top culture tube with Teflon cap and mixed with water to a total vol of 1 mL; then 2.5 mL of methanol and 1.25 mL of chloroform were added in the same tube and vortexed. The contents of the culture tube were kept at room temperature for 60 min with every 10 min vortexing. After 1 h, 1.25 mL of chloroform, 1.15 mL of water and 0.1 mL of 3 M HCl were added, vortexed and centrifuged at 1,200×g for 3 min at room temperature. The acid (i.e., 3 M HCl) was added to ensure the pH of the extract was acidic. The chloroform layer (bottom phase) containing lipid was removed using 2 Pasteur pipettes, one inserted into other. The methanol-water phase was extracted with an additional 1.25 mL of chloroform and the chloroform phases were combined, dried over anhydrous Na₂SO₄, filtered and then transferred into a 4-mL vial. Chloroform was removed from the vial under a stream of N₂ and 3 drops of benzene were added and vortexed. The lipid content in the vial was methylated by adding 200 µL of NaOCH₃ (0.5 M solution in methanol, Sigma-Aldrich, St. Louis, MO) (Cruz-Hernandez *et al.*, 2004; Muller *et al.*, 2005). The vials were kept at room temperature for 25 min. Then, 1 mL of 1 N methanolic sulfuric acid (2.8 mL of 96% sulfuric acid in 100 mL methanol) was added. After vortexing, the vials were heated at 50°C for 15 min. After cooling at -20°C for 3 min, 1.0 mL of water and 1.0 mL of hexane were added, vortexed and centrifuged. The upper portion (i.e., hexane layer) containing Fatty Acid Methyl Esters (FAME) was transferred into another vial for GLC analysis.

Table 1: Ingredients and chemical analysis of experimental diets

Ingredients	Dry matter (%)	
	Control	Fishmeal supplemented
Mixed grain (barley, oats and corn)	16.34	16.34
Alfalfa hay	24.72	24.72
Alfalfa pellets	47.26	47.26
Protein supplement		
Soybean meal	6.62	--
Fishmeal	--	4.73
Feather meal	2.36	1.47
Wheat grain	0.71	2.36
Wheat shorts	--	1.68
Calcium mono phosphate	0.66	0.25
Calcium carbonate	0.57	0.42
Salt	0.42	0.38
Magnesium oxide	0.19	0.23
Minerals and vitamins ¹	0.16	0.16
Chemical composition		
Dry matter (%)	90.70	90.70
Crude protein (N × 6.25)	21.60	21.60
A Neutral detergent fiber	22.70	22.50
Neutral detergent fiber	35.30	36.30
Lignin	4.70	4.80
Crude fat	2.60	2.80
NFC ²	37.60	37.10
ME ³ , Mcal/kg	2.10	2.10

¹Floradale Feed Mill Limited, Floradale, ON, Canada; ²Nonfiber carbohydrates = 100 - [(neutral detergent fiber - neutral detergent insoluble protein) + crude protein + crude fat + ash]. ³Calculated using the Cornell Net Carbohydrate and Protein System for sheep (CNCPS-Sheep v. 1.0.21, Cornell University, Ithaca, NY) using forage chemical analysis.

Table 2: The fatty acid composition of experimental diets (Dry matter basis)

	(g / kg total Dry matter)	
	Fishmeal	Fatty acids
Control supplemented		
10:0	0.04	0.04
12:0	0.09	0.10
14:0	0.23	0.32
14:1	0.01	0.01
16:0	5.39	5.75
9c-16:1	0.17	0.25
18:0	0.93	0.89
9c-18:1	2.44	2.39
11c-18:1	0.24	0.30
18:2n6	7.02	6.98
20:0	0.29	0.30
20:1	0.05	0.09
18:3n3	8.51	9.35
22:0	0.28	0.30
20:4n6	0.01	0.02
20:5n3	nd	0.20
24:0	0.31	0.33
22:5n3	nd	0.02
22:6n3	nd	0.36

