

## INFLUENCE OF SELECTED CEREALS IN DIETS OF DAIRY COWS ON THE FATTY ACID COMPOSITION OF MILK

M. ŠÍPALOVÁ<sup>1</sup>, O. HANUŠ<sup>2\*</sup>, R. VÍCHA<sup>3</sup>, T. D. PHAN<sup>1</sup>, J. POZDÍŠEK<sup>4</sup>, S. KRÁČMAR<sup>1</sup> and P. ROUBAL<sup>2</sup>

<sup>1</sup> Tomas Bata University in Zlín, Department of Analysis and Food Chemistry, Faculty of Technology, 760 01 Zlín, Czech Republic

<sup>2</sup> Dairy Research Institute, 160 00 Prague, Czech Republic

<sup>3</sup> Tomas Bata University in Zlín, Department of Chemistry, Faculty of Technology, 760 01 Zlín, Czech Republic

<sup>4</sup> Research Institute for Cattle Breeding, Rapotín, 788 13 Vikýřovice, Czech Republic

### Abstract

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The study is dealing with the comparison of milk from three groups of cows which were fed with three different cereals (maize, wheat, triticale) focused on fatty acid (FA) profile of milk fat. During six weeks, 26 milk samples were obtained from Czech Fleckvieh dairy cows. These groups were balanced in terms of milk yield, stage and number of lactation as much as possible. The analysis of FAs was performed using esterification and following GC-FID determination in the samples of tested grains as well as in milk. The significant differences in contents of FAs were observed: – for C15:0 which content was higher in milk from group which was fed by maize ( $1.81 \pm 0.04\%$ ) in comparison to milk from group which was fed by triticale ( $1.67 \pm 0.024\%$ ) – for C16:1 which content in milk was higher in the case of triticale group ( $2.18 \pm 0.026\%$ ) as compared to wheat ( $2.08 \pm 0.036\%$ ) group. From all points of view this is possible to conclude that triticale seems to be more suitable replacement.

**Key words:** dairy cow; maize; wheat; triticale; fatty acid

**Abbreviations:** ANOVA – analysis of variance; DM – dry matter; FA – fatty acid; GC – gas chromatography; GPS – global position system; PUFA – polyunsaturated fatty acid; VFA – volatile fatty acid

### Introduction

From nutritional point of view fatty acid profile of milk fat is important quality indicator of various biological kinds of milk and also milk products as yoghurt or cheese (Ivanova et al., 2011; Pajor et al., 2012). The impact of dairy cow nutrition on milk fat content and its fatty acid (FA) profile has been extensively studied (Sutton, 1989; Grummer, 1991; Palmquist et al., 1993; Kennelly, 1996; Ashes et al., 1997;

Jensen, 2002; Lock and Shingfield, 2004). Diets which are consumed by lactating dairy cows are usually low in their fat content. In general this content is only about 4–5% of lipids. The predominant PUFA in ruminant diets are linolenic acid and linoleic acid. The former is derived principally from forage crops (Samková et al., 2009; Frelich et al., 2009, 2012) and the latter being a major component of the oilseeds and concentrates that are fed to dairy cows (Lock and Bauman, 2004).

\*E-mail: hanus.oto@seznam.cz

According to Palmquist et al. (1993) at low rates of feeding, increased grain (fermentable starch) intake increases total milk production and milk protein content. At higher intakes (usually more than 50% of feed DM), increasing starch intake depresses milk fat percentage. When milk fat depression occurs, changes in milk fat FA profile also take place. In most cases, concentration of short-chain FA is decreased, and *cis* FAs are increased, depending on cereal source and its fat content (Palmquist et al., 1993). At carbohydrates in the concentrate can be expected that these affect the pattern of fatty acids in the rumen (Khorasani et al., 1993). Many studies (Kibon and Holmes, 1987; Sloan et al., 1987; Jackson et al., 1991; Schwarz et al., 1995) showed that concentrates rich in crude fibre with those high in starch lead to no significant effects on milk fat content. On the other hand, in some experiments a higher fat content was found after feeding the components high in fibre (De Visser et al., 1990; Valk et al., 1990).

