

Milk yield, milk composition, fatty acid profile and indices of milk fat quality as affected by feeding with extruded full-fat soybean

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Abstract

The aim of the study was to evaluate the effect of extruded full-fat soybean in diets of dairy cows on nutrient intake, milk yield, content and daily production of milk components, fatty acid (FA) profile and indices of milk fat quality. Four lactating Holstein cows were divided into control group fed a diet containing extruded rapeseed cake (R) and the experimental group fed a diet in which a part of extruded rapeseed cake was substituted by extruded full-fat soybean (RS). Dry matter intake (DMI), milk yield and milk composition were recorded. Milk FA profile was determined with subsequent calculation of atherogenicity index (AI), peroxidisability index (PI), desaturation index (DI) and spreadability index (SI) characterising quality of milk fat. Cows fed diet containing RS had higher DMI (17.8 kg/d) than cows fed diet containing R (16.8 kg/d, $P < 0.05$). Milk yield in RS (19.5 kg/d) was higher compared to R (17.6 kg/d, $P < 0.05$). Content of protein and casein was lower in RS than in R while content of lactose and urea was higher in RS than in R ($P < 0.05$). Experimental diet (RS) compared to control (R) had higher ($P < 0.05$) content of unsaturated FA (31.64 % and 30.69 %, respectively). Content of polyunsaturated FA in RS (4.03 %) was significantly higher ($P < 0.05$) than in R (3.66 %) mainly due to differences in C 18:2n6c, C 18:3n6 and C 18:3n3 FA ($P < 0.05$). Proportion of short-, medium- and long-chain FA was similar in both groups ($P > 0.05$). PI was higher ($P < 0.05$) in RS than in R being 5.54 and 5.06, respectively.

Key words: dairy cows, extruded rapeseed cake, extruded full-fat soybean, milk fatty acid profile

Introduction

Milk and dairy products contribute significantly to the consumption of essential nutrients in human populations (Drewnowski, 2011). Milk also provides various physiologically active compounds

such as bioactive peptides, antioxidants, vitamins, minerals, and nutritionally desirable fatty acids (FA), including α -linolenic acid (C 18:3n3), conjugated linoleic acids (CLA - C 18:2c9t11) and oleic acids (C 18:1n9c) (Haug et al., 2007; Mills et al., 2011).

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Nutrition of dairy cows is one of the factors significantly affecting composition, nutritional value, sensory and technological properties of milk. The proportion of milk constituents is not constant and the largest variations are occurring in milk fat content (Kennelly, 1996). Milk fat usually contains a high proportion of saturated fatty acids (SFA, 70-75 %), largely as a consequence of hydrolysis and subsequent biohydrogenation of dietary fat in the rumen, monounsaturated fatty acids (MUFA; 20-25 %) and small amounts of polyunsaturated fatty acids (PUFA; 5 %; Lock and Shingfield, 2004). According to Shingfield et al. (2008) it is possible to significantly reduce the content of SFA and enhance the concentration of different desirable lipid components several fold in milk through changes in the ruminant diet. The effect of raw or technologically processed oilseeds included into the diets of dairy cows on FA profile has been described and reviewed in many studies (e. g. Kennelly, 1996; Chilliard and Ferlay, 2004). As reviewed by Glasser et al. (2008) supplementation of the cow's diet with oilseeds decreased the proportion of SFA, including atherogenic ones (C 12:0, C 14:0, and C 16:0) by decreasing de novo FA synthesis in the mammary gland. While above mentioned atherogenic FA may be responsible for the increase in blood plasma cholesterol concentration (Wales et al., 2009) and for the increased incidence of coronary heart disease (Williams, 2000) potential benefits of some UFA such as C 18:2n-7 or ω -3 FA include lowering of total blood cholesterol content, anticarcinogenic and antidiabetic effects (Mills et al., 2011) or prevention of heart disease and improved immune response (Palladino et al., 2010). So it is necessary to use additional criteria such as atherogenicity index (AI; Ulbricht and Southgate, 1991) or other indices to compare proportion of certain SFA and UFA in milk fat.

