Effects of oilseed supplements on milk production and quality in dairy cows fed a hay-based diet

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ABSTRACT

The influence of rapeseed (*Brassica napus* L.) and linseed (*Linum usitatissimum* L.) on feed consumption, milk yield, composition and fatty acid (FA) profile was investigated in lactating dairy cows. According to a Latin square design, twelve cows were assigned to three experimental settings which received a hay-based diet supplemented with one of the following mixtures: cracked barley (1.0 kg) and cracked maize (0.5 kg) as Control diet (C-diet); full fat ground rapeseed (1.0 kg), cracked barley (1.0 kg) and cracked maize (0.5 kg) as Rapeseed diet (R-diet) and extruded linseed (1.2 kg), cracked barley (1.0 kg) and cracked maize (0.5 kg) as Linseed diet (L-diet). Diet supplemented with rape- and linseed did not affect dry matter intake. As result of the high amount of dietary ether extract R- and L-diet showed higher crude fat intake as compared to the control. Despite the higher intake of crude fat and, in particular, of the polyunsaturated fraction, milk yield and composition resulted similar among treatments. Compared to C-diet, R-diet resulted in milk containing significantly lower amounts of saturated fatty acids (SFA) (60.9 vs 66.9% of total detected SFA) as well as higher amounts of monounsaturated fatty acids (MUFA) (30.6 vs 24.2%). However, R-diet had no effect on polyunsaturated fatty acid content (PUFA). With respect to C-diet, L-diet also significantly reduced the content of SFA (59.8 vs 66.9%) and increased both MUFA (29.1 vs 24.2%) and PUFA (4.7 vs 4.1%) contents. Feeding about 1 kg of whole oilseeds per head per day had no clear effects on milk vaccenic acid and conjugated linoleic acid contents. The inclusion of rapeseed in the diet significantly reduced the content of n-6 FA in the milk, whereas the linseed-supplemented diet significantly increased the content of n-3 FA. In comparison to C-diet, n-6/n-3 ratio was lower in L-diet milk and intermediate in R-diet milk. Feeding lactating dairy cows with oilseeds rich in unsaturated fatty acids (UFA) did not exert any effects on productive performance. Considering the increase in PUFA and the reduction of n-6/n-3 ratio as a desirable enrichment of milk fat for human health, under our experimental condition feeding linseed appears to improve the nutritional value of milk better than rapeseed.

Key words: Dairy cow, Oilseeds, Milk quality, Fatty acid composition, CLA.
RIASSUNTO
EFFETTO DELL’INTEGRAZIONE CON SEMI OLEOSI SU PRODUZIONE E QUALITÀ DEL LATTE IN VACCHE DA LATTE ALIMENTATE CON UNA DIETA A BASE DI FIENO

