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# Analysis of factors predisposing dairy Assaf ewes to marine lipid-induced milk fat depression

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ABSTRACT

In sheep, diet-induced milk fat depression (**MFD**) results from ruminal alterations when ewes are fed marine lipids to modulate milk fatty acid profile and secretion. However, there is little available information on the relationship between characteristics existing before the consumption of MFD-inducing diets and response to those diets. Understanding predisposing factors for this condition may provide additional insight into the mechanisms of MFD and allow better predictions of its occurrence. Thus, a meta-analytical approach was used to identify traits that may predetermine the extent of marine lipid-induced MFD in individual Assaf sheep (a dairy breed with high genetic potential for milk production). Data were collected from 10 trials in which 160 ewes were fed 16 diets that included fish oil or marine algae until sustained MFD was observed. The decrease in milk fat concentration and yield was calculated using three approaches: absolute change (final – initial), relative change as a percent of pre-trial value, and potential change relative to the maximal expected MFD (assuming a maximal decrease to 3% milk fat). First, using bivariate analyses, there was a relationship between initial milk fat concentration and the absolute ($R^2$ = 0.46; partial $R^2$ of 0.08), relative ($R^2$ = 0.39; partial $R^2$ of 0.06), and potential changes in milk fat concentration during MFD ($R^2$ = 0.17; partial $R^2$ = 0.03). This finding was supported by a second approach that categorised ewes by initial milk fat concentration and yield and additionally by multivariate analyses. In addition, bivariate and multivariate analyses suggested that high MFD responsiveness in Assaf ewes was related to pre-trial milk yield and protein concentration, and to milk concentrations of candidate antilipogenic metabolites and the sum of preformed fatty acids. However, relationships were weaker than for initial milk fat concentration. Overall, when Assaf ewes were fed marine lipids, the higher the initial milk fat concentration, the greater the extent of MFD. The role of other performance traits and milk fatty acids as predisposing factors for marine lipid-induced MFD remains unclear.



## Implications

Much research effort in dairy nutrition is focused on understanding milk fat depression, a syndrome that raises major concerns due to potential economic losses. Identifying predisposing factors for milk fat depression may allow to predict its occurrence, but we know very little about which traits predetermine individual susceptibility to the syndrome. Overall, this meta-analysis identified a relationship between milk fat concentration and milk fat depression in Assaf sheep: the higher the initial milk fat concentration, the greater the extent of the syndrome. Results also supported the involvement of mammary fatty acid uptake, a metabolic pathway that has received little attention.

## Introduction

Milk fat depression (**MFD**) is a complex syndrome, which involves the interaction of multiple factors that alter the ruminal environment, leading to the production of biohydrogenation intermediates with antilipogenic activity in the mammary gland (Harvatine et al., 2009; Shingfield et al., 2010). In cows, MFD is commonly caused by feeding diets rich in grains, plant oils, or both, and the main isomers related to this condition, but not the only ones, are the *trans*-10,*cis*-12 conjugated linoleic acid (**CLA**) and possibly *trans*-10 18:1 (Bauman et al., 2006; McCarthy et al., 2018). Conversely, ewes are not prone to develop MFD when fed

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diets rich in grain and plant oils (Mele et al., 2006; Shingfield et al., 2010; Toral et al., 2020), but often experience severe decreases in milk fat when fed marine lipids (Toral et al., 2015; Frutos et al., 2017). A meta-analysis conducted by Toral et al. (2020) failed to find a clear relationship between MFD and milk fat *trans*-10,*cis*-12 CLA and *trans*-10 18:1 in dairy sheep fed marine lipids, suggesting that other biohydrogenation intermediates or factors may be responsible for marine lipid-induced MFD in this species. Della Badia et al. (2023a) reported that the individual severity of MFD in ewes supplemented with fish oil was associated with ruminal increases in *cis*-9 16:1, *cis*-11 18:1, and 20:5n-3 concentration. The biological mechanism or relationship with MFD for these fatty acids (**FA**) is not known.

Previous studies have investigated the mechanism of the decrease in milk fat concentration and yield in ewes randomly assigned to marine lipid treatments (Bichi et al., 2013; Carreño et al., 2016), but the magnitude of the MFD varies between individuals and the factors contributing to this variation are not clear. In dairy cows, an association between MFD and level of milk production (Harvatine and Allen, 2006; Rico et al., 2014; Baldin et al., 2018) or ruminal pH (Dewanckele et al., 2019) has been reported. Della Badia et al. (2021) suggested that there might be a relationship with individual milk fat concentration during the pre-trial period in a study with goats and sheep. Also, Padilha et al. (2022) recently modelled milk fat concentration and yield in does and ewes experiencing MFD due to exogenous *trans*-10,*cis*-12 CLA supply and suggested that production traits and biohydrogenation intermediates could be implicated in increasing response in animals with high susceptibility.

Understanding predisposing factors for diet-induced MFD may provide additional insight into the mechanisms underlying this syndrome and allow better predictions of its occurrence or selection of less susceptible animals. In addition, the aggregation of data collected in multiple trials allows a more robust examination of relationships between potential predisposing factors and MFD extent. On this basis, the goal of this study was to use a meta-analytical approach to identify pre-trial characteristics of ewes that are related to the change in milk fat concentration or yield experienced when fed diets that contain marine lipids (i.e., MFD-inducing diets). Preliminary results have been published in abstract form (Della Badia et al., 2023b).

## Material and methods

### *Database preparation*

#### *Selection of publications*

A database of individual animal observations from published studies in dairy ewes experiencing diet-induced MFD (i.e., diet supplementation with marine lipids), excluding those in which lambs were kept with their mothers during the trial, to avoid possible confounding effects on performance (Capper et al., 2007; Gallardo et al., 2014), was created. Within some of the selected studies, we also excluded treatments in which ewes experienced MFD due to exogenous *trans*-10,*cis*-12 CLA supply (e.g., Toral et al., 2015). As a result, the 10 selected studies (corresponding to 16 treatments inducing MFD) were all conducted at Instituto de Ganadería de Montaña (León, Spain) using Assaf ewes (the list of references is given in Supplementary Material S1). Different sheep were used in the different trials. Overall, this allowed the compilation of a database of individual observations, as opposed to previous animal nutrition meta-analyses that are generally based on published treatment means (e.g., Matamoros et al., 2020; Vargas-Bello-Pérez et al., 2021). In this context, and given the high individual variation observed in the response to marine oil supplementation in ewes (e.g., Frutos et al., 2017; Della Badia et al., 2021), the use of individual data fits better to our aim. Despite the use of only our own data and one ovine breed, an adequate number of observations was obtained (up to 160). In addition, Assaf sheep are a specialised dairy breed and have received considerable research attention because of its increasingly widespread distribution in intensive dairy farms (Milán et al., 2011; Riveiro et al., 2013). Due to their high milk yields, the milk fat content of Assaf ewes is usually lower than that of autochthonous ovine breeds (Milán et al., 2011). Any further decrease in milk fat content due to MFD would therefore have a negative impact on milk marketability in this breed.

#### *Experimental data*

The database was compiled using data from individual animals that received MFD-inducing diets, including their initial pre-treatment values as well as observations during MFD (i.e., decreases in milk fat concentration and yield at the end of the MFD induction period, when sustained MFD was observed). Data from animals fed dietary treatments that did not cause MFD (e.g., with no supplementation or supplemented only with plant oils) and group observations [i.e., DM intake (**DMI**) expressed as a pen mean] were excluded. Individual animal observations included, when available, were BW (kg), DMI (kg/d), milk yield (kg/d), milk fat, protein and lactose concentration and yield (g/kg), and milk FA concentrations (g/100 g of FA; Table 1). Data on parity and days in milk are also reported in Table 1.

The 10 studies were conducted between 2007 and 2021 and included 16 different diets formulated to induce MFD (Supplementary Table S1). Control (pre-trial) diets were fed as a TMR for 3–5 weeks. Forage type (alfalfa hay) and sources of starch and protein (mainly whole corn grain, whole barley grain and soybean meal) were similar across experiments, and the main difference in the composition of basal diets was the forage to concentrate ratio, which ranged from 21:79 to 50:50. The MFD induction diets were based on the same control basal diets with the addition of marine microalgae and fish oils, and were fed from 27 to 54 days (Supplementary Table S1). The different unprotected lipid sources and levels used across experiments included: fish oil or marine algae alone or in combination with sunflower oil or a prill rich in stearic acid (18:0) at 0.8 to 4% DM. Diet ingredients and chemical composition, including FA profile, are reported in Supplementary Table S2.

In each trial, milk samples were collected immediately before inducing MFD and at the end of the MFD induction period (when sustained MFD was observed). Milk fat, protein and lactose concentration was determined in all experiments by infrared spectrophotometry (ISO 9622:1999). Energy-corrected milk (**ECM**) was calculated according to INRA (2018) equations for sheep [ECM = L/d of milk yield × [(0.0071 × g/L of milk fat) + (0.0043 × g/L of milk protein) + 0.2224] and transformed to kg/d assuming a mean density of 1.036 kg/L. Milk FA profile was determined by gas chromatography using the same methodology across experiments (Toral et al., 2015; Frutos et al., 2017).

Milk FA were quantified both as a percent of total FA (g/100 g) and as a proportion of their biosynthetic origin group (g/100 g of FA), as described by Andreen et al. (2021). Briefly, *de novo* synthesised FA included straight chain even carbon FA with < 16 carbons (**< C16**), preformed FA included straight chain even carbon FA with > 16 carbons (> C16), and mixed-source FA included 16 carbon straight chain FA (C16). Total odd- and branched-chain FA (**OBCFA**) was also included in the database. Total FA content and yield were calculated as described by Glasser et al. (2007).