nd: not detected

The hexane containing FAME was analyzed by GLC and subsequently identified as described by Cruz-Hernandez *et al.* (2004) and Odongo *et al.* (2007) with a different temperature program. Briefly, FAME analysis was performed using an Agilent 6890N GLC (Agilent Technologies, Palo Alto, CA) equipped with a split-splitless injector at 250°C, a flame-ionization detector at 250°C and a CP Sil 88 column (100 m×0.25 mm, 0.2 µm of film thickness; Varian Inc., Mississauga, ON, Canada). Hydrogen was used as the carrier gas at a constant flow rate of 1 mL/min. The temperature of the GLC oven was set to 45°C for 4 min, increased at 13°C/min to 173°C and held for 28 min and increased at the rate of 4 °C/min to a final temperature of 215°C and held for 43 min. Agilent Technologies Chemstation software (Rev. B.01.01) was used for data analysis. A 1-µL sample was injected at splitless mode. Peaks were routinely identified by comparison of retention times with fatty acid methyl ester standards (GLC #463, #UC-59-M, C21:0, C23:0 and C26:0; NuCheck Prep Inc., Elysian, MN). In addition, some peaks of 18:1 and CLA and branched-chain FA, for which standard FAME were not available, were identified by comparison to published data as described by Kramer *et al.* (2002), Cruz-Hernandez *et al.* (2004) and Odongo *et al.* (2007).

The samples from the experimental diets were also analyzed for FA as shown in Table 2. The FM-supplemented diet provided 20:5n3, 22:5n3 and 22:6n3 of 0.20 g/kg total DM, 0.02 g/kg total DM and 0.36 g/kg total DM, respectively.

Statistical analysis: The data were analyzed as a completely randomized design using the PROC MIXED procedure of SAS (v. 9.1; SAS Inst., Inc., Cary, NC) using the model $Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha \times \beta)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} = the dependent variable, μ = overall mean, α_i = effect of diet ($i = 1, 2$), β_j = effect of day ($j = 1, 2, 3, 4$), $(\alpha \times \beta)_{ij}$ = effect of diet by day interaction ($ij = 1, 2, 3, 4, 5, 6, 7, 8$) and ε_{ijk} = random residual error. The effects of diet and day were considered as fixed effects. Day of experiment was used as a repeated measurement with ewe within dietary treatment as the subject. For each analyzed variable, animal was subjected to five covariance structures: compound symmetry, heterogeneous compound symmetry, autoregressive order 1, heterogeneous autoregressive order 1 and unstructured. The covariance structure that gave the smallest Bayesian information criterion was used (Littell *et al.*, 1996). Orthogonal polynomial contrast was used to describe the linear and quadratic terms of the day effect and diet by day interaction.

The FA contents of lamb plasma were analyzed as a completely randomized design using the PROC MIXED procedure of using the model $Y_j = \mu + \beta_j + \varepsilon_{jk}$;

where μ = overall mean, β = effect of diet ($j = 1, 2$) and ε_{jk} = random residual error. Effects were considered significant at a probability of $p < 0.05$.

RESULTS

The mean percentages of the different FA in ewe plasma are given in Table 3-5. In the present study, the major FA in plasma of sheep were palmitic (16:0), stearic (18:0), oleic (9c-18:1), linoleic (18:2n6) and linolenic (18:3n3) acids. The ewes from both groups had a similar FA profile prior to supplementation (100 d, $p > 0.05$, Table 3-5).

Among Saturated Fatty Acids (SFA), the percentages of 12:0, 13:0, 14:0, 15:0, 17:0, 21:0, 22:0, 23:0, 24:0 and 26:0 were not significantly different ($p > 0.05$) between the two experimental groups (Table 3). Among these FA, there was no diet by day (100, 114, 128 and 142 d of gestation) interaction effect ($p > 0.05$); but some of them, such as 12:0, 14:0, 23:0 and 26:0, increased ($p < 0.04$) over time during gestation. The percentage of palmitic acid (16:0) was not influenced ($p > 0.05$) by diet and day, but there was a significant interaction ($p = 0.02$, diet by day). On the other hand, 18:0 was decreased ($p = 0.02$) over time, regardless of diet; but the FM-supplemented diet did not make any greater reduction in 18:0 ($p = 0.57$, diet by day). The percentage of total SFA was decreased ($p = 0.001$) over time but not influenced by the type of diet.

