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Milk production and milk fatty acids in dairy cows fed crushed rapeseed or rapeseed oil

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ABSTRACT

The effect of crushed full fat rapeseed or its free oil on milk yield and milk fatty acid composition was assed using three groups of mid-lactating Holstein dairy cows, assigned to a randomized 3 × 3 Latin square design. Dietary treatments included a control (CTL, ether extract 2.3% of dry matter, DM) and two experimental treatments (ether extract 4.2% of DM) supplemented with the same amount of fat either supplied as 4.9% of DM crushed full fat rapeseed, RCor2.2% of DM free rapeseed oil RO. Both experimental treatments increased DM intake ($P < 0.005$) and daily milk yield ($P < 0.001$). Milk fat and protein concentration was decreased in the supplemented diets ($p < 0.001$). A reduced ($p < 0.001$) yield of saturated fatty acids (FA) and an increased yield of long chain and unsaturated FA ($p < 0.001$ for mono unsaturated FA and $p < 0.05$ for poly unsaturated FA) in milk fat was observed for both experimental treatments. The results confirmed a reduced *de novo* synthesis and an increased carry-over of dietary FA upon feeding dairy cow diets supplemented with long chain and unsaturated FA. The lower degree of saturation in milk fat from cows fed RC compared to RO indicates a partial protection of the crushed rapeseed against ruminal hydrogenation.

1. Introduction

Supplementation of diets for dairy cows with plant oils results in higher energy intake, although dry matter intake (DMI) could be reduced at greater dietary fat levels (Schroeder et al., 2004). Additionally, fat supplements are used to modify the fatty acid (FA) composition of milk fat such as enrichment with mono- and poly unsaturated FA (MUFA and PUFA) including conjugated linoleic acids (CLA), which are known to be beneficial for consumers' health (Kliem and Shingfield, 2016). Considerable efforts have been made to increase the proportion of beneficial dietary FA ($\geq$ C18) in milk fat, including oleic acid (OA, C18:1c9), linoleic acid (LA, C18:2c9,12) and linolenic acid (LNA, C18:3c9,12,15).

Unprotected dietary fats are exposed to ruminal metabolism resulting in lipolysis, isomerisation and biohydrogenation (BH) (Harfoot and Hazlewood,1997). These changes are reflected in altered milk FA composition (Grummer, 1991). However, results on potential effects of the physical form of fat supplements such as free oil *vs*. crushed oilseed are still equivocal. Compared to their free oils, full fat seeds provide a natural protection of their oils due to the seeds' surrounding cell wall structures (Chilliard et al., 2000). Thus, oils from full fat seeds are less exposed to ruminal BH processes in comparison to free oils, resulting in greater amounts of unsaturated FA leaving the rumen. Contrary, Grummer (1991) assumed that oil from whole seeds is gradually released during microbial fermentation ensuring a constant supply for ruminal BH and therefore a more complete BH, whereas free oil present in the rumen might exceeding the hydrogenation capacity of rumen microbes. In general, the use of fat supplements varying in source and form in diets for dairy cow, is well documented as reviewed by Kliem and Shingfield (2016), and according to the results of a meta-analysis by Glasser et al. (2008). However, the number of studies pertaining to direct comparisons of crushed full fat rapeseed with its pure oil is rather limited. Thus, the current objective was to assess the effect of these dietary fat supplements fed to dairy cows on milk production and milk FA composition.

2. Materials and methods

2.1. Experimental design, animals and diets

The 18 Holstein dairy cows (5 primi- and 13 multiparous, 648 ± 61 kg BW; 144 ± 68 d postpartum) were allotted to three dietary treatments (six cows each) in a 3 × 3 Latin square design.

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Table 1.
Ingredients and chemical composition of control and experimental diets.

Diet[a]	CTL	RC	RO
Ingredients (g/kg DM)			
Maize silage	198	198	198
Grass silage	149	149	149
Hay	149	149	149
Field beans	99	99	99
Maize grain	136	99	99
Wheat grain	84	50	50
Peas	74	50	50
Potato starch	15	–	–
Corn gluten	23	–	–
Soybean meal	49	–	–
Rapeseed meal	–	140	167
Rapeseed, crushed	–	49	–
Rapeseed oil	–	–	22
Mineral + Vitamin Premix	24	17	17
Chemical composition (g/kg DM, if not otherwise stated)			
Dry matter (g/kg)	411	407	408
Organic matter	925	925	922
Ether extract, EE	23	42	42
Crude protein, CP	147	151	156
Crude fiber, CF	167	181	184
NDF[b]	371	378	379
ADF[b]	192	209	214
NFC[c]	371	340	330
ME MJ/kg DM (calculated)[d]	11.2	11.2	11.3
Main fatty acids (g/ kg DM)			
C16:0	2.8	3.2	3.5
C18:0 SA	0.5	0.8	0.8
C18:1c9 OA	5.3	17.9	16.2
C18:2c9,12 LA	11.7	13.2	13.1
C18:3c9,12,15 LNA	1.9	3.8	4.1

[a] CTL control; RC crushed rapeseed; RO rapeseed oil.
[b] NDF neutral detergent fiber; ADF acid detergent fiber.
[c] Non-fiber-carbohydrates.
[d] Calculated based on the analytical values of the single feed ingredients added to the diets; ME content of diet ingredients was estimated using the Hohenheim Gas Test (Steingass and Menke, 1986).