#### *Calculation of the extent of milk fat depression*

The decrease in milk fat synthesis due to MFD was calculated using 3 different methods that characterised the absolute, relative,

**Table 1**
Distribution analysis of performance traits and changes in milk fat concentration and yield during MFD in Assaf sheep.

| Variable | n | Mean | Median | Skewness | SD | Percentiles 10th | Percentiles 90th |
|---|---|---|---|---|---|---|---|
| Number of lactation | 160 | 2.49 | 2.00 | 0.267 | 1.203 | 1.00 | 4.00 |
| Days in milk | 160 | 77.1 | 87.0 | −0.50 | 24.01 | 42.9 | 105.0 |
| Performance traits (pre-trial) | | | | | | | |
| BW, kg | 160 | 79.9 | 79.2 | 0.28 | 12.43 | 63.9 | 96.0 |
| DMI, kg/d | 48 | 2.87 | 3.08 | −0.48 | 0.732 | 1.98 | 3.74 |
| Milk, kg/d | 158 | 2.40 | 2.53 | 0.12 | 0.872 | 1.20 | 3.49 |
| ECM, kg/d | 154 | 2.04 | 2.12 | −0.005 | 0.704 | 1.04 | 2.80 |
| Initial fat, g/kg | 154 | 57.2 | 56.0 | 0.36 | 9.01 | 46.8 | 70.7 |
| Initial fat, g/d | 154 | 134.8 | 138.6 | −0.01 | 45.98 | 71.6 | 194.9 |
| CP, g/kg | 157 | 51.1 | 50.5 | 0.44 | 4.24 | 46.6 | 56.5 |
| CP, g/d | 157 | 122.2 | 126.5 | 0.08 | 43.88 | 57.5 | 170.3 |
| Lactose, g/kg | 158 | 49.4 | 49.5 | −0.92 | 2.80 | 45.9 | 52.8 |
| Lactose, g/d | 158 | 119.7 | 124.4 | 0.13 | 45.66 | 59.7 | 172.9 |
| Total FA, g/d | 38 | 109.7 | 110.7 | 0.12 | 46.72 | 44.1 | 181.1 |
| Performance traits (MFD period) | | | | | | | |
| Final fat, g/kg | 154 | 48.3 | 47.2 | 0.74 | 7.67 | 39.9 | 57.3 |
| Final fat, g/d | 154 | 98.2 | 97.9 | 0.33 | 36.55 | 49.7 | 145.9 |
| Absolute change in milk fat during MFD | | | | | | | |
| Concentration, g/kg | 154 | −8.89 | −8.76 | 0.12 | 9.098 | −19.6 | 2.52 |
| Yield, g/d | 154 | −36.6 | −34.7 | −0.42 | 31.13 | −68.2 | −2.55 |
| Relative change in milk fat during MFD | | | | | | | |
| Concentration, % | 154 | −14.3 | −15.4 | 1.03 | 15.23 | −32.2 | 5.09 |
| Yield, % | 154 | −25.2 | −27.7 | 0.67 | 19.18 | −49.5 | −3.70 |
| Potential change in milk fat during MFD | | | | | | | |
| Concentration, % | 154 | −27.5 | −33.7 | 3.12 | 39.04 | −65.9 | 12.2 |
| Yield, % | 154 | −39.8 | −50.3 | 4.82 | 57.48 | −79.9 | −10.8 |

Abbreviations: DMI = DM intake; ECM = energy-corrected milk; FA = fatty acids; MDF = milk fat depression; n = number of observations.

and potential change in milk fat concentration and yield relative to initial (pre-trial) levels.

i. Absolute change in milk fat concentration and yield:

$$\text{Absolute change in concentration (g/kg)} = \text{Final milk fat concentration (g/kg)} - \text{Initial milk fat concentration (g/kg)}$$

$$\text{Absolute change in yield (g/d)} = \text{Final milk fat yield (g/d)} - \text{Initial milk fat yield (g/d)}$$

ii. Relative change in milk fat concentration and yield (%):

$$\text{Relative change in concentration (\%)} = [\text{Final milk fat concentration (g/kg)} / \text{Initial milk fat concentration (g/kg)} \times 100] - 100$$

$$\text{Relative change in yield (\%)} = [\text{Final milk fat yield (g/d)} / \text{Initial milk fat yield (g/d)} \times 100] - 100$$

iii. Potential change in milk fat concentration and yield (%):

This change represents the percent variation relative to the maximal MFD response expected in sheep, which is considered the portion responsive to dietary changes (see Fig. 1). The maximal MFD response was estimated assuming a maximum decrease to 30 g/kg of milk fat, a threshold established based on individual raw data in Assaf sheep (minimum milk fat content in the database = 31 g/kg; Bichi et al., 2013). It is important to note that the potential change in milk fat yield was calculated assuming that basal milk fat yield scales with changes in total milk yield during the trial.

$$\text{Potential change in concentration (\%)} = [(\text{Final milk fat concentration (g/kg)} - \text{Initial milk fat concentration (g/kg)}) / (\text{Initial milk fat concentration (g/kg)} - 30)] \times 100$$

$$\text{Potential change in yield (\%)} = [\text{Final milk fat yield (g/d)} - \text{Initial milk fat yield (g/d)}] / [(\text{Initial milk fat yield (g/d)} - (\text{Final milk yield (g/d)} \times 30)] \times 100$$

*Categorisation of individuals by pre-trial milk fat concentration and yield*

Because initial (pre-trial) milk fat is used in calculating the change in milk fat (i.e., extent of the MFD), the latter is not independent of initial milk fat data. Thus, an alternative approach was taken to categorise individuals by milk fat level into two categories (Low and High) within each experiment based on the median (Supplementary Table S1). These categorical variables were then used to test the relationship between initial milk fat and response to the MFD diets.

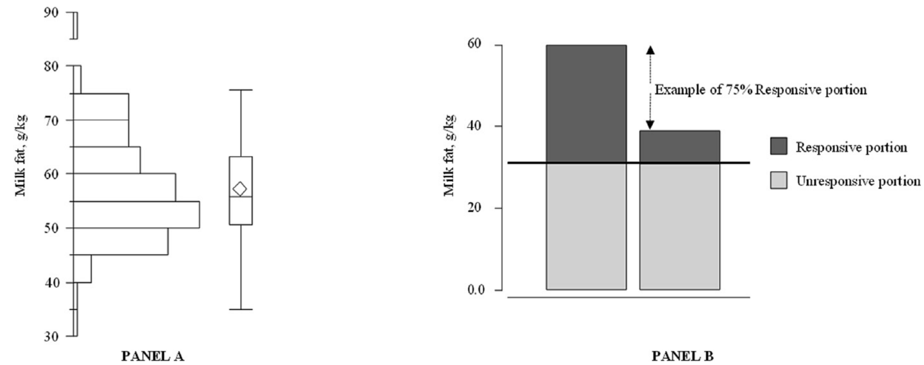


**Fig. 1.** A novel approach to analyse the portion of milk fat responsive to diet-induced milk fat depression (MFD) in sheep. Panel A: Distribution of milk fat concentration in the database showing no ewes below 30 g/kg (n = 154); Panel B: Illustration of the portions of milk fat responsive and non-responsive to MFD assuming that milk fat cannot decrease below 30 g/kg.

*Statistical analysis*

All analyses were conducted using JMP Pro 16 (SAS Institute Inc., Cary, NC, USA).

Differences were declared significant at $P \leq 0.05$ and considered a trend towards significance at $0.05 < P \leq 0.10$. Observations with studentised residuals outside of ± 3.0 were considered outliers and removed. Linear and quadratic relationships were analysed, and the quadratic effect was removed when $P > 0.10$. Furthermore, decreases in milk fat concentration and yield plotted in figures were adjusted for the random effect of the trial as outlined by St-Pierre (2001).

In Supplementary Material S1, an outline is presented to facilitate the follow-up of all the analyses carried out, as well as the respective result tables or figures.

*Distribution analysis*

A distribution analysis of pre-trial performance traits and MFD extent (changes in milk fat concentration and yield during MFD) was conducted to characterise the dataset and calculate metrics of variability.

**Table 2**
Bivariate analysis of performance traits and major milk FA groups (mean ± SE) in relation to absolute change in milk fat concentration and yield in Assaf sheep.

