Effect of Cow Energy Status on the Hypercholesterolaemic Fatty Acid Proportion in Raw Milk

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Abstract

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We evaluated the proportion of fatty acid groups, with an emphasis on hypercholesterolaemic fatty acids, in the milk of 25 Holstein cows during the 1st period of lactation in relation to their negative energy balance (NEB). Sampling of each cow's milk started on the 7th day after calving. Milk samples (n = 425) were collected at 7-day periods during the first 17 weeks of lactation. The proportion (%) of saturated (SFA), hypercholesterolaemic (HCFA), volatile (VFA), unsaturated (UFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids in the milk fat was determined. Body condition score and fat to protein ratio in milk were applied for precise determination of the NEB breakpoint during the observed period. The effects of parity, NEB, regression on lactation week and fat to protein ratio were evaluated using SAS 9.3. Milk contained a lower proportion of SFA as well as equally higher UFA (± 2.13%; $P < 0.01$) during the NEB period. The overcoming of NEB caused an increase in SFA, however, and simultaneously a significant decline in total HCFA (–1.86%; $P < 0.01$) as well as main MUFA (–1.81%, $P < 0.05$). The results document the necessity of increasing Holstein cow robustness to meet the production conditions in dairy farms in relation to the requirement of higher nutrient quality as well as the potential health benefits of cow's raw milk for consumers.

Keywords: consumer; dairy cow; health; negative energy balance; milk fat

Cow's milk is the basic product of animal origin used for the nutrition of born calves (Innis 2007) and for human consumption as well (Haug *et al.* 2007). Capability of dairy cows to increase feed intake during the postpartum period could be predicted with respect to the polymorphism of selected genes (Kadlecová *et al.* 2014a). Milk production increases in early lactation (Szencziová *et al.* 2013) and despite simultaneously increased feed intake, the cow's metabolism goes through the negative energy balance (NEB) phase of lactation (Kadlecová *et al.* 2014b). This standard course of the postpartum period of all dairy cows evokes body fat reserve degradation as an additional source of energy, concurrently followed by a decline in live weight (Řehák *et al.* 2012), body condition score (BCS) (Stádník *et al.* 2002; Ducháček *et al.* 2012a) causing a simultaneous decrease of reproduction (Louda & Stádník 2000; Ducháček *et al.* 2012b) and impairment of health (Vacek *et al.* 2007), and/or changes in milk composition (Gellrich *et al.* 2014). Lipomobilisation increases free fatty acid plasma content and triacylglycerol accumulation in the liver tissue affecting its structure and function (Shibano & Kawamura 2006; Jóźwik *et al.* 2012). Milk fat consists of individual fatty acids (FA) and their triglycerides as the main components influenced by animal species or rather breed (Pešek *et al.* 2005; Marounek *et al.* 2012), cow's individuality (Stádník & Louda 1999; Soyeurt *et al.* 2006), parity (Stádník *et al.* 2013),

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nutrition level (Kupczyński *et al.* 2012), milk yield (Kay *et al.* 2005), and season as well as milking time (Toušová *et al.* 2013).

Groups of FA in milk fat have a positive and/or negative effect on the consumer health. Especially saturated (SFA) and unsaturated (UFA) fatty acids are known for their important influence on the human health (Staňková *et al.* 2013). Wróblewska and Kaliszewska (2012) determined significant allergenicity of food according to the composition of bovine milk. Therefore, it is necessary to observe and evaluate bovine products from a wide range of all particular aspects. SFA have a negative effect on the cardiovascular system, especially the part represented by the hypercholesterolaemic FA (HCFA), which increase deposition of fat in the vascular walls, and are related to atherosclerotic diseases (Jensen 2002). In addition, there is another part of SFA, volatile fatty acids (VFA), which are produced in the rumen, thus indicating the intensity of rumen fermentation and the efficiency of nutrient utilisation (Bhagwat *et al.* 2012). UFA have a positive effect and represent important sources for the synthesis of biologically active substances improving the course of metabolism processes (Bauman & Lock 2010).

Changes in the milk content of individual FA or their groups in relation to NEB depth and NEB length have been evaluated in many studies (Gross *et al.* 2011). The findings usually agree in a higher proportion of UFA and/or lower content of SFA early *postpartum* under NEB with a subsequent UFA decline and/or SFA increase with regard to balancing the current energy status and continued lactation. Therefore, this period seems to be very important for milk composition directly affecting the human health via the proportion of FA groups. No research currently studies changes in FA group content, particularly with regard to HCFA, between negative and positive energy balance of cows, changes which directly affect the health quality of milk as an essential part of human food. Thus, the objective of this study was to evaluate the proportion of FA groups in the milk of Holstein dairy cows during the 1st period of lactation in relation to negative energy balance, with an emphasis on its potential importance for the consumer health.

