TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2015) 39: 357-363
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doi:10.3906/vet-1411-43

Relationships of fatty acid group contents in milk and reproductive performance in Holstein cows

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Received: 21.11.2014 • **Accepted/Published Online:** 03.03.2015 • **Printed:** 10.06.2015

Abstract: The aim of this study was to evaluate the relationships between milk fatty acid group contents and subsequent reproductive performance in Holstein cows. A total of 27 cows in the 1st to 4th lactations were included in the evaluation. Cows were divided into primiparous and multiparous groups. Daily milk yields increased in the first 8 weeks of lactation. Body condition score decreased until the third month of lactation. As the cows gradually recovered from the negative energy balance, the contents of saturated fatty acids (SFAs) increased, whereas the contents of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) decreased. The contents of SFAs and MUFAs were significantly ($P < 0.01–0.05$) correlated ($r = –0.279$ to $r = 0.275$) with all the reproductive traits evaluated (calving to first insemination interval, days open, and insemination index). PUFA was only correlated ($P < 0.01$) with calving to first insemination interval ($r = –0.141$). High milk SFA contents were associated with improved values of days open and insemination index, whereas opposite tendencies were observed for MUFA. It was concluded that the relationship was confirmed between the changes of different milk fatty acid group contents in early lactation, used as negative energy balance indicators, and subsequent reproduction results.

Key words: Days open, fatty acids, negative energy balance, insemination interval, insemination index

1. Introduction

The intensity of the dairy cow's metabolism considerably changes in early lactation. However, it is important to monitor its changes during the entire transition period (1). This period is characterized by the deficiency of energy and the reduction of feed intake, which is insufficient for maintenance requirements, milk production, and reproductive performance (2). Consequently, cows enter a stage of negative energy balance (NEB) indicated by fundamental changes in the endocrine, metabolic, and physiological status of an animal (3). During the cow's reproductive cycle, profound changes occur in the body condition score (BCS) that can be used to assess the status of energy balance in the animal (4). Cows with severe BCS reduction after calving produce more milk with higher fat contents and higher fat/protein ratios (5). Milk fat is mostly (97% to 98%) composed of glycerol and fatty acid (FA) esters (6). Approximately half of milk FA from ruminants ($C_{4:0}$ to $C_{14:0}$ and half of $C_{16:0}$) is synthesized de novo in the mammary gland from short-chain FA with two-carbon units (acetyl CoA) (7). The second half of FA (half of $C_{16:0}$ and $C_{18:0}$ and longer-chain FA) is transported to the mammary gland by the blood, especially as its highly labile β-lipoprotein fraction, in the form of nonesterified FA (NEFA) derived directly from the diet (8) or released from the adipose tissue (9). Less than 10% of FA originates from the adipose tissue during lactation with the exception of the NEB period, when this proportion considerably increases (9). The most changes in the composition and content of FA are realized during the first part of lactation (10,11).

A number of factors influence the reproductive performance and conception rate in cattle (12), such as genetics (3), parity of lactation (13), NEB (14), and others. The fundamental reason for reduced fertility can be seen in the likely additive effect of the combination of various physiological and management factors (15). According to Rossi et al. (16), the length of NEB is the main nutritional factor influencing the decline of reproductive efficiency in high-yielding dairy cows. The occurrence of NEB extends the period between calving and first ovulation (17), and increases embryonic mortality and the risk of uterine diseases. Milk FA contents and composition are influenced by NEB and may therefore be associated with

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the subsequent reproductive performance. Correlations were previously calculated between milk FA contents and the length of days open (DO) (18). In that study, positive genetic correlations were determined between unsaturated fatty acid (UFA) as well as monounsaturated fatty acid (MUFA) contents in the first 100 days of lactation and the length of DO. In contrast, positive genetic correlations were observed between saturated fatty acid (SFA) contents and DO in the first 100 lactation days.

We can suppose that there are relations between FA group content and reproductive parameters in Holstein cattle. Therefore, the objective of this paper was to evaluate the relationships between milk FA group contents measured in the first 120 days of lactation and selected reproductive traits in Holstein cows.