| Variables[1] | | | | | | | Fit statistics[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $R^2$ | | |
| Y | X | Intercept | SE | Slope | SE | $P$ | Full | Partial | RMSE |
| Absolute change in milk fat concentration, g/kg | | | | | | | | | |
| | BW, kg | −4.10 | 5.153 | −0.053 | 0.0615 | 0.382 | 0.30 | 0.003 | 7.80 |
| | DMI, kg DM/d | −8.04 | 3.796 | −0.37 | 1.294 | 0.787 | 0.002 | 0.002 | 6.39 |
| | Milk, kg/d | −14.3 | 2.93 | 2.44 | 0.971 | 0.013 | 0.33 | 0.02 | 7.68 |
| | ECM, kg/d | −11.5 | 3.01 | 1.51 | 1.216 | 0.216 | 0.31 | 0.01 | 7.80 |
| | Initial fat, g/kg | 28.7 | 4.19 | −0.66 | 0.073 | <0.001 | 0.46 | 0.08 | 6.71 |
| | Final fat, g/kg | −38.5 | 5.65 | 0.64 | 0.112 | 0.003 | 0.59 | 0.01 | 6.08 |
| | CP, g/kg | −1.62 | 8.127 | −0.13 | 0.154 | 0.396 | 0.31 | 0.004 | 7.81 |
| | CP, g/d | −13.6 | 2.97 | 0.042 | 0.0194 | 0.032 | 0.32 | 0.02 | 7.74 |
| | Lactose, g/kg | −21.6 | 14.28 | 0.27 | 0.285 | 0.352 | 0.30 | 0.002 | 7.80 |
| | Lactose, g/d | −14.3 | 2.87 | 0.048 | 0.0189 | 0.011 | 0.33 | 0.02 | 7.67 |
| | FA yield, g/d | −4.68 | 2.479 | −0.043 | 0.0208 | 0.043 | 0.11 | 0.11 | 5.92 |
| | < C16, g/100 g FA | −16.8 | 8.64 | 0.19 | 0.235 | 0.494 | 0.02 | 0.02 | 5.35 |
| | C16, g/100 g FA | −21.3 | 11.73 | 0.43 | 0.423 | 0.321 | 0.03 | 0.03 | 6.19 |
| | > C16, g/100 g FA | 5.50 | 8.621 | −0.50 | 0.286 | 0.089 | 0.08 | 0.08 | 6.02 |
| | OBCFA, g/100 g FA | −9.99 | 10.797 | 0.11 | 2.252 | 0.963 | 0.001 | 0.001 | 6.27 |
| Absolute change in milk fat yield, g/d | | | | | | | | | |
| | BW, kg | −61.3 | 15.60 | 0.36 | 0.177 | 0.042 | 0.43 | 0.02 | 22.3 |
| | DMI, kg DM/d | −34.7 | 47.80 | 1.76 | 14.722 | 0.925 | 0.47 | 0.06 | 15.0 |
| | Milk, kg/d | 16.8 | 10.42 | −20.3 | 3.23 | 0.002 | 0.56 | 0.02 | 21.4 |
| | ECM, kg/d | 34.6 | 14.30 | −32.3 | 6.00 | 0.038 | 0.63 | 0.32 | 19.9 |
| | Initial fat, g/kg | 48.5 | 17.75 | −0.59 | 0.122 | 0.011 | 0.67 | 0.06 | 18.7 |
| | Final fat, g/kg | −44.3 | 10.01 | 0.11 | 0.072 | 0.140 | 0.39 | 0.01 | 25.0 |
| | CP, g/kg | 5.42 | 24.014 | −0.75 | 0.451 | 0.101 | 0.42 | 0.01 | 22.6 |
| | CP, g/d | 19.2 | 9.82 | −0.42 | 0.054 | <0.001 | 0.57 | 0.18 | 21.1 |
| | Lactose, g/kg | −48.8 | 42.43 | 0.33 | 0.842 | 0.700 | 0.41 | 0.001 | 22.7 |
| | Lactose, g/d | 12.5 | 9.74 | −0.37 | 0.055 | <0.001 | 0.54 | 0.15 | 21.8 |
| | FA yield, g/d | 40.4 | 20.96 | −0.55 | 0.180 | 0.048 | 0.71 | 0.11 | 14.7 |
| | < C16, g/100 g FA | −44.4 | 43.25 | 0.36 | 1.112 | 0.747 | 0.48 | 0.005 | 20.0 |
| | C16, g/100 g FA | −107.6 | 45.60 | 2.85 | 1.650 | 0.094 | 0.52 | 0.05 | 19.2 |
| | > C16, g/100 g FA | 8.26 | 31.118 | −1.31 | 0.984 | 0.191 | 0.51 | 0.03 | 19.5 |
| | OBCFA, g/100 g FA | 20.8 | 44.47 | −10.6 | 8.84 | 0.240 | 0.50 | 0.02 | 19.7 |

Abbreviations: < C16 = *de novo* synthesised fatty acids; C16 = mixed-source fatty acids; >C16 = preformed fatty acids; DMI = DM intake; ECM = energy-corrected milk; FA = fatty acid; OBCFA = odd- and branched-chain fatty acids.

[1] Y = dependent variable in the first heading; X = independent variables in the subheading.

[2] Fit statistics of the prediction model: $R^2$ for the linear relationships between studentised residuals of observed and predicted values and RMSE.

*Bivariate analysis*

A series of three bivariate analyses were performed to study the relationship between pre-trial variables (animal performance traits and major milk FA groups) and the absolute, relative, and potential change in milk fat concentration or yield during MFD (Bivariate analysis 2.1., 2.2., and 2.3., respectively, in Supplementary Material S1), using the REML method with the following model:

$$Y_{ij} = S_i + b_i S_j + \beta_0 + \beta_1 X_j + \beta_2 X_j^2 + \epsilon_{ij}$$

where $Y_{ij}$ is the decrease in milk fat concentration or yield (absolute, relative, and potential changes) for the *i*-th study and *j*-th animal and $S_i$ and $b_i$ are the random intercept and slope for each study, $\beta_0$ is the overall intercept, $\beta_1$ *and* $\beta_2$ are the linear and quadratic slopes associated with the pre-trial observation for each animal ($X_j$), and $\epsilon_{ij}$ is the residual error. Since the variation accounted for by the random effect of study was included in the "full model $R^2$", the coefficient of partial determination for the predictor variables was determined by running the model a second time, with study as a fixed effect allowing calculation of the sums of squares for each term and the calculation of the partial $R^2$ by dividing sum of squares of the predictor variables of interest by the total sum of squares.

An additional analysis was conducted using data from individuals categorised by pre-trial milk fat concentration and yield. The aim of this categorical analysis was to investigate the relationship between the change in milk fat concentration or yield during MFD and pre-trial milk fat concentration and yield by categorising animals (top and bottom half of the study; Bivariate analysis 2.4 in Supplementary Material S1). The following model was used:

$$Y_{ij} = S_i + C_j + \epsilon_{ij}$$

where $Y_{ij}$ is the decrease in milk fat concentration or yield during MFD (absolute, relative, and potential changes) for the *i*-th study and *j*-th animal and $S_i$ is the random effect of the study, $C_j$ is the fixed effect of milk fat production category (i.e., Low and High), and $\epsilon_{ij}$ is the residual error.

*Multivariate analysis (backward stepwise elimination)*

Two multivariate analyses were performed to test the relationship between pre-trial variables and the change in milk fat concentration and yield during MFD using backwards stepwise elimination. Variables were removed from the model when $P > 0.05$.

The first multivariate analysis (Multivariate analysis 3.1 in Supplementary Material S1) included the performance traits available for all experiments in the database, namely BW, DMI, and milk production and composition (e.g., milk lactose concentration and yield). The second multivariate analysis (Multivariate analysis 3.2. in Supplementary Material S1) included the same traits and the main milk FA groups (< C16, C16, > C16, and OBCFA), using a

**Table 3**
Bivariate analysis of performance traits and major milk FA groups (mean ± SE) in relation to relative change in milk fat concentration and yield in Assaf sheep.

| Variables[1] | | | | | | | Fit statistics[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $R^2$ | | |
| Y | X | Intercept | SE | Slope | SE | P | Full | Partial | RMSE |
| Relative change in milk fat concentration, % | | | | | | | | | |
| | BW, kg | −9.00 | 7.768 | −0.074 | 0.0946 | 0.431 | 0.20 | 0.002 | 12.1 |
| | DMI, kg DM/d | −12.5 | 6.32 | −0.95 | 2.155 | 0.663 | 0.004 | 0.004 | 10.7 |
| | Milk, kg/d | −22.2 | 4.21 | 2.99 | 1.478 | 0.046 | 0.22 | 0.02 | 11.9 |
| | ECM, kg/d | −19.6 | 4.37 | 2.28 | 1.815 | 0.211 | 0.21 | 0.01 | 12.0 |
| | Initial fat, g/kg | 121.7 | 43.22 | | | | 0.39 | 0.01 | 11.9 |
| | Linear | | | −3.87 | 1.446 | 0.009 | | | |
| | Quadratic | | | 0.026 | 0.0122 | 0.040 | | | |
| | Final fat, g/kg | −71.9 | 10.76 | 1.22 | 0.219 | 0.001 | 0.66 | 0.01 | 8.83 |
| | CP, g/kg | −18.2 | 12.69 | 0.063 | 0.2439 | 0.795 | 0.20 | 0.001 | 12.1 |
| | CP, g/d | −22.4 | 4.27 | 0.060 | 0.0295 | 0.044 | 0.22 | 0.02 | 12.0 |
| | Lactose, g/kg | −34.9 | 21.98 | 0.40 | 0.440 | 0.361 | 0.20 | 0.003 | 12.1 |
| | Lactose, g/d | −22.2 | 4.13 | 0.059 | 0.0288 | 0.040 | 0.22 | 0.02 | 11.9 |
| | FA yield, g/d | −12.7 | 3.55 | −0.039 | 0.0294 | 0.188 | 0.05 | 0.05 | 8.10 |
| | < C16, g/100 g FA | −6.30 | 7.746 | −0.28 | 0.229 | 0.608 | −0.14 | 0.02 | 8.46 |
| | C16, g/100 g FA | −43.4 | 21.33 | 0.99 | 0.767 | 0.414 | −0.14 | 0.01 | 7.87 |
| | > C16, g/100 g FA | −7.09 | 11.966 | −0.31 | 0.398 | 0.438 | −0.13 | 0.03 | 8.37 |
| | OBCFA, g/100 g FA | −11.1 | 20.92 | −1.09 | 4.362 | 0.818 | 0.09 | 0.0004 | 7.89 |
| Relative change in milk fat yield, % | | | | | | | | | |
| | BW, kg | −26.3 | 10.39 | 0.048 | 0.1204 | 0.689 | 0.41 | 0.002 | 15.2 |
| | DMI, kg DM/d | −31.4 | 12.57 | 3.75 | 3.773 | 0.327 | 0.17 | 0.05 | 10.1 |
| | Milk, kg/d | −15.4 | 6.42 | −2.89 | 1.943 | 0.139 | 0.42 | 0.01 | 15.1 |
| | ECM kg/d | −11.9 | 6.37 | −5.11 | 2.325 | 0.030 | 0.42 | 0.02 | 15.0 |
| | Initial fat, g/d | 13.6 | 14.31 | −0.26 | 0.099 | 0.046 | 0.54 | 0.04 | 13.7 |
| | Final fat, g/d | −61.1 | 12.22 | 0.34 | 0.118 | 0.034 | 0.67 | 0.03 | 11.0 |
| | CP, g/kg | −4.50 | 23.774 | −0.36 | 0.458 | 0.472 | 0.45 | 0.004 | 14.9 |
| | CP, g/d | −13.9 | 6.49 | −0.068 | 0.0386 | 0.077 | 0.42 | 0.01 | 15.1 |
| | Lactose, g/kg | −67.2 | 27.99 | 0.90 | 0.556 | 0.108 | 0.42 | 0.01 | 15.1 |
| | Lactose, g/d | −16.8 | 6.37 | −0.046 | 0.0381 | 0.229 | 0.41 | 0.01 | 15.1 |
| | FA yield, g/d | 6.09 | 9.234 | −0.23 | 0.072 | 0.007 | 0.45 | 0.14 | 11.5 |
| | < C16, g/100 g FA | −28.4 | 29.40 | 0.21 | 0.769 | 0.791 | 0.30 | 0.002 | 13.2 |
| | C16, g/100 g FA | −64.6 | 29.95 | 1.63 | 1.097 | 0.147 | 0.35 | 0.05 | 12.7 |
| | > C16, g/100 g FA | 6.12 | 19.763 | −0.90 | 0.641 | 0.170 | 0.34 | 0.05 | 12.7 |
| | OBCFA, g/100 g FA | 23.1 | 40.99 | −8.88 | 8.384 | 0.344 | 0.43 | 0.05 | 12.2 |

Abbreviations: < C16 = *de novo* synthesised fatty acids; C16 = mixed-source fatty acids; > C16 = preformed fatty acids; DMI = DM intake; ECM = energy-corrected milk; FA = fatty acid; OBCFA = odd- and branched-chain fatty acids.

