# Influence of supplementary fish oil and rumen-protected methionine on milk yield and composition in dairy cows

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Summary. The effects of a daily supplement of 300 ml fish oil and 20 g rumen-protected methionine, alone or in combination, were investigated in mid-lactation cows receiving a maize silage-based diet *ad lib*. Fish oil significantly decreased feed intake, increased milk yield, decreased protein and casein concentrations, and especially fat concentration (by 13·1 g/kg) and output. Fat concentration decreased more in primiparous than in multiparous cows. Methionine supplementation increased protein and casein concentrations and outputs. No significant interaction between oil and methionine supplementation was found on milk composition. Treatments did not modify live weight or body condition changes, or lactose and non-casein nitrogenous compounds in milk. Oil plus methionine supplementation made it possible to decrease milk fat content without changing protein content.

The new constraints on dairy production in the European Union tend to favour the production of a milk richer in protein to facilitate cheesemaking, and poorer in fat to avoid exacerbating the present excess dairy fat production. Genetic improvement of cows to promote higher milk protein is accompanied by a concomitant increase in milk fat. Nutritional manipulation may enable the protein:fat ratio in milk to be increased, by considerably increasing the amount of cereals in the diet (Nelson *et al*. 1968). However, this option is not convenient in forage-based systems. Moreover, there are some risks of metabolic disturbances such as acidosis.

Modifications in the protein:fat ratio in milk can be attained by the combined use of two supplements: most fish oils are known to decrease fat concentration (Jarrige & Journet, 1959; Christie, 1981; Opstvedt, 1984) and rumen-protected methionine to increase protein concentration (Rulquin & Vérité, 1993). Their combined use has not been previously studied.

## MATERIALS AND METHODS

### *Animals and experimental design*

Eight lactating Holstein cows, of which four were primiparous and four multiparous, were studied after peak lactation in a 4 × 4 Latin square design. Each period lasted 4 weeks; the results presented here were obtained in the fourth week of each period. Cows had been accustomed to being fed on fish oil before the beginning of the experiment.

Table 1. *Composition of diets used in this experiment*

| | Diet | |
|---|---|---|
| Composition, g/kg | Control and control + methionine | Fish oil and fish oil + methionine |
| Organic matter | 940 | 941 |
| Neutral detergent fibre | 396 | 389 |
| Acid detergent fibre | 218 | 214 |
| Crude protein | 141 | 139 |
| Ether extract | 20 | 36 |

*Dietary treatments*

All cows received a diet containing (g/kg) maize silage 650, concentrates 350, on a dry matter (DM) basis, given *ad lib.* so that the proportion of refusals was ~ 10%. Concentrates contained (g/kg) wheat 200, barley 200, beet pulp 300, rapeseed meal 150, soyabean meal 70, beet molasses 50, dicalcium phosphate 10, limestone 10, magnesium oxide 5, sodium chloride 5. Cows also received daily 0·85 kg DM of hay, 220 g mineral and vitamin premix and 100 g urea. The four treatments were: no supplement (control), 300 ml fish oil of the menhaden type, 20 g rumen-protected methionine (Smartamine-M™; Rhône-Poulenc Animal Nutrition, F-92160 Antony, France), or 300 ml fish oil plus 20 g rumen-protected methionine.

Oil, methionine, mineral and vitamin premix and urea were mixed with 5 kg of the concentrates. This mixture was given at 08.45, maize silage and hay were given at 09.15, the other portion of concentrates was given at 16.00. Concentrates and forages were given in two separate parts of the feeding trough so that the composition of the refusals could be identified. (In fact, all refusals were of maize silage.) The amount of silage and concentrates offered was adjusted daily according to the intake of the previous day. Under these conditions the mean proportion of concentrates in the total diet, including hay, was between 0·333 and 0·335 according to the treatments.

The composition of the diets is given in Table 1. The fatty acid composition of the fish oil, determined by gas–liquid chromatography (Delsi Instruments, Model 300 chromatograph; F-95100 Argenteuil, France) using a glass capillary column coated with free fatty acid phase, was (g/kg) 14:0 76, 16:0 171, 16:1 92, other $C_{16}$ fatty acids 57, 18:0 27, 18:1*n*–9 80, 18:1*n*–7 33, 18:4*n*–3 29, other $C_{18}$ fatty acids 35, 20:5*n*–3 215, 22:6*n*–3 71, other $C_{20}$ and $C_{22}$ fatty acids 100.