2. Materials and methods

A total of 27 Holstein cows were included in the analysis (11, 8, 4, and 4 cows in the 1st, 2nd, 3rd, and 4th lactation, respectively). A total of 415 milk samples were collected weekly during the first 17 weeks of lactation. A lower number of milk samples was caused by practical conditions such as mastitis incidences and other health problems. The cows calving within a month (end of June to middle of July) were included in the evaluation. The average daily milk yield in different weeks ranged from 23.95 to 31.29 l with standard deviations ranging from 7.59 to 12.62 l. The cows were loose-housed in a cubicle straw-bedded barn. They were fed a total mixed ratio (TMR) consisting of corn silage (22 kg, 40.51%), alfalfa silage (20 kg, 36.83%), alfalfa hay (1 kg, 1.84%), straw (0.5 kg, 0.92%), molasses (1 kg, 1.84%), concentrates (9.5 kg, 17.50%), and mineral supplements (0.3 kg, 0.56%). The energy level of the TMR was 162.88 MJ NEL. The ingredient composition of the diet during the first 30 days was the same for all the animals. In the following period (5 to 16 weeks of lactation) the feeding ration had constant energy content but it was same for all the animals as well. The diet was in full agreement with the lactation curve with no FA enrichment. Two aliquot milk samples from each cow were collected in accordance with the official methodology of the milk performance recording system (19). The first sample with a preservative was heated to 39 ± 1 °C and used to determine basic components of milk fat (F, %) and milk protein (P, %) using a Milkoscan 133B (N. Foss Electric, Denmark). The second sample without a preservative was used for the extraction of F and determination of FA contents. The gravimetric (reference) method was used in accordance with standard protocol in the Czech Republic (20) for milk F extraction. The extract was obtained using a water-based-solution of ammonia, ethanol, diethyl ether, and petroleum ether. FA methyl esters were prepared by potassium hydroxide catalyzed methylation and extracted into heptane. Gas chromatography (GC) of FA methyl esters was performed using a Master GC (DANI Instruments S.p.A., Italy) (split regime, FID detector) on a column with polyethylene glycol stationary phase (FameWax: 30 mm × 0.32 mm × 0.25 µm). Helium was used as the carrier gas at a flow rate of 5 mL min^{-1}. The temperature program used for GC was as follows: 50 °C (2 min), after which the temperature was increased to 230 °C at 10 °C min^{-1} (8 min), the temperature of the detector being 220 °C. Contents (mg 100 g^{-1}) and proportions (%) were determined for 34 individual FAs and 4 FA groups (SFAs, UFAs, MUFAs, and PUFAs). BCS of cows was determined monthly in accordance with the methodology of linear description and type evaluation of Holstein cattle. A body condition index (a 5-point scale with 0.25 point increments) was used to evaluate BCS (21).

The reproduction factors of calving to first insemination (CFI), days open (DO), and insemination index (II) were observed. All the cows were examined sonographically 60 to 74 days after parturition.

The data were evaluated with statistical software SAS 9.3 (22). The MEANS and UNIVARIATE procedures were used to calculate descriptive statistics. The CORR procedure was applied to calculate correlation coefficients, evaluating the relationships between milk FA group contents and reproductive parameters. The GLM procedure was used for detailed analysis of the influence of FA groups on reproductive traits. The cows were divided for statistic evaluation only into primiparous and multiparous groups. A model including effects of parity, FA group (SFA, MUFA, PUFA) and regressions on lactation week and on milk yield at sampling day was developed to evaluate reproductive parameters (CFI, DO, and II) in Holstein cows. The animals were grouped into 4 classes according to parity (1st, 2nd, 3rd, and 4th and following lactations). FA category groups were divided into three classes according to their means ± standard deviations (<$\bar{x}\bar{x}$-1/2s; $\bar{x}\bar{x}$-1/2s to $\bar{x}\bar{x}$+1/2s; >$\bar{x}\bar{x}$+1/2s). The model equation used for the evaluation of reproductive parameters was as follows:

$$Y_{ijk} = \mu + A_i + B_j + b_1*(WEEK) + b_2*(MILK) + e_{ijk},$$

where:

Y_{ijk} = dependent variable (calving to first insemination interval, days open, insemination index);

µ = mean value of dependent variable;

A_i = fixed effect of *i*th parity of lactation (i = 1 – first lactation = primiparous, n = 154; 2 – second and subsequent parities = multiparous, n = 261);

B_j = fixed effect of *j*th FA group (SFA– <71.81%, n = 111; 71.81%–78.05%, n = 156; >78.05%, n=143), (MUFA– <19.31%, n = 155; 19.31%–24.07%, n = 140; >24.07%, n = 115), (PUFA– <3.00%, n = 135; 3.00%–3.76%, n = 151; >3.76%, n = 122);

b_1*(WEEK) = regression on lactation week;

b_2*(MILK) = regression on milk yield at sampling day;

e_{ijk} = random error.

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

3. Results

Milk samples contained, on average, 12.51% dry matter (DM), 3.83% fat (F), 3.16% protein (P), and 4.84% lactose (L). BCS decreased from 3.05 points at calving to 2.47 points in the third month of lactation (12th week of lactation) and then slightly increased in the following month (Figure 1).

The developments of F and P contents are presented in Figure 2. The content of F decreased from 4.89% in the 1st week to 3.27% in the 7th week. After this period, when the animals were in NEB according to the decreasing values of the F/P ratio, the contents of F continually increased until the 14th week (4.06%). The contents of P were also reduced between weeks 1 and 7, but to a lesser extent than F contents. After week 7, P contents were stable and slightly increased. The average P contents ranged from 3.04% to 3.54%. The contents of other basic milk constituents were also determined.

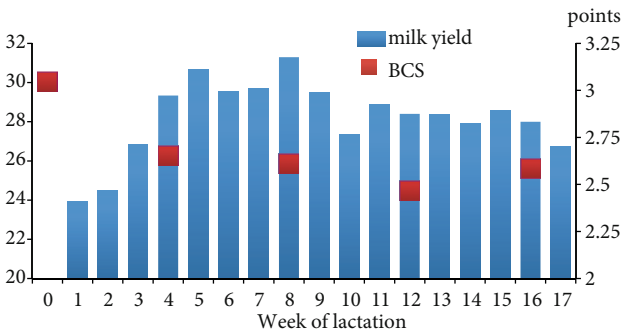
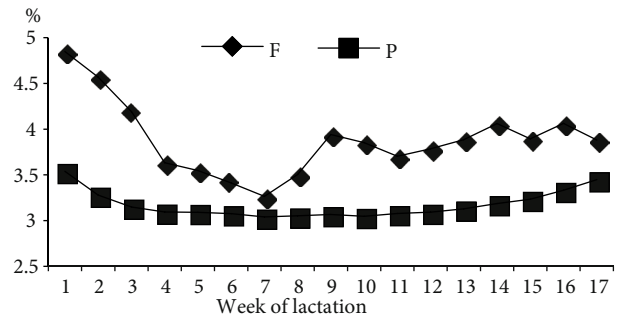


Figure 1. The development of average daily milk yields and BCS in Holstein cows.



F = % content of fat in milk; P = % content of protein in milk.

Figure 2. The development of F and P contents in milk of Holstein cows.

Pearson correlation coefficients were used to describe relationships among NEB indicators and reproductive traits mutually (Table 1). Significant relationships ($P < 0.05–0.01$) were observed among BCS, milk component contents, FA groups, and reproductive parameters. BCS significantly correlated ($P < 0.05–0.01$) with P contents and FA groups. F contents significantly correlated with all the other milk components evaluated. The content of P significantly correlated ($P < 0.05–0.01$) with the content of DM, F/P ratio, SFA, MUFA, DO, and II. The content of DM and the F/P ratio were significantly correlated only with other milk components ($P < 0.01$). FA groups correlated mainly with each other ($P < 0.01$). Low correlation coefficients ($P < 0.05–0.01$) were calculated between SFA and the reproductive parameters evaluated (CFI, DO, and II). Similarly low correlations but of opposite sign were determined between MUFA and reproductive performance ($P < 0.05–0.01$). PUFA contents were only weakly negatively correlated with CFI ($P < 0.05$).