Soybean and rapeseed products are used in many European countries as excellent sources of high-quality protein and energy (Chouinard et al. 1997, Brito and Broderick, 2007; Třináctý et al., 2016). However, FA profile of these two feeding components is different. While rapeseed products in general contain mainly high proportion of C 18:1 (60.0 %) and they are lower in C 18:2 (20.6 %) and C 18:3 (8.9 %) soybean products are rich C 18:2 (52.5 %) and lower in C 18:1 (20.3 %) and of C 18:3 (6.8 %) (Siurana and Calsamiglia, 2016).

Thus, the aim of the study was to compare the short term effect of diets for mid-lactating dairy cows containing either extruded rapeseed cake or extruded full-fat soybean and rapeseed mixture on nutrient intake, milk yield, content and daily production of milk components, milk fatty acid profile and indices of milk fat quality.

Material and methods

Design of experiment

The experiment was carried out on four lactating Holstein cows (lactation 2, 22-26th week of lactation) with average milk production of 18.0 ± 1.1 kg/day that were divided into 2 groups with similar milk yield. Cows were fed individually twice daily (6.30 and 16.30 h) *ad libitum* the conventional diet based on maize silage, lucerne hay and supplemental mixture. The control diet contained extruded rapeseed cake (R) while in the experimental diet part of extruded rapeseed cake was substituted by extruded full-fat soybean (RS). Almost isonitrogenous and isoenergetic diets were optimised using INRA 2007 system (Agabriel et al., 2007; Table 1). The experiment was carried out in the form of a cross-over design and was divided into 2 periods of 14 days. Each period consisted of 10 days adaptation period and 4 days experimental period.

Sampling and analyses of feed and milk

Feed intake and respective refusals were monitored daily during the experiment. An aliquot of feed was taken for subsequent analyses. Dry matter (DM) was determined by drying at 55 °C for 24 h, followed by milling through a 1 mm screen and drying for another 4 h at 103 °C. Content of crude protein (CP), crude fibre (CF), ash and fat were estimated according to AOAC (1984). Neutral detergent fibre (NDF, with α -amylase) was estimated according to Van Soest et al. (1991), ash-free acid detergent fibre (ADF) was estimated according to Goering and Van Soest (1970). The NE_L was calculated according to Sommer et al. (1994).

Cows were milked twice a day (07.00 a.m. and 05.00 p.m.). Milk yield was recorded at each milking. During the experimental period, samples of milk were taken from each milking (8 samples per

cow per period). Samples for determination of basic components were conserved by 2-bromo-2-nitropropane-1,3-diol (Bronopol; D&F Control Systems, Inc. USA), cooled to the 6 °C and kept refrigerated until analyses by infrared analyser (Bentley Instruments 2000, Bentley Instruments Inc., USA). The urea content was determined using UREAKVANT apparatus (AGROSLUŽBY Olomouc, s.r.o., Czech Republic). Samples for determination of FA profile were taken at each milking, centrifuged and milk fat was kept frozen at -20 °C until subsequent analyses.

FA profile was determined as follows: extracted milk fat (50-60 mg) was dissolved in isooctane and homogenised in ultrasound. After the addition of sodium methanolate the mixture was heated under a reverse cooler. FA were released in the form of fatty acid methyl esters (FAMES) which were sepa-

rated using a gas chromatograph HP 4890D (Hewlett-Packard, USA) with capillary column DB-23 (60 m × 0.25 mm × 0.25 μm). FAMES were detected with the flame ionisation detector (FID) and identified according to the retention times using external standards of fatty acids Supelco 37 component FAME Mix (Supelco, USA) and linoleic acid conjugated methyl ester (Sigma-Aldrich, Germany).

Calculation of indices of milk fat quality

For the calculation of indices of milk fat quality, the following equations were used:

Atherogenicity index (Ulbricht and Southgate, 1991):

$$AI = (C12 + 4 \times C14 + C16) / \text{sum of UFA}$$

Table 1. Ingredients and chemical composition of diets (g/kg, dry matter basis)

