

Effects of lactation number, milk yield and milk composition on freezing point of milk of Polish Holstein-Friesian cows

Wpływ numeru laktacji, wydajności i składników mleka na temperaturę zamrażania mleka krów rasy polskiej holsztyńsko-fryzyjskiej

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Abstract

The objective of the study was to assess the influence of lactation number, daily milk yield, somatic cell count and milk composition on the freezing point of milk of Polish Holstein-Friesian cows. The data comprised 3,067,343 test day milk samples collected in 2014 from 865,198 first seven lactations of 714,018 Polish Holstein-Friesian cows, made available by the Polish Federation of Cattle Breeders and Dairy Farmers. The cows calved in 20,043 herds in 2013 and 2014. Four lactation classes were created: first, second, third, and fourth to seventh (≥ 4 class). Analysis of variance using the MIXED procedure in SAS was applied to milk freezing point (MFP). The following factors were included in the analysis: lactation class, daily milk yield (≤ 16 kg, 16.1–24 kg, > 24 kg), fat content ($< 3.6\%$, 3.6–4.4%, $> 4.4\%$), protein content ($< 3.1\%$, 3.1–3.5%, $> 3.5\%$), urea content (≤ 150 mg·L⁻¹, 151–269 mg·L⁻¹, ≥ 270 mg·L⁻¹), lactose content ($< 4.75\%$, 4.75–5%, $> 5\%$), somatic cell count ($< 400,000$, $\geq 400,000$) and dry matter in milk ($\leq 12.5\%$, 12.6–13.4% and $\geq 13.5\%$), with interactions between lactation and each of the factors mentioned above. MFP decreased with increasing daily milk yield, protein, urea, lactose content and dry matter content, while MFP slightly increased with increasing fat content and somatic cell count. The differences in means of MFP between daily milk yield classes, fat, protein, urea and lactose content, dry matter and somatic cell count were highly significant ($P < 0.001$).

Keywords: Holstein-Friesian cows, milk constituents, milk freezing point, milk yield, somatic cell count

Streszczenie

Celem pracy było oszacowanie wpływu numeru laktacji, dziennej wydajności oraz składników mleka na temperaturę zamrażania mleka krów rasy polskiej holsztyńsko-fryzyjskiej. Dane obejmowały 3 067 343 próbnich udojów pochodzących z 865 198 pierwszych siedmiu laktacji 714 018 krów. Dane zostały udostępnione przez Polską Federację Hodowców Bydła i Producentów Mleka. Krowy cielżyły się w 20 043 stadach w latach 2013-2014. Laktacje pogrupowano w cztery klasy: pierwsze, drugie, trzecie i czwarte do siódmych (klasa ≥ 4). Do obliczeń użyto procedury MIXED z pakietu statystycznego SAS. Przeprowadzono wieloczynnikową analizę wariancji według modelu liniowego, w którym poza klasą laktacji uwzględniono dzienną wydajność mleka (≤ 16 kg, 16,1–24 kg, > 24 kg), zawartość tłuszczu ($< 3,6\%$, 3,6–4,4%, $> 4,4\%$), zawartość białka ($< 3,1\%$, 3,1–3,5%, $> 3,5\%$), zawartość mocznika (≤ 150 mg·L⁻¹, 151–269 mg·L⁻¹, ≥ 270 mg·L⁻¹), zawartość laktozy ($< 4,75\%$, 4,75–5%, $> 5\%$), liczbę komórek somatycznych (< 400 000, ≥ 400 000) oraz zawartość suchej masy w mleku ($\leq 12,5\%$, 12,6–13,4%, $\geq 13,5\%$). W modelu uwzględniono interakcje między klasą laktacji i pozostałymi czynnikami. Temperatura zamrażania mleka (TZM) malała wraz ze wzrostem dziennej wydajności mleka, zawartości białka, mocznika, laktozy i suchej masy, natomiast TZM nieznacznie podwyższała się gdy rosła zawartość tłuszczu oraz liczba komórek somatycznych w mleku. Stwierdzono wysoce istotny wpływ ($P < 0.001$) wszystkich badanych czynników na temperaturę zamrażania mleka.