The effects of diet and day on Monounsaturated Fatty Acids (MUFA) are shown in Table 3 and 4. The FM-supplemented diet did not affect ($p > 0.05$) the average percentages of all MUFA (e.g., 9c-16:1, 9c-20:1, all *trans* isomers of 18:1 and all *cis* isomers of 18:1) compared to control except 11c-20:1. There was an increase ($p < 0.04$) over time in 9c-16:1, 11t-18:1, 12t-18:1, 13-14t-18:1, 12c-18:1, 13c-18:1 and 16t-18:1, regardless of diet. However, there was no significant interaction (diet by day) on these FA except 11t-18:1 ($p = 0.01$). Type of diet and day did not change ($p > 0.05$) the average percentages of total *trans*-18:1 and total *cis*-18:1, although there was a significant interaction ($p = 0.01$, diet by day) on total *trans*-18:1. There was no diet, day and/or diet by day interaction effect on oleic acid (9c-18:1), 11c-18:1 and 10t-18:1 and total MUFA.

The average percentage of linoleic acid (18:2n6) in ewe plasma of the FM-supplemented group was not changed ($p = 0.16$) compared with the control

group. However, this FA was increased ($p = 0.001$) over time across both diets. The FM-supplementation did not change ($p > 0.05$) the percentages of 20:2n6, 20:3n6, 20:4n6 and 22:5n6 in ewe plasma compared with control, but were affected ($p < 0.001$) by day. The percentages of γ -linolenic acid (18:3n6), 22:2n6 and 22:4n6 were not changed ($p = 0.08$) by diet or day. The FM-supplementation did not change the content of total omega-6 PUFA compared to control, but the content was changed ($p = 0.001$) over time.

The percentages of α -linolenic acid (18:3n3), 20:3n3 and 22:5n3 were changed ($p < 0.05$) over time, but were not altered by diet and diet by day interaction (Table 3). The FM-supplementation in the diet of ewes increased ($p < 0.001$) the percentages of eicosapentaenoic acid (20:5n3) and docosahexaenoic acid (22:6n3) compared to control. The contents of these FA were also increased ($p < 0.002$) over time and were greater for FM-supplemented group ($p < 0.002$, diet by day interaction). Type of diet and day had significant influence ($p < 0.03$) on the percentages of total n3-PUFA and total VL_n3-PUFA (20:5n3 plus 22:5n3 plus 22:6n3). There was also a significant interaction ($p < 0.03$, diet by day) on total n3-PUFA and total VL_n3-PUFA.

The percentages of 9c11t-CLA, 9t11c-CLA, 10t12c-CLA, 9t11t + 10t12t-CLA and total CLA in plasma of ewes fed FM-supplemented diet were not changed compared to control Table 5. The contents of these CLA isomers were also not changed over time. On the other hand, the percentage of 11t13t-CLA was increased ($p = 0.01$) over time, regardless of diet; but there was no significant interaction ($p > 0.05$, diet by day).

Table 6 shows some selected FA in the plasma of lambs at birth from ewes fed either a control or FM-supplemented diet. The percentages of the main SFA, such as 16:0, 18:0, were not different ($p > 0.05$) between the two treatments. Among MUFA (e.g., 10t-18:1, 11t-18:1 and 9c-18:1), there was an increase ($p < 0.05$) in plasma 11t-18:1 in lambs from the maternal FM-supplemented group compared with the control group. The percentages of PUFA, such as 18:2n6, 18:3n3, 20:5n3 and 22:6n3, were greater ($p < 0.05$) in FM-supplemented group. The 9c11t-CLA isomer was greater ($p < 0.05$) in lambs from the maternal FM-supplemented group compared with the control group, whereas no difference in 10t12c-CLA content between two treatment groups was observed.