[1] Y = dependent variable in the first heading; X = independent variables in the subheading.

[2] Fit statistics of the prediction model: $R^2$ for the linear relationships between studentised residuals of observed and predicted values and RMSE.

**Table 4**
Bivariate analysis of performance traits and major milk FA groups (mean ± SE) in relation to potential change in milk fat concentration and yield in Assaf sheep.

| Variables[1] | | | | | | | Fit statistics[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $R^2$ | | |
| Y | X | Intercept | SE | Slope | SE | P | Full | Partial | RMSE |
| Potential change in milk fat concentration, % | | | | | | | | | |
| | BW, kg | −20.4 | 15.82 | −0.14 | 0.193 | 0.475 | 0.16 | 0.002 | 24.7 |
| | DMI, kg DM/d | −30.9 | 10.76 | −0.74 | 3.653 | 0.840 | 0.001 | 0.001 | 17.9 |
| | Milk, kg/d | −43.8 | 5.11 | 8.43 | 3.022 | 0.094 | 0.16 | 0.005 | 24.5 |
| | ECM, kg/d | −41.6 | 8.72 | 4.96 | 3.700 | 0.183 | 0.16 | 0.001 | 24.6 |
| | Initial fat, g/kg | 33.7 | 25.02 | −1.15 | 0.435 | 0.043 | 0.17 | 0.03 | 25.2 |
| | Final fat, g/kg | −261.1 | 39.19 | 7.04 | 1.529 | <0.001 | 0.65 | 0.08 | 16.0 |
| | CP, g/kg | −0.70 | 27.06 | | | | 0.16 | 0.01 | 24.6 |
| | Linear | | | −0.59 | 0.524 | 0.266 | | | |
| | Quadratic | | | −0.045 | 0.0147 | 0.003 | | | |
| | CP, g/d | −44.9 | 7.82 | −0.023 | 0.0531 | 0.658 | 0.22 | 0.002 | 21.2 |
| | Lactose, g/kg | −140.8 | 47.03 | 2.21 | 0.939 | 0.021 | 0.18 | 0.01 | 24.1 |
| | Lactose, g/d | −43.7 | 8.26 | 0.10 | 0.059 | 0.087 | 0.17 | 0.01 | 24.5 |
| | FA yield, g/d | −23.5 | 6.43 | −0.12 | 0.053 | 0.033 | 0.13 | 0.13 | 13.6 |
| | < C16, g/100 g FA | −16.3 | 25.87 | −0.55 | 0.704 | 0.439 | 0.02 | 0.02 | 16.0 |
| | C16, g/100 g FA | −126.3 | 62.60 | 3.42 | 1.528 | 0.110 | 0.35 | 0.07 | 17.6 |
| | > C16, g/100 g FA | −26.9 | 23.83 | −0.31 | 0.793 | 0.703 | −0.02 | 0.01 | 16.2 |
| | OBCFA, g/100 g FA | −5.69 | 27.386 | −6.43 | 5.710 | 0.277 | 0.04 | 0.04 | 15.9 |
| Potential change in milk fat yield, % | | | | | | | | | |
| | BW, kg | −45.2 | 19.22 | −0.033 | 0.2439 | 0.907 | 0.29 | 0.004 | 19.8 |
| | DMI, kg DM/d | −43.7 | 24.72 | 1.88 | 7.722 | 0.811 | 0.13 | 0.02 | 17.7 |
| | Milk, kg/d | −40.9 | 7.48 | −2.56 | 2.523 | 0.313 | 0.27 | 0.003 | 19.8 |
| | ECM, kg/d | −44.6 | 7.87 | −1.57 | 3.244 | 0.629 | 0.22 | 0.003 | 21.1 |
| | Initial fat, g/d | −42.5 | 7.60 | −0.034 | 0.0466 | 0.460 | 0.27 | 0.0002 | 19.8 |
| | Final fat, g/d | −100.5 | 17.07 | 0.52 | 0.163 | 0.023 | 0.56 | 0.03 | 20.36 |
| | CP, g/kg | −59.2 | 22.55 | 0.23 | 0.433 | 0.603 | 0.22 | 0.0004 | 21.2 |
| | CP, g/d | −44.9 | 7.81 | −0.023 | 0.0531 | 0.658 | 0.22 | 0.003 | 21.2 |
| | Lactose, g/d | −42.8 | 7.42 | −0.040 | 0.0494 | 0.471 | 0.27 | 0.001 | 19.9 |
| | FA yield, g/d | −10.7 | 13.71 | −0.25 | 0.107 | 0.036 | 0.36 | 0.07 | 17.2 |
| | < C16, g/100 g FA | −17.9 | 40.44 | −0.61 | 1.058 | 0.569 | 0.27 | 0.004 | 18.5 |
| | C16, g/100 g FA | −108.9 | 41.57 | 2.51 | 1.523 | 0.108 | 0.35 | 0.07 | 17.6 |
| | > C16, g/100 g FA | −28.7 | 28.90 | −0.40 | 0.942 | 0.670 | 0.28 | 0.01 | 18.4 |
| | OBCFA, g/100 g FA | 14.5 | 40.61 | −11.3 | 8.19 | 0.175 | 0.31 | 0.03 | 18.0 |

Abbreviations: < C16 = *de novo* synthesised fatty acids; C16 = mixed-source fatty acids; > C16 = preformed fatty acids; DMI = DM intake; ECM = energy-corrected milk; FA = fatty acid; OBCFA = odd- and branched-chain fatty acids.
[1] Y = dependent variable in the first heading; X = independent variables in the subheading.
[2] Fit statistics of the prediction model: $R^2$ for the linear relationships between studentised residuals of observed and predicted values and RMSE.

smaller number of experiments for which milk FA profile was available (Supplementary Table S1).

*Multivariate analysis (principal component analysis)*

Two principle component analyses (**PCA**) were then conducted with the aim of reducing dimensionality of the data. Similar to the previous multivariate analysis, the first PCA was performed using only the performance traits available across all experiments (PCA 4.1 in Supplementary Material S1), whereas the second PCA included the same traits and the main milk FA groups (PCA 4.2 in Supplementary Material S1). However, DMI was excluded from the first PCA, and ECM and lactose concentration and yield from the second PCA, because the model did not converge due to correlation among variables.

*Analysis using individual milk fatty acids*

Detailed milk FA profiles from each ewe were available for five experiments (Supplementary Table S1). Thus, additional analyses were conducted using data from those five experiments to test the association between pre-trial concentrations of 86 individual milk FA and the change in milk fat concentration or yield during MFD. Individual milk FA concentrations were expressed as a percent of total FA and as a proportion of their biosynthetic group.

A preliminary ANOVA was conducted to examine the fixed effect of changes in milk fat concentration or yield while accounting for the random effect of experiment. Only those individual milk FA concentrations that were significantly affected in this preliminary analysis ($P < 0.05$) were included in the subsequent bivariate and multivariate analyses of milk FA (analysis 5.3 and 5.4, respectively, in Supplementary Material S1), which were conducted as described above for performance traits.

## Results

*Distribution analysis*

Table 1 reports the results from the distribution analysis (i.e., *n*, mean, median, skewness, SD and the 10th and 90th percentiles) for pre-trial performance traits and for the absolute, relative, and potential changes in milk fat concentration and yield in our database. Substantial variation existed in pre-trial performance parameters and in MFD extent and, as mentioned above, no individual ewes fed the MFD diets were below 30 g of milk fat/kg of raw milk (Fig. 1).