Components (g/kg)	R	RS
Maize silage	508	508
Lucerne hay	92	92
Barley	106.4	106.4
Oat	106.4	106.4
Sugarbeet chippings	60.0	49.2
Extruded full-fat soya		67.2
Extruded rapeseed cake	112.8	56.4
Rapeseed oil	4.2	2.1
Sodium chloride (NaCl)	2.2	1.9
Dicalciumphosphate (DCP)	3	4.3
Limestone (CaCO ₃)	4.2	4.4
Sodium bicarbonate (NaHCO ₃)	0.4	1.1
Magnesiumphosphate (MgP)		0.2
Blend-s minerals	0.2	0.2
Blend-s vitamins	0.2	0.2
Nutrient composition (g/kg)		
Crude protein	146.8	150.9
Fat	30.4	35.6
Ash	72.8	76.1
Crude fibre	180.5	178.4
NDF ¹	361.7	351.3
ADF ²	199.1	188.8
NE _L ³	5.7	5.8

R - diet containing extruded rapeseed cake; RS - diet containing mixture of extruded rapeseed cake and extruded full-fat soybean
¹NDF = Neutral Detergent Fibre, ²ADF = Acid Detergent Fibre, ³NE_L = Net Energy of Lactation

Peroxidisability index (Castellini et al., 2001):
 $PI = 0.025 \times \text{Mono} + \text{Di} + 2 \times \text{Tri} + 4 \times \text{Tetra} + 6 \times \text{Penta} + 8 \times \text{Hexa}$

where: Mono, Di, Tri, Tetra, Penta and Hexa represent the weight percentages of monoenoic, dienoic, trienoic, tetraenoic, pentaenoic and hexaenoic FA, respectively.

Desaturation index (Chilliard and Ferlay, 2004):

$$DI = C\ 18:1n9c / (C\ 18:0 + C\ 18:1n9c)$$

Spreadability index (Timmen, 1990):

$$SI = C\ 18:1n9c / C\ 16:0$$

Statistical analyses

Data obtained in the experiment were analysed using the GLM procedure of the Statgraphics 7.0 package (Manugistics Inc. and Statistical Graphics Corporation, Rockville, Maryland, USA) according to the following model:

$$Y_{ijkl} = \mu + T_i + C_j + P_k + D_l + \varepsilon_{ijkl}$$

where μ = general mean, T_i = treatment effect ($i = 2$), C_j = cow effect ($j = 4$), P_k = period effect ($k = 2$), D_l = day of sampling effect ($l = 4$) and ε_{ijkl} = error term.

Table 2. Average daily nutrient intake in cows fed diets containing extruded rapeseed cake (R) and extruded full-fat soybean (RS)

	Units	R	RS	SEM
Dry matter	kg/d	16.8*	17.8*	0.28
Crude protein	kg/d	2.5*	2.6*	0.05
Fat	kg/d	0.5*	0.6*	0.01
Ash	kg/d	1.2*	1.4*	0.02
Crude fibre	kg/d	3.0	3.1	0.06
NDF ¹	kg/d	5.9	6.2	0.11
ADF ²	kg/d	3.3	3.4	0.06
NE _L ³	MJ/d	97.2*	103.7*	1.52

* $P < 0.05$; R - diet containing extruded rapeseed cake; RS - diet containing mixture of extruded rapeseed cake and extruded full-fat soybean; ¹NDF = Neutral Detergent Fibre; ²ADF = Acid Detergent Fibre; ³NE_L = Net Energy of Lactation; SEM - standard error of the mean

Table 3. Milk yield and content and yield of milk components in cows fed diets with extruded rapeseed cake (R) and extruded full-fat soybean (RS)

	Units	R	RS	SEM
Milk yield	kg/d	17.6*	19.5*	0.50
4 % FCM ¹	kg/d	19.0	20.9	0.68
Fat	g/kg	44.9	44.9	1.11
Protein	g/kg	36.5 *	34.4*	0.20
Casein	g/kg	29.0 *	27.4*	0.16
Lactose	g/kg	46.5*	48.0*	0.22
Urea	mg/100 mL	16.5*	19.4*	0.48
Yield of milk components				
Fat	g/d	798.1	872.9	34.14
Protein	g/d	638.8	672.3	16.98
Casein	g/d	507.9	534.2	13.46
Lactose	g/d	816.5*	934.4*	24.72

* $P < 0.05$; R - diet containing extruded rapeseed cake; RS - diet containing mixture of extruded rapeseed cake and extruded full-fat soybean; ¹4 % FCM - fat corrected milk; SEM - standard error of the mean

Results and discussion

Nutrient intake and milk yield and composition

Nutrient intake is given in Table 2. Average daily DM intake and intake of CP, fat, ash and NE_L was higher in RS in comparison to R ($P < 0.05$). On the other hand, Veselý et al. (2009) or Kudrna and Marounek (2006) did not find differences in DM intake between cows consuming diets containing extruded rapeseed cake and extruded soybeans. Differences may be due to the varying composition of diets between our and above mentioned studies.