Table 3: Least square means for fatty acids (g/100 g total FA) in the plasma of ewes fed a basal diet supplemented with either a control or fishmeal supplement during gestation¹

FA/day of gestation	Control				FM-supplemented				SEM (P-value)	Diet (P-value)	Day (P-value)		Diet X Day (P-value)	
	100 d	114 d	128 d	142 d	100 d	114 d	128 d	142 d			² L	³ Q	² L	³ Q
12:0	0.09	0.09	0.11	0.12	0.09	0.10	0.10	0.10	0.01	0.770	0.040	0.880	0.360	0.820
iso-13:0	0.04	0.04	0.03	0.03	0.04	0.04	0.03	0.02	0.00	0.440	0.002	0.160	0.890	0.780
anteiso-13:0	0.08	0.09	0.10	0.06	0.09	0.10	0.08	0.04	0.01	0.760	0.001	0.001	0.070	0.740
13:0	0.03	0.04	0.04	0.03	0.04	0.04	0.04	0.03	0.01	0.820	0.550	0.040	0.320	0.870
iso-14:0	0.16	0.17	0.20	0.18	0.16	0.17	0.20	0.15	0.03	0.850	0.630	0.090	0.640	0.550
14:0	0.56	0.59	0.61	0.66	0.52	0.55	0.71	0.66	0.07	0.990	0.030	0.680	0.490	0.390
iso-15:0	0.42	0.44	0.36	0.32	0.39	0.41	0.35	0.30	0.06	0.760	0.010	0.030	0.760	0.890
anteiso-15:0	0.54	0.57	0.45	0.42	0.52	0.54	0.56	0.41	0.08	0.910	0.010	0.001	0.560	0.130
15:0	1.00	1.05	0.98	0.84	0.94	0.99	1.14	0.82	0.14	0.970	0.140	0.001	0.510	0.190
iso-16:0	0.62	0.65	0.76	0.64	0.57	0.60	0.90	0.62	0.09	0.980	0.120	0.002	0.490	0.110
16:0	17.40	16.90	15.60	15.60	15.70	16.10	16.40	16.20	0.52	0.650	0.160	0.870	0.020	0.240
iso-17:0	0.83	0.87	0.71	0.61	0.77	0.81	0.68	0.60	0.06	0.530	0.001	0.010	0.560	0.800
anteiso-17:0	0.89	0.93	0.95	0.84	0.89	0.93	1.25	0.71	0.11	0.650	0.600	0.010	0.870	0.140
9c-16:1	0.35	0.37	0.44	0.45	0.35	0.37	0.41	0.38	0.05	0.720	0.040	0.330	0.250	0.560
17:0	1.40	1.48	1.42	1.25	1.35	1.42	1.53	1.26	0.08	0.990	0.160	0.001	0.450	0.510
iso-18:0	0.20	0.21	0.19	0.20	0.22	0.24	0.18	0.21	0.03	0.720	0.430	0.870	0.510	0.660
anteiso-18:0	0.35	0.37	0.44	0.40	0.37	0.38	0.29	0.27	0.04	0.290	0.470	0.100	0.010	0.750
18:0	24.30	21.80	20.20	21.50	26.30	22.90	20.70	21.90	1.74	0.640	0.020	0.010	0.570	0.800
9t,12c-18:2	0.09	0.09	0.11	0.07	0.08	0.08	0.13	0.07	0.01	0.760	0.770	0.001	0.290	0.220
11t,15c-18:2	0.13	0.14	0.16	0.07	0.09	0.09	0.12	0.08	0.03	0.330	0.360	0.060	0.310	0.470
18:2n6	18.90	19.90	25.20	24.00	19.00	20.60	21.00	21.80	0.92	0.160	0.001	0.180	0.070	0.550
20:0	0.23	0.24	0.20	0.17	0.23	0.31	0.50	0.26	0.04	0.010	0.790	0.010	0.090	0.020
18:3n6	0.18	0.18	0.24	0.20	0.19	0.20	0.16	0.15	0.03	0.540	0.940	0.110	0.060	0.590