It is important to note that other factors as breed, stage of lactation, season, and herds were all major sources of variation in fatty acid (FA) profile (Palmquist et al., 1993; Pešek et al., 2005, 2006; Samková et al., 2012). Grain processing was also reported to be beneficial for lactation performance (Crocker et al., 1994). Ruminal starch digestion affects the supply of energy which yields substrates for milk synthesis (Nocek and Tamminga, 1991). The processing of grains to increase ruminally available carbohydrate improved milk yield (Chen et al., 1994; Simas et al., 1995; Knowlton et al., 1996). Milk protein percentage and yield were also reported to increase when steamflaked grains were fed (Chen et al., 1994; Simas et al., 1995; Plascencia et al., 1996). However, extensive processing induced a depression in milk fat percentage because of the effects on ruminal volatile fatty acid (VFA) patterns (Simas et al., 1995; Knowlton et al., 1996; Plascencia et al., 1996). McCartney and Vaage (1994) suggested that the economic value of cereal forage for cattle feeding is dependent on its yield and feeding value (i.e., chemical composition, digestibility and annual performance).

Maize, wheat, oat or barley are the most common cereal crops for dairy cattle feeding either like the concentrated component of feeding ration or the silage. However, there has been increased interest in triticale (as possible maize replacement in feeding rations) growing for which only little information exists in terms of possible impact on milk fat FA profile by dairy cow feeding.

The goal of this paper was to obtain information about the effect of triticale on milk fat FA by dairy cow feeding using a comparison of milk which was obtained from three groups of cows fed with different cereals (maize, wheat and triticale).

## Materials and Methods

### Tested grain samples

Winter wheat (cultivar Sulamit), winter triticale (cultivar Kitaro) and maize. All plants were grown in the field in the Moravia district (Agroječmínek Ltd., Chropyně, Czech Republic). Cultivars were selected according to results of previous investigation in terms of evaluation of grain feeding properties (Pozdíšek and Vaculová, 2008; Pozdíšek et al., 2008).

### Design of the feeding experiment

The experimental design comprised three feeding groups of cows. The experiment was proceeded for six weeks (April and May) on farm Klas Nekoř a.s. (total land 2072 ha and arable land 1502 ha; east Bohemia; altitude 496 m; GPS 50°3'12.359"N, 16°33'34.962"E; annual precipitation 855 mm). 26 Czech Fleckvieh cows ( $n_1 = 8$ ;  $n_2 = 9$ ;  $n_3 = 9$ ) were included into the feeding experiment. The dairy cow groups were fed with the same total mixed ration on the basis of maize and clover silage and hay. The group feeding rations differed only in concentrate portion as can be seen in the Table 1. During the experiment, one cow from the first group was excluded because of mastitis illness occurrence. Other cows were relatively healthy in terms of occurrence of milk secretion disorders. The feed groups were balanced in terms of milk yield, stage of lactation (cows in groups were from 10 to 90 days in milk at the beginning of experiment) and number of lactations (from first to tenth) as much as possible (three or four primiparous dairy cows were included in each group). The tie stable and pipeline milking equipment were used in the experiment.

### Milk sampling and sample preparation

Dairy cows were milked twice a day and sampled at morning milking in intervals of about seven days. On the whole, there were obtained 182 individual milk samples in the seven sampling terms using Flow milk meter (Tru-Test Ltd., New Zealand). Within groups, the individual milk samples were combined into bulk samples (three groups multiplied by seven sampling periods,  $n = 21$ ). The milk samples were transported under cold conditions (< 10°C) to the laboratory. Then they were deep-frozen at -80°C overnight. Afterwards, the samples were freeze-dried (-40°C, 120 Pa, 48 h; Alpha 1-4 LSC, Christ, Germany).

### Reagents

Used chemicals as DMF puriss, disodium hydrogen citrate purum, sodium methoxide, methanol p.a., 1, 4-dioxane p.a., n-pentane p.a. were purchased from Sigma-Aldrich.

**Table 1****Components of feeding ration for three experimental groups (kg) and analysis of feeding ration (Pozdišek et al., 2008)**

Component of feeding ration	1 maize (control group)	2 wheat (tested group)	3 triticale (tested group)
clover silage	21.3	21.3	21.3
maize silage	13.1	13.1	13.1
hay	1.0	1.0	1.0
maize silage grain	3.0	3.0	3.0
squeezing corn	3.0	3.0	3.0
feeding mixture	5.8	5.8	5.8
maize	<b>1.5</b>	—	—
wheat	—	<b>2.0</b>	—
triticale	—	—	<b>2.0</b>
Analysis of feeding ration			
NEL/ kg of dry matter	6.524	6.512	6.491
NL % in dry matter	17.9	18.2	17.9
Fibre % in dry matter	15.96	15.74	15.72
PDIN/PDIE	1.189	1.189	1.191

Food Industry FAME Mix standard was acquired from Restek.