The results of reproductive performance depending on parity and FA group contents are presented in Table 2. CFI tended to decrease with parity (from 112.29 in primiparous to 107.01 days in multiparous cows, $P < 0.01$). Nonsignificantly, the longest period of DO was determined for the animals in the 1st lactation (62.48 days) compared to animals in 2nd and subsequent parities. Significantly lower values of II were determined for multiparous cows (–1.18, $P < 0.01$).

The effect of SFA contents on reproductive performance is shown in Table 2. CFI tended to increase with increasing milk SFA contents (from 97.34 to 118.20 days). The group with the lowest SFA content significantly differed ($P < 0.01$) from the remaining groups. The highest DO, 222.46 days, was detected for the lowest SFA content (<71.81%). In contrast, the lowest DO (193.22 days) was observed in the SFA group of 71.81%–78.05%. The differences between all groups were insignificant ($P > 0.05$). The parameter of II decreased with increasing SFA from 2.89 to 2.42, with significant differences (0.47, $P < 0.05$) between lowest SFA content and of SFA 71.81%–78.05% only. Generally, the cows with higher milk SFA contents were inseminated later but needed fewer inseminations to conceive.

Milk MUFA contents were associated with reproductive parameters in an opposite manner compared to SFA (Table 2). The parameter of CFI significantly ($P < 0.01$) decreased (from 117.35 to 98.60 days) with increasing MUFA contents in milk. DO tended to increase with MUFA contents from 192.26 to 219.80 days. No significant differences in DO were detected between MUFA content groups ($P > 0.05$). The values of II increased with increasing MUFA contents from 2.37 to 2.84. However, the differences between groups were insignificant ($P > 0.05$).

Table 1. Correlations between selected NEB indicators and reproductive parameters in Holstein cows.

		DM	F	P	F/P ratio	SFA	MUFA	PUFA	CFI	DO	II
BCS	r	0.164	0.067	0.304	–0.075	–0.259	0.249	0.239	0.067	0.107	0.065
	P	0.079	0.475	0.001	0.422	0.005	0.007	0.010	0.508	0.352	0.571
DM	r		0.923	0.311	0.744	–0.171	0.160	0.179	0.034	–0.035	–0.069
	P		<0.001	<0.001	<0.001	<0.001	0.001	<0.001	0.525	0.5712	0.259
F	r			0.137	0.912	–0.285	0.276	0.234	–0.034	0.012	–0.048
	P			0.006	<0.001	<0.001	<0.001	<0.001	0.526	0.851	0.434
P	r				–0.270	0.125	–0.126	–0.068	–0.054	–0.218	–0.149
	P				<0.001	0.012	0.011	0.171	0.315	<0.001	0.014
F/P ratio	r					–0.326	0.319	0.238	–0.005	0.086	0.001
	P					<0.001	<0.001	<0.001	0.923	0.161	0.988
SFA	r						–0.995	–0.643	0.275	–0.130	–0.149
	P						<0.001	<0.001	<0.001	0.033	0.014
MUFA	r							0.559	–0.279	0.136	0.154
	P							<0.001	<0.001	0.025	0.012
PUFA	r								–0.141	0.045	0.067
	P								0.008	0.464	0.271
CFI	r									0.168	0.006
	P									0.005	0.920
DO	r										0.929
	P										<0.001

BCS = body condition score; F = % content of fat in milk; P = % content of protein in milk; DM = % content of dry matter in milk; F/P = fat to protein ratio in milk; SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; CFI = calving to first insemination interval; DO = days open; II = insemination index.

