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Original article

Effects of ruminal or postruminal fish oil supplementation on intake and digestion in dairy cows

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Summary — The effect of fish oil supplementation on intake, digestibility and the volatile fatty acid profile in dairy cows was investigated in two trials. In each of the two trials, six cows received a diet based on maize silage in a latin square design. In the first trial, the cows were fitted with ruminal and duodenal cannulae and their diet was either supplemented or not with 300 mL fish oil and infused either into the rumen or the duodenum. In the second trial, the cows were not cannulated and their diet was either supplemented or not with 200 or 400 mL fish oil given orally. In both trials, the fish oil reaching the rumen decreased ($P < 0.01$) feed intake, increased ($P < 0.01$) the organic matter and fibre digestibility, and the percentage of propionate in the volatile fatty acid profile. These increases were higher for the 400 mL supplement than for the 200 mL one. The long-chain fatty acid pattern in the duodenum showed a high degree of hydrogenation of 20- and 22-carbon fatty acids. All these results demonstrated the particular action of fish oil on ruminal digestion when compared to other lipid sources.

cow / intake / digestion / fish oil / rumen / VFA

Résumé — Effets sur l'ingestion et la digestion d'une supplémentation en huile de poisson au niveau du rumen ou du duodénum chez la vache laitière. Les effets d'une supplémentation en huile de poisson sur l'ingestion, la digestibilité et le profil des acides gras volatils du rumen ont été étudiés au cours de deux essais menés chacun avec six vaches selon un schéma en carré latin 3 x 3, sur des régimes à base d'ensilage de maïs. Dans le premier essai, les vaches étaient munies de canules du rumen et du duodénum, et la ration était soit non supplémentée, soit supplémentée avec 300 mL par jour d'huile de poisson infusée soit dans le rumen, soit dans le duodénum. Dans le second essai, les vaches ne portaient pas de canule et la ration était soit non

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supplémentée, soit supplémentée par la bouche avec 200 ou 400 mL par jour d'huile de poisson. Dans les deux essais, l'huile de poisson parvenant au rumen a réduit ($p < 0,01$) les quantités ingérées, accru ($p < 0,01$) la digestibilité de la matière organique et des parois végétales de la ration, ainsi que le pourcentage de propionate dans le mélange des acides gras volatils du rumen. Ces accroissements ont été plus prononcés avec 400 mL qu'avec 200 mL d'huile de poisson. Le profil des acides gras longs dans le contenu duodénal a mis en évidence un niveau élevé d'hydrogénation des acides gras à 20 et 22 carbones. Ces différents résultats montrent, par rapport aux données recueillies avec d'autres sources de lipides, une action spécifique de l'huile de poisson sur la digestion ruminale.

vache / ingestion / digestion / huile de poisson / rumen / AGV

INTRODUCTION

The effects of fish oil supplementation in dairy cow diets were studied years ago, mainly between 1970 and 1975. Most of the experiments from this period showed that adding ca 300 g/day cod liver oil to the diet led to significant decreases in milk fat concentration (Brumby et al, 1972; Storry et al, 1974; Pennington and Davis, 1975). For this reason, the practical use of such sources of fat has been rather limited and fish fat studies were halted. However, the new economic conditions of dairy production in many countries, especially in the European Community, have resulted in economic pressure for limits in milk fat production. On the other hand, the nutritional implications for human consumption of *n*-3 20- and 22-carbon long chain fatty acids (FA), present in fish oils, is attracting increasing attention (Sanders, 1993). There is renewed interest in the use of fish fat supplements for dairy cows. However, lipid supplements have side effects which can limit their use: eg, a decrease in intake and a decrease in digestibility of the rest of the diet, especially due to modifications of ruminal digestion, and hydrogenation of FA in the rumen (Doreau et al, 1997). Few experiments have studied the effects of fish oils on intake and digestion. In order to answer practical questions relating to this supplementation, two objectives were selected. The first was to determine if the specific effects of fish oil were due to the native FA of fish oil, such as polyunsaturated 20- or 22-carbon FA, or to the FA that could be produced when the fish oil was hydrogenated by the rumen microorganisms. To this end, a first trial was conducted in which the same dose of fish oil was infused either in the rumen or in the duodenum. A second trial compared the effects of two different doses of fish oil added to the diets of dairy cows. These trials included measurements of intake, digestibility, ruminal volatile fatty acid (VFA) profile, and duodenal FA composition. Preliminary results on milk production and FA composition were also obtained and have been presented elsewhere (Chilliard and Doreau, 1997b).