MATERIAL AND METHODS

Animals and herd management. A total of 25 Holstein cows (9 primiparous, 9 in the second, and 7 in the third and subsequent parity) calved within one month were included in the study. Therefore, breed, year as well as seasonal effects were excluded. The average daily milk yield ranged from 11.34 l to 47.2 l of milk with an average of 28.60 l. All cows selected for observations were without reproduction and health disorders in previous lactations. Body condition was evaluated monthly in relation to the habit practiced by breeders. A body condition index (BCS; a 5-point scale with 0.25 point increments) was used for body condition evaluation (Ferguson *et al.* 1994). BCS ranged from 1.5 to 3.75 points with an average of 2.69. The cows were loose housed in a straw-bedded cubicle barn. All the animals were fed a total mixed ration (TMR) consisting of maize silage, lucerne silage, straw, grass hay, lucerne hay, concentrates, brewery draff, bakery waste, and mineral supplements. The ingredient composition of the diet corresponded to the current daily milk yield of individual cows, and feed rations were completely balanced for energy, protein and fat as well as mineral and vitamin content. Feed rations consisted of the same ingredients throughout the entire experimental period.

Sample collection and analyses. Sampling of each dairy cow's milk started on the 7th day after calving. Milk samples (n = 425) were collected at 7-day periods during the first 17 weeks of lactation. Two aliquot milk samples were collected from each cow in accordance with the milk recording system on every sampling day. The first sample with a preservative was heated to 39 ± 1°C and applied for fat (F) and protein (P) percentage content determination using Milkoscan 133B (N. Foss Electric, Hillerød, Denmark). Subsequently the fat to protein ratio (FPR) was computed. The second sample, without a preservative, was used for the fat extraction and fatty acid (FA) content determination in accordance with the methodology described by Ducháček *et al.* (2012c). The content (mg/100 g) of individual FA (28) and six FA groups (SFA, its parts HCFA and VFA; UFA, its parts MUFA and PUFA) was investigated. Subsequently, the proportions (%) of the six FA groups observed in milk fat were determined and evaluated. The HCFA group included lauric, myristic and palmitic FA in accordance with Kontkanen *et al.* (2011). The VFA group was represented by the content of butyric, caproic, caprylic, and capric FA in accordance with the study by Pešek *et al.* (2006).

Statistical analysis. The data were evaluated by the SAS 9.3 statistical software (SAS/STAT® 9.3, 2011) using UNIVARIATE, CORR, and MIXED procedures. The best model for evaluation was selected in accordance with the values of the Akaike information criterion (AIC). A model including the effects of parity, negative energy balance, and regression on lactation week corresponding with milk yield recorded as well as on milk FPR both specifying the energy status of dairy cow (Moallem *et al.* 2007) was designed for evaluation of FA group (SFA, HCFA, VFA, UFA, MUFA, PUFA) proportions in the total milk fat content. The main effect of NEB was represented by two levels (YES – within the NEB period, NO – NEB period overcome) expressed according to the individual BCS changes and additionally by the course of current milk yield as well as milk FPR values during weeks of observation used as a regression. Cows with BCS decline were considered within the NEB period, while those with balanced or increased BCS were labelled as NEB overcome. The length of average NEB was 10.12 weeks, when average BCS continually declined from 3.13 points in calving to 2.52 points in the 12th week. Subsequently, BCS slowly, however continuously increased to 2.55 points in the 16th week *post partum*. The NEB course was specified on the level of individual dairy cows using their current milk yield and corresponding FPR during three weeks before balancing/increasing BCS. In accordance with Duffield *et al.* (1997) and Vacek *et al.* (2011), the threshold of FPR on the level 1.3 was taken as a criterion for within NEB (> 1.3) and/or NEB overcome (< 1.3). Regressions on lactation week and milk FPR applied within the model clarified determination of the NEB breakpoint because of the monthly period of BCS evaluation. The Tukey-Kramer method was used for evaluation of differences in the least squares means. The model equation used for the evaluation was as follows:

$$Y_{ijk} = \mu + PAR_i + NEB_j + b_1*(WEEK) + b_2*(FPR) + e_{ijk}$$

where: Y_{ijk} – dependent variable (SFA, HCFA, VFA, UFA, MUFA, PUFA in %); μ – mean value of dependent variable; PAR_i – fixed effect of i^{th} number of lactation (i = 1st lactation, n = 153; 2nd lactation, n = 153; 3rd and subsequent lactations, n = 119); NEB_j – fixed effect of j^{th} NEB period occurrence (j = YES – within NEB period, n = 242; NO – NEB period overcome, n = 183); b_1*(WEEK) – regression on the lactation week order; b_2*(FPR) = regression on the fat to protein ratio in milk sample; e_{ijk} – random error