È stato valutato l’effetto dell’integrazione con semi di colza (Brassica napus L.) e lino (Linum usitatissimum L.) di una dieta per vacche da latte a base foraggera affienata sull’ingestione di SS, sulla produzione di latte e sulla composizione e profilo acidico di questo ultimo. Secondo un disegno sperimentale a quadrato latino, dodici vacche (106 ± 52 giorni di lattazione) alimentate con una dieta “a secco” (fieno di prato stabile, premix proteico-vitaminico-minerale e derivati dei cereali) sono state assegnate a tre gruppi alimentari che hanno ricevuto una integrazione a base di orzo (1,0 kg) e mais (0,5 kg) schiacciati per la tesi dieta Controllo (C); semi macinati di colza (1,0 kg) e orzo (1,0 kg) e mais (0,5 kg) schiacciati nella tesi dieta Colza (R); semi di lino estrusi (1,2 kg), orzo (1,0 kg) e mais (0,5 kg) schiacciati nella tesi dieta lino (L). Il trattamento alimentare non ha influenzato l’ingestione di sostanza secca; in relazione al maggiore tenore di estratto etereo della razione (3,2, 5,8 e 5,7% SS rispettivamente per le tesi C, R e L), per le diete R e L si è verificata una significativa maggiore assunzione di lipidi alimentari. Tale maggiore ingestione di lipidi e, ancor più, di acidi grassi polinsaturi non ha influenzato negativamente produzione e composizione del latte rispetto alla dieta C probabilmente in relazione al fatto che l’integrazione è stata somministrata in forma di semi interi (macinati o estrusi). Rispetto alla dieta C, per quella R il latte si è caratterizzato per un minore contenuto di acidi grassi (AG) saturi (66,9 vs 60,9% del totale degli AG rilevati) e uno maggiore in AG monoinsaturi (24,2 vs 29,1%) che dei polinsaturi (4,1 vs 4,7%). In riferimento a singoli AG, si sono osservate delle variazioni a carico dei livelli di C16:0, C18:0, C18:1n-9 e C18:3n-3, per il quale però si sono registrati valori simili tra le tesi C e R. Il ricorso all’integrazione con circa 1 kg/d di semi oleosi non ha avuto significativi effetti sul tenore in acido vaccenico e CLA (isomeri coniugati dell’acido linoleico). L’inclusione di semi di colza ha significativamente ridotto il contenuto di AG n-6 nel latte, mentre quella con semi di lino ha determinato un aumento degli n-3 e quindi una riduzione del rapporto n-6/n-3. L’integrazione con semi oleosi ricchi in AG polinsaturi in una dieta a base di fieno per vacche da latte ha comportato un rilevante cambiamento del profilo acidico del latte senza compromettere le prestazioni produttive delle bovine in lattazione. Considerando che un aumento degli AG polinsaturi e una riduzione del rapporto n-6/n-3 nella frazione lipidica del latte sono auspicabili ai fini nutraceutici, i risultati ottenuti in questa prova evidenziano che, rispetto al colza, il ricorso ai semi di lino sembra valorizzare maggiormente il valore dietetico-nutrizionale del latte.

Parole chiave: Vacca da latte, Semi oleosi, Qualità latte, Profilo acidi grassi, CLA.

Introduction

Fatty acid composition of milk has been of great interest in recent years in order to produce dairy products of higher nutritional quality. Milk fat has been criticised due to its higher content of SFA compared to vegetable fat or fish oil. Moreover, milk fat contains considerable amounts of myristic (C14:0) and palmitic acid (C16:0) and low concentrations of monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids (Kennelly, 1996).

Fatty acid composition of milk influences several aspects of human health: butyrate and isomers of conjugated linoleic acid (CLA) have an antiatherogenic, antiobesity or anticarcinogenic potential, whereas the medium-chain SFA and trans-fatty acids (trans-FA) show a potential negative effect (Parodi, 1999; Dhiman et al., 2000; MacDonald, 2000; Chilliard et al., 2001). Since milk fat is a major source of SFA in human diets (Gravert, 1983), dairy products have developed a negative image due to the increased frequency of coronary
heart diseases (Eyer, 1997). On the contrary, within the group of PUFA, n-3 fatty acids possess a protecting effect against cardiovascular diseases (Hagemeister et al., 1988; Schacky, 2004). Therefore, an enrichment of milk fat with n-3 fatty acids is considered desirable from a human health perspective. Furthermore, they play an important role on postnatal brain growth and are therefore important in the diet of infants (Weber, 1986). It is also known that plasma cholesterol level decreases with the presence of n-3 fatty acids and increases with the presence of trans-FA in the diet (Pfeuffer, 1992).

Supplementing dairy rations with oilseed and/or other dietary fat sources has a potential for changing milk yield, fat content and fatty acid composition (Chilliard et al., 2001). Feeding trials indicate that lipids available as supplementary energy sources for dairy cattle markedly influence the fatty acid composition of milk fat, thus improving milk quality (Murphy et al., 1990; Jahreis et al., 1995; Kennelly, 1996; Stoll et al., 2001; Secchiari et al., 2003; Collomb et al., 2004). As rapeseed shows high content of C18:1n-9 (Jahreis et al., 1996) and linseed is rich in C18:3n-3 (Collomb et al., 2004), these two oilseeds could be used as dietary supplements in order to increase CLA and PUFA percentage (Ryhänen et al., 2005). Little information is available on the influence of oilseeds added to hay-based diet on milk composition.