9c-20:1	0.07	0.07	0.06	0.06	0.09	0.10	0.15	0.12	0.02	0.080	0.470	0.230	0.110	0.360
11c-20:1	0.05	0.05	0.05	0.02	0.05	0.05	0.13	0.04	0.01	0.040	0.840	0.001	0.070	0.070
18:3n3	5.09	5.35	4.91	6.78	4.32	4.55	4.15	5.75	0.42	0.120	0.002	0.001	0.760	0.760
21:0	0.02	0.03	0.01	0.02	0.01	0.01	0.02	0.02	0.01	0.620	0.770	0.910	0.150	0.440
20:2n6	0.05	0.05	0.08	0.09	0.05	0.06	0.07	0.09	0.01	0.950	0.001	0.030	0.520	0.610
22:0	0.15	0.16	0.11	0.12	0.12	0.16	0.24	0.16	0.03	0.270	0.880	0.190	0.120	0.220
20:3n6	0.22	0.23	0.38	0.39	0.22	0.23	0.25	0.22	0.03	0.080	0.001	0.310	0.001	0.520
20:3n3	0.05	0.06	0.08	0.07	0.05	0.05	0.10	0.10	0.02	0.520	0.010	0.790	0.280	0.910
20:4n6	2.90	3.05	2.44	2.26	3.17	3.33	2.50	2.22	0.28	0.720	0.001	0.010	0.290	0.690
23:0	0.12	0.12	0.09	0.02	0.08	0.09	0.11	0.02	0.02	0.460	0.001	0.002	0.230	0.730
22:2n6	0.03	0.03	0.02	0.03	0.02	0.02	0.03	0.03	0.01	0.770	0.370	0.170	0.220	0.330
20:5n3	1.03	1.09	0.93	1.08	1.07	1.79	2.07	1.83	0.12	0.001	0.002	0.002	0.002	0.001
24:0	0.15	0.16	0.11	0.10	0.13	0.13	0.14	0.10	0.03	0.930	0.070	0.160	0.350	0.710
22:3n3	0.05	0.05	0.02	0.02	0.05	0.05	0.04	0.04	0.01	0.280	0.001	0.080	0.030	0.370
22:4n6	0.10	0.11	0.16	0.17	0.11	0.12	0.08	0.07	0.02	0.080	0.350	0.580	0.001	0.430
22:5n6	0.24	0.25	0.15	0.06	0.14	0.15	0.12	0.04	0.04	0.240	0.001	0.020	0.230	0.810
22:5n3	1.82	1.91	1.97	2.27	1.83	1.92	1.84	1.91	0.23	0.730	0.050	0.370	0.120	0.240
26:0	0.20	0.21	0.09	0.11	0.24	0.25	0.14	0.10	0.03	0.370	0.001	0.360	0.540	0.2400
22:6n3	1.51	1.58	1.32	1.34	1.62	2.93	3.41	3.44	0.21	0.001	0.001	0.010	0.001	0.010
⁴ Total SFA	49.70	47.10	43.80	44.20	49.60	47.20	46.30	44.90	1.48	0.590	0.001	0.250	0.640	0.540
⁵ Total MUFA	17.40	18.30	17.70	16.50	18.00	16.20	17.20	16.90	0.99	0.720	0.350	0.770	0.880	0.080
⁶ Total n6 PUFA	22.70	23.90	28.70	27.20	22.91	24.70	24.40	24.70	1.04	0.200	0.001	0.090	0.060	0.630
⁷ Total n3 PUFA	9.54	10.00	9.22	11.56	8.94	11.31	11.62	13.07	0.47	0.030	0.001	0.440	0.030	0.030
⁸ Total CLA	0.47	0.50	0.45	0.46	0.40	0.43	0.43	0.41	0.03	0.240	0.620	0.200	0.420	0.5700
Total trans-18:1	3.05	3.21	2.75	2.46	2.43	2.55	4.20	3.25	0.46	0.690	0.410	0.020	0.010	0.310
Total cis-18:1	13.90	14.60	14.40	13.48	15.09	13.14	12.26	13.11	1.08	0.600	0.190	0.530	0.380	0.030
⁹ Total VL_n3-PUFA	4.35	4.58	4.22	4.69	4.52	6.65	7.32	7.17	0.40	0.010	0.001	0.010	0.001	0.001