Table 2. The effect of NEB indicators on reproductive performance in Holstein cows.

	Group description	CFI	DO	II
		LSM ± SE	LSM ± SE	LSM ± SE
Parity	Primiparous	112.29 ± 2.084	235.42 ± 8.908^{A}	3.17 ± 0.122^{A}
	Multiparous	107.01 ± 1.767	172.94 ± 6.763^{B}	1.99 ± 0.093^{B}
SFA	<71.78%	97.34 ± 2.695^{A}	222.46 ± 11.454	2.89 ± 0.157^{a}
	71.78%–78%	113.40 ± 2.098^{B}	193.22 ± 8.563	2.42 ± 0.117^{b}
	>78%	118.20 ± 2.175^{B}	196.85 ± 9.177	2.42 ± 0.126
MUFA	<18.85%	117.35 ± 2.123^{A}	192.26 ± 8.847	2.37 ± 0.121
	18.85%–24.59%	113.37 ± 2.257^{C}	199.86 ± 9.149	2.51 ± 0.125
	>24.59%	98.60 ± 2.657^{B,D}	219.80 ± 11.154	2.84 ± 0.153
PUFA	<3.01%	116.37 ± 2.327^{a}	206.24 ± 9.309	2.53 ± 0.129
	3.01%–3.79%	109.05 ± 2.184	185.36 ± 8.688^{a}	2.39 ± 0.120
	>3.79%	107.12 ± 2.571^{b}	217.35 ± 10.012^{b}	2.74 ± 0.138

CFI = calving to first insemination interval; DO = days open; II = insemination index; SFA = saturated fatty acid; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; $^{A, B, C...}$ = $P < 0.01$; $^{a, b}$ = $P < 0.05$.

The effects of PUFA contents on reproductive parameters are also summarized in Table 2. The highest (116.37 days) and lowest (107.12 days) values of CFI were determined in the groups of <3.01% and >3.79% PUFA, respectively. Significant difference ($P < 0.05$) in CFI was found between the groups with the lowest and the highest content of PUFA. The relationship between PUFA content and DO had no clear tendency. The highest (217.35 days) and lowest (185.36 days) DO values were identified in the groups of >3.01% and 3.01%–3.79% PUFA, respectively. This difference was significant ($P < 0.05$). On the contrary, II had a clear tendency. The highest II (2.74) was found in the group with PUFA of >3.79%. The lowest II (2.39) was detected in the group with PUFA in the range of 3.01%–3.79%. However, no significant differences were detected between PUFA groups ($P > 0.05$). Therefore, cows with low PUFA should achieve improved reproductive parameters, as was confirmed for II in our study.

4. Discussion

The contents of milk constituents (F, P) determined in early lactation in our study corresponded to those reported previously in Holstein cows (23). The absolute change of BCS in the initial 12 weeks of lactation also generally corresponded to the results of Maršálek et al. (24), who, however, observed permanently decreasing BCS from 3.59 at calving to 2.43 in the sixth month of lactation. In our study, the reduction of BCS occurred until the third month instead of the fourth month of lactation as reported by Maršálek et al. (24).

F and P contents and development in the first part of lactation can be used as energy balance indicators, as previously reported (25). The development of F and P contents in our study were confirmed by Sojková et al. (26), who described the influence of milk production and lactation stage on F contents (26). Similar P contents, as in our study, were reported previously (23). Our results agreed with those of Čejna and Chládek (25), who observed decreasing F and slightly increasing P contents in early lactation.

Frequent correlations were found among NEB indicators mutually and among NEB indicators and reproductive parameters. These correlations among indicators of NEB mutually were confirmed, for example, in the study published by Doležalová et al. (4). The correlations between FA contents and reproduction results were reported also by Petit (27).

The results for effect of parity are in disagreement with those of Ježková et al. (13), who observed similar tendencies but lower average values of all reproductive parameters. Compared to the national milk recording data (28) for CFI (80.5 days) and DO (121 days), considerably higher values were observed in our study. Similarly to our study, higher II for cows in the 1st and 2nd lactations were observed by Ježková et al. (13). However, mean values found in that study profoundly differed from our results.