Słowa kluczowe: dzienna wydajność mleka, krowy rasy polskiej holsztyńsko-fryzyjskiej, liczba komórek somatycznych, składniki mleka, temperatura zamrażania mleka

Introduction

The freezing point of milk (MFP) is relatively constant. Depending on the country, reported mean values of MFP vary between -0.521 °C and -0.539 °C, with -0.521 °C reported in the Netherlands (Slaghuis, 2001) and -0.539 °C in the United Kingdom (Zagorska and Ciprovica, 2013). In Poland a mean MFP of -0.533 °C was noted (Otwinowska-Mindur et al., 2017). MFP is determined by the osmolarity of the milk: that is, the concentration of water-soluble constituents (Bjerg et al., 2005). Cow's milk typically consists of approximately 87% water and 13% total solids, and changes when the amount of water present in milk varies. Milk with extraneous water has a higher freezing point than raw milk (Kedzierska-Matysek et al., 2011). The freezing point of milk can be used to verify the quality of raw milk, because added water is the most significant factor affecting MFP. Water can enter milk from milking machines or as the result of negligence or bad milking practices (Slaghuis, 2001; Hanus et al., 2010). Automated milking systems can increase the milk freezing point, so monitoring of MFP may be useful to farmers and technicians when trying to determine the efficiency of milking and cleaning systems (Sanchez et al., 2007).

The milk freezing point is affected not only by the presence of extraneous water but also by the milk constituents. Lactose and chlorides play the most important role in depression of MFP, as they are responsible for 75–80% of the MFP value. The

remaining 20–25% of the MFP value is affected by other milk constituents: calcium, magnesium, lactates, phosphates, citrates and urea (Navratilova et al., 2006). Brouwer (1981) reported that up to 53.8% of MFP depression depended on lactose content, and observed that MFP depression was related to some extent with potassium (by 12.7%), chloride (by 10.5%), sodium (by 7.2%) and citrates (by 4.3%). An increase of protein and urea content in milk also resulted in decreased MFP (Henno et al., 2008; Kedzierska-Matysek et al., 2011; Senevirathne et al., 2016). Brzozowski and Zdziarski (2006) and Hanus et al. (2010) indicated a dependence of MFP on daily milk yield. The latter authors concluded that MFP increased with increasing daily milk yield.

The objective of the present study was to assess the influence of lactation number, daily milk yield, somatic cell count and milk composition on the freezing point of milk of Polish Holstein-Friesian cows.

Material and methods

The data comprised 3,067,343 test day (TD) milk samples collected in 2014 from 865,198 first seven lactations of 714,018 Polish Holstein-Friesian cows, made available by the Polish Federation of Cattle Breeders and Dairy Farmers. The study analyzed milk freezing point (MFP), which was measured, like the content of milk constituents were, using a MilkoScanTM FT+ automatic milk analyzer (FOSS, Hillerod, Denmark). Cows calved in 20,043 herds in 2013 and 2014. The number of test days per cow per lactation ranged up to 12. Average herd size was 36 cows (standard deviation: 55), with the number of cows per herd ranging from 1 to 1,338.

Four lactation classes were created: first, second, third, and fourth to seventh (≥ 4 class). The number of lactations and number of TD milk samples, together with characteristics of daily milk yield, fat, protein, urea, lactose and dry matter content and somatic cell count in each lactation class are presented in Table 1. Daily milk yield was divided into three classes: (1) ≤ 16 kg, (2) 16.1–24 kg and (3) > 24 kg. Three classes were created for each milk constituent: fat content – (1) $< 3.6\%$, (2) 3.6–4.4% and (3) $> 4.4\%$; protein content – (1) $< 3.1\%$, (2) 3.1–3.5% and (3) $> 3.5\%$; urea content – (1) $\leq 150 \text{ mg}\cdot\text{L}^{-1}$, (2) 151–269 $\text{mg}\cdot\text{L}^{-1}$ and (3) $\geq 270 \text{ mg}\cdot\text{L}^{-1}$; and lactose content – (1) $< 4.75\%$, (2) 4.75–5% and (3) $> 5\%$. Somatic cell count was divided into two classes: (1) $< 400,000$ and (2) $\geq 400,000$ in 1 mL, following the Polish standard for raw milk quality. Dry matter in milk was also divided into three classes: (1) $\leq 12.5\%$, (2) 12.6–13.4% and (3) $\geq 13.5\%$.