*Measurements and analyses*

On the fourth week of each period, feed intake was measured over 4 d. Milk yield was recorded every day. Representative samples of milk were taken over 4 d at each milking. In these samples, fat, protein and lactose concentrations were determined by i.r. spectrophotometry. Samples from two consecutive days were analysed for nitrogen fractions: crude protein by Kjeldahl, protein by i.r. spectrophotometry, casein by precipitation with 1 M-sodium acetate buffer, pH 4·6 containing 100 g acetic acid/l (Amariglio, 1986).

At the beginning and end of each experimental period, cow live weight was recorded and body condition score was estimated on a scale of 0 (very thin) to 5 (very fat). Live weight changes have been corrected for variations in the contents of the digestive tract assuming that 1 kg of change in intake leads to 4 kg of change in digestive tract contents (Chilliard *et al.* 1987).

Statistical analysis was performed using an analysis of variance (ANOVA) according to the model

$$Y_{ijklm} = \mu + PA_i + C(PA)_{ij} + PE_k + M_l + O_m + M \times O_{lm} + PA \times M_{il} + PA \times O_{im} + e_{ijklm}$$

where $\mu$ is overall mean, $PA$ parity (1 df), C($PA$) cow nested within parity (6 df), $PE$ period (3 df), $M$ methionine (1 df), $O$ oil (1 df), $M \times O$ interaction between methionine and oil (1 df), $PA \times M$ interaction between parity and methionine (1 df), $PA \times O$ interaction between parity and oil (1 df) and $e$ is residual error (16 df). The effect of parity was tested against C($PA$). Differences were taken to be significant at $P < 0{\cdot}05$. This analysis made it possible to assess the significance of the respective main effects of the two factors (oil and methionine), together with their interaction. This was completed by a comparison of the means of the four treatments using the Student–Newman–Keuls $t$ test.

## RESULTS AND DISCUSSION

### *Feed intake, live weight and body condition score variations*

Incorporation of fish oil in the diet decreased ($P < 0{\cdot}01$) total feed intake by 1·6 kg DM daily on average, whereas methionine supplementation did not alter feed intake (Table 2). The effect of fish oil was more pronounced than that generally found with other sources of lipids (see Chilliard, 1993). This trend is consistent with the results of Wonsil *et al*. (1994), and could be due to properties common to all fatty acids. However, this effect was probably not a direct effect of fish oil fatty acids, because the depression of intake was higher when fish oil was infused into the rumen than into the duodenum (Doreau & Chilliard, 1997). The effect of specific fatty acids could be due more to a metabolic effect of fatty acids produced by ruminal biohydrogenation than to a negative effect of fatty acids of fish oil on rumen function. Indeed, fish oil does not disturb the ruminal digestion of carbohydrates (Wonsil *et al*. 1994; Chilliard & Doreau, 1997).

No differences in live weight and body condition score were found when diets were supplemented with methionine or oil. This could be due to the relatively short experimental periods, but is consistent with the mean effect of lipids after peak lactation (Chilliard, 1993). No attempt was made to calculate energy balances, because it was not possible to give an accurate energy value to fish oil.

### *Milk yield and lactose*

Addition of fish oil increased milk yield by 2·0 kg/d on average ($P < 0{\cdot}01$, Table 3). This was not found in previous experiments with similar amounts of cod liver oil (Brumby *et al*. 1972; Storry *et al*. 1974; Pennington & Davis, 1975) or menhaden oil (Wonsil *et al*. 1994) and the effect was more marked than generally found with lipid supplements in mid-lactation cows (Østergaard *et al*. 1981; Chilliard, 1993). As body reserves apparently did not vary, this increase in milk yield suggests that the decrease in intake was compensated for by an increase in dietary energy value due to the fish oil.

No effect of methionine on milk yield was found, as in most experiments with diets based on maize silage (Rulquin & Vérité, 1993). Lactose concentration did not vary significantly between treatments.

### *Milk fat*

Fish oil supplementation sharply decreased ($P < 0{\cdot}01$) milk fat concentration, on average by 13·1 g/kg; there was no interaction with methionine supplementation.