#### Determination of total fat

The determination of total fat of feedstuff samples was made according to Commission Regulation No 152/2009. The results of milk fat were presented in the related study Pozdišek et al. (2008).

#### Determination of FAs

0.5 g of homogenous sample of milk (1 g of homogenous sample of grain) was accurately weighted. Determination of FAs was performed according to Suter et al. (1997). In the case of grain the pretreatment of the samples before transesterification was performed as described Suter et al. (1997). The pretreatment included the heating in DMF: 2.5 ml of DMF were added and the slurry refluxed whilst it was stirring, for 15 min. Before transesterification, samples were cooled to ambient temperature. The next steps were accomplished according to the above mentioned method. Each sample was analysed four times.

The pentane solution was used for GC-FID analysis which was performed with Shimadzu GC 2010 using L × I.D. 30 m × 0.25 mm,  $d_f$  0.20 µm capillary column SPB-PUFA. The injection volume was 1.0 µl with split ratio 1:100. During injection, the oven temperature was 50°C, then programmed at 20°C/min to 220°C; the total GC programme took about 30 min.

Nitrogen 5.0 served as the mobile phase. Software GC Solution was used for data analysis. Quantitative analysis of

FAs in samples was performed by comparison with FAME Mix standard. The conversion from FAMEs to fatty acids was performed using coefficient which was calculated as the ratio of the molecular weight of fatty acid to the molecular weight of fatty acid methyl ester.

#### Statistical methods

The data were statistically analysed by means of the statistical programme Unistat v 5.5.05 (©Copyright 1984–2003 UNISTAT Ltd., London, England), using analysis of variance (ANOVA). Tukey's test at  $\alpha = 0.05$  was used to determine statistically significant differences between means.

## Results and Discussion

#### Fat content

The fat contents which were determined in used feedstuffs are presented in Table 2. As it is shown in this table, the content of fat in feedstuffs ranged from  $1.48 \pm 0.003$  g/100 g to  $1.51 \pm 0.037$  g/100 g. From this point of view there are no statistically significant differences between the tested feedstuffs. It is well known (Zeman et al., 1995) that maize contains more fat than other two tested grains, therefore the amount of maize in feeding ration was lower (1.5 kg) than by wheat or triticale (2.0 kg). The fat content in milk samples is showed also in Table 2. There is a statistically significant difference between milk fat content from group which was fed by maize ( $3.27 \pm 0.055$  g/100 g) and milk fat content from group which was fed by wheat ( $3.47 \pm 0.061$  g/100 g).

**Table 2**

**Fat content determined in feedstuff and milk samples (g/100 g)**

	1 maize (control group) mean ± SE	2 wheat (tested group) mean ± SE	3 triticale (tested group) mean ± SE
feedstuff	1.51 ± 0.037 <sup>a</sup>	1.48 ± 0.003 <sup>a</sup>	1.48 ± 0.011 <sup>a</sup>
milk	3.27 ± 0.055 <sup>a</sup>	3.47 ± 0.061 <sup>b</sup>	3.44 ± 0.042 <sup>a,b</sup>

<sup>a-b</sup> Means followed by the same letter in the row are not significantly different ( $P \leq 0.05$ )

SE – standard error

#### Fatty acid profile

There were determined five fatty acids in the tested feedstuffs (C16:0, C18:0, C18:1, C18:2 and C18:3). The content of these FAs is described in Table 3. The results show that the content of C16:0 and C18:0 did not show any difference, so the amount of saturated FAs is balanced in the all tested grains. However, there are statistically significant differences in the case of unsaturated fatty acids. The highest content of C18:1 was analysed in maize (23.53±2.215%) which was statistically higher than in triticale (16.39±0.512%). Wheat contained the statistically highest amount of C18:2 (57.48±0.952%) in comparison to maize (50.44±1.091%) that was also poor on C18:3 (1.94±0.277%) as compared with wheat (3.21±0.546%) or triticale (3.58±0.237%).