*Bivariate analyses*

In bivariate analyses, the fixed effect of the predictor (pre-trial animal performance traits and major milk FA groups) was tested against the absolute change (Table 2), relative change (Table 3),

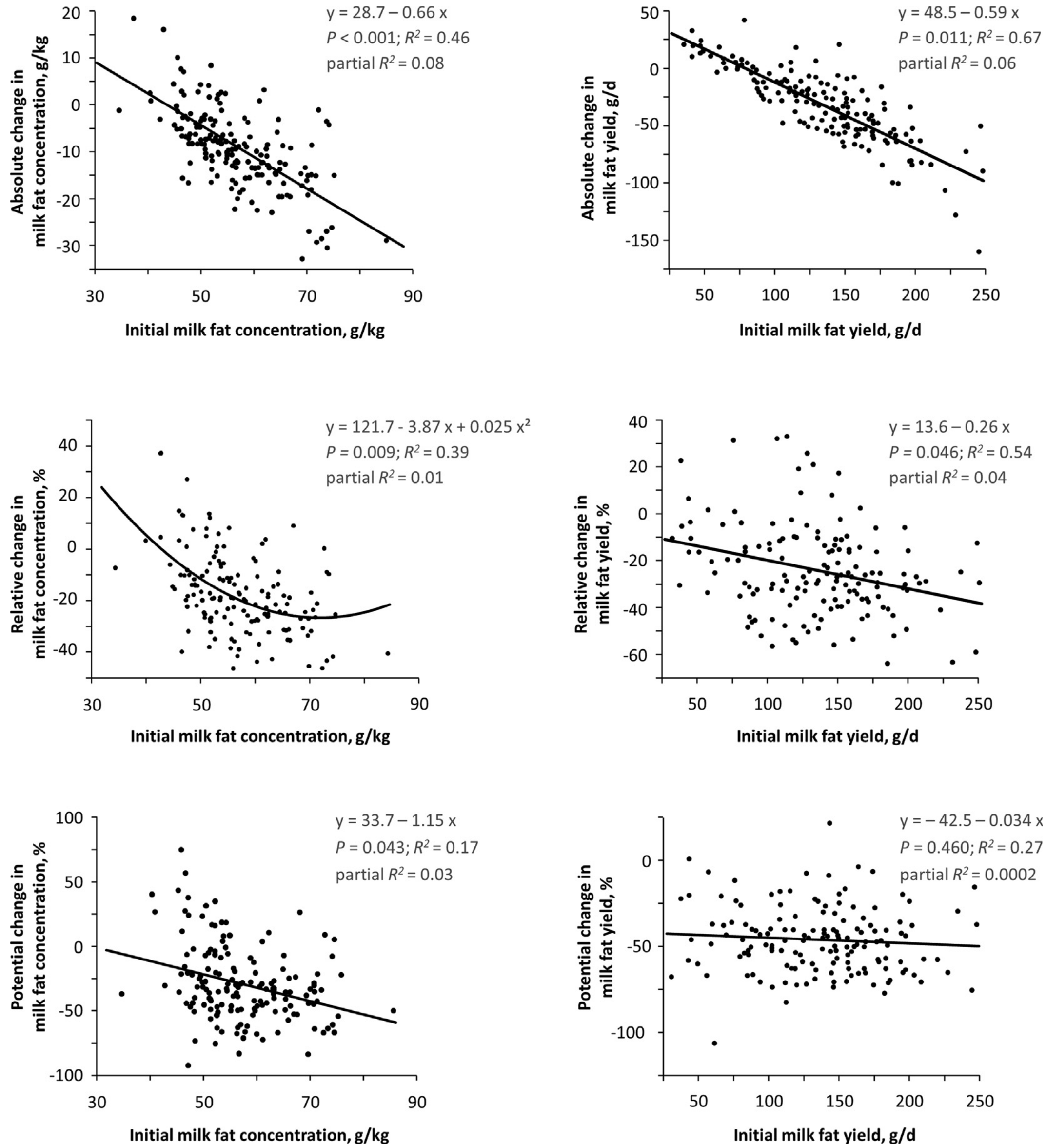


**Fig. 2.** Observations and graphical representation of regression equations for absolute, relative and potential changes in milk fat concentration and yield in relation to respective pre-trial milk fat concentration and yield in Assaf sheep. Each point represents an individual observation.

and potential change (Table 4) in milk fat concentration and yield, while accounting for the random effect of the experiment. In the tables, the full $R^2$ is the same as the partial $R^2$ when the random effect of the experiment was not significant and was removed from the model.

As summarised in Fig. 2, as pre-trial milk fat concentration and yield increased, there was a decrease in the absolute, relative, and potential change in milk fat concentration and yield during MFD ($P < 0.05$), except for the potential change in milk fat yield in relation to pre-trial milk fat yield ($P = 0.460$). For example, when expressed as an absolute change, for each g/kg increase in pre-trial milk fat concentration, final milk fat concentration decreased an additional 0.66 g/kg ($P < 0.001$) and final milk fat yield decreased by 0.59 g/d ($P = 0.013$; Table 2).

Table 5 reports the results from the categorical analysis. In general, ewes in the High category for pre-trial milk fat concentration (top half) showed a larger decrease in milk fat concentration during MFD (absolute, relative and potential change) than ewes in the Low category ($P < 0.001$), except for the potential change in milk fat yield ($P = 0.91$).

Regarding other production traits, there was no significant relationship between pre-trial BW, DMI and milk lactose with absolute, relative, or potential change in milk fat concentration or yield during MFD (Tables 2, 3 and 4). However, there were negative linear relationships between milk yield and the absolute and relative change in milk fat concentration ($P < 0.05$; Tables 2 and 3 respectively), the potential decrease in milk fat concentration ($P = 0.094$; Table 4), and the absolute change in milk fat yield (kg/d; $P = 0.002$; Table 2).

Negative relationships were also found for pre-trial milk protein concentration and the potential change in milk fat concentration ($P = 0.003$; Table 4), and for milk protein yield and absolute ($P < 0.001$; Table 2) and relative ($P = 0.077$; Table 3) changes in milk fat yield. However, there was a positive linear relationship between milk protein yield and absolute ($P = 0.032$; Table 2) and relative ($P = 0.0442$; Table 3) changes in milk fat concentration.

**Table 5**
Bivariate analysis of absolute, relative and potential change in milk fat concentration and yield in ewes categorised by initial or final milk fat level (Low and High) within each experiment based on the median[1] in Assaf sheep.

| Variable[2] | | Categories | | | |
|---|---|---|---|---|---|
| Y | X | Low | High | SEM | $P$ |
| Absolute change in milk fat concentration, g/kg | | | | | |
| | Initial fat, g/kg | −4.7 | −12.0 | 2.92 | <0.001 |
| | Final fat, g/kg | −12.1 | −4.5 | 2.90 | <0.001 |
| Absolute change in milk fat yield, g/d | | | | | |
| | Initial fat, g/d | −21.9 | −45.2 | 9.78 | <0.001 |
| | Final fat, g/d | −35.1 | −30.2 | 9.90 | 0.19 |
| Relative change in milk fat concentration, % | | | | | |
| | Initial fat, g/kg | −10.5 | −19.1 | 4.28 | <0.001 |
| | Final fat, g/kg | −21.1 | −8.1 | 4.11 | <0.001 |
| Relative change in milk fat yield, % | | | | | |
| | Initial fat, g/d | −19.5 | −25.6 | 6.49 | 0.011 |
| | Final fat, g/d | −29.0 | −16.6 | 5.99 | <0.001 |
| Potential change in milk fat concentration, % | | | | | |
| | Initial fat, g/kg | −25.5 | −36.9 | 8.51 | 0.004 |
| | Final fat, g/kg | −45.5 | −16.0 | 8.01 | <0.001 |
| Potential change in milk fat yield, % | | | | | |
| | Initial fat, g/d | −48.1 | −47.7 | 7.73 | 0.91 |
| | Final fat, g/d | −55.4 | −38.9 | 7.56 | <0.001 |

[1] Medians are reported in Supplementary Table S1.
[2] Y = dependent variable in the first heading; X = independent variables in the subheading.

Pre-trial proportion of preformed FA in milk fat showed a tendency for a negative linear relationship with the absolute change in milk fat concentration ($P$ = 0.089; Table 2). On the contrary, there was a tendency for a positive relationship between pre-trial milk fat C16 FA and the absolute change in milk fat yield ($P$ = 0.094; Table 2). No significant relationships were found for pre-trial milk FA < C16 FA or OBCFA (Tables 2, 3 and 4).

*Multivariate analysis (backward stepwise elimination)*

According to this multivariate analysis, pre-trial milk fat concentration would determine the absolute, relative, and potential change in milk fat concentration and yield during MFD (Table 6). In particular, the absolute change in milk fat concentration was relatively well predicted by a model that included only the linear effect of initial milk fat concentration ($P$ < 0.001), whereas the partial $R^2$ was lower for other models including both linear and quadratic effects of this variable.

Few additional performance traits appeared in the prediction models and their partial $R^2$ were lower than that of initial milk fat content, which may explain some inconsistent findings. In this regard, milk lactose concentration was related with the relative and potential changes in milk fat yield ($P$ = 0.023 and 0.001, respectively).

Milk FA groups according to their biological origin were included in the second multivariate analysis, but for all models, performance traits represented again the most significant variables and only small contributions of milk FA groups were found (Table 7). For example, OBCFA were related to the potential decrease in milk fat yield ($P$ = 0.009).

*Multivariate analysis (principal component analysis)*

In the first PCA (including the 9 main production variables available across all experiments), PC1 explained 56.5% of the variation, with major influences of yields of milk and milk components, whereas PC2 had major influences of milk fat and protein concentrations (15.7%; Fig. 3). The second PCA also included the concentrations of major milk FA groups. The most relevant result was that milk preformed FA (> C16) loaded close to milk fat content and opposite to milk *de novo* FA (< C16; Fig. 4). Milk OBCFA showed only moderate correlations.

*Analysis using individual milk fatty acids*

Few of the 86 individual milk FA were significant in the bivariate regression analysis (Table 8). Pre-trial concentrations of unsaturated FA supplied by marine lipids (e.g., *cis*-9 16:1, *cis*-11 18:1, and 20:5n-3) were not related with changes in milk fat concentration, except for a quadratic relationship between 22:5n-3 and the relative change in milk fat concentration ($P$ = 0.018; Table 8). In addition, pre-trial milk fat *trans*-10 18:1 content was quadratically and positively related to the absolute change and relative change in milk fat yield (Table 8). The sum of *trans*-10,*cis*-15 18:2 + *trans*-11,*cis*-15 18:2 showed a linear and negative relationship with the absolute change in milk fat yield ($P$ = 0.0128). Table 8 reports other significant relationships for minor unsaturated FA (e.g., *trans*-9 16:1 or *trans*-16 + *cis*-14 18:1) and OBCFA (e.g., iso 13:0, iso 16:0 or 17:0). In general, multivariate analysis using individual milk FA did not offer additional insight (Supplementary Table S3).