Significance levels $P < 0.05$, $P < 0.01$, and $P < 0.001$ were used to evaluate the differences between groups.

RESULTS AND DISCUSSION

The average F was 3.80% with standard deviation 0.84. The average FPR was 1.21 with standard deviation 0.29. FA groups were represented in milk fat as follows: SFA 75.10%, HCFA 43.03%, VFA 20.43%, UFA 24.90%, MUFA 21.50%, and PUFA 3.39%. The basic characteristics of the applied model presented in Table 1 documented its suitability for evaluation of all FA groups.

The results from the MIXED model are given in Tables 2 and 3. The significantly higher value of SFA (+2.13%; $P < 0.01$) and/or equally lower UFA (–2.13%; $P < 0.01$) was evaluated after the NEB period. This fact corresponds with the findings of Soyeurt *et al.* (2007), Stoop *et al.* (2009), Gross *et al.* (2011), and Ducháček *et al.* (2013).

SFA, primarily HCFA as its part, are considered to be harmful mainly in relation to an increased plasma cholesterol level (Bauman & Lock 2010). A higher proportion of HCFA group in blood causes the deposition of fat in the blood vessel walls and leads to the atherosclerotic disease (Haug *et al.* 2007). Kirchnerová *et al.* (2013) found a gradual increase in HCFA values during lactation expressed by the correlation coefficient $r = 0.584$. However, they evaluated its content in different breeds of cattle kept on farms using the pasture system. On the other hand, our results, detected in high-yielding dairy cows

Table 1. The basic characteristics of the model designed

Traits	Model		Parity		NEB		Lactation week		FPR	
	r^2	P	F-test	P	F-test	P	F-test	P	F-test	P
SFA	0.38	< 0.0001	14.22	< 0.0001	7.68	0.006	5.28	< 0.0001	32.63	< 0.0001
HCFA	0.30	< 0.0001	25.11	< 0.0001	9.13	0.003	6.64	< 0.0001	20.95	< 0.0001
VFA	0.18	< 0.0001	1.52	0.2200	27.47	< 0.0001	3.29	< 0.0001	17.65	< 0.0001
UFA	0.38	< 0.0001	14.22	< 0.0001	7.68	0.0059	5.28	< 0.0001	32.63	< 0.0001
MUFA	0.35	< 0.0001	12.38	< 0.0001	6.13	0.014	4.75	< 0.0001	29.17	< 0.0001
PUFA	0.28	< 0.0001	9.98	< 0.0001	9.19	0.003	3.29	< 0.0001	18.09	< 0.0001

NEB – negative energy balance; FPR – fat to protein ratio; SFA, HCFA, VFA, UFA, MUFA, PUFA – saturated, hypercholesterolaemic, volatile, unsaturated, monounsaturated, and polyunsaturated fatty acids, respectively

Table 2. Effect of cow negative energy balance (NEB) on the proportion of saturated (SFA) and unsaturated (UFA) fatty acids

Factor	Level	SFA (%)	UFA (%)
		LSM ± SE	
NEB	YES	73.80 ± 0.398^{A}	26.20 ± 0.398^{A}
	NO	75.93 ± 0.516^{B}	24.07 ± 0.516^{B}

YES – within the NEB period; NO – NEB period overcome; ^{A,B}different superscript letters confirm statistical significance of differences between rows at $P < 0.01$; *LSM* – Least Square Mean