Table 1. Description of the data set

Item	Lactation class			
	1	2	3	≥4
Number of lactations	284,776	214,608	155,107	210,707
Number of test-day records	1,052,478	734,71	537,286	742,869
Daily milk yield [kg]				
Mean	24.33	26.83	27.35	25.68
SD ¹⁾	7.62	9.68	9.96	9.53
Fat content [%]				
Mean	4.05	4.11	4.16	4.21
SD ¹⁾	0.84	0.9	0.93	0.93
Protein content [%]				
Mean	3.23	3.32	3.29	3.29
SD ¹⁾	0.35	0.38	0.38	0.39
Urea content [mg·L ⁻¹]				
Mean	215.47	210.26	205.36	200.66
SD ¹⁾	87.72	89.7	89.44	90.11
Lactose content [%]				
Mean	4.88	4.77	4.73	4.67
SD ¹⁾	0.21	0.23	0.25	0.27
Somatic cell count				
Mean	399,383	558,353	683,088	871,927
SD ¹⁾	1,120	1,360	1,521	1,783
Dry matter [%]				
Mean	12.79	12.84	12.84	12.84
SD ¹⁾	0.96	1.05	1.07	1.08

¹⁾ SD – standard deviation

The following linear model was fitted using the MIXED procedure in SAS (2014):

$$Y_{ijklmnop} = \mu + L_i + MY_j + F_k + P_l + U_m + LA_n + SCC_o + DM_p + (L \times MY)_{ij} + (L \times F)_{ik} + (L \times P)_{il} + (L \times U)_{im} + (L \times LA)_{in} + (L \times SCC)_{io} + (L \times DM)_{ip} + \varepsilon_{ijklmnop}$$

where:

$Y_{ijklmnop}$ – milk freezing point,

μ – overall mean,

L_i – effect of i-th lactation class ($i = 1, 2, 3, 4$),

MY_j – effect of j-th daily milk yield class ($j = 1, 2, 3$),

F_k – effect of k-th fat content class ($k = 1, 2, 3$),
 P_l – effect of l-th protein content class ($l = 1, 2, 3$),
 U_m – effect of m-th urea content class ($m = 1, 2, 3$),
 LA_n – effect of n-th lactose content class ($n = 1, 2, 3$),
 SCC_o – effect of o-th somatic cell count class ($o = 1, 2$),
 DM_p – effect of p-th dry matter class ($p = 1, 2, 3$),
 $(L \times MY)_{ij}$ – effect of interaction between lactation and daily milk yield classes,
 $(L \times F)_{ik}$ – effect of interaction between lactation and fat content classes,
 $(L \times P)_{il}$ – effect of interaction between lactation and protein content classes,
 $(L \times U)_{im}$ – effect of interaction between lactation and urea content classes,
 $(L \times LA)_{in}$ – effect of interaction between lactation and lactose content classes,
 $(L \times SCC)_{io}$ – effect of interaction between lactation and somatic cell count classes,
 $(L \times DM)_{ip}$ – effect of interaction between lactation and dry matter classes,
 $\epsilon_{ijklmnopr}$ – residual effect.

The significance of differences between least squares means (LSMEAN) was determined by the Tukey–Kramer test. The correlations between all analyzed factors were calculated using the CORR procedure in SAS (2014).

Results

Table 2 presents the effect of lactation number on milk freezing point. MFP increased with lactation number, being lowest in the first lactation (LSMEAN = -0.5351 °C) and highest in fourth and later (≥ 4 class) lactations (LSMEAN = -0.5329 °C). The differences between means of MFP in each pair of lactations (first, second, third and ≥ 4 classes) were highly significant ($P < 0.001$).