Yield and composition of milk is presented in Table 3. Milk yield was higher in RS than in R ($P < 0.05$), however yield of milk expressed in 4 % FCM did not differ significantly between groups ($P > 0.05$). Similar findings were reported in other studies, e.g. Veselý et al. (2009), while Kudrna and Marounek (2006) did not find a difference in milk yield between cows fed diet supplemented with rapeseed cake and extruded soybeans. Although milk fat content and yield in our study did not differ significantly between groups ($P > 0.05$), fat yield was numerically higher, by 9 %, in RS than in R. This is in accordance with findings of Jacobs et al. (2011) or Kudrna and Marounek (2006). The contents of total proteins and casein were lower in RS than in R, while concentration of lactose and urea was higher in RS than in R ($P < 0.05$). Yield of milk components was not affected by the treatment ($P > 0.05$) except of lactose yield that was higher in RS in comparison to R ($P < 0.05$).

Fatty acid profile and indices of milk fat quality

Profile of FA and indices characterizing quality of milk fat are presented in Table 4. With regards to saturation, all groups of FA were affected ($P < 0.05$) by the treatment except MUFA. Feeding experimental diet (RS) resulted in lower content of SFA in milk fat ($P < 0.05$) in comparison to feeding control diet (R) mainly due to numerically lower concentrations of C 16:0 and C 18:0 ($P > 0.05$) and significantly lower concentration of C 20:0 ($P < 0.05$) in RS than in R. Our results are in discrepancy with findings reported by Veselý et al. (2009) who did not find significant differences in SFA content of milk. However, in their study significant differences in

individual SFA were observed, mainly an increase in C 16:0 and a decrease in C 18:0 after feeding diet with extruded full-fat soybean compared to diet containing extruded rapeseed cake. This discrepancy was probably caused by differences in the composition of diets in the above mentioned study.

The concentration of PUFA was higher in RS than in R ($P < 0.05$). Similarly, Kudrna and Marounek (2006) found positive effect of feeding extruded soybean on PUFA content in milk fat. Content of short-, medium- and long-chain FA was similar in both groups and was not affected by the treatment ($P > 0.05$). Among individual FA, significant differences were observed in long-chain and/or unsaturated FA C 18:2n6c, C 18:3n6 and C 18:3n3 that were higher in RS than in R ($P < 0.05$). Similar results were also reported by Veselý et al. (2009) or Kudrna and Marounek (2006). Content of C 20:0 and C 20:3n3 was lower in RS than in R ($P < 0.05$) and the concentration of C 18:1n9c tended to be higher in RS than in R ($P > 0.05$).

The quality of milk fat is influenced by the length of the carbon chain of FA, their degree of (un)saturation and their positional distribution within the triacylglycerol molecules. AI proposed by Ulbricht and Southgate (1991) is based on information about the effect of various FA on serum cholesterol and low- and high-density lipoprotein concentrations in humans. The typical AI value of milk fat is about 2 (Bobe et al., 2004). Improvements in FA profile have been demonstrated by feeding unsaturated lipids to cows. In our study, the AI was not affected by the treatment similarly to findings reported by Kudrna and Marounek (2006). On the other hand, Veselý et al. (2009) found higher AI after feeding diet with extruded rapeseed cake compared to diet with extruded full-fat soybean. In above mentioned studies that were performed on similar types of diets the AI values ranged between 1.89 and 2.91. Considerably higher AI values ranging from 4.08 to 5.13 were calculated by Nantapo et al. (2014) for different stages of lactation on pasture-based diets.

The PI characterising the oxidative stability of milk fat was higher in RS than in R ($P < 0.05$). The same findings were reported by Veselý et al. (2009) or by Kudrna and Marounek (2006).