MATERIALS AND METHODS

Trial 1

Animals, experimental design and treatments

Six multiparous Holstein dairy cows, fitted with permanent cannulae of the rumen and the proximal duodenum, were studied after their lactation peak. The ruminal cannulae were made of polyvinyl chloride, and had an internal diameter of 140 mm. The duodenal cannulae were made of plastisol, and were of the T-type with a gutter-type base. At 84 ± 22 days of lactation, the cows were assigned to a replicated 3 x 3 latin square design for three periods of 4 weeks. The cows

weighed 671 ± 62 kg and produced 24.9 ± 7.0 kg/day of milk at the beginning of the experiment.

All cows received ad libitum a complete diet composed, on a dry matter (DM) basis, of 70% maize silage and 30% concentrates. The concentrate contained 33.3% formaldehyde-treated soyabean–rapeseed (80:20) meal, 13.3% wheat, 13.3% barley, 20.0% beet pulp, 10.0% rapeseed meal, 4.6% soya bean meal, 3.5% beet molasses, 0.7% limestone, 0.7% dicalcium phosphate, 0.3% magnesium oxide and 0.3% sodium chloride. In addition, they received daily 850 g DM hay, 200 g mineral-vitamin premix and 100 g urea. The ration was then mixed and given once daily at 0900 hours. Three treatments were compared: the control diet without oil infusion (C1); the control diet with a continuous infusion of 300 mL (276 g) fish oil of menhaden type infused in the rumen (R); the control diet with a continuous infusion of 300 mL fish oil infused in the duodenum (D). The infusions were performed using a peristaltic pump (Minipuls 2™, Gilson, Villiers-le-Bel, France).

Measurements and analyses

On the fourth week of each period, feed intake was recorded for 5 days by weighing the amount of feeds delivered to cows and the refusals. Daily samples were dried at 80 °C for 48 h, for determination of DM. A representative sample of each feed was taken. Organic matter (OM) was determined from the ashes after treatment at 550 °C for 6 h. The crude protein content was obtained from Kjeldahl analysis. Fibre was obtained from neutral detergent fibre (NDF) and acid detergent fibre (ADF) analyses (Goering and Van Soest, 1970). Ether extract was determined using ether petroleum extraction after acid hydrolysis. The effective chemical composition of the diets, taking into account feed intake, is given in table I. Oil composition was analysed on methyl esters by gas-liquid chromatography with a glass capillary column coated with free FA phase (Ferlay et al, 1992). The chromatographic analysis showed 92 peaks.

Digestibility was measured by a total faeces collection for the same 5 day period. The faecal samples were dried for 48 h at 80 °C for determination of DM. On these samples pooled for 1 week, OM, NDF, ADF and ether extract were determined as mentioned earlier.

Three ruminal liquid samples were collected in the ventral sac by a tube, at 0830, 1100 and 1500 hours for 2 consecutive days. For a given hour, samples of the 2 days were pooled before analysis. The pH was measured using a glass electrode. The VFA concentration and composition were determined by gas-liquid chro-

Table I. Chemical composition of diets * (trials 1 and 2).

	Trial 1			*Trial 2*		
	C1	*R*	*D*	*C2*	*L*	*H*
Organic matter (%)	92.8	92.8	92.9	94.0	94.1	94.2
Neutral detergent fibre (%)	43.4	42.8	42.7	39.6	39.4	38.8
Acid detergent fibre (%)	22.9	22.7	22.6	21.8	21.7	21.4
Crude protein (%)	15.6	15.5	15.5	14.1	14.0	13.7
Ether extract (%)	1.7	3.4	3.2	2.0	3.0	4.4

* C1: control diet; R: diet supplemented with 300 mL fish oil infused in the rumen; D: diet supplemented with 300 mL fish oil infused in the duodenum; C2: control diet; L: diet supplemented with 200 mL fish oil; H: diet supplemented with 400 mL fish oil.

matography (Jouany, 1982) using isocaproate as the internal standard.