Table 2. Effect of lactation number on milk freezing point

Lactation class	Milk freezing point [°C]	
	LSMEAN ¹⁾	SE ²⁾
1	-0.5351 ^A	0.000012
2	-0.534 ^B	0.000015
3	-0.5334 ^C	0.000018
≥ 4	-0.5329 ^D	0.000016

¹⁾ LSMEAN – least squares mean

²⁾ SE – standard error

A, B, C, D values bearing different letters differ highly significantly ($P < 0.001$).

The effect of daily milk yield, milk constituents and somatic cell count (SCC) on MFP are presented in Table 3. MFP decreased with increasing daily milk yield. When daily milk yield changed from less than 16 kg to more than 24 kg, the LSMEAN of MFP decreased about 0.001 °C from -0.5333 °C to -0.5343 °C. MFP increased with increasing fat content in milk: the lowest MFP (-0.5341 °C) was observed for milk with fat content less than 3.6%, and highest (-0.5335 °C) for milk with the highest fat content (above 4.4%). The trend of changes of MFP in subsequent protein content classes was opposite to that for fat content classes, indicating that an increase of protein content from less than 3.1% to more than 3.5% was connected with a decrease of MFP of about 0.0035 °C (from -0.5321 °C to -0.5356 °C).

The effect of urea content class on MFP was very similar to the effect of protein content: MFP decreased with increasing urea concentration from -0.5321 °C in the first class (urea content ≤ 150 mg·L⁻¹) to -0.5357 °C in the third class (urea content ≥ 270 mg·L⁻¹). MFP decreased from -0.5271 °C for milk with lactose content less than 4.75% to -0.5403 °C for milk with more than 5% lactose.

SCC of up to 400,000 is allowed in raw bovine milk. MFP slightly increased with increasing SCC (from -0.534 °C to -0.5337 °C). Increased dry matter in milk caused more conspicuous changes in MFP. The highest MFP (-0.5314 °C) was associated with the lowest dry matter (up to 12.5%), and the lowest MFP (-0.5366 °C) with dry matter above 13.5%. For each factor included in the analysis the differences in MFP were highly significant ($P < 0.001$). It is worth noting that the differences between lactose content classes were at least three times higher than those between urea or protein classes. All interactions – that is, between lactation and daily milk yield, each of the milk components, and SCC – were also highly significant ($P < 0.001$), indicating different courses of changes in MFP for different daily milk yield or milk components or SCC classes in consecutive lactations.

The correlations between MFP and daily milk yield, milk constituents and somatic cell count are presented in Table 4. All correlations were highly significant ($P < 0.001$). The relationship between MFP and other factors was low or moderate and in most cases negative. Only the correlations between MFP and SCC were positive but low (from 0.08 to 0.17). Milk yield was the factor most weakly correlated with MFP (from -0.1 to -0.06), and lactose content the one most strongly correlated with MFP (from -0.58 to -0.51). The correlations between MFP and the majority of other factors (daily milk yield, fat content, lactose content, SCC) trended slightly upward with lactation number. Only the correlations of MFP with protein content trended lower with lactation number.

Table 3. Effects of daily milk yield, fat, protein, urea and lactose content, somatic cell count and dry matter content on milk freezing point

Item	Number of test-day records	Milk freezing point [°C]	
		LSMEAN ¹⁾	SE ²⁾
Daily milk yield [kg]			
≤16	420,090	-0.5333 ^A	0.000014
16.1–24	1,000,579	-0.5339 ^B	0.00001
>24	1,646,674	-0.5343 ^C	0.000008
Fat content [%]			
<3.6	828,338	-0.5341 ^A	0.000015
3.6–4.4	1,224,884	-0.5339 ^B	0.000011
>4.4	1,014,121	-0.5335 ^C	0.000013
Protein content [%]			
<3.1	1,014,673	-0.5321 ^A	0.000012
3.1–3.5	1,240,978	-0.5339 ^B	0.000009
>3.5	811,692	-0.5356 ^C	0.000011
Urea content [mg·L⁻¹]			
≤150	825,675	-0.5321 ^A	0.00001
151–269	1,525,895	-0.5338 ^B	0.000009
≥270	715,773	-0.5357 ^C	0.000012
Lactose content [%]			
<4.75	1,220,980	-0.5271 ^A	0.000008
4.75–5	1,377,774	-0.5342 ^B	0.000009
>5	468,589	-0.5403 ^C	0.000016
Somatic cell count			
<400,000	2,221,582	-0.534 ^A	0.000007
≥400,000	845,761	-0.5337 ^B	0.000011
Dry matter [%]			
≤12.5	1,217,952	-0.5314 ^A	0.000013
12.6–13.4	1,132,493	-0.5336 ^B	0.000011
≥13.5	716,898	-0.5366 ^C	0.000017