## Discussion

Feeding diets supplemented with marine lipids decreases milk fat synthesis in sheep, goats and cows, but the predisposing factors involved in this specific MFD condition remain uncertain (Bauman and Griinari, 2001; Dewanckele et al., 2020). In our previous study (Della Badia et al., 2021), the ewes with greater sensitivity to fish oil-induced MFD showed higher initial milk fat concentration, but it was unclear whether this finding could be extrapolated to other studies or if other traits further affect MFD extent. Thus, we applied a meta-analytic approach to determine whether pre-trial parameters influenced the magnitude of individual responses of ewes to diets that induce MFD. A priori, a limitation of our analysis would be that all studies included in the database were conducted using the same breed, although animal selection criteria

**Table 6**
Multivariate analysis of performance traits (mean ± SE) in relation to absolute, relative and potential change in milk fat concentration and yield in Assaf sheep.

| Variables[1] | | | | | Fit statistics[2] | | |
|---|---|---|---|---|---|---|---|
| | | | | | $R^2$ | | |
| Y | X | Estimate | SE | *P* | Full | Partial | RMSE |
| Absolute change in milk fat concentration, g/kg (n = 154) | | | | | 0.40 | 0.40 | 7.02 |
| | Intercept | 28.6 | 4.62 | <0.001 | – | – | |
| | Linear initial fat, g/kg | −0.66 | 0.080 | <0.001 | – | 0.40 | |
| Absolute change in milk fat yield, g/d (n = 154) | | | | | 0.44 | 0.08 | 24.1 |
| | Intercept | 225.7 | 71.28 | <0.001 | – | – | |
| | Linear initial fat, g/kg | −8.30 | 2.407 | <0.001 | – | 0.04 | |
| | Quadratic initial fat, g/kg | 0.064 | 0.0201 | <0.001 | – | 0.04 | |
| Relative change in milk fat concentration, % (n = 45) | | | | | 0.57 | 0.57 | 7.22 |
| | Intercept | 393.2 | 68.72 | <0.001 | – | – | |
| | Linear initial fat, g/kg | −14.9 | 2.39 | <0.001 | – | – | |
| | Quadratic initial fat, g/kg | 0.12 | 0.020 | <0.001 | – | – | |
| | Linear DMI, kg DM/d | 38.9 | 10.76 | 0.001 | – | – | |
| | Quadratic DMI, kg DM/d | −7.36 | 1.973 | 0.001 | – | – | |
| Relative change in milk fat yield, % (n = 153) | | | | | 0.51 | 0.12 | 14.1 |
| | Intercept | 93.3 | 51.49 | 0.072 | – | – | |
| | Linear initial fat, g/kg | −6.01 | 1.666 | <0.001 | – | 0.04 | |
| | Quadratic initial fat, g/kg | 2.12 | 0.768 | 0.001 | – | 0.03 | |
| | Linear lactose, g/kg | −0.63 | 0.273 | 0.023 | – | 0.02 | |
| | Linear lactose, g/d | 31.4 | 16.13 | 0.055 | – | 0.02 | |
| | Linear ECM, kg/d | 0.040 | 0.0129 | <0.001 | – | 0.01 | |
| Potential change in milk fat concentration, % (n = 151) | | | | | 0.15 | 0.03 | 25.4 |
| | Intercept | 161.9 | 77.42 | 0.038 | – | – | |
| | Linear initial fat, g/kg | −5.70 | 2.625 | 0.032 | – | 0.02 | |
| | Quadratic initial fat, g/kg | 0.040 | 0.0219 | 0.072 | – | 0.01 | |
| Potential change in milk fat yield, % (n = 153) | | | | | 0.31 | 0.22 | 49.6 |
| | Intercept | 468.5 | 179.12 | 0.010 | – | – | |
| | Linear initial fat, g/kg | −23.4 | 5.77 | <0.001 | – | 0.06 | |
| | Quadratic initial fat, g/kg | 0.16 | 0.045 | 0.010 | – | 0.04 | |
| | Linear lactose, g/kg | 6.90 | 2.647 | 0.001 | – | 0.03 | |
| | Linear lactose, g/d | −2.98 | 0.953 | <0.001 | – | 0.05 | |
| | Linear ECM, kg/d | 160.9 | 56.28 | <0.001 | – | 0.04 | |

Abbreviations: DMI = DM intake; ECM = energy-corrected milk.
[1] Y = dependent variable in the first heading; X = independent variables in the subheading.
[2] Fit statistics of the prediction model: $R^2$ for the linear relationships between studentised residuals of observed and predicted values and RMSE.

and genetic background differed between experiments, and substantial variation existed in pre-trial performance parameters and in MFD extent (Table 1). All studies were also conducted at the same research institute. Therefore, findings obtained in this meta-analysis require confirmation before being extrapolated to other breeds or ruminant species that are also affected by marine lipid-induced MFD (e.g., Churra sheep, Holstein cows and Alpine goats; Gallardo et al., 2014; Fougère et al., 2018; Dewanckele et al., 2020).

*Pre-trial milk fat concentration and yield*

Della Badia et al. (2021) suggested that sheep experiencing a stronger MFD when fed fish oil had higher initial milk fat content. Overall, our meta-analysis supports that this trait (and milk fat yield) would be a determinant of MFD extent regardless of the statistical approach (bivariate or multivariate analyses), the method to calculate the extent of MFD (in particular the absolute and relative changes in milk fat concentration and yield), and the categorisation or not of the sheep by initial milk fat concentration and yield within each experiment. Therefore, the higher the initial milk fat concentration, the higher the reduction in milk fat concentration and yield upon MFD induction.

In this meta-analysis, we introduced the concept of maximal response to MFD, which is novel in sheep and may have relevant implications when investigating the syndrome, because an ewe that has lower milk fat concentration during the pre-trial is expected to have less potential to respond to a MFD diet. However, the existence of a maximal response to diet-induced MFD is well −recognised in cows. Maximal mean decreases in milk fat yield of approximately 50% can be reached in Holstein cows by infusion of exogenous *trans*-10,*cis*-12 CLA (Chouinard et al., 1999; Bauman and Griinari, 2001; Mackle et al., 2003), although diet-induced MFD rarely exceeds 35–40% reduction (Shingfield et al., 2010). The scientific literature in sheep suggests a similar scenario, but with smaller MFD extents on average. Mean drops in milk fat yield

**Table 7**
Multivariate analysis of performance traits and major milk FA groups (mean ± SE) in relation to absolute, relative and potential change in milk fat concentration and yield in Assaf sheep.

| Variables[1] | | | | | Fit statistics[2] | | |
|---|---|---|---|---|---|---|---|
| | | | | | $R^2$ | | |
| Y | X | Estimate | SE | P | Full | Partial | RMSE |
| Absolute change in fat concentration, g/kg (n = 38) | | | | | 0.73 | 0.09 | 3.61 |
| | Intercept | 93.8 | 35.73 | 0.013 | – | – | |
| | Linear initial fat, g/kg | −5.26 | 1.135 | <0.001 | – | 0.02 | |
| | Quadratic initial fat, g/kg | 0.034 | 0.0094 | 0.001 | – | 0.01 | |
| | Linear lactose, g/kg | 1.45 | 0.379 | 0.001 | – | 0.0002 | |
| | Linear lactose, g/d | −0.96 | 0.262 | 0.001 | – | 0.03 | |
| | Quadratic lactose, g/d | 0.0013 | 0.00051 | 0.017 | – | 0.02 | |
| | Linear ECM, kg/d | 38.7 | 11.23 | 0.002 | – | 0.005 | |
| | Linear C16, g/100 g FA | 0.89 | 0.317 | 0.009 | – | 0.007 | |
| Absolute change in milk fat yield, g/d (n = 38) | | | | | 0.75 | 0.04 | 14.6 |
| | Intercept | −73.7 | 39.05 | 0.068 | – | – | |
| | Linear ECM, kg/d | 231.6 | 107.70 | 0.039 | – | 0.01 | |
| | Quadratic ECM, kg/d | −51.2 | 23.98 | 0.040 | – | 0.003 | |
| | Linear FA, g/d | −3.45 | 1.642 | 0.043 | – | 0.0001 | |
| | Quadratic FA, g/d | 0.010 | 0.0055 | 0.084 | – | 0.0001 | |
| | C16, g/100 g FA | 2.21 | 1.290 | 0.097 | – | 0.02 | |
| Relative change in milk fat concentration, % (n = 38) | | | | | 0.30 | 0.06 | 12.2 |
| | Intercept | 107.5 | 36.51 | 0.004 | – | – | |
| | Linear initial fat, g/kg | −3.42 | 1.243 | 0.007 | – | 0.04 | |
| | Quadratic initial fat, g/kg | 0.022 | 0.0104 | 0.040 | – | 0.02 | |
| Relative change in milk fat yield, % (n = 38) | | | | | 0.50 | 0.03 | 11.3 |
| | Intercept | −28.8 | 29.83 | 0.341 | – | – | |
| | Linear initial fat, g/kg | −0.82 | 0.278 | 0.006 | – | 0.01 | |
| | Linear C16, g/100 g FA | 1.98 | 0.977 | 0.050 | – | 0.02 | |
| Potential change in milk fat concentration, % (n = 27) | | | | | 0.15 | 0.09 | 25.4 |
| | Intercept | 161.9 | 77.42 | 0.03 | – | – | |
| | Linear initial fat, g/kg | −5.70 | 2.625 | 0.031 | – | 0.06 | |
| | Quadratic initial fat, g/kg | 0.040 | 0.0219 | 0.072 | – | 0.03 | |
| Potential change in milk fat yield, % (n = 29) | | | | | 0.55 | 0.12 | 14.8 |
| | Intercept | −3.87 | 55.114 | 0.944 | – | – | |
| | Linear CP, g/kg | 6.04 | 1.821 | 0.002 | – | 0.04 | |
| | Linear initial fat, g/kg | −3.69 | 1.163 | 0.004 | – | 0.03 | |
| | Linear OBCFA, g/100 g FA | −21.8 | 7.23 | 0.009 | – | 0.01 | |
| | Linear CP, g/d | −2.83 | 1.083 | 0.014 | – | 0.03 | |
| | Linear ECM, kg/d | 153.0 | 64.88 | 0.025 | – | 0.01 | |