Table 4. Content of fatty acids (%) and indices of milk fat quality in cows fed diets containing extruded rapeseed cake (R) and extruded full-fat soybean (RS)

Fatty acid	R (%)	RS (%)	SEM
C 4:0	0.52	0.58	0.042
C 6:0	0.33	0.36	0.033
C 8:0	0.24	0.27	0.023
C 10:0	1.23	1.23	0.070
C 11:0	0.04	0.04	0.003
C 12:0	2.77	2.71	0.081
C 13:0	0.12	0.11	0.006
C 14:0	11.55	11.63	0.154
C 14:1	1.09	1.21	0.041
C 15:0	1.42	1.32	0.051
C 15:1	0.02	0.02	0.001
C 16:0	38.96	38.25	0.471
C 16:1	2.13	2.20	0.060
C 17:1	0.64	0.64	0.019
C 17:0	0.28	0.27	0.016
C 18:0	11.42	11.23	0.330
C 18:1n9c	22.82	23.31	0.345
C 18:2n6t	0.18	0.18	0.008
C 18:2n6c	2.32*	2.60*	0.044
C 18:3n3	0.37*	0.43*	0.012
C 18:3n6	0.06*	0.07*	0.003
C 20:0	0.18*	0.15*	0.007
C 20:1n9	0.23	0.21	0.009
C 20:2	0.17	0.15	0.021
C 20:3n3	0.19*	0.17*	0.004
C 20:3n6	0.05	0.06	0.023
C 20:5n3	0.01	0.01	0.001
C 21:0	0.10	0.09	0.004
C 22:0	0.14	0.12	0.007
C 22:2	0.32	0.35	0.057
FA according to saturation			
SFA ¹	69.31 *	68.36*	0.314
MUFA ²	27.03	27.60	0.305
PUFA ³	3.66 *	4.03*	0.106
UFA ⁴	30.69 *	31.64*	0.314
FA according to chain length			
Short-chain ⁵	5.26	5.29	0.222
Medium-chain ⁶	56.20	55.55	0.582
Long-chain ⁷	38.55	39.16	0.637

Indices of milk fat quality			
AI ⁸	2.91	2.78	0.058
PI ⁹	5.06 *	5.54*	0.136
DI ¹⁰	0.67	0.68	0.004
SI ¹¹	0.60	0.61	0.015

*P<0.05; R - diet containing extruded rapeseed cake; RS - diet containing mixture of extruded rapeseed cake and extruded full-fat soybean; SEM - standard error of the mean; ¹SFA = saturated fatty acids; ²UFA = unsaturated fatty acids; ³MUFA = monounsaturated fatty acids; ⁴PUFA = polyunsaturated fatty acids; ⁵fatty acids with carbon length from C4 to C12; ⁶fatty acids with carbon length from C14 to C16; ⁷fatty acids with carbon length C18 and more; ⁸AI = atherogenicity index; ⁹PI = peroxidisability index; ¹⁰DI = desaturation index; ¹¹SI = spreadability index

The introduction of a cis- double bond between carbon 9 and 10 of SFA with a chain length of 10 to 18 carbons by $\Delta 9$ -desaturase enzyme is an important step in the synthesis of UFA (Bauman et al., 1999; Ntambi and Miyazaki, 2004). The $\Delta 9$ -desaturase enzyme regulates mainly the production of the major isomer of CLA and the conversions of C 14:0 into C 14:1 cis-9, C 16:0 into C 16:1 cis-9, and C 18:0 into C 18:1 cis-9 (Bauman et al., 1999). According to Soyeurt et al. (2008) $\Delta 9$ -desaturation contributes to more than 50 % of C 18:1 cis-9 secreted in bovine milk. There are several indices describing the $\Delta 9$ -desaturase activity. These indices in general are defined as ratios product/substrate (Lock and Garnsworthy, 2003), substrate/product (Chouinard et al., 1999), or product/(substrate + product) (Chilliard and Ferlay, 2004) and are affected by lipid supplementation (Chouinard et al., 1999), season (Lock and Garnsworthy, 2003), breed (Kelsey et al., 2003; Soyeurt et al., 2006a) and days in milk (Soyeurt et al., 2008). In the present study the DI values describing the conversion of C 18:0 into C18:1 cis-9 (Chilliard and Ferlay, 2004) were not affected by the treatment (P>0.05). These results were expected because the contents of C 18:0 and C 18:1n9c in both groups did not differ significantly (P>0.05). On the other hand, Veselý et al. (2009) found significant differences between these two FA in cows fed either extruded rapeseed or extruded full-fat soybean, however DI in their study was not affected by the type of feeding and was similar to our results.