kept in an intensive stable system, documented a significantly ($P < 0.01$) lower value of HCFA after the NEB period (–1.86%). Thus, the early overcoming of NEB is important for the respective cows' health and reproduction ability (Patton *et al.* 2007; Podpečan *et al.* 2008) as well as for the quality of milk used for human consumption (Pešek *et al.* 2006; Hanuš *et al.* 2010). It is possible to state that cows with the earlier overcoming of NEB started to produce milk healthier for human consumption sooner after calving. Although a higher proportion of SFA was detected after the NEB period, simultaneously a significantly lower HCFA proportion was determined as well ($P < 0.01$). The higher SFA proportion does not necessarily mean the worse quality of milk with respect to the paradoxically declined HCFA proportion. The reason for the opposite results for SFA and HCFA has a physiological background. Approximately half of the fatty acids in ruminant milk fat (FA from $C_{4:0}$ to $C_{14:0}$ and half of $C_{16:0}$) is synthesised in the mammary gland "*de novo*" from short-chain FA with two carbon segments (acetyl CoA) (Kaylegian & Lindsay 1995; Bauman & Griinari 2003). The rest of fatty acids (half of $C_{16:0}$ and $C_{18:0}$ and FA with more carbons) is transported to the mammary gland by blood, especially in the form of the highly labile ß-lipoprotein fraction of non-esterified FA absorbed directly from the feed ration (Harvatine *et al.* 2009) or mobilised from tissue and fat depots (Bauman *et al.* 2006; Samková *et al.* 2008). The different content of $C_{16:0}$ as the most represented milk SFA is also the highest in percentage proportion of HCFA. Thus, a relatively large part of this FA group comes from body fat reserve lipomobilisation, thereby overcoming the NEB period and stopping the fat depot mobilisation leading to its decline while SFA content rises. Cows overcoming NEB also produced significantly ($P < 0.01$) higher VFA content (+5.88%), similarly to the SFA proportion. Stoop *et al.* (2009) detected slightly higher levels of butyric acid (+0.031%) and lower values of caproic, caprylic, and capric acids (–0.301%) under the NEB effect. Lower VFA content during the NEB period agrees with our findings. These results, detected in HCFA and VFA proportions, indicate the lower effectiveness of nutrient utilisation from the feed ration and higher intensity of fat reserve lipomobilisation for increasing milk production during the NEB period. However, the VFA content increase after NEB does not have any basic importance for human nutrition.

Similar results like for UFA were found for MUFA and PUFA content. Significantly ($P < 0.01–0.05$) higher values for these FA groups were determined in the NEB period (+1.81, resp. +0.33%). Thus, an opposite trend to SFA and VFA in relation to the energy balance course and development was observed. A more considerable decrease in UFA was detected especially in MUFA content, which corresponded to the balancing of energy status (Bauman & Griinari 2003; Ducháček *et al.* 2012c, 2013). The incidence of milk UFA is desirable for the consumer health, especially in relation to the content of conjugated linoleic acid (CLA) (Dhiman *et al.* 1999; Domagala *et al.* 2013) and other essential FA (Innis 2007). These affect metabolism as biologically active substances. The UFA and mainly PUFA decline is a physiologically natural state, although undesirable from the aspect of nutrient quality of milk (Gross *et al.* 2011). The above-mentioned relationships could be in direct

Table 3. Effect of cow negative energy balance (NEB) on the proportion of hypercholesterolaemic (HCFA), volatile (VFA), monounsaturated (MUFA), and polyunsaturated fatty acids (PUFA) (*LSM* ± SE)

Factor	Level	SFA		UFA	
		HCFA (%)	VFA (%)	MUFA (%)	PUFA (%)
NEB	YES	43.54 ± 0.318^{A}	17.72 ± 0.578^{A}	22.63 ± 0.376^{a}	3.58 ± 0.056^{A}
	NO	41.68 ± 0.412^{B}	23.60 ± 0.750^{B}	20.82 ± 0.488^{b}	3.25 ± 0.073^{B}

YES – within the NEB period; NO – NEB period overcome; $^{a,b;\ A,B}$different superscript letters confirm statistical significance of difference between rows at $P < 0.05$; resp. $P < 0.01$; *LSM* – Least Square Mean conflict with the EU legislation continuously emphasising and requiring an increase in the quality, health, and safety of agricultural and food products (Velčovská & Sadílek 2014). However, the human organism acquires these FA from other biological sources as well (Jokić *et al.* 2013; Staňková *et al.* 2013). Therefore, we can consider their decline in milk during lactation as an issue of lower importance compared to the HCFA proportion.

CONCLUSIONS

The finding that the overcoming of NEB caused a significantly lower proportion of HCFA is very important. The shorter the NEB, the longer the rest of lactation when cows will produce milk of higher quality. Therefore, the shortening of the NEB period will be positively reflected in the production of healthier milk with lower HCFA content. The results are applicable in the framework of dairy farm management, selection and breeding of dairy cattle breeds at the population level as well as in dairy plants processing raw cow's milk as the basic human food.

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