Abbreviations: C16 = mixed-source fatty acids; ECM = energy-corrected milk; FA = fatty acid; OBCFA = odd- and branched-chain fatty acids.
[1] Y = dependent variable in the first heading; X = independent variables in the subheading.
[2] Fit statistics of the prediction model: $R^2$ for the linear relationships between studentised residuals of observed and predicted values and RMSE.

can exceed 30% in response to exogenous *trans*-10,*cis*-12 CLA in sheep (Oliveira et al., 2012; Fernandes et al., 2014; Ticiani et al., 2016) but not with the use of dietary marine lipids (e.g., Bichi et al., 2013; Carreño et al., 2016; Frutos et al., 2017). In terms of individual responses, greater variation is expected and, thus, 20.8% of the animals in our dataset showed relative decreases in milk fat yield over 40%, but only 1.9% of them (i.e., 3 sheep) exceeded a 40% reduction in milk fat concentration, and the nadir of milk fat concentration was 31 g/kg. This is compatible with an inhibition of milk fat secretion below which only milk fat yield (and total milk yield), but not concentration, could fall. Furthermore, Matamoros et al. (2020), when modelling the relationship between milk fat concentration and *trans*-10 18:1 in Holstein cows, observed that this trait was not expected to decrease below 25.1 g/kg of raw milk during MFD induced by high-starch diets and plant oils. This has been interpreted as milk fat having two portions: a dietary-responsive portion and a constitutive portion that is not responsive to MFD regulators (Matamoros et al., 2020). Based on our dataset, we proposed a value of 30 g of milk fat/kg of raw milk as a potential basal level that would not be responsive to MFD factors in Assaf sheep (Fig. 1). Which factors regulate the relative size of the non-responsive pool of milk fat synthesis to MFD in Holstein cows and Assaf ewes is relatively unknown and warrants further research. In addition, results from MFD induced by exogenous *trans*-10,*cis*-12 CLA suggest that the responsive pool would mostly be composed of de novo synthesised FA (Bauman and Griinari, 2001). However, on a molar basis, dietary marine lipids may affect mammary preformed FA uptake more strongly than de novo FA

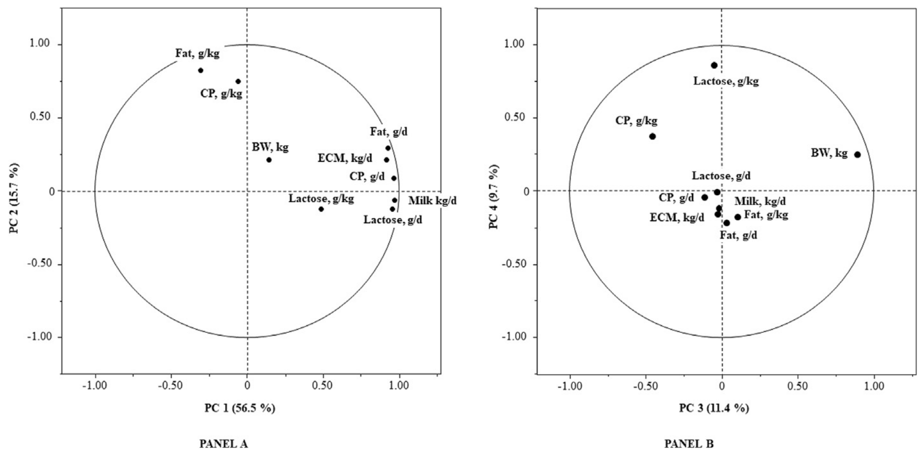


**Fig. 3.** Principal component analysis including production variables reported across all the experiments (n = 160 ewes): BW (kg), milk yield (kg/d), initial milk fat concentration (g/kg) and yield (g/d), CP and lactose concentration (g/kg) and yield (g/d), and energy-corrected milk (ECM; kg/d). Loading plot projected on the basis of principal component 1 × principal component 2 (Panel A), and principal component 3 × principal component 4 (Panel B).

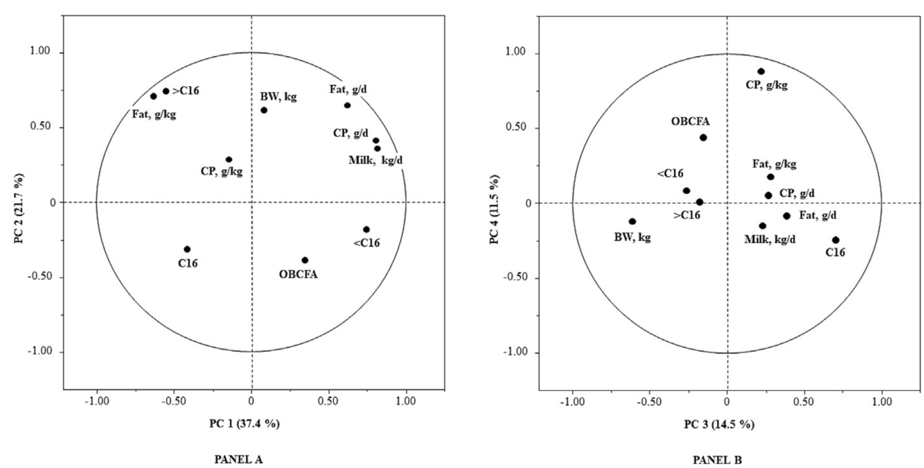


**Fig. 4.** Principal component analysis including selected production variables (BW, milk production and composition) and sums of milk fatty acids (g/100 g of fatty acids) by biosynthetic source (n = 38 ewes). Loading plot projected on the basis of principal component 1 × principal component 2 (Panel A), and principal component 3 × principal component 4 (Panel B). OBCFA = odd- and branched-chain fatty acids.

synthesis (Shingfield et al., 2003; Dewanckele et al., 2020; Della Badia et al., 2021). A better understanding of the physiological mechanisms underlying both MFD conditions may also contribute to unravel the nutritional regulation of the responsive pool of milk fat.

*Other pre-trial performance traits*

The meta-analysis showed a weak link between pre-trial milk yield and marine lipid-induced MFD in Assaf sheep, whereas no relationship was found in our previous study (Della Badia et al., 2021). Certain associations may exist in cattle, but available reports are conflicting (Bradford and Allen, 2004; Harvatine and Allen, 2005; Baldin et al., 2018). Similarly, a greater intake in higher–producing cows may change rumen microbial populations or ruminal passage rates and favour the *trans*-10 shift and diet-induced MFD (Baldin et al., 2018), but DMI was not related to MFD in the current analysis. The different number of observations in milk yield and DMI in our database (155 vs. 45 observations, respectively; Supplementary Table S1) might partly explain this inconsistency.

The lack of relationship between BW and MFD would also suggest that this trait (and indirectly DMI) would not have an influence on the magnitude of MFD in Assaf ewes, which might differ from cattle too. In Holstein cows, rumen biohydrogenation is expected to be responsive to changes in rumen turnover rate, which are influenced by DMI and BW in cows (Harvatine and Allen, 2006). A similar relationship between BW, DMI and rumen turnover rate has been reported in sheep (e.g., Cannas et al., 2003), but the relationship with rumen biohydrogenation seems largely unknown in this species.

Diet-induced MFD is normally defined as a decrease in milk fat with no changes in milk protein and lactose synthesis (Bauman and Griinari, 2001; Dewanckele et al., 2020). However, marine oil-induced MFD has been associated with lower milk protein content in cows and sheep (Shingfield et al., 2006; Pirondini et al., 2015; Frutos et al., 2017). Our bivariate analysis would contribute to this controversy, as results support either a negative or a positive relationship between milk protein content and MFD. Similarly, the apparent lack of relationship between milk lactose content and MFD responsiveness in bivariate analysis would support some reports in the literature (e.g., Angulo et al., 2012; Bichi et al., 2013; Pirondini et al., 2015). However, it contrasts with our previous findings (Frutos et al., 2017) and with prediction models obtained in the backward stepwise elimination analysis.

**Table 8**
Bivariate analysis of selected milk FA showing significant differences in the ANOVA (mean ± SE) in relation to changes in milk fat concentration and yield in Assaf sheep.