A representative sample of duodenal content was obtained from five subsamples taken at different hours of the day. For treatment D, the infusion was temporarily stopped 5 min before samplings. The samples for the six cows and the three periods were mixed so that one sample per treatment was analysed. Lipids were extracted by a chloroform:methanol (2/1, vol/vol) mixture. After evaporation, the lipids were saponified by potassium hydroxide in an ethanolic solution. Fatty acids were released by hydrochloric acid, extracted by hexane and then methylated with a methanol-hydrochloric acid mixture. The fatty acid determination was performed on methyl esters by gas-liquid chromatography under the same conditions as for the oil.

Statistical analyses were performed by a three-way analysis of variance (cow, period and treatments). The means of the three treatments were compared by the Student-Newman-Keuls' t-test.

Trial 2

Animals, experimental design and diets

Six Holstein dairy cows, of which three were primiparous, were studied after their lactation peak. The cows were separated into three groups of two (one primiparous and one multiparous in each) and each group was assigned to a replicated 3 x 3 latin square design, beginning at 86 ± 17 days of lactation, for three periods of 4 weeks. The cow live weight was 637 ± 81 kg and they produced 33.6 ± 3.8 kg/day milk at the beginning of the experiment.

All cows received ad libitum a diet composed, on a DM basis, of 65% maize silage and 35% concentrate. The composition of the concentrate was similar to that of trial 1. In addition, the cows received daily 850 g hay, 220 g mineral-vitamin premix and 100 g urea. Three treatments were compared: the earlier described control diet alone (C2), the control diet with 200 mL (185 g) fish oil of menhaden type (low level, L), and the control diet with 400 mL (370 g) fish oil (high level, H). The cows had been accustomed to fish oil consumption before the experiment. Fish oil was mixed with 5 kg concentrate and was given at 0845 hours. Maize silage, hay, premix and urea were given at 0915 hours and the rest of the concentrates was given at 1600 hours. No refusals of the fish oil were observed. Refusals of forages and concentrates were weighed so that the amount of silage and concentrates offered was adjusted daily according to the intake of the previous day.

Measurements and analyses

Digestibility analysis was performed, and feed and faeces analyses were determined as in trial 1. The composition of the diets, taking into account the effective feed intake, is given in table I. A sample of ruminal liquid was taken by puncture through the rumen wall using a trocar, under local anaesthesia (xylocaine, Roger Bellon, Neuilly, France) on the last day of each period at 1400 hours. VFA composition was determined as in trial 1 and statistical analyses were performed as in trial 1.

RESULTS

Trial 1

Ruminal oil infusion decreased total DM intake (table II). Duodenal oil infusion nonsignificantly tended to decrease intake. The DM and OM digestibilities were significantly higher for diet R than for diet C1, with diet D being intermediate and not significantly different from the other two diets (table II). These differences between diets C1 and R can be attributed to nonsignificant variations in ether extract digestibility, and to variations in fibre digestibility, which were only significant for NDF.

No significant pH variations were observed between diets (table III). The total concen-

Table II. Total intake and digestibility of diets in cows receiving a control diet (C1) or diets supplemented with 300 mL fish oil infused in the rumen (R) or in the duodenum (D) (trial 1).

	Treatment			*Residual standard deviation*
	C1	*R*	*D*	
Dry matter intake (kg/day)*	19.8^{A}	16.2^{B}	18.0^{AB}	1.5
Digestibility (%)				
Dry matter	70.2^{A}	73.5^{B}	71.8^{AB}	1.4
Organic matter	72.6^{a}	75.5^{b}	74.2^{ab}	1.4
Neutral detergent fibre	59.4^{a}	64.5^{b}	60.2^{a}	2.7
Acid detergent fibre	56.5	59.3	55.8	2.7
Ether extract	69.7	77.1	80.6	8.3

* Including oil infusion. $^{a, b}$ Means on the same row with different superscripts differ ($P < 0.05$); $^{A, B}$ Means on the same row with different superscripts differ ($P < 0.01$).

trations of VFA did not vary whatever the time of sampling. Ruminal oil infusion significantly decreased the acetate proportion and the acetate: propionate ratio, and increased the propionate proportion at the three sampling times. The butyrate and valerate proportions were only decreased by the oil infusion at 1500 hours. For all samples, the oil infusion increased the isovalerate proportions and decreased the caproate, whereas that of isobutyrate did not vary. As expected, no effect due to the duodenal infusion was observed on the proportions of individual VFA.