In South Tyrol most of the dairy cattle farms are located at high altitudes. Climatic conditions as well as territorial characteristics make cultivation of crops such as maize extremely difficult. Therefore, most farms rely on dried grassland forage as the main food component in the diet for lactating dairy cows. Thus, the present study was undertaken to evaluate the effects of feeding rapeseed or linseed as full fat oilseed supplements on productive performance, milk quality and fatty acid profile in dairy cows fed a hay-based diet.

Material and methods

Livestock and treatments

The study was carried out at the experimental farm “Mair am Hof” of the Research Centre for Agriculture and Forestry Laimburg (Teodone/Brunico, South Tyrol, Italy, 850 m a.s.l.), between November 2002 and March 2003. Twelve cows, eight Brown Swiss and four Friesian, two primiparous and ten multiparous (642 ± 46 kg), were randomly assigned to a Latin square design, according to milk yield (28.6 ± 5.8 kg/d) and days of lactation (106 ± 52 d). Animals were grouped into six homogeneous groups of two individuals each and allocated to one of the three type of diets. The trial lasted twenty weeks in total and consisted of three consecutive trial periods of three weeks each. In an adaptation period (2 weeks) cows were adapted from a dry forage, maize- and grass silage diet to a hay-based diet. Each trial period was preceded by a three-week adjustment period in which the animals were gradually accustomed to the diet composition of the subsequent trial period (Figure 1). The control diet consisted of hay (2/3 of permanent meadow 1st cut and 1/3 of permanent meadow 3rd cut), grain-vitamin-mineral premix, cereal mix and supplement (Table 1). The basal diet was supplemented with three mixtures formulated as follows: 1 kg of cracked barley and 0.5 kg of cracked maize (1.0:0.5) in the so-called Control diet (C-diet); 1 kg of ground full fat rapeseed added to the control supplement (1.0:1.0:0.5) in the so-called Rapeseed diet (R-diet); 1.2 kg of extruded linseed added to the control supplement (1.2:1.0:0.5) in the so-called Linseed diet (L-diet). The main
characteristics of the three experimental diets and supplements are summarised in Table 2. Ration per meal was as follows: 2.5 kg hay 1st cut with one hour feeding time, 3 kg hay 3rd cut with the experimental supplement based on barley and maize (C-diet)
plus rapeseed (R-diet) or linseed (L-diet) with one hour feeding time, hay 1st cut ad libitum. Cows were housed in a loose housing stable and were fed individually by means of a Calan Feeding Control System (Am. Calan, Inc., Northwood, New Hamster, USA). Concentrate, grain-vitamin-mineral premix and cereal mix were fed to the animals with an automated system (Westfalia, Codatron DP5, D-58313 Herdeck, Germany).

Sampling and analyses

Samples of feed and milk were collected over six days of each experimental period (end of the second and beginning of the third week). Part of the forage of the diet (permanent meadow 1st and 3rd cut) was fed ad libitum. In order to determine the DM intake, the daily amount consumed was calculated for each cow by subtracting the weight of residual forage from the initial amount. For each trial period two samples of hay, concentrates and treatment diets and forage residues were collected and analysed for proximate composition. Dry matter (DM), crude ash (CA), crude protein (CP), and crude lipids (CL) were determined by Weender analysis using standard procedures (VDLUFA, 1976). The content of neutral detergent fibre (NDF) was determined with a Fiber Analyzer (ANKOM/2000, ANKOM Technology, New York). Net energy lactation (NE\textsubscript{L}) was calculated by a regression equation according to RAP, (1999).

Cows were milked twice a day with an automated milking plant (Rohrmelkanlage Westfalia, M-6001, D-58131 Herdeck,