The contents of twelve determined FAs in milk samples which were obtained from the three observed groups of dairy cows are described in Table 4. Major part of analysed FAs are saturated FAs. The significant

difference in content was observed by C15:0 which content was higher in milk in case of group fed by maize (1.81±0.04%) in comparison to milk from group fed by triticale (1.67±0.024%). There were found three unsaturated FAs (C16:1, C18:1 and C18:2). The significant difference was observed in C16:1 which content was higher in the case of triticale (2.18±0.026%) as compared to wheat (2.08±0.036%).

The FA profile in used cereals is in agreement with report by Price and Parsons, 1975 with the exception that the lower amount of C14:0 was determined in their work. In our work we have determined higher amount of C16:0 in used cereals in comparison to studies published by Price and Parsons, 1975 and Ryan et al., 2007. In case of C16:0 amount in wheat we gained similar results as Liu, 2011 (20.78%). The cereals used in our experiment showed higher amount of C18:0 in comparison to Price and Parsons, 1975 (corn – 1.93%, wheat – 1.44%, triticale – 0.62%), lower amount of C18:0 in maize determined by Ryan et al., 2007 (1.96%) as well as Liu, 2011 in wheat (1.02%). Our tested grains contained the similar amount of C18:1 compared with Price and Parsons, 1975 (corn – 23.99%, wheat – 20.38%, triticale – 13.66%). The content of C18:2 and C18:3 acids in maize was determined in accordance with Ryan et al., 2007 (52.99%, 1.62%, resp.). In case of wheat we reached lower content of C18:2 and C18:3 acids as reported by Liu, 2011 (57.85%, 4.12%, resp.) as well as lower content of triticale as published by Price and Parsons, 1975 (63.78%, 4.99%, resp.). The differences in content of FAs could be especially caused by another genotype, soil and climate conditions in which the grains were grown.

**Table 3**

**Fatty acid composition of used grains in feedstuff within the observed groups (expressed as % relative to total fatty acids)**

	1 maize (control group) mean ± SE	2 wheat (tested group) mean ± SE	3 triticale (tested group) mean ± SE
Palmitic acid	21.22 ± 2.411 <sup>a</sup>	18.43 ± 1.435 <sup>a</sup>	24.99 ± 1.752 <sup>a</sup>
C16:0			
Stearic acid	2.87 ± 0.285 <sup>a</sup>	2.07 ± 0.523 <sup>a</sup>	2.34 ± 0.451 <sup>a</sup>
C18:0			
Oleic acid	23.53 ± 2.215 <sup>a</sup>	18.81 ± 2.762 <sup>a,b</sup>	16.39 ± 0.512 <sup>b</sup>
C18:1			
Linoleic acid (ω6)	50.44 ± 1.091 <sup>a</sup>	57.48 ± 0.952 <sup>b</sup>	52.69 ± 2.369 <sup>a,b</sup>
C18:2			
Linolenic acid (ω3)	1.94 ± 0.277 <sup>a</sup>	3.21 ± 0.546 <sup>b</sup>	3.58 ± 0.237 <sup>b</sup>
C18:3			

<sup>a-b</sup> Means followed by the same letter in the row are not significantly different ( $P \leq 0.05$ )

SE – standard error

**Table 4**

**Fatty acid compositions of analyzed milk samples within the observed groups (expressed as % relative to total fatty acids)**