Chromatographic analyses of the duodenal contents revealed 105 peaks. The main peaks were identified. The FA of cows fed the control diet were mainly C16:0, C18:0 and isomers of C18:1, which represented 82% of total FA (table IV); 20- and 22-carbon FA represented 2.2% of FA. The ruminal infusion of fish oil totally modified this composition: 20- and 22-carbon FA represented 18.5% of total FA; the two most important of these FA present in oil, C20:5 *n*-3 and C22:6 *n*-3, were observed in very low amounts. Among the 18-carbon FA, the percentage of C18:0 was low compared to the isomers of C18:1. For the duodenal infusion, the FA pattern was, as expected, close to that observed in cows fed the control diet, although slight differences were observed.

Trial 2

Total intake was lower for treatment H than for treatments C2 and L (table V). Fish oil supplementation significantly increased the DM, OM, fibre and ether extract digestibility with treatment H (table V). When treatment L was supplied, only the DM and ether extract digestibilities were significantly higher than in the C2 diet; OM and fibre digestibilities tended to increase.

When fish oil was supplied, the proportion of propionate increased and that of acetate and the acetate:propionate ratio decreased in the ruminal VFA mixture (table VI). This increase was significant only when 400 g fish oil were added to the diet. Among the minor VFA, the isovalerate proportion increased and that of caproate decreased due to the fish oil supplementation.

Table III. Ruminal pH, volatile fatty acid concentration and composition in cows receiving a control diet (C1) or diets supplemented with 300 mL fish oil infused in the rumen (R) or in the duodenum (D) (trial 1).

	Treatment			*Residual standard deviation*
	C1	*R*	*D*	
pH				
0830 h	6.84	6.87	6.81	0.09
1100 h	6.35	6.42	6.40	0.11
1500 h	6.17	6.27	6.20	0.12
Volatile fatty acids (mM)				
0830 h	75.5	73.9	79.2	8.4
1100 h	106.0	99.7	107.0	7.9
1500 h	120.6	109.1	120.9	9.3
Acetate (mol/100 mol)				
0830 h	67.1^{A}	61.5^{B}	66.5^{A}	1.9
1100 h	63.5^{A}	59.7^{B}	63.5^{A}	0.9
1500 h	63.0^{A}	58.9^{B}	62.4^{A}	1.1
Propionate (mol/100 mol)				
0830 h	17.1^{A}	22.0^{B}	17.8^{A}	1.9
1100 h	20.1^{A}	24.8^{B}	21.0^{A}	1.0
1500 h	19.4^{A}	25.0^{B}	20.6^{A}	1.6
Isobutyrate (mol/100 mol)				
0830 h	0.8	0.9	0.9	0.1
1100 h	0.6	0.6	0.6	0.1
1500 h	0.5	0.5	0.5	0.1
Butyrate (mol/100 mol)				
0830 h	12.0	12.1	11.7	0.7
1100 h	12.8	11.5	12.0	0.8
1500 h	13.6^{a}	11.9^{b}	13.1^{ab}	0.9
Isovalerate (mol/100 mol)				
0830 h	1.5^{A}	2.3^{B}	1.5^{A}	0.3
1100 h	1.3^{A}	2.1^{B}	1.3^{A}	0.2
1500 h	1.4^{A}	2.3^{B}	1.3^{A}	0.4
Valerate (mol/100 mol)				
0830 h	1.0	1.0	1.1	0.1
1100 h	1.1	1.1	1.1	0.1
1500 h	1.4^{A}	1.2^{B}	1.4^{A}	0.1
Caproate (mol/100 mol)				
0830 h	0.5^{A}	0.2^{B}	0.5^{A}	0.1
1100 h	0.5^{A}	0.2^{B}	0.5^{A}	0.1
1500 h	0.7^{a}	0.2^{b}	0.7^{a}	0.2
Acetate:propionate				
0830 h	3.97^{A}	2.81^{B}	3.76^{A}	0.45
1100 h	3.18^{A}	2.42^{B}	3.04^{A}	0.16
1500 h	3.26^{A}	2.37^{B}	3.05^{A}	0.23

$^{a, b}$ Means on the same row with different superscripts differ ($P < 0.05$); $^{A, B}$ means on the same row with different superscripts differ ($P < 0.01$).