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Table 2. *Feed intake, live weight and body condition score variations for cows given a control diet or diets supplemented with oil, methionine or oil plus methionine*

| | Diet | | | | Residual SD |
|---|---|---|---|---|---|
| | Control | Oil | Methionine | Oil + methionine | |
| Feed intake, kg dry matter† | 19·2[ab] | 17·6[c] | 19·7[a] | 18·1[bc] | 1·0 |
| Live weight change, kg | +13 | +2 | +18 | +7 | 18 |
| Corrected live weight change, kg | +8 | +4 | +11 | +3 | 13 |
| Body condition score change | +0·22 | +0·22 | +0·09 | +0·11 | 0·22 |

[a, b, c] Means in the same row without a common superscript were significantly different: $P < 0{\cdot}01$.
† Significant main effect of oil: $P < 0{\cdot}01$.

This decrease was greater in primiparous than in multiparous cows (significant interaction between oil and parity, $P < 0{\cdot}05$). Fat content was 38·7, 24·0, 38·4 and 22·6 g/kg for primiparous cows, and 38·4, 26·7, 38·5 and 28·3 g/kg for multiparous cows receiving control diet or diets supplemented with oil, methionine, or oil plus methionine respectively. The same trend was found for daily fat secretion, although this was not significant, so that the interaction between fish oil and parity on milk fat concentration cannot be explained by a dilution effect due to a lower milk yield in primiparous cows. The decrease in fat concentration was more pronounced than that (5–10 g/kg) found with similar amounts of cod liver oil (Nicholson & Sutton, 1971; Brumby *et al.* 1972; Storry *et al.* 1974; Pennington & Davis, 1975), menhaden oil (Wonsil *et al.* 1994) or fishmeal (Hussein & Jordan, 1991). We suggest that the decrease in fat content was due to (1) low incorporation in milk fat of $C_{20}$ and $C_{22}$ fatty acids from fish oil, (2) decreased mammary uptake of other fatty acids due to these long-chain fatty acids, as suggested by Storry *et al.* (1974) and (3) a decrease in *de novo* fatty acid synthesis or esterification consequent to a shortage in acetate (Nicholson & Sutton, 1971; Storry *et al.* 1974) or to the ruminal formation of significant amounts of *trans* fatty acids (Wonsil *et al.* 1994; Chilliard & Doreau, 1997). The large magnitude of the decrease was perhaps due to the higher concentration of 20:5$n$–3 in the oil used in this experiment (215 g/kg) compared with cod liver oil (~ 120 g/kg) and/or to the inclusion of primiparous cows. Despite an increase in milk yield, fish oil supplementation significantly ($P < 0{\cdot}01$) decreased fat output (by 285 g/d).

We found no effect of methionine on fat concentration. This confirmed previous reports, although a slight positive effect has sometimes been found (Rulquin & Vérité, 1993).

*Milk protein*

Fish oil supplement significantly decreased ($P < 0{\cdot}01$) milk protein concentration, by 1·2 g/kg on average (Table 3). This decrease was almost entirely due to the casein fraction (Table 4), and was probably a dilution effect since protein and casein output were not decreased, as has generally been found in most experiments with different kinds of lipids (see reviews by Doreau & Chilliard, 1992; Wu & Huber, 1994). Few experimental data are available on the specific effect of fish oils on protein concentration. In a trial by Varman *et al.* (1968) neither safflower oil nor cod liver oil changed milk yield or milk protein concentration. However, in a trial by Wonsil *et al.* (1994) menhaden oil plus stearic acid decreased milk protein secretion without changing milk protein content. More research is needed in this area.

Methionine supplementation significantly increased ($P < 0{\cdot}01$) milk protein

Table 3. *Milk production and composition for cows given a control diet or diets supplemented with oil, methionine or oil plus methionine*

| | Diet | | | | Residual SD |
|---|---|---|---|---|---|
| | Control | Oil | Methionine | Oil + methionine | |
| Milk yield, kg/d† | 26·5[cd] | 28·0[c] | 25·8[d] | 28·2[c] | 1·7 |
| Fat-corrected milk (to 40 g/kg), kg/d† | 26·0[a] | 22·1[b] | 25·3[ab] | 22·1[b] | 2·4 |
| Fat, g/kg‡ | 38·6[a] | 25·3[b] | 38·5[a] | 25·5[b] | 3·0 |
| Protein, g/kg§ | 28·8[a] | 27·9[a] | 30·7[b] | 29·1[a] | 1·0 |
| Lactose, g/kg | 48·1 | 47·6 | 47·6 | 47·4 | 0·8 |
| Fat, g/d† | 1024[a] | 724[b] | 994[a] | 723[b] | 118 |
| Protein, g/d‖ | 764 | 780 | 794 | 820 | 42 |
| Lactose, g/d¶ | 1276 | 1332 | 1230 | 1336 | 97 |

[a, b, c, d] Means in the same row without a common superscript were significantly different: a, b, $P < 0·01$; c, d, $P < 0·05$.