¹Average of 4 measurements; ²L: linear effect; ³Q: quadratic effect; ⁴Total SFA: all saturated fatty acids (without any double bond, 12:0 to 26:0). ⁵Total MUFA: all monounsaturated fatty acids with a single double bond (16:1 to 20:1). ⁶Total n-6 polyunsaturated fatty acids (PUFA): 18:2n6; 18:3n6; 20:2n6; 20:3n6; 20:4n6; 22:2n6; 22:4n6; and 22:5n6. ⁷Total n-3 polyunsaturated fatty acids (PUFA): 18:3n3; 20:3n3; 20:5n3; 22:3n3; 22:5n3; and 22:6n3. ⁸Total conjugated linoleic acid (CLA): 9c,11t 18:2; 9t,11c 18:2; 10t,12c 18:2; 11t13t 18:2; and 9t,11t plus 10t12t 18:2. ⁹Total very long chain n-3 PUFA (VL_n3-PUFA, >18C): 20:5n3; 22:5n3; and 22:6n3

Table 4: Positional isomers of 18:1 monoenes (g/100 g total FA) in the plasma of ewes fed a basal diet supplemented with either a control or fishmeal supplement during gestation¹

FA / day of gestation	Control				FM-supplemented				SEM	Diet (P-value)	Day Diet (P-value)		X Day (P-value)	
	100 d	114 d	128 d	142 d	100 d	114 d	128 d	142 d			² L	³ Q	² L	³ Q
4 <i>t</i> -18:1	0.01	0.02	0.06	0.05	0.03	0.03	0.02	0.04	0.02	0.93	0.120	0.820	0.26	0.62
5 <i>t</i> -18:1	0.05	0.05	0.07	0.03	0.05	0.06	0.10	0.03	0.02	0.51	0.560	0.030	0.85	0.52
6-8 <i>t</i> -18:1	0.18	0.19	0.18	0.21	0.18	0.19	0.23	0.22	0.03	0.70	0.150	0.760	0.71	0.36
9 <i>t</i> -18:1	0.26	0.28	0.24	0.21	0.24	0.25	0.30	0.24	0.02	0.74	0.230	0.010	0.10	0.41
10 <i>t</i> -18:1	0.18	0.19	0.18	0.18	0.19	0.20	0.27	0.23	0.03	0.36	0.290	0.190	0.31	0.31
11 <i>t</i> -18:1	1.78	1.87	1.10	0.93	1.19	1.26	1.67	1.22	0.25	0.79	0.040	0.050	0.01	0.49
12 <i>t</i> -18:1	0.25	0.26	0.32	0.27	0.22	0.23	0.58	0.48	0.06	0.20	0.002	0.110	0.01	0.68
13-14 <i>t</i> -18:1	0.23	0.24	0.39	0.38	0.20	0.21	0.74	0.53	0.10	0.32	0.001	0.220	0.13	0.32
9 <i>c</i> -18:1	12.92	13.59	12.89	12.16	14.08	12.07	10.62	11.83	1.09	0.58	0.080	0.320	0.39	0.02
11 <i>c</i> -18:1	0.70	0.74	0.80	0.84	0.74	0.78	0.90	0.79	0.06	0.66	0.070	0.260	0.56	0.22
12 <i>c</i> -18:1	0.22	0.23	0.67	0.43	0.24	0.25	0.68	0.42	0.05	0.91	0.001	0.001	0.75	0.78
13 <i>c</i> -18:1	0.04	0.04	0.04	0.04	0.04	0.04	0.06	0.08	0.01	0.16	0.001	0.150	0.00	0.31
16 <i>t</i> -18:1	0.12	0.12	0.21	0.20	0.10	0.11	0.30	0.27	0.04	0.48	0.001	0.440	0.09	0.77