| Variables[1] | | | | | | | Fit statistics[2] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $R^2$ | | |
| Y | X | Intercept | SE | Slope | SE | $P$ | Full | Partial | RMSE |
| Absolute change in milk fat concentration, g/kg | | | | | | | | | |
| | iso 16:0[3] | 12.4 | 14.03 | | | 0.384 | 0.09 | 0.09 | 5.21 |
| | Linear | | | −149.7 | 86.71 | 0.093 | – | – | |
| | Quadratic | | | 240.3 | 131.57 | 0.077 | – | – | |
| | 17:0[4] | 8.42 | 6.751 | −0.67 | 0.243 | 0.009 | – | 0.18 | 4.90 |
| Absolute change in milk fat yield, g/d | | | | | | | | | |
| | iso 13:0[3] | −68.3 | 15.18 | | | 0.001 | 0.56 | 0.20 | 15.1 |
| | Linear | | | 2 984.3 | 1 062.01 | 0.008 | – | 0.12 | |
| | Quadratic | | | −41 019.8 | 17 611.28 | 0.026 | – | 0.08 | |
| | *trans*-9 16:1[3] | 12.7 | 22.24 | | | 0.575 | 0.65 | 0.24 | 16.8 |
| | Linear | | | −937.3 | 326.93 | 0.007 | – | 0.10 | |
| | Quadratic | | | 3 972.9 | 1 162.94 | 0.002 | – | 0.14 | |
| | *trans*-9 16:1[4] | 11.3 | 20.02 | | | 0.579 | 0.66 | 0.29 | 16.6 |
| | Linear | | | −241.1 | 74.94 | 0.003 | – | 0.12 | |
| | Quadratic | | | 26 243.2 | 7 131.13 | 0.001 | – | 0.16 | |
| | 17:0[4] | 22.8 | 24.39 | −1.84 | 0.853 | 0.039 | – | 0.50 | 16.0 |
| | *trans*-10 18:1[3] | −6.19 | 19.59 | | | 0.756 | 0.62 | 0.15 | 17.5 |
| | Linear | | | −136.2 | 66.80 | 0.049 | – | 0.06 | |
| | Quadratic | | | 117.9 | 46.53 | 0.016 | – | 0.09 | |
| | *trans*-10 18:1[4] | −14.4 | 20.33 | | | 0.488 | 0.60 | 0.09 | 17.9 |
| | Linear | | | −30.0 | 21.57 | 0.174 | – | 0.03 | |
| | Quadratic | | | 878.7 | 460.38 | 0.065 | – | 0.06 | |
| | *trans*-16 + *cis*-14 18:1[4] | −15.8 | 17.34 | | | 0.379 | 0.62 | 0.10 | 17.4 |
| | Linear | | | −19.0 | 11.58 | 0.111 | – | 0.03 | |
| | Quadratic | | | 303.7 | 133.81 | 0.030 | – | 0.06 | |
| | *trans*-11,*cis*-15 + *trans*-10,*cis*-15 18:2[3] | −9.48 | 9.658 | −242.0 | 91.11 | 0.012 | 0.52 | 0.10 | 15.6 |
| | *trans*-11,*cis*-15 + *trans*-10,*cis*-15 18:2[4] | −9.19 | 10.429 | −76.6 | 31.90 | 0.022 | 0.53 | 0.07 | 15.4 |
| | 20:0 + 18:3n-6[3] | −430.9 | 148.17 | | | 0.007 | 0.54 | 0.15 | 14.4 |
| | Linear | | | 2 985.0 | 1 157.63 | 0.014 | – | 0.08 | |
| | Quadratic | | | −5 379.3 | 2 231.31 | 0.021 | – | 0.07 | |
| | 22:5n-6[4] | −20.1 | 20.34 | | | 0.349 | 0.58 | 0.08 | 18.3 |
| | Linear | | | −260.7 | 189.35 | 0.178 | – | 0.02 | |
| | Quadratic | | | 80 329.5 | 39 731.99 | 0.050 | – | 0.06 | |
| Relative change in milk fat concentration, % | | | | | | | | | |
| | 22:5n-3[4] | 27.8 | 24.39 | | | 0.262 | 0.26 | 0.26 | 9.10 |
| | Linear | | | −252.5 | 118.09 | 0.040 | – | – | |
| | Quadratic | | | 3 3817.3 | 13 641.44 | 0.018 | – | – | |
| Relative change in milk fat yield, % | | | | | | | | | |
| | *trans*-9 16:1[3] | −2.76 | 14.411 | | | 0.849 | 0.44 | 0.16 | 12.0 |
| | Linear | | | −405.2 | 234.23 | 0.092 | – | 0.06 | |
| | Quadratic | | | 1808.4 | 832.86 | 0.037 | – | 0.10 | |
| | *trans*-9 16:1[4] | −2.48 | 12.716 | | | 0.846 | 0.44 | 0.20 | 11.9 |
| | Linear | | | −107.8 | 54.06 | 0.054 | – | 0.08 | |
| | Quadratic | | | 12 178.8 | 5 142.10 | 0.023 | – | 0.12 | |
| | *trans*-10 18:1[3] | −3.07 | 11.899 | | | 0.799 | 0.46 | 0.22 | 11.8 |
| | Linear | | | −92.4 | 44.96 | 0.047 | – | 0.09 | |
| | Quadratic | | | 75.3 | 31.31 | 0.022 | – | 0.13 | |
| | *trans*-16 + *cis*-14 18:1[4] | −10.3 | 10.19 | | | 0.324 | 0.44 | 0.11 | 11.9 |
| | Linear | | | −12.3 | 7.983 | 0.133 | – | 0.04 | |
| | Quadratic | | | 183.6 | 92.17 | 0.055 | – | 0.07 | |
| Potential change in milk fat concentration, % | | | | | | | | | |
| | iso 16:0[3] | 26.7 | 21.26 | | | 0.217 | 0.12 | 0.12 | 7.88 |
| | Linear | | | −282.7 | 131.04 | 0.038 | – | – | |
| | Quadratic | | | 437.7 | 198.83 | 0.035 | – | | |
| | *trans*-10 18:1[4] | −45.7 | 6.27 | 7.29 | 4.165 | 0.089 | 0.17 | 0.14 | 15.1 |
| Potential change in milk fat yield, % | | | | | | | | | |
| | *trans*-16 + *cis*-14 18:1[4] | −28.2 | 14.25 | | | 0.062 | 0.43 | 0.10 | 16.8 |
| | Linear | | | −15.5 | 11.25 | 0.178 | – | 0.03 | |
| | Quadratic | | | 238.9 | 129.89 | 0.075 | – | 0.06 | |
| | 22:5n-6[4] | −25.9 | 15.83 | | | 0.140 | 0.45 | 0.15 | 16.4 |
| | Linear | | | −302.9 | 163.84 | 0.075 | – | 0.05 | |
| | Quadratic | | | 84 470.6 | 35 322.42 | 0.022 | – | 0.10 | |

Abbreviations: FA = fatty acid.
[1] Y = dependent variable in the first heading; X = independent variables in the subheading.
[2] Fit statistics of the prediction model: $R^2$ for the linear relationships between studentised residuals of observed and predicted values and RMSE.
[3] FA expressed as a percent of total FA.
[4] FA expressed as a percent of their biosynthetic origin group.

In any event, compared with pre-trial milk fat content, these results suggest that other performance traits would not have a key role in MFD susceptibility in Assaf ewes.

*Pre-trial milk fatty acids*

Diets that cause a *trans*-10 biohydrogenation shift consistently decrease the yield of FA synthesised *de novo* in the mammary gland (< C16; Harvatine et al., 2009; Bernard et al., 2009; Tsiplakou and Zervas, 2013). Contrary to cows, small ruminants often offset this reduction by increasing > C16 FA uptake, thus preventing MFD (Parente et al., 2018; Toral et al., 2020; Manso et al., 2022). However, the addition of marine lipids affects preformed FA uptake, thus causing MFD in sheep and goats (Toral et al., 2015; Fougère et al., 2018). Overall, the bivariate analysis and the first PCA would support this hypothetical key role of mammary FA uptake in marine lipid-induced MFD (Dallaire et al., 2014; Toral et al., 2020).

Milk OBCFA concentration shows relatively consistent decreases during plant oil-induced MFD in cows (Rego et al., 2009; Saliba et al., 2014), but responses are less clear for dietary marine oils (Shingfield et al., 2003; Boeckaert et al., 2008; Fougère et al., 2018), consistent with the poor relationship with MFD in our study.

Relationships between individual FA and MFD extent were also detected, but they seemed generally weak, probably because pre-trial milk FA profile was only available on 38 animals. In this regard, milk *trans*-10 18:1 has been proposed as a diagnostic tool to predict MFD in Holstein cows (Matamoros et al., 2020), and our meta-analysis suggests that it may also have a certain role in predetermining the extent of MFD in Assaf sheep. Another *trans*-10 FA associated with biohydrogenation-induced MFD is the *trans*-10,*cis*-15 18:2, which very often coelutes with *trans*-11,*cis*-15 18:2 (Alves and Bessa, 2014). In the bivariate analysis, the sum of both FA in the pre-trial showed a negative relationship with milk fat yield, which might be another indication of link between the *trans*-10 shift and MFD in Assaf ewes.

Finally, sheep showing a greater extent of fish oil-induced MFD have been reported to have higher concentrations of FA supplied by the marine lipid, such as *cis*-9 16:1, *cis*-11 18:1, and 20:5n-3, in both milk and ruminal fluid (Della Badia et al., 2021; 2023a). However, pre-trial concentrations of these dietary FA were often low (in line with basal diet composition) and not related with responsiveness to the syndrome.

## Conclusion

Overall, there is a relationship between pre-trial milk fat concentration and the magnitude of MFD (expressed as absolute, relative, or potential change) when Assaf ewes of high production potential are fed marine lipids: the higher the initial milk fat concentration, the higher the reduction in milk fat concentration and yield upon MFD induction. A weaker relationship may also exist between the extent of MFD and pre-trial milk yield and protein concentration. Although the use of specific milk FA as markers of the predisposition of individual animals to diet-induced MFD remains unclear, results from this meta-analysis support the involvement of preformed FA uptake in the syndrome and warrant further investigation. Additional research is also necessary to elucidate the physiological basis underlying the greater responsiveness to MFD of Assaf ewes with higher milk fat concentration, and the possible roles of energy balance, diet composition (e.g., forage:concentrate ratio) and nutrient intake (e.g., total lipid or non–fiber carbohydrates) in determining individual responses.

## Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101517) can be found at the foot of the online page, in the Appendix section.

## Ethics approval

Not applicable.

## Data and model availability statement

The data and models that support this study were not deposited in an official repository, but they are available from the corresponding author upon request.

## Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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**A. Della Badia:** Writing – original draft, Visualisation, Methodology, Investigation, Formal analysis, Data curation, Conceptualisation. **K.J. Harvatine:** Writing – original draft, Visualisation, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualisation. **P.G. Toral:** Writing – original draft, Validation, Supervision, Investigation, Data curation, Conceptualisation. **C. Matamoros:** Writing – review & editing, Visualisation, Validation, Methodology, Formal analysis. **P. Frutos:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualisation. **G. Hervás:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualisation.

## Declaration of interest

None.

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