According to Hillbrick and Augustin (2003) and Couvreur et al. (2006) spreadability of milk fat is positively correlated to the percentage of UFA. Because in our study contents of C 16:0 and C 18:1n9c were similar in both groups the SI did not differ significantly ($P>0.05$). This is in discrepancy with Veselý et al. (2009) who found higher SI in extruded rapeseed cake-based diet than in extruded soybean-based diet due to significant differences in the contents of C 16:0 and C 18:1n9c between groups found in their study.

Conclusion

Despite the limited number of cows and short-term experimental periods, results of this experiment suggest that partial substitution of extruded rapeseed cake with extruded full-fat soybean had positive effect on intake of DM, CP, fat and NE_L and on milk yield but negative effect on the content of protein and casein in milk. Although milk fat content did not differ significantly between groups and content of short-, medium- and long-chain FA was not affected by the treatment, significant differences in the content of SFA and UFA in milk were noted. Concerning to individual FA, positive effect of experimental diet was observed on the content of C 18:2n6c, C 18:3n6 and C 18:3n3. There was not much difference in the indices of milk fat quality. Further investigation with greater number of animals and longer experimental period is necessary.

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Mliječnost, sastav mlijeka, profil masnih kiselina i indeksi kvalitete mliječne masti kao utjecaj hranidbe ekstrudiranom punomasnom sojom

Sažetak

Cilj ovog istraživanja bio je procijeniti utjecaj ekstrudirano punomasnog zrna soje u hranidbi mliječni krava na unos hranjivih tvari, proizvodnju mlijeka, sastav i dnevnu proizvodnju mlijeka, profil masnih kiselina (FA) i indeksa kvalitete mliječne masti. Četiri krave pasmine holstein su u razdoblju laktacije bile raspoređene u kontrolnu skupinu hranjenu obrokom koji sadrži ekstrudirane pogače (R) i eksperimentalnu skupinu hranjenu obrokom u kojem je dio ekstrudirane pogače zamijenjen s ekstrudiranom punomasnom sojom (RS). Utvrđivani su unos suhe tvari (DMI), proizvodnja i sastav mlijeka. Profil masnih kiselina mlijeka određen je naknadnim izračunavanjem indeksa aterogenosti (AI), osjetljivosti na oksidaciju (PI), stupanj nezasićenost (DI) i mazivost (SI) koji određuju kvalitetu mliječne masti. Krave hranjene obrokom koji sadrži ekstrudiranu punomasnu soju imaju veći unos suhe tvari (17,8 kg/d) od krava hranjenih obrokom koji sadrži ekstrudirane pogače (16,8 kg/d, $P<0,05$). Mliječnost u skupini RS (19,5 kg/d) bila je veća u odnosu na skupinu R (17,6 kg/d, $P<0,05$). Udjel proteina i kazeina bio je niži u RS nego u R skupini a sadržaj laktoze i uree bio je veći u RS negoli u skupini R ($P<0,05$). Eksperimentalna skupina (RS) u usporedbi s kontrolnom (R) imala je veći ($P<0,05$) udjel nezasićenih FA (31.64 % i 30.69 %, respektivno). Udjel višestruko nezasićenih FA u RS (4,03 %) bio je značajno veći ($P<0,05$) u odnosu na R (3,66 %), uglavnom zbog razlika u C 18:2n6c, C 18:3n6 i C 18:3n3 FA ($P<0,05$). Udio kratko-, srednjo- i dugolančanih FA bio je sličan u obje skupine ($P>0,05$). PI bio je veći ($P<0,05$) u RS nego u R, 5,54 i 5,06, respektivno.

Ključne riječi: mliječne krave, ekstrudirane pogače, ekstrudirana punomasna soja, profil mliječnih masnih kiselina

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