| Table 2. Formulation and fatty acid composition of the three experimental supplements. |
|-------------------------------------------------|------------|---------|
| Treatment | Control | Rapeseed | Linseed |
| Crude barley (%DM): | 67 | 39 | 36 |
| Crushed maize | 33 | 20 | 18 |
| Fullfat ground rapeseed | 41 | | |
| Extruded linseed | | | 46 |
| Fatty acid profile (% of the total detected fatty acids): | | | |
| C16:0 | 15.47 | 10.96 | 11.02 |
| C18:0 | 1.59 | 1.74 | 2.68 |
| C18:1 n-9 | 18.61 | 35.27 | 18.48 |
| C18:1 n-7 | 0.64 | 1.61 | 0.64 |
| C18:2 n-6 | 54.57 | 39.75 | 36.72 |
| C18:3 n-3 | 5.16 | 6.32 | 27.47 |
| C18:3 n-6 | 0.04 | 0.03 | 0.03 |
| Saturated fatty acids | 18.12 | 13.61 | 14.75 |
| Monounsaturated fatty acids | 19.71 | 37.43 | 19.65 |
| Polyunsaturated fatty acids | 60.18 | 46.45 | 64.55 |
| Total n-3 | 5.31 | 6.44 | 27.62 |
| Total n-6 | 54.73 | 39.89 | 36.84 |
Every day a milk sample containing morning and afternoon milk at a 1:1 ratio was collected, refrigerated at 4°C, conserved using Bronopol tablets and analysed for fat, protein and lactose by infrared analysis (Milcoscan FT 6000). The urea content was determined using an enzymatic method (Skalar method, EUROCHEM, Roma). For the determination of the milk fatty acid composition, two milk samples were taken from the two cows of each group per experimental period. Samples were stored at -80°C until analyses.

Fatty acid composition of feed and diets was determined after lipid extraction by chromatography using a dichloromethane/methanol solution (2:1 v/v) as described by Nourroz-Zadeh and Appelqvist (1988). Aliquots of the extracts were then trans-esterified according to the Christie (1982) procedure and fatty acid methyl esters (FAME) were detected as described. An aliquot of 100 mL was homogenised in a 100 mL solution of anhydrous sodium sulphate (0.47 M), centrifuged for 10 min at 4000 xg, and 100 mg of surfaced fat was mixed with 4 mL methanol and 4 mL n-heptane and centrifuged again (4000 xg, 5 min, 4°C). Two mL of the upper phase containing the ether extract was trans-esterified with sodium methoxide and FAME were quantified by gas chromatography (Shimatzu GC17A, equipped with FID detector, using a Omegawax 250 column). Obtained data were reported as percentage of the total detected fatty acids.

Homogeneity of variance was tested with the Cochran’s Test (Sachs, 1999), normal distribution of residuals with the Kolmogorov-Smirnov’s Test (Sachs, 1999). Tukey-HSD was used as post hoc test for multiple comparisons.

**Results and discussion**

**Feed and diet composition**

Compared with the C-diet, R-diet one showed a higher percentage of MUFA due to the higher content of oleic acid (C18:1n-9). As expected, L-diet showed the highest percentage of α-linolenic acid (C18:3n-3) leading to a content of PUFA around 65% (Table 2). The tested oilseed supplements (R-diet and L-diet) caused a content of crude fat higher than the Control (Table 1).

**Productive performance and milk composition**

Dietary treatment had no effect on DM intake and feed consumption. According to the different dietary content, crude fat intake was significantly higher for R- and L-diets (Table 3). Despite the high crude fat content of R- and L-diets, milk yields and milk composition were not affected by treatments in the same way. Large amounts of oil such as soybean oil in free form at 3.6% or linseed oil at 4.4% of dietary DM had no negative influence on feed intake (Dhiman et al., 2000) but oilseed supplements such as full fat soybeans or ground full fat rape-seeds significantly reduced milk protein (Lawless et al., 1998). However, Mohamed et al. (1988) observed negative effects of feeding 4% oil in the diet on feed intake, probably related to a reduction in digestibility of DM by free oil. The amount of dietary fat transferred directly to milk fat is influenced by ruminal biohydrogenation, absorption (digestibility), and deposition in adipose tissue (Palmquist et al., 1993). The present
trial shows that feeding ground whole oilseeds, as sources of PUFA, does not significantly affect milk yield and milk composition; this is also supported by findings of Collomb et al. (2004).

