Effect of supplementing sunflower cake in dairy cow diet on milk production and composition

Objective. Sunflower cake (SFC) is one of the co-products of biodiesel production chain, which can be supplemented in the diet of lactating cows, substituting a portion of soybean meal (SBM). The aim of this experiment was to evaluate the possibility of substituting a portion of SBM with SFC in the diet of lactating cows, by estimating the effects on production and on some milk chemical and physical characteristics.

Materials and methods. The experiment was carried out in two experimental farms in which two groups of sixteen Italian Holstein cows were allotted into two groups, fed SBM and SFC, respectively. Composition and chemical characteristics of the diets, daily production, gross composition and fat globule size distribution of milk, fatty acid composition of both milk and sunflower cake were determined. Results. SFC supplementation did not affect milk production, but it reduced milk protein percentage; milk fat concentration was only decreased in mid lactation; if the whole lactation is considered, milk fat percentage was not influenced by SFC substitution for partial SBM. Total unsaturated fatty acids significantly increased through SFC supplementation. Both vaccenic and conjugated linoleic acid were higher in milk of cows fed SFC.

Conclusions. Introduction of SFC in dairy cow diet is a feasible strategy to make biodiesel production more profitable. It also seems able to improve the milk nutritional properties.

Keywords: sunflower cake, bovine milk, production, milk composition, fatty acids

Riassunto

Obiettivo. Il panello di girasole (SFC) è uno dei coprodotti della catena di produzione del biodiesel, che può essere integrato nella dieta delle bovine, in parziale sostituzione della farina di soia (SBM). In questa ricerca è stata verificata la possibilità di sostituire una quota di SBM con SFC nella dieta delle bovine, stimando gli effetti sulla produzione e su alcune caratteristiche del latte.

Materiali e metodi. l’esperimento è stato condotto in due aziende sperimentali in cui due gruppi di 16 bovine di razza Frisona Italiana sono state divise in due gruppi, alimentati rispettivamente con SBM e SFC. Sono stati determinati: composizione e caratteristiche chimiche delle diete, produzione giornaliera e macro-composizione del latte, distribuzione delle dimensioni dei globuli di grasso, composizione in acidi grassi del latte e del panello di girasole. Risultati. L’integrazione con SFC non ha influito sulla produzione di latte, ma ha ridotto la sua percentuale di proteine; il contenuto in grasso ha subito una riduzione solo a metà del periodo di lattazione; se si considera l’intera lattazione, la percentuale di grasso del latte non è stata influenzata dalla parziale sostituzione di SBM con SFC. Gli acidi grassi insaturi hanno mostrato un significativo aumento a seguito dell’integrazione con SFC. Sia l’acido linoleico coniugato che il vaccenico sono risultati più elevati nel latte di bovine alimentate con SFC.

Conclusioni. L’introduzione dell’SFC nella dieta delle bovine da latte è una strategia percorribile per rendere la produzione di biodiesel più redditizia. Inoltre, sembra influenziare positivamente le proprietà nutrizionali del latte.

Parole chiave: Panello di girasole, Latte vaccino, Produzione, Composizione del latte, Acidi grassi
INTRODUCTION
Utilization of biodiesel is considered one of the strategies for reducing fossil fuel consumption and decreasing the concentration of greenhouse gases in the atmosphere (1). This fuel can be obtained from different vegetable and animal fats and from yellow grease as well (2). The main sources of vegetable oils in the world are soybean and canola; however, sunflower seed are considered suitable in Mediterranean agro-climatic condition (3). According to FAO, in 2017 about 48 Mt of sunflower seeds were produced in the world; Italian production was estimated to be about 213 Kt in the same year (4). About 60% of the sunflower oil is destined for the food chain and the remaining 40% is used in non-food chain, i.e. of biodiesel production and cosmetics (4). According to ISTAT (5) sunflower for biodiesel oil is cropped on 3000 ha in Italy. In addition to oil, several co-products are obtained from sunflower oil industry; solvent extracted meal is the most common because of its characteristics that make it easier to transport and stock and it is widely used in livestock and poultry industries. An alternative to solvent extracted sunflower meal is cake, that is obtained through the mechanical squeezing of oil seeds, and has a lipid content several times higher than solvent extract meal (7). This characteristic might limit its utilization in lactating dairy cow feeding because, in some experiments it appeared that fat supplements from oilseed crops could affect negatively dry matter intake (DMI), milk production, and milk composition (8).