	1 maize (control group) mean ± SE	2 wheat (tested group) mean ± SE	3 triticale (tested group) mean ± SE
Butyric acid C4:0	1.90 ± 0.124 <sup>a</sup>	1.90 ± 0.077 <sup>a</sup>	2.10 ± 0.077 <sup>a</sup>
Caproic acid C6:0	1.76 ± 0.114 <sup>a</sup>	1.86 ± 0.084 <sup>a</sup>	2.00 ± 0.077 <sup>a</sup>
Caprylic acid C8:0	1.53 ± 0.086 <sup>a</sup>	1.66 ± 0.067 <sup>a</sup>	1.70 ± 0.062 <sup>a</sup>
Capric acid C10:0	4.47 ± 0.196 <sup>a</sup>	4.90 ± 0.166 <sup>a</sup>	4.77 ± 0.144 <sup>a</sup>
Lauric acid C12:0	6.46 ± 0.206 <sup>a</sup>	6.98 ± 0.168 <sup>a</sup>	6.60 ± 0.129 <sup>a</sup>
Myristic acid C14:0	0.53 ± 0.013 <sup>a</sup>	0.53 ± 0.012 <sup>a</sup>	0.52 ± 0.010 <sup>a</sup>
Pentadecylic acid C15:0	1.81 ± 0.040 <sup>a</sup>	1.78 ± 0.022 <sup>a</sup>	1.67 ± 0.024 <sup>b</sup>
Palmitic acid C16:0	44.48 ± 0.384 <sup>a</sup>	44.77 ± 0.255 <sup>a</sup>	44.78 ± 0.416 <sup>a</sup>
Palmitoleic acid C16:1	2.11 ± 0.028 <sup>a,b</sup>	2.08 ± 0.036 <sup>a</sup>	2.18 ± 0.026 <sup>b</sup>
Stearic acid C18:0	9.24 ± 0.092 <sup>a</sup>	9.26 ± 0.093 <sup>a</sup>	8.99 ± 0.151 <sup>a</sup>
Oleic acid C18:1	20.57 ± 0.262 <sup>a</sup>	20.83 ± 0.343 <sup>a</sup>	21.02 ± 0.311 <sup>a</sup>
Linoleic acid (ω6) C18:2	2.50 ± 0.087 <sup>a</sup>	2.52 ± 0.045 <sup>a</sup>	2.55 ± 0.049 <sup>a</sup>

<sup>a-b</sup> Means followed by the same letter in the row are not significantly different ( $P \leq 0.05$ ).

SE – standard error

The fatty acids of milk fat originated from two sources. The 4:0 – 14:0 acids and approximately one-half of 16:0 are synthesized *de novo* in the mammary gland from short-chain fatty acids which come from microbial digestion of carbohydrates in the rumen. The remaining 16:0 and virtually all of the C18 acids are derived from circulating blood lipids. Blood lipids may come from the diet or from fatty acids mobilized from triacylglycerol stores within adipose tissue (Grummer, 1991; Hawke and Taylor, 1995; Wijesudera et al., 2003). Because of this thesis the lack of significant dietary effects on 4:0 – 14:0 acids in milk fat from tested groups is not surprising considering fact that short-chain fatty acids are synthesized entirely within the mammary gland from acetate and β-hydroxybutyrate (Kennelly, 1996).

According to results of our work there were detected statistically significant differences ( $P \leq 0.05$ ) in contents of C15:0 and C16:1 in milk fat. The milk from cows fed by triticale contained statistically lower amount of C15:0

as compared to milk from another two groups ( $P \leq 0.05$ ). Conversely the amount of C16:1 in milk from cows fed by triticale was significantly higher ( $P \leq 0.05$ ) in comparison to group fed by wheat. The higher amount of C16:0 was detected in milk from all groups as compared with Palmquist et al., 1993 or Wijesudera et al., 2003. This could be caused by a high content of this fatty acid in used grains. However, the content of C18:1 and C18:2 was in milk lower than it was expected according to Jenkins and McGuire, 2006 who conveyed that grain feeding increases the proportion 18-carbon unsaturated fatty acids.

## Conclusion

This experiment was dealing with the comparison of milk which was obtained from three groups of cows which were fed with three different cereals (maize, wheat and triticale). The study was focused on fatty acid profile of milk fat.

The tested grains used in feeding rations did not influence milk fat content, however, there were shown statistically significant differences ( $P \leq 0.05$ ) for C15:0 and C16:1 fatty acid contents in milk fat. The result of this investigation indicated that maize (the control grain) was slightly superior to triticale and wheat. Simultaneously according to our results wheat and triticale are comparable replacements of maize. However, in all views it is possible to conclude that triticale (cultivar Kitaro) seems to be more suitable replacement of maize because of its higher content of C16:1 ( $P \leq 0.05$ ). Also the higher contents of C18:1 and C18:2 were investigated but these differences were statistically not significant. The conclusion of the study is in accordance with our previous published works that were focusing on another indicators (e.g. amino acid composition – Šípalová et al., 2010, total milk quality – Pozdíšek et al., 2008).

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