Milk fatty acids profile

Significant differences among treatments were found in the fatty acid profile of milk (Table 4). Treatments with oilseeds significantly increased MUFA concentration compared to the C-diet. Feeding L-diet resulted in an increase in the level of PUFA (4.1, 3.7 and 4.7% of total FA, for diet C, R and L, respectively). As expected, the higher concentration of \(\alpha\)-linolenic acid in the L-diet led to an increased concentration of this FA in milk fat. Rapeseed supplemented diet significantly decreased the content of linoleic acid, resulting in a lower n-6 FA content compared to the Control diet. In dairy cows fed with hay and fodder beet supplemented with either ground rapeseed or ground linseed a reduction in SFA and an increase in both MUFA and PUFA were also observed (Collomb et al., 2004). In the R-diet group, oleic acid was either not hydrogenated or hydrogenated to stearic acid (C18:0) in the rumen and only a small amount was isomerised to vaccenic acid. Considering the L-diet group, the high content of \(\alpha\)-linolenic acid led to increased concentration of this FA in milk but did not modify vaccenic acid content. The lack of effect of linseed supplementation on vaccenic acid percentage is probably consistent with the slow release of UFA from oilseed in the rumen that may reduce the

| Intake (day⁻¹ cow⁻¹): | Control | Rapeseed | Linseed |
|-----------------------|---------|----------|---------|
| Dry matter kg         | 17.7    | 18.0     | 18.2    | ns      |
| Crude lipids g        | 574*    | 1040a    | 1031a   | ***     |
| NDF "                 | 8103    | 7770     | 7832    | ns      |
| NFC "                 | 5156    | 5104     | 5168    | ns      |
| NE MJ                 | 105b    | 114a*    | 122a    | *       |
| Yield (day⁻¹ cow⁻¹):  |         |          |         |
| Milk kg               | 23.28   | 23.99    | 23.45   | ns      |
| ECM "                 | 23.89   | 24.76    | 24.11   | ns      |
| Protein "             | 0.83    | 0.86     | 0.84    | ns      |
| Fat "                 | 0.94    | 0.99     | 0.96    | ns      |
| ECM/fodder intake     | 1.34    | 1.37     | 1.32    | ns      |
| Milk components (w/v):|         |          |         |
| Fat %                 | 4.09    | 4.15     | 4.15    | ns      |
| Protein "             | 3.61    | 3.60     | 3.61    | ns      |
| Lactose "             | 4.87    | 4.80     | 4.83    | ns      |
| Urea mg/100 ml        | 22.21   | 23.88    | 25.04   | ns      |

ECM: Energy Corrected Milk calculated according to RAP, 1999. Within a row means with different letters differ significantly (*: P<0.05; ***: P<0.001; ns: not significant).
The content of CLA in milk fat was unaffected by oilseed supplements. However, Chilliard et al. (2002) have shown that the addition of plant oils rich in PUFA to dairy diets increases the total CLA content of milk. Furthermore, linseed oil supplementation to a hay-based diet principally increased rumenic acid (cis-9, trans-11 CLA) but also different isomers of CLA (e.g., cis-9, cis-11 CLA and trans-11, trans-13 CLA). Results of this trial for the L-diet confirmed that the pathway for the hydrogenation of α-linolenic in the rumen allowed the accumulation of oleic and stearic acids (Focant et al., 1998), but had only a limited effect on the production of CLA as an intermediate (Collomb et al., 2004; Rhyänen et al., 2005). Trans-vaccenic acid is the main FA responsible for the formation of the CLA by its desaturation in the mammary gland (Griinari et al., 2000). Thus, in our trial the same vaccenic acid content observed among dietary treatments could explain that CLA in the milk was not influenced. In addition, the CLA concentration observed in milk from C-diet fed cows is in good agreement with CLA contents reported in milk from dairy cows fed hay-based diets enriched with extruded or toasted soybean (Bailoni et al., 2004) or obtained by animals fed with full fat extruded soybean or cottonseed oil.

Table 4. Effect of the experimental diet on fatty acid composition of milk (% of the total detected FA).

|                  | Treatment        | Control | Rapeseed | Linseed |
|------------------|------------------|---------|----------|---------|
| C4:0             |                  | 2.30    | 2.38     | 2.28    | ns      |
| C6:0             |                  | 1.77    | 1.69     | 1.64    | ns      |
| C8:0             |                  | 1.09    | 1.01     | 0.97    | ns      |
| C10:0            |                  | 2.53    | 2.05     | 2.09    | ns      |
| C12:0            |                  | 3.12    | 2.55     | 2.47    | ns      |
| C14:0            |                  | 11.50   | 9.89     | 9.83    | ns      |
| C16:0            |                  | 34.21   | 25.77    | 25.16   | **      |
| C18:0            |                  | 8.69    | 13.89    | 13.77   | **      |
| C18:1 n-9        |                  | 19.27   | 26.55    | 24.97   | **      |
| C18:1 n-7        |                  | 1.24    | 1.23     | 1.37    | ns      |
| C18:2 n-6        |                  | 2.27    | 1.99     | 2.25    | *       |
| C18:3 n-6        |                  | 0.14    | 0.13     | 0.10    | ns      |
| C18:3 n-3        |                  | 0.60    | 0.56     | 1.18    | ***     |
| CLA              |                  | 0.71    | 0.68     | 0.80    | ns      |
| C20:4 n-6        |                  | 0.10    | 0.10     | 0.09    | ns      |
| Saturated fatty acids |            | 66.92   | 60.88    | 59.75   | **      |
| Monounsaturated fatty acids |           | 24.19   | 30.56    | 29.05   | *       |
| Polyunsaturated fatty acids |           | 4.05    | 3.68     | 4.66    | **      |
| Total n-6        |                  | 2.65    | 2.35     | 2.57    | **      |
| Total n-3        |                  | 0.68    | 0.64     | 1.28    | ***     |
| n-6/n-3          |                  | 3.89    | 3.68     | 2.03    | ***     |

Within a row means with different letters differ significantly (*: P<0.05; **: P<0.01; ***: P<0.001; ns: not significant).
A significant increase in CLA content was observed feeding large supplement of ground sunflowerseed (Collomb et al., 2004) or linseed oil (Dhiman et al., 2000).

Even though milk fat composition is mainly an effect of dietary lipid supplements, with forage being less important (Chilliard et al., 2001), feeding hay from mountains leads to an increase in CLA and other PUFA content (excluded n-3 FA) compared to hay from lowlands (Collomb et al., 2002). As described above, these results are mainly due to the process of biohydrogenation of linoleic and α-linolenic in the rumen and the subsequent synthesis of trans-11 C18:1 from which CLA is endogenously synthesised (Grinari et al., 2000). Kraft et al. (2003) showed α-linolenic acid to be the primary FA in grass of the Alps (about 50% of the total FAME). Hay fed in our trials was characterised by a high content of α-linolenic acid (35.3 and 50.6% of total detected FA, for permanent meadow 1st cut and permanent meadow 3rd cut, respectively) and according to Shingfield et al. (2005) the amount of C18:2 n-6 was lower (17.6 vs 14.1% of total detected FA, for permanent meadow 1st cut and 3rd cut, respectively). For this reason, the influence of oilseed supplements on CLA content was very limited while L-diet increased total n-3 FA percentage.

The supplement of oilseeds in hay-based diet may enhance the milk quality from a nutritional point of view. A ratio of essential fatty acids C18:2n-6/C18:3n-3 lower than 5 is recommended by human nutritional guidelines, which advise an increase in n-3 PUFA intake as prevention for cardiovascular disease and cancer (Weill et al., 2002). Thus, considering the highest PUFA content and the lowest n-6/n-3 FA ratio, L-diet seems to enhance the nutritional value of milk better than rapeseed diet.

Conclusions

Feeding around 1kg per day of oilseed as a supplement of a hay-based diet had no effect on overall milk yield and milk composition. In comparison to the Control-diet, the Rapeseed- and Linseed-diets had a stronger effect on the milk FA profile. The increase in unsaturated FA in the diet was associated with an increase in MUFA in the R-diet and both MUFA and PUFA in the L-diet. Indeed, the content of n-3 FA could also be increased, resulting in a lower n-6/n-3 ratio. However, feeding linseed seems to be more effective in increasing n-3 FA, while a reduction of n-6 FA was achieved by feeding rapeseed. Finally, even though no significant influence on CLA content was observed in this study, oilseeds can be used as a dietary strategy to increase the content of human health-promoting FA in dairy products without negative effects on productive performance of the lactating cows.

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