The use of sunflower oil or seeds are interesting in animal nutrition, because they are potential energy and protein sources, but they could be limited in ruminant diet, because of the high content of linoleic acid (LA) if compared with other vegetable oils (9).

Sarrazin et al. (10) found a decrease of DMI of lactating dairy cows as consequence of sunflower seed supplementation; however, other authors (11-15) did not find any effect on DMI. Results about milk production were more inconsistent: in some cases, milk production was decreased by whole sunflower supplementation in lactating dairy cattle diet (11-13); in other cases, it was increased (14,15), and in some others, there was no effect (10,11). Variable results were also observed about milk protein percentage, which was decreased according to Petit et al. (13) and Stegeman et al. (15); instead, it was not affected by supplementation with sunflower seeds according with other authors (9,11,12,14). When fed to lactating dairy cows, sunflower seeds were associated with a reduction of milk fat percentage in the experiments of Sarrazin et al. (10) and Casper et al. (11), but no effect was observed in other trials (12-15). Effects of sunflower oil as fat supplement in lactating dairy cow diet were studied by Cruz-Hernandez et al. (16), who found no effect on DMI, milk production and fat and protein concentrations. On the contrary, Rego et al. (17) showed that energy corrected milk production and milk fat concentration were negatively affected by sunflower oil. Supplementation of sunflower seeds or oil of dairy cow diet invariably determines a modification of milk fatty acid (FA) profile; with a reduction percentage of saturated fatty acids (SFA) and an increase of mono- and polyunsaturated fatty acids (MUFA and PUFA respectively) (10-12,15-17).

A very promising consequence of supplementation of dairy cow diet with vegetable oils is the possibility to increase the content of cis-9,trans-11 C18:2 (CLA), the main conjugated linoleic acid. Some animal studies suggested that CLA has some health benefits, including anticarcinogenic and antiatherogenic effects and improvements in blood lipid concentrations, although most of these are not unequivocally supported by consistent data from human studies (18). Sarrazin et al. (10) and Cruz-Hernandez et al. (17) obtained a significant increase in CLA concentration in milk fat as dairy cow diets were supplemented with sunflower seeds or oil respectively. Supplementation of diet of lactating cows with sunflower seeds or oil is considered one of the most effective strategies for increasing milk CLA content, because it is well known that primary source of CLA is LA through rumen isomerization and biohydrogenation and through desaturation of vaccenic acid (VA) in mammary gland (19). There is little information about cakes deriving from a simple mechanical extraction of vegetable oils. Compared with solvent-extract oilseed meal, cakes have a higher and more variable lipid content (20), with possible effects on ruminant performances and on quality of animal products. So far, we do not know any experiment aiming at utilizing sunflower cake (SFC) as supplement in dairy cow diet. Objective of this experiment was to evaluate the possibility of substituting a portion of soybean meal (SBM) with SFC in the diet of lactating dairy cows, by estimating the effects on production and on some milk chemical and physical characteristics.

MATERIAL AND METHODS

Experiment organization
Two groups of sixteen Italian Holstein cows were kept in two experimental farms for evaluating the effect of partial substitution of SBM with SFC, obtained from mechanical squeezing. All cows were of second or third lactation and of mid (between 80 and 160 days from calving) or late (between 160 and 240 days from calving) stage of lactation. In both farms, cows were allotted into two groups, according to age and stage of lactation; cows of half a group were fed SBM and the others were fed SFC. The diets, administrated as total mixed ration, were isonitrogenous. The rations were distributed on a group-basis to obtain less than 5% refusals.
The experimental design was a change-over, replicated in each farm, with periods of three weeks: the first two for adaptation and the third week for data and sample collection.

**Diets and chemical characteristics**

The cake was obtained from a regular type of sunflower; chemical composition of SFC are reported in Table I. Cakes were obtained in an Italian oil industry. Chemical composition of both sunflower cake and diets were determined by applying the AOAC methods (21); the nitrogen free-extract was determined by subtracting the percentage of moisture, crude protein, oil, ash, and crude fibre from 100. The lipid matrix was extracted with diethyl ether under reflux, for 6 hours. Transmethylation and gas chromatographic analysis were carried out applying the procedures described in Contarini et al. (22). Cake FA composition is shown on Table II. In the two farms diets were identical, i.e. the same diet and the same concentrates were used. Composition of SBM and SFC diet are reported in Table III and their chemical characteristics are shown in Table IV.