Cows had an average energy corrected milk yield (ECM; 4.0% fat and 3.4% protein, 3.28 MJ NEL (net energy for lactation)/kg milk; ECM kg =(kg milk × (0.38 × fat%+0.21 × protein %+1.05))/3.28), GfE, 2001) of 32.5 ± 4.24 kg/d. The cows were allocated at random to the treatments according to milk yield, lactation number and lactation day. Each experimental period comprised 21 days, including14 days adaptation and seven days for sample collection (feed and milk). Diets were fed as a total mixed ration and comprised a non-supplemented control (CTL) and supplemented diets with crushed full fat rapeseed (RC) or free rapeseed oil (RO) (Table 1). The RC blend was prepared by milling (hammer mill 3 mm screen size) the whole rapeseed with wheat (50:50 w/w).

Diets were prepared according to requirements of dairy cows with an average BW of 680 kg, and producing 30 kg/d of ECM at a DMI of 20 kg/d (GfE, 2001) and to contain 440 g of rapeseed fat based on this assumption of DMI.

2.2. Housing, feeding and sampling

The cows were housed in a free stall barn, and had continuous access to fresh water. Cows were milked twice daily at 0600 and 1600 h. Milk production was recorded daily and three individual milk samples were in the last week of every experimental period. The milk contents of fat, protein, lactose and urea were analyzed using near infrared spectroscopy (Bentley FTS, Chaska, MN, USA). Subsamples were stored at −20 °C for analysis of FA.

Diets were prepared every day and fed *ad libitum* once daily at 0730 h, allowing feed refusals of approximately 5%. Daily feed intake was recorded. Samples of the diets were collected daily and stored at −20 °C for further analysis.

2.3. Chemical analyses

Methods of VDLUFA (VDLUFA, 2006) were used to determine proximate nutrients and fiber fractions in the diets. Analyses included determination of DM (method 3.1), crude ash (method 8.1), crude protein (method 4.1.1, multiplied by 6.25), ether extract (method 5.1.1), crude fiber (method 6.1.1), NDF assayed with a heat stable amylase (method 6.5.1) and ADF (method 6.5.2), each expressed exclusive of residual ash. The ether extract was used for FA analysis, performed as described for milk FA.

The frozen milk samples were pooled by period and cow. The milk fat for determination of FA composition was obtained by centrifugation at 12000g for 30 min at 4 °C in a Heraeus Multifuge (Thermo Scientific, Langenselbold, Germany). The fat layer was transferred to microtubes (2 mL), and stored at room temperature for 15 min. The microtubes were centrifuged at 14000g for 30 min at 36 °C. The resulting top layer was used for analysis of FA. The feed and milk lipids were *trans*-esterified according to the method described by Chouinard et al.(1999). Fatty acid methyl esters (FAME) dissolved in hexane were analyzed using an Agilent 6890 gas chromatograph (Agilent Technologies, Waldbronn, Germany). Separation of FAME was performed using a Supelco SP-2380 fused silica capillary column (30 m × 250 μm, 0.2 μm film thickness) (Sigma-Aldrich Chemie GmbH, Germany) with a column head pressure of 81.4 kPa and helium as carrier gas. The column temperature was 50 °C for 2 min post injection, then heated up to 130 °C (4 °C/min), to 210 °C (2 °C/min), finally to 250 °C (30 °C/min), and held for 15 min. Injector temperature was 250 °C, and detector temperature 260 °C. Isomers C18:1 t6 and C18:1 t9 could not be separated during analysis and were pooled as C18:1 t6+ t9. Standards of FAME consisted of a mix of 37 components (Sigma-Aldrich Chemie GmbH, Germany), including FA from C4:0 to C24:1. This mix was completed by adding FAMEs of C18:1 t6, t11, c6, c7, c11, c12; C18:2 c9t11, t9t11, t10c12 and C19:0 as internal standard, purchased from Nu-Chek Prep (Nu-Chek Prep, Elysian, MN, USA).

2.4. Calculations and statistical analysis

Daily FA intake and milk FA production was calculated using the dietary FA content and daily DMI or milk FA composition and the daily milk fat yield, respectively. Milk FA were corrected for glycerol fraction, assuming the analyzed FA were present as triacylglycerol.

The data were tested for homogeneity of variance and normal distribution. When necessary, data were transformed using a log or square root transformation and corrected for outliers. Analysis of variance was performed using the procedure for linear mixed models (PROC MIXED) of the software package SAS for Windows (version 9.1, SAS Institute Inc., Carry, NC, USA, 2003) according to the following model:

$$y_{ijkl} = \mu + \alpha_i + \beta_j + \gamma_k + a_l + e_{ijkl}$$

where y_{ijkl} is the observation, μ is the overall mean, α_i is the fix effect of the diet, β_j is the fix effect of the period, γ_k is the fix effect of the group of cows and e_{ijkl} is the residual error. Cows allocated to the treatments (a_l) were included in the random statement. If for a specific effect the *F*-test was significant, differences between treatments were evaluated using a two tailed *t*-test for comparison of the least square means (LSM) of fix effects for each FA. Significant level for the fixed parameter was set at $P \leq 0.05$. Least square means, respective pooled standard error of means and *P*-values were given for fixed effect of the diet.