Most of the earlier studies focused on the relationship between cows' FA intake and their reproductive performance or health (27). The increase of SFA content in later stages of lactation was associated with the recovery of animals from NEB. Thus, the group with higher SFA content should exhibit the best reproductive performance. These assumptions were confirmed for II and partly for DO in our study. However, a different tendency was described for the parameter of CFI. The cows with higher SFA contents had later signs of estrus. Conversely, the animals with low SFA content in early lactation suffered from severe NEB and had difficulty to come to heat. It reduced the CFI in the group with low SFA contents. It is concluded that high SFA contents were associated with better reproductive performance. The content of SFA probably had no influence on the onset of ovulation activity, but it may have affected the quality and size of ovulated oocytes (27). However, a longer period between calving and first insemination indicates a prolonged energy deficiency (16).

Bastin et al. (18) determined genetic correlations between milk FA and DO. They found a positive correlation between milk UFA contents and DO, which was generally in agreement with our results. A number of authors (26,29) reported that high contents of oleic acid and UFA in early lactation were associated with intensive NEB. As for SFA, our results confirmed those of Bastin et al. (18), who found positive correlations between milk MUFA contents and the period of DO. The contents of UFA and MUFA continually decreased with advancing lactation as the severity of NEB was reduced. Therefore, it is suggested that cows with lower MUFA contents suffered a minor NEB. Animals with lower MUFA contents were also characterized by lower II and DO values. In addition, the variation of MUFA compared to SFA in early lactation was considerably greater, also as a consequence of the recovery from NEB (29). On the contrary, animals with low MUFA contents exhibited signs of estrus later and thus had longer CFI compared to the animals with high milk MUFA. It is concluded that the effect of MUFA on reproductive performance was quite opposite that of SFA. The cows with low MUFA contents were inseminated later after calving but needed fewer inseminations to conceive.

Different effects of PUFA and MUFA contents can be explained by a markedly lower PUFA content in milk (9). The variation in PUFA contents was considerably larger due to individual cow effects (29). The content of PUFA decreased with advancing lactation (26) as a consequence of the recovery from NEB.

Our results confirmed the conclusions of Stoop et al. (30) that FA contents may be good indicators of NEB

and thus reliable predictors of reproductive parameters. In particular, the percentage contents of MUFA as well as SFAs can be employed as reproductive performance predictors. On the other hand, it is concluded that PUFA contents were not reliable indicators of NEB and had considerably lower influence on reproduction and health parameters compared to the other FA groups.

In conclusion, based on the traits observed, NEB occurred in Holstein cows in the first weeks of lactation. The average milk yields increased until week 8, whereas BCS decreased until the 3rd month of lactation. The reduction of BCS was associated with changes in milk FA groups and deteriorated reproductive performance. It was confirmed by the analysis of relationships between NEB indicators and reproductive parameters. SFA and MUFA contents were especially significantly correlated ($P < 0.001–0.05$) with all the reproductive performance parameters observed (CFI, DO, and II). PUFA contents were significantly correlated ($P < 0.01$) only with CFI ($r = –0.141$). The effect of FA groups on reproduction was further confirmed using ANOVA. High SFA contents evidencing only mild NEB were associated with improved values of DO and II. On the contrary, high MUFA contents in milk were associated with increasing DO and II. Similarly to MUFA, high PUFA contents tended to be associated with increased II, but the differences lacked statistical significance ($P > 0.05$). It was concluded that relationships were found between changes in milk FA contents (especially SFA and MUFA) in early lactation, which can be used as NEB indicators, and subsequent reproductive performance. The practical importance of this study lies in the possibility of determining milk FA contents in the first weeks after calving as a preselection criterion for cows with potential reproduction problems. Special care should then be given to these cows by herd managers.

Acknowledgment
This research was funded by an "S" grant of MSMT CR, by Projects NAZV QI91A061 and NAZV QH91270.

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