**Milk production and composition, FA analysis and fat globule size**

Milk production was recorded every two day, two times a day, through lactometers in both farms. Milk composition was estimated through MIR spectrophotometry. The milk fat was extracted by applying the ISO standard 14156 (23). Methyl esters of FAs were obtained according to ISO 15884 (24) and analyzed by using a 100 m GC column, according to Contarini et al. (22). Size of fat globules was evaluated according to Cabassi et al. (25), by adopting ISO 13320 (26) and using a Mastersizer 2000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK) granulometer equipped with a 633 nm laser source. Fatty globule size was expressed as surface weighted mean, according to Sautern mean – $D(3,2)$; where

$$D(3,2)_{\mu m} = \frac{\sum_i d_i^3 N_i}{\sum_i d_i^2 N_i}$$

**Statistical analysis**

Distribution normality was ascertained by estimating skewness and kurtosis; homogeneity of variance was assessed through F test. Data have been analyzed through GLM procedure of SAS (Release 9.2; SAS Institute Inc., Cary NC), using the following model: trait = farm period(farm) cow(farm) stage treatment stage x treatment. Differences were considered significant for $P < 0.05$ and highly significant for $P < 0.001$. The effects of farm and period are neither presented or discussed and the effect of stage of lactation is considered when there is a significant interaction with treatment.

**RESULTS**

Statistical analysis showed that there were no significant interactions between feeding treatment and stage of lactation, except for milk fat concentration, UFAs C10:1 to C14:1, C18, and sum C20 to C22, expressed in percentage on total milk FAs (Tab. V and VI). Partial substitution of SBM with SFC (Tab. III) did not influence milk production and milk fat content; on the contrary, it determined a significant ($P < 0.005$) reduction of milk protein content (Table V). Feeding effect on milk fat concentration was influenced by stage of lactation (Fig. 1).

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**Table I. Chemical composition of sunflower cake**

| Tabella I. Composizione chimica del panello di girasole |
|--------------------------------------------------------|
| **Dry matter (DM)** | (% weight) | 95.2 |
| **Ether extract** | (% DM) | 13.4 |
| **Fiber** | " | 31.7 |
| **Crude protein** | " | 25.3 |
| **Nitrogen-free extract** | " | 23.2 |
| **Ash** | " | 5.9 |

**Table II. Fatty acid composition of sunflower cake**

| Tabella II. Composizione in acidi grassi del panello di girasole |
|---------------------------------------------------------------|
| **Fatty acid** | % on total FAs |
| C14 | 0.12 |
| C16 | 6.57 |
| C17 iso | 0.02 |
| C17 anteiso | 0.12 |
| C17 | 0.03 |
| C18 | 3.32 |
| C18:1 | 32.30 |
| C18:2 | 56.48 |
| C20 | 0.20 |
| C18:3 | 0.20 |
| C23 | 0.01 |
| C24 | 0.15 |
Composition of milk FAs has been reported on Table VI. Supplementation of SFC modified the proportion of several FAs, decreasing \( P<0.001 \) percentage of palmitic acid (C16) and of total SFAs. Sunflower cake increased \( P<0.001 \) UFAs C10:1 to C14:1, C16:1cis7 + C16:1cis 9, stearic acid (C18), oleic acid (C18:1), VA, CLA, sum C20 to C22, total UFA, MUFA, and PUFA. Supplementation of SFC did not influence concentration of C4 to C14, C17:1, arachidonic acid (C20:4n6), and n-3 fatty acids (\( \omega-3 \)). Analysis of variance pointed out a significant \( P<0.05 \) effect of the interaction between feeding treatment and stage of lactation for the UFAs C10:1 to C14:1 (Fig. 2), C18 (Fig. 3), and sum C20 to C22 (Fig. 4).

Partial substitution of SMB with SFC (Tab. III) did not modify size \( (d_{32}) \) of milk fat globules (3.24 \( \mu \) in SBM versus 3.22 \( \mu \) in SFC).

### DISCUSSION

Cows fed SFC had the same daily milk production of cows on SBM diet, in both mid and late lactation (Tab. V). Dry matter intake is considered to be one of the main drivers of milk production. The effect of administration of sunflower seeds to lactating cows on DMI is controversial, because some authors (10,11) observed a reduction of DMI and others (11-15) that did not. In the present experiment, we did not measured individual DMI so that we do not know if there was a difference in this parameter between the two groups of animals; however we cannot exclude that the substitution of SFC for a portion of SMB could have lowered DMI, because in the literature, increases of milk production, as consequence of administration of diets containing sunflower seeds, were normally associated with a neutral effect on DMI (11,15,14).
The experimental design does not allow to solve the problem whether a possible reduction of DMI associated with a stable milk production can determine an excessive mobilization of body reserves. However, in his review, Chilliard (27) reported that dietary fats do not influence body weight gain after peak of lactation, but can increase body weight losses in the early stage and we have no reason to exclude that substitution of SFC for a portion of SBM can affect body reserve recovery during mid and late lactation.