Table IV. Fatty acid composition of fish oil and of duodenal contents (in % of weight of methyl esters) in cows receiving a control diet (C1) or diets supplemented with 300 mL fish oil infused in the rumen (R) or in the duodenum (D) (trial 1).

		Treatment		
	Oil	*C1*	*R*	*D*
C14:0	7.13	1.44	2.56	4.29
iC15:0 + aiC15:0*	0.25	1.32	1.51	1.42
C15:0	0.35	0.82	0.95	1.08
C16:0	17.26	14.18	19.29	16.83
Sum of C16:1	10.72	0.58	2.43	1.55
C16:2 n-4	1.25	0.09	0.08	0.03
C16:3 n-4	1.35	0.05	0.13	0.15
C16:4 n-1	2.22	0.02	0.03	0.11
iC17:0 + aiC17:0	0.42	0.68	1.02	0.76
C17:0	0.37	0.50	0.68	0.64
C18:0	2.94	54.48	7.88	46.19
Sum of C18:1	12.83	13.39	36.02	12.98
C18:2 n-6	0.88	5.54	2.56	4.38
C18:3 n-3	0.62	0.48	0.25	0.44
C18:4	2.68	0.10	0.06	0.18
C20:0	0.40	0.37	0.45	0.59
Sum of C20:1	2.55	0.13	1.09	0.32
C20:4 n-6	0.82	0.01	0.04	0.05
C20:4 n-3	0.69	0.03	1.23	0.05
C20:5 n-3	17.72	0.03	0.33	0.77
C22:0	0.28	0.17	0.80	0.49
Sum of C22:1	2.52	0.02	0.82	0.20
C22:5 n-3	1.41	0.02	0.53	0.10
C22:6 n-3	6.17	0.10	0.51	0.42
Sum of NI < C20:0†	4.28	4.21	6.07	4.08
Sum of NI > C20:0	1.88	1.26	12.84	1.96

* i: iso; ai: anteiso; † NI: nonidentified.

DISCUSSION

Intake

Duodenal infusion of fish oil (300 mL/day) tended to reduce DM intake, although not significantly. Such a result had been found by Hagemeister et al (1988) with an abomasal infusion of 420 g/day of menhaden oil and could be due to a metabolic negative effect of fatty acids on intake, as previously shown by Gagliostro and Chilliard (1991) and Ottou et al (1995) with duodenal infusions of rapeseed oil at higher doses.

Ruminal infusion strongly decreased intake. It has often been suggested that the negative effect of dietary lipids on intake is mainly due to a depressive effect on ruminal digestion or to a low palatability of fat supplements. It was not the case in the present work, since digestibility was enhanced, and oil was infused by the ruminal cannula. In the same way, protected lipids which do not decrease ruminal digestion have a negative

Table V. Total intake and digestibility of diets in cows receiving a control diet (C2), 200 mL fish oil (L) or 400 mL fish oil (H) daily (trial 2).

	Treatment			*Residual standard deviation*
	C2	*L*	*H*	
Dry matter intake (kg/day)*	19.2^{A}	19.0^{A}	15.5^{B}	1.6
Digestibility (%)				
Dry matter	66.8^{A}	69.9^{B}	71.2^{B}	0.7
Organic matter	69.2^{A}	72.2^{AB}	73.5^{B}	0.7
Neutral detergent fibre	47.0^{A}	51.8^{AB}	52.7^{B}	0.7
Acid detergent fibre	42.7^{a}	46.2^{ab}	47.3^{b}	0.9
Ether extract	63.2^{A}	79.8^{B}	85.3^{C}	3.1

* Including oil supply. ^{a,b} Means on the same row with different superscripts differ ($P < 0.05$); ^{A,B} means on the same row with different superscripts differ ($P < 0.01$).

Table VI. Composition of ruminal volatile fatty acids (mol/100 mol) and acetate/propionate ratio in cows receiving a control diet (C2), 200 mL fish oil (L) or 400 mL fish oil (H) daily (trial 2).