¹Average of 4 measurements; ²L: linear effect; ³Q: quadratic effect

Table 5: Positional isomers of conjugated linoleic acid (g/100 g total FA) in the plasma of ewes fed a basal diet supplemented with either a control or fishmeal supplement during gestation¹

FA / day of gestation	Control				FM-supplemented				SEM	Diet (P-value)	Day (P-value)		Diet X Day (P-value)	
	100 d	114 d	128 d	142 d	100 d	114 d	128 d	142 d			² L	³ Q	² L	³ Q
9 <i>c</i> , 11 <i>t</i> -CLA	0.27	0.28	0.26	0.26	0.25	0.26	0.24	0.26	0.02	0.60	0.78	0.80	0.56	0.63
9 <i>t</i> , 11 <i>c</i> -CLA	0.02	0.02	0.01	0.03	0.01	0.01	0.01	0.02	0.00	0.29	0.09	0.07	0.96	0.22
10 <i>t</i> , 12 <i>c</i> -CLA	0.02	0.03	0.02	0.02	0.02	0.02	0.04	0.01	0.01	0.97	0.52	0.16	0.71	0.16
11 <i>t</i> , 13 <i>t</i> -CLA	0.05	0.06	0.05	0.04	0.05	0.05	0.04	0.04	0.01	0.31	0.01	0.06	0.68	0.41
9 <i>t</i> , 11 <i>t</i> +10 <i>t</i> , 12 <i>t</i> -CLA	0.11	0.12	0.11	0.12	0.08	0.09	0.10	0.09	0.01	0.17	0.58	0.30	0.75	0.47

¹Average of 4 measurements; ²L: linear effect; ³Q: quadratic effect

Table 6: Some selected fatty acids (g/100 g FA) in the plasma of lambs at birth from ewes fed a basal diet supplemented with either a control or fishmeal supplement¹

Fatty acid	Control	FM-supplemented	SEM
16:0	26.40	24.40	1.020
9 <i>c</i> -16:1	3.08	2.25	0.140
18:0	11.80	14.00	0.850
10 <i>t</i> -18:1	0.13	0.14	0.080
11 <i>t</i> -18:1	0.39 ^a	0.49 ^b	0.100
9 <i>c</i> -18:1	34.30	32.60	1.480
18:2n6	2.86 ^a	3.93 ^b	1.080
18:3n3	0.99 ^a	1.30 ^b	0.260
9 <i>c</i> ,11 <i>t</i> -CLA	0.25 ^a	0.36 ^b	0.050
10 <i>t</i> ,12 <i>c</i> -CLA	0.01	0.01	0.003
20:5n3	0.39 ^a	0.70 ^b	0.110
22:6n3	0.93 ^a	1.59 ^b	0.150
Total VL _n 3-PUFA ²	2.05 ^a	3.27 ^b	0.270

¹Average of 6 measurements; ²Total very long chain n-3 PUFA (VL_n3-PUFA, >18C): 20:5n3; 22:5n3; and 22:6n3; ^a^b Means within a row with different superscripts differ (p<0.05)

DISCUSSION

The aim of the current study was to establish whether feeding a diet supplemented with FM to ewes during gestation resulted in changes in the FA composition of their own plasma and subsequently in their lamb's plasma at birth. The major SFA in ewe plasma collected from both groups, were 16:0, 18:0,

9*c*-18:1, 18:2n6 and 18:3n3, which was consistent with the results of Leat (1966). In the present study, the isomers of *trans*-18:1 FA and 9*c*11*t*-CLA in ewe-plasma were not increased with the supply of the FM-supplemented diet. It was reported that the proportion of plasma 18:3n3 was higher in sheep supplemented with fish oil compared to control (Sinclair *et al.*, 2005; Capper *et al.*, 2006). According to them, tissue elongation and desaturation of 18:3n3 to 20:5n3 and 22:6n3 in sheep was inhibited by the VL_n3-PUFA, such as DHA and EPA, in fish oil. However, we did not find any significant difference in ewe plasma 18:3n3, when sheep were fed a FM-supplemented diet compared to control.