† Significant main effect of oil: $P < 0·01$.

‡ Significant main effects of oil $P < 0·01$ and of interaction between oil and parity: $P < 0·05$.

§ Significant main effects of oil and of methionine: $P < 0·01$.

‖ Significant main effect of methionine: $P < 0·05$.

¶ Significant main effect of oil: $P < 0·05$.

Table 4. *Nitrogen fractions of milk for cows given a control diet or diets supplemented with oil, methionine or oil plus methionine*

| | Diet | | | | Residual SD |
|---|---|---|---|---|---|
| | Control | Oil | Methionine | Oil + methionine | |
| Crude protein, g/kg† | 30·8[a] | 29·3[a] | 32·7[b] | 30·7[a] | 1·1 |
| Protein, g/kg† | 29·0[a] | 27·4[a] | 30·8[b] | 28·9[a] | 1·1 |
| Casein, g/kg† | 22·9[a] | 21·7[a] | 24·5[b] | 23·0[a] | 0·9 |
| Whey protein, g/kg | 6·1 | 5·7 | 6·3 | 6·0 | 1·0 |
| Non-protein N compounds, g/kg | 1·8 | 1·9 | 1·8 | 1·8 | 0·2 |
| Casein: protein ratio | 0·788 | 0·794 | 0·795 | 0·794 | 0·020 |
| Casein secretion, g/d‡ | 608 | 608 | 633 | 648 | 42 |

[a, b] Means in the same row without a common superscript were significantly different: $P < 0·01$.

† Significant main effects of oil and of methionine: $P < 0·01$.

‡ Significant main effect of methionine: $P < 0·05$.

concentration (by 1·5 g/kg on average). The difference between control diet and the diet supplemented with methionine alone was higher (+1·9 g/kg) than the difference between the diet supplemented with oil and that supplemented with oil plus methionine (+1·2 g/kg), but the interaction between oil and methionine was not significant (Table 3). On average, protein and casein outputs were significantly ($P < 0·05$) increased by methionine supplementation (by 35 and 32 g/d respectively). The positive effect of protected methionine on milk protein concentration was greater than usually found: an increase of 0·4 g/kg was quoted in the review by Rulquin & Vérité (1993). In the present trial, nitrogen balance, expressed as protein digestible in the intestine (INRA, 1989), was largely positive, +422, +456, +497 and +540 g/d for control diet and diets supplemented with oil, methionine, or oil plus methionine respectively. However, the proportion of methionine (g/kg protein) digestible in the intestine was 0·117 for the diets without methionine supplementation, and 0·233 for the diets with methionine. This suggests a relative limitation in methionine with unsupplemented diets, since the recommendation for maximizing milk protein content is 25 g methionine/kg protein digestible in the intestine (Rulquin *et al.* 1993).

There was a suggestion in the present trial of a negative interaction between oil and methionine supplementations on milk protein concentration, although this was not significant. In a previous trial (Chilliard & Doreau, 1991) we found an increase in milk protein concentration by 1·3 g/kg with a duodenal infusion of methionine and lysine, and also a non-significant trend for a negative interaction between rapeseed oil and methionine plus lysine on milk protein concentration. These observations differ from those of Canale *et al*. (1990) and Karunanandaa *et al*. (1994), who found no interaction between a blend of fats and protected lysine and methionine. Chow *et al*. (1990), using yellow fat, found a positive interaction between fat and protected lysine and methionine. In this last experiment, however, fat supplementation was accompanied by a modification of basal diet so that the supply of limiting amino acids should differ for the two diets.

In conclusion, the incorporation of 300 ml fish oil in dairy cow diet succeeded in increasing milk yield and sharply decreasing milk fat concentration. The addition of methionine to the oil-supplemented diet counterbalanced the negative effect of oil alone on protein concentration.

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