	Treatment			*Residual standard deviation*
	C2	*L*	*H*	
Acetate	63.4^{A}	62.1^{AB}	59.0^{B}	1.7
Propionate	16.4^{A}	18.5^{AB}	21.5^{B}	1.7
Isobutyrate	0.4	0.4	0.5	0.1
Butyrate	16.0	15.3	15.3	1.9
Isovalerate	1.4^{a}	1.9^{b}	2.0^{b}	0.4
Valerate	1.5	1.3	1.4	0.2
Caproate	0.6^{A}	0.5^{A}	0.2^{B}	0.1
Acetate:propionate	3.90^{A}	3.37^{AB}	2.77^{B}	0.35

^{a,b} Means on the same row with different superscripts differ ($P < 0.05$); ^{A,B} means on the same row with different superscripts differ ($P < 0.01$).

effect on DM intake, total energy intake being modified to a lesser degree (Chilliard et al, 1993). This suggests a metabolic effect of lipids. A specific effect of some FA which are produced by the hydrogenation of fish oil in the rumen is suggested.

The decrease in intake with fish oil supply was significant for 400 mL/day (–3.7 kg DM/day) but not for 200 mL/day in trial 2, whereas a decrease by 3.6 kg DM/day was found in trial 1 for treatment R (300 mL/day). This decrease was higher than in a previous experiment (Chilliard and Doreau, 1997a) in which a daily supply of 300 mL/day of the same fish oil given orally decreased intake by only 1.6 kg DM/day.

Although other references are lacking, it can be hypothesized that a continuous infusion has a more negative effect on intake than a distribution per os once a day.

Ruminal volatile fatty acids

No difference in the pH values and total VFA concentration was observed between cows receiving the control diet and the oil ruminal infusion treatment. Conversely, in both trials, the fish oil supply significantly decreased the acetate and increased the propionate proportion. These results have often been observed for most lipid sources. In particular, with fish oil, almost all experiments have shown an increase in the propionate concentration and a decrease in acetate (among others Nicholson and Sutton, 1971; Storry et al, 1974). This result is thought to be due to a modification of the ruminal microbial ecosystem, as occurs with 18-carbon polyunsaturated FA. A decrease in cellulolytic and methanogenic bacteria is observed with most fat sources. The increase in propionate was also due to the competition for metabolic hydrogen between methane and propionate production pathways (Demeyer and Van Nevel, 1995; Doreau and Ferlay, 1995).

The shift in acetate and propionate proportions was significant at 400 mL supply, a nonsignificant tendency being found at 200 mL supply. This result is intermediate between that of Nicholson and Sutton (1971) who found a variation in VFA proportions as soon as 125 mL of cod liver oil were given daily, and that of Tanaka (1970) in which VFA proportions remained constant until 200 mL of cod oil daily, then were modified from 300 mL daily. Collectively, these results do not indicate the occurrence of a lower level of fish oil supply which modifies the ruminal VFA proportions.

The decrease in the acetate:propionate ratio probably contributed to the decrease in mammary de novo FA synthesis, which requires mainly acetate as precursor. In trial 1, the milk fat content was 35.4 g/kg for diet C1 and 25.1 g/kg for diet R. The decrease in short- and medium-chain FA de novo synthesis accounted for less than half the total decrease in milk fat (Chilliard and Doreau, 1997b).

Digestibility

The increase in digestibility when fish oil was added is highly surprising. The fact that results of the two experiments are consistent gives reliability to this assessment. Generally, the addition of lipids to ruminant diets decreases DM and especially cell-wall digestibility, or does not modify them (Palmquist and Jenkins, 1980; Jenkins, 1993). In some cases, slight increases in digestibility have been observed with different lipid supplements (Mir, 1988; Elmeddah et al, 1991). With hydrogenated fish oil, DM and/or fibre digestibility was either depressed (Sundstøl, 1974) or unmodified (Doreau, 1992). Curiously, digestibility was not measured in most experiments in which crude fish oil was given to ruminants, except by Sutton et al (1975) who did not observe any variations in OM and fibre digestibility and ruminal digestion in sheep fed a diet supplemented or not with 20 g/day of cod liver oil. In continuous cultures of rumen contents, Hoover et al (1989) did not find variations of DM and NDF digestibilities between fish meals defatted and rich in fat, whereas ADF digestibility was higher for fish meals rich in fat, in uncontrolled pH conditions.