¹⁾ LSMEAN – least squares mean

²⁾ SE – standard error

A, B, C values that differ highly significantly ($P < 0.001$) for a given factor bear different letters

Table 4. Correlations¹⁾ between milk freezing point (MFP) and daily milk yield (MY), fat (F), protein (P), urea (U) and lactose (LA) concentrations, somatic cell count (SCC) and dry matter (DM) content, by lactation class

Lactation class	MFP-MY	MFP-F	MFP-P	MFP-U	MFP-LA	MFP-SCC	MFP-DM
1	-0.07	-0.12	-0.23	-0.16	-0.51	0.08	-0.26
2	-0.06	-0.13	-0.19	-0.15	-0.54	0.12	-0.25
3	-0.08	-0.15	-0.16	-0.14	-0.55	0.14	-0.27
≥4	-0.1	-0.16	-0.13	-0.15	-0.58	0.17	-0.28
Total	-0.06	-0.12	-0.16	-0.16	-0.57	0.15	-0.25

¹⁾ All correlations highly significant ($P < 0.001$).

Discussion

The milk freezing point increased with lactation number in the Polish Holstein-Friesian population; the effect of lactation number on MFP was highly significant. Kedzierska-Matysek et al. (2011), Senevirathne et al. (2016) and Otwinowska-Mindur et al. (2017) also reported significant effects of lactation number on MFP. The first-mentioned authors found MFP to be lowest in first lactations (-0.539 °C) and highest for milk from very old cows, that is, in eleventh lactations (-0.525 °C), and concluded that cow age and production level were associated with an increase of MFP. Senevirathne et al. (2016) showed a significant effect ($P < 0.05$) of lactation class on MFP. They noted lower MFP in the first three lactations (-0.5318 °C) and higher MFP in later (4–8) lactations (-0.5216 °C).

A highly significant effect of daily milk yield on MFP was found, with MFP decreasing with increasing daily milk yield. Brzozowski and Zdziarki (2006) reported fluctuations in MFP dependent on daily milk yield class. They observed lowest MFP (-0.5334 °C) when daily milk yield was higher than 24 kg, and highest MFP (-0.5328 °C) when daily milk yield was low (8.1–16 kg). They also reported a significant effect ($P < 0.01$) of daily milk yield class on MFP. Hanus et al. (2010) came to different conclusion: in their work, MFP increased with increasing daily milk yield, and they obtained a closer (positive) dependence between those two factors ($r = 0.4$) in both Holstein and Czech Fleckvieh populations.

MFP fell with increasing fat content in milk, and the effect of fat content class on MFP was highly significant. Brzozowski and Zdziarski (2005) also found that MFP decreased with the increase of fat content. They calculated a similar correlation between MFP and fat content ($r = -0.113$). Hanus et al. (2010) determined a slightly higher correlation between MFP and fat content in Holsteins ($r = -0.163$) and a substantially higher one in Czech Fleckvieh ($r = -0.458$) populations. In a comparison of the freezing points of raw skimmed milk and raw milk, Zagorska and Ciprovica (2013) found the lowest MFP (-0.533 °C) in milk with higher fat content (4.21%). Senevirathne et al. (2016) reported the opposite trend of changes: MFP increased with increasing fat content. In that work the effect of fat content class on MFP was not significant.