The FM-supplementation to ewes, in the present research, increased plasma 22:6n3 by 3.3 times and 20:5n3 by 1.8 times compared to control. The significant increase in plasma 20:5n3 and 22:6n3 in ewes offered diets containing fishmeal were a direct result of the increased dietary supply of preformed VL_n3-PUFA and in agreement with Capper *et al.* (2007). However, our study was carried out with a limited number of experimental units. The plasma of the control group also contained 20:5n3 and 22:6n3, but in much lower percentages compared to FM-supplemented group. Endogenous synthesis of 20:5n3

and 22:6n3 from 18:3n3 likely occurs to a limited extent (Wachira *et al.*, 2002; Chikunya *et al.*, 2004). The present study also revealed that the proportions of total VL_n3-PUFA in ewe plasma were increased and the proportions of total n6-PUFA were decreased by feeding the FM-supplemented diet. In an experiment conducted by Rooke *et al.* (1998), feeding tuna oil to sows increased the VL_n3-PUFA, especially 20:5n3 and 22:6n3, primarily at the expense of 18:2n6.

Although maternal ewe-plasma contained large amounts of 18:2n6 and a considerable amount of 18:3n3, very small amounts of these FA (15.8% of 18:2n6 and 19.2% of 18:3n3) were present in the plasma of newborn lambs. The same phenomenon was also observed by Leat (1966) in calves and lambs. Leat (1966) had also shown that newborn piglets contained much more 18:2n6 in their plasma than did the newborn ruminant. It appeared that, in contrast with the non-ruminant, the ruminant placenta was relatively impermeable to 18:2n6 and 18:3n3 (Hansen *et al.*, 1964; Leat, 1966). In addition, it has been shown that the placentas of rats, rabbits and sheep are relatively impermeable to plasma cholesterol and phospholipids, whereas plasma non-esterified FA can pass the placental barrier into the fetus (Duyne *et al.*, 1960; Popjak, 1954; McBride and Korn, 1964). Leat (1966) reported that sow-plasma non-esterified FA contained 4.8-10:0 times more 18:2n6 than ruminants and suggested that less 18:2n6 acid would pass into the ruminant fetus than into the non-ruminant fetus in this way. It was also observed that supplementing diets of ruminants with protected PUFA during late gestation would improve the essential FA status of newborn ruminants (Soares, 1986). In the diet of ruminants, protected lipids containing PUFA can by-pass the rumen without biohydrogenation and concomitantly increase the concentration in maternal plasma as well as the fetus (Scott *et al.*, 1971; Soares, 1986; Hobson and Stewart, 1997). In an experiment, Capper *et al.* (2006) found that lambs with increased plasma status of 20:5n3 and 22:6n3 had reduced latency of standing and suckling, which may have beneficial effects on lamb survival rate. According to O'Connor and Lawrence (1992) and Capper *et al.* (2006), the reduced latency of suckling might be due in part to increased visual acuity, which improved the ability of the lambs to successfully locate the udder. Our study confirmed that the plasma of lambs born to ewes fed the FM-supplemented diets during late gestation was characterized by high content of 20:5n3 and 22:6n3 compared with plasma obtained from the lambs born to the ewes that received control diet. However, further work is required to determine the survival rate of lambs born to ewes supplemented with FM. On the other hand, the present study revealed that the contents of 20:5n3 and 22:6n3 were enhanced in

lambs born to ewes fed a FM-supplemented diet, suggesting specific FA can preferentially cross the placenta in late pregnancy.

CONCLUSION

The present study has clearly shown that when ewes are fed a FM-supplemented diet during late gestation, the percentages of EPA (20:5n3) and DHA (20:6n3) are significantly increased compared to control. Lambs born to FM-supplemented ewes had greater plasma concentrations of EPA, DHA and VL_n3-PUFA than lambs born to control ewes.

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