No comprehensive explanation can be proposed to understand the variation in digestibility observed in our trials. The decrease in feed intake may partially explain an increase in digestibility (Chilliard et al, 1995). The modification of the VFA profile suggests a change in the ruminal microbial ecosystem but, contrary to the general trend when the diets are enriched in lipids, this does not result in a lower digestibility. Indeed, the magnitude of

the increase in propionate in VFA is often related to that of the decrease in digestibility (Palmquist and Jenkins, 1980). Increasing the proportion of lipids in the diet, especially when they are rich in polyunsaturated FA, generally decreases both OM and fibre digestibility as well as the acetate:propionate ratio. In rare experiments, however, lipid supplements may decrease digestibility without any simultaneous change in the VFA profile (Pantoja et al, 1994), or digestibility may be unchanged whereas the proportion of propionate is strongly increased (Cottyn et al, 1971). In the present study, one or several FA present in the fish oil but not in other fat sources could have a specific action on the metabolism and/or the nature of microbes involved in cell-wall digestion. It can be hypothesized that among cellulolytic species, those which produce acetate decrease in number and those which produce succinate, and thus indirectly propionate, increase. It was shown by Wolin and Miller (1988) that *Selenomonas ruminantium* in coculture with *Bacteroides succinogenes,* which is a cellulolytic species, can produce propionate from succinate. Such modifications of the ecosystem or between-species interactions remain to be demonstrated with an addition of fish oil to the diet.

Duodenal fatty acid pattern

With treatment C1, FA appeared to be hydrogenated to a large extent by ruminal bacteria, as reviewed by Doreau and Ferlay (1994). Dietary polyunsaturated 18-carbon FA produced mainly stearic acid, and *cis-* and *trans-*octadecenoic isomers. This FA composition was similar to the composition determined in a previous trial with a similar diet (Ben Salem et al, 1993).

The differences between treatments C1 and D were low because of the experimental design. The small increases in some FA typical of fish oil was probably due to contamination by the infused oil, even though the infusion was stopped 5 min before sampling.

The ruminal infusion of fish oil resulted in considerable hydrogenation of FA in the rumen. This has been especially observed for 20- and 22-carbon FA, which are hydrogenated in several components but do not end up completely saturated. According to Spain et al (1995), a ruminal infusion of 50 g fish oil in cows moderately altered in vivo FA composition of duodenal contents, but these authors did not give the results for 20- and 22-carbon FA. Ashes et al (1992) found in 24 h incubations with rumen contents that unsaturated 20- and 22-carbon FA were not hydrogenated at all, and in vitro data of Palmquist and Kinsey (1994) suggested the lack of hydrogenation of C20:5 and C22:6. However, Van Nevel and Demeyer (in preparation), in accordance with our work, recently demonstrated in 6 h incubations with rumen contents an extensive hydrogenation of 20- and 22-carbon FA from fish oil. Further experiments are necessary to evaluate the extent of biohydrogenation of very long-chain FA.

With treatment R, the proportion of C18:0 in duodenal contents was low and the level of C18:1 was very high. The C18:1 isomers were largely made up of the *trans* n-7 isomer. This was also shown by Wonsil et al (1994) with a mixture of menhaden oil and stearic acid. This could mean that the supply of 20- and 22-carbon FA disturbs the classical mechanisms of biohydrogenation of dietary C18:2 and C18:3 (including those from forages and concentrates) by stopping the process before these FA are completely saturated. A similar result, although to a lesser extent, was mentioned by Pennington and Davis (1975) in the ruminal contents of cows receiving cod liver oil.

The differences in duodenal FA composition between treatments D and R had marked consequences on milk FA secretion. With treatment D and R, the milk fat yield was 46 and 216 g/day lower than with treatment C1, respectively (Chilliard and Doreau, 1997).

For treatment R, it can be supposed that the large amount of duodenal *trans* FA inhibited the synthesis of short-and medium-chain FA in the mammary gland (Griinari et al, 1996).

CONCLUSION

The effects of fish oil on ruminal metabolism appear to be very different from those of other oils or fats. However, more research is needed in this area because very few data are available concerning digestibility and ruminal biohydrogenation. In particular, results on the fate of 20- and 22-carbon FA are very scarce. It would be interesting to have a better knowledge of the effect of fish oil on the microbial ecosystem.

If the results of these trials are confirmed by further experiments, then the digestive effects of fish oil should not be a limiting factor for a putative development of the use of fish oil in dairy cow rations, especially when economical constraints require a reduction of milk butterfat content.

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