Similarly to fat percentage, MFP decreased with increasing protein content in milk, and the effect of this factor on MFP was highly significant, what is consistent with the findings of Kedzierska-Matysek et al. (2011), Senevirathne et al. (2016) and Ule et al. (2016). Senevirathne et al. (2016) reported a significant effect ($P < 0.05$) of protein content class on MFP. Ule et al. (2016) observed that MFP decreased by $0.00069\text{ }^{\circ}\text{C}$ when protein content increased by 0.1% . Importantly, those authors used not individual but bulk tank samples. Brzozowski and Zdziarski (2005) confirmed that an increase of protein content in milk caused a decrease of MFP, and obtained a similar correlation between milk freezing point and protein content in milk ($r = -0.165$).

A highly significant effect of urea content class on MFP was observed. As with fat and protein content, increased urea content in milk was associated with decreased MFP. Henno et al. (2008) found that an increase of milk urea content by $20\text{ mg}\cdot\text{L}^{-1}$ resulted in a decrease of MFP by $0.0004\text{ }^{\circ}\text{C}$. Hanus et al. (2010) also found that high urea content could decrease MFP; the negative correlation they obtained between these two factors ($r = -0.08$) for milk from Holstein cows was slightly lower than those in Table 4.

Lactose as a major component of milk can be used to identify cows at risk of poor reproduction, whereas the ratio of fat to protein is used for estimation of the energy balance, especially in large dairy herds. Milk with high lactose content had lower MFP. Again, the differences in MFP between lactose content classes were highly significant. Brzozowski and Zdziarski (2005) noted a highly significant effect ($P < 0.01$) and Senevirathne et al. (2016) a significant effect ($P < 0.05$) of lactose content class on MFP. Both sets of authors reported that MFP decreased with increasing lactose content, consistent with results presented in this paper. The former found the lowest MFP ($-0.5368\text{ }^{\circ}\text{C}$) for milk with lactose content higher than 5% , and the highest MFP ($-0.5297\text{ }^{\circ}\text{C}$) for milk with lactose content lower than 4.5% . They also found a slightly weaker relation between MFP and lactose content ($r = -0.213$). In a study of goat's milk bulk tank samples, Sanchez et al. (2007) obtained a highly significant ($P < 0.0001$) effect of lactose content class on MFP, and stated that MFP depended on the concentration of water-soluble constituents in milk, among others lactose content, the factor that most affected variation of MFP. Macek et al. (2008) found that increased lactose content was associated with lower MFP in cows ($r = -0.35$) and higher MFP in sheep ($r = 0.39$). Hanus et al. (2010) reported the opposite tendency for the relations between MFP and lactose content in Holsteins ($r = 0.16$) and Czech Fleckviehs ($r = -0.35$).

Somatic cell count is the main indicator of subclinical mastitis but also is used to evaluate the hygienic quality of milk. Milk freezing point slightly increased with increasing somatic cell count. The differences in MFP between SCC classes were highly significant. Kedzierska-Matysek et al. (2011) observed that increased SCC caused a small decrease of MFP: from $-0.537\text{ }^{\circ}\text{C}$ when SCC was up to $400,000$ to $-0.538\text{ }^{\circ}\text{C}$ when SCC was over $400,000$. They did not find significant difference in MFP between SCC classes. Brzozowski and Zdziarski (2005) saw a similar tendency for the relation between MFP and SCC: MFP slightly increased when SCC increased. They calculated a lower correlation than shown in Table 4 between these two factors ($r = 0.035$). Macek et al. (2008) concluded that using SCC as an indicator of secretion disorders could reduce lactose content and consequently elevate sodium and potassium concentrations, which could improve (decrease) the milk freezing point.

Results presented in this paper are in agreement with findings from Hanus et al. (2010) that MFP could be improved by increasing SCC in milk from Holstein ($r=-0.36$) and Czech Fleckvieh ($r=-0.07$) cows.

The last analyzed factor, dry matter in milk, also highly significantly affected MFP, with MFP lowering with increasing dry matter. Hanus et al. (2010) observed that high dry matter in milk reduced the freezing point of milk from Holsteins ($r=-0.22$