Substitution of a portion SBM with SFC significantly decreased milk protein concentration in both mid and late lactation (Tab. V). A similar result was observed in the experiments of Petit et al. (13) and Stegeman et al. (15) with sunflower seeds; whereas no effects on milk protein content were recorded by Sarrazin et al. (10), Casper et al. (11), McGuffy and Schingoethe (12), Rafałowski and Park (14) with sunflower seeds and by Cruz-Hernandez et al. (16) and Rego et al. (17) with sunflower oil. However, negative effects of fat introduction in the diets of lactating cows on milk protein concentration are well documented in several reviews (28,29). In another review, Doureau and Chilliard (30) reported that dairy cows experienced a reduction of milk protein concentration as consequence of an increase of dietary fat content after peak of lactation; they also reported that this reduction mainly interested casein content. Some experiments showed that the depressing effect of lipid administration were independent from the nature of lipids (9,31,32). The causes of the impairing effect of diet fat on milk protein concentration are still unclear. The decrease of protein content seems to be explained by a reduction in casein content, that is synthetized in the mammary gland (30). In the cases where there was an increase of milk production (15), the negative effect on protein concentration might be due to the dilution of protein in a greater volume of milk. However, depressing effect seems to be more general and can be observed also when the production was not modified (13). Several other explanations were advanced: i. glucose deficiency; ii. insulin resistance; iii. increased energy efficiency in milk production; iv. somatotropin shortage (33). Any case, according to Sutton (34), the causes should be extra-ruminal, because the reduction of milk protein concentration was found with rumen-protected as well as unprotected lipids.

Main effect of SFC supplementation on milk fat concentration was not significantly different in respect to SBM diet (Tab. V). However, a significant interaction between lactation stage and dietary treatment was observed, if considering the effect separately in mid and late lactation (Fig. 1). In mid lactation, there was no difference in milk fat concentration between cows fed SBM and SFC; whereas, SFC supplementation determined a significant reduction of milk fat concentration in late lactation. There is a wide variability among the experiments where the effects of the supplementation of sunflower seed or oil on milk fat concentration were assessed. Some authors (10,11) found a reduction of milk fat percentage as consequence of the supplementation of sunflower seeds or oil; on the contrary, in other studies no effects were found (12-15). The discrepancies among these experiments can be explained by differences in kind

**Table V. Milk production and milk fat and protein concentration of cows fed on control (SBM) and experimental (SFC) diets**

| Diet         | Mean | SE  |
|--------------|------|-----|
| SBM          | 36.5 | 0.37|
| SFC          | 37.3 | 0.37|
| Fat concentration§ | 4.03 | 3.82 |
| Protein concentration** | 3.52 | 3.42 |

**Significant main effect (p <0.001); § significant interaction between treatment and stage of lactation (p <0.05)
of lipids fed to cows or lactation stage: Casper et al. (11) pointed out that milk fat concentration was depressed by regular sunflower seeds (rich in linoleic acid), whereas administration of high-oleic sunflower seeds did not have this effect. He et al. (35) confirmed this observation by finding that administration of high LA sunflower oil had a stronger effect than low LA sunflower compared with a no-fat diet on milk fat production and percentage. For what concerns distance from calving, in the two experiments where cows in early lactation were used, milk fat concentration was not influenced by feeding treatment (13,14).

Partial substitution of SBM with SFC significantly modified lipid composition of milk fat (Tab.VI), with an increase of UFA and a decrease of SFA. De novo synthetized FAs (C4 to C14) were not influenced by feeding treatment. Palmitic acid, which derives from de novo synthesis, but also from body fat mobilization and directly from feed, was decreased by SFC supplementation; on the contrary, percentage of stearic acid, that is not synthetized in mammary gland, was increased. Regarding this FA, we also observed a significant interaction between dietary treatment and lactation stage, with a higher difference between dietary treatment in mid than in late lactation (Fig. 3).

Similiar results have recently been obtained by Goiri et al. (36) who substituted hydrogenated palm fat with SFC and observed a significant decrease of C12 and C16 together with an increase of C18, C18:1 cis12, C18:1 trans10, C18:2 and C18:2 cis9,trans11. Consistently to Glasser et al. (9) in the milk fat of the cows fed SBM there was a lower percentage of minor FAs than in that of cows receiving SFC. However, the differences of UFAs C10:1 to C14:1 (Fig. 2) and sum C20 to C22 (Fig. 4), between the two treatments, were higher in mid than in late lactation. Oleic acid concentration was higher in the milk fat of cows fed SFC than in that of SBM cows (Tab.VI). Main source of C18:1 is desaturation of C18 through activity of Δ9desaturase in the mammary gland (19). This finding could explain also the results obtained by He et al. (35), who reported increases of C18:1 with both high-oleic low-linoleic sunflower oil and low-oleic high-linoleic oil supplementation. Sunflower cake supplementation determined also increase of VA, CLA, LA and the sum C20 to C22. Lack of effects on ω-3 was expected, since sunflower oil has a little percentage of α-linolenic acid (19).

The diet fed to the cows of this experiment was rich in rapidly fermentable carbohydrates, that are present in maize silage and cereal grains. Even if the supply of roughages can be considered adequate, the increase of UFA in the diet makes the condition favorable for the reduction of milk fat percentage in late lactation. There are several reviews which discussed the milk fat depression syndrome; in one of them, Bauman and Griinari (37) explained that a high-grain with a low-roughage diet is a requisite for altering rumen microbial processes; but it does not determine any reduction of milk fat concentration, unless there is a sufficient amount of PUFA in the diet. Polyunsaturated fatty acids, such as C18:2, are toxic for microbial bacteria, which have to rapidly biohydrogenate them to SFA. Accor-
This experiment evidenced that a partial substitution of SMB with SFC in the diet of lactating cows can be considered a feasible strategy for an economic exploitation of this co-product of biodiesel production chain.

Sunflower cake did not influence milk production, milk fat percentage and fat globule size on whole lactation. The reduction in milk fat percentage, observed in late lactation, is to be attributed to the impairing effect of LA on the de novo FA synthesis in mammary gland. We observed a reduction of milk protein concentration, according to several other experiments where vegetable oils were fed to lactating cows.

The abundance of CLA in milk is strongly dependent on the presence of LA in the diet, as said above, so that feeding SFC seems to be a very effective strategy to increase its content in milk.

Table VI. Fatty acid composition (% on total FA) of milk of cows fed on SBM or SFC diets

| Fatty acid          | SBM | SFC | SE  |
|---------------------|-----|-----|-----|
| ∑ SFA C4 to C14     | 26.4| 26.1| 0.28|
| ∑ UFA C10 to C14:1**§| 1.15| 1.36| 0.01|
| C16**               | 31.5| 28.2| 0.20|
| C16:1cis 7 e C16:1cis 9**| 1.70| 1.39| 0.23|
| C17:1               | 0.15| 0.19| 0.02|
| C18**§              | 8.66| 10.31| 0.12|
| C18:1**             | 21.3| 22.9| 0.24|
| C18:1trans11 (VA)**| 0.87| 1.21| 0.04|
| C18:2cis9,trans11 (CLA)**| 0.47| 0.63| 0.02|
| C18:2cis9,cis12 (LA)**| 2.59| 2.95| 0.04|
| ∑ C20 to C22**§     | 0.11| 0.13| 0.002|
| C20:4n6             | 0.13| 0.13| 0.002|
| Others              | 3.28| 3.16| 0.05|
| Total SFA**         | 70.0| 67.9| 0.31|
| Total UFA**         | 30.0| 32.1| 0.31|
| Total MUFA**        | 25.61| 27.2| 0.27|
| Total PUFA**        | 4.39| 4.90| 0.50|
| Total ω-3 acids     | 0.39| 0.38| 0.05|

**Significant main effect (p <0.001); § significant interaction between treatment and stage of lactation (p <0.05)

CONCLUSIONI
This experiment evidenced that a partial substitution of SMB with SFC in the diet of lactating cows can be considered a feasible strategy for an economic exploitation of this co-product of biodiesel production chain. The decrease of SFA and the increase of CLA, lent some positive nutritional characteristics to milk, and make this feeding strategy very interesting.

From this perspective, introduction of SFC in dairy cow diet is not only a strategy for an economic use of this by-product, but it is also a mean for improving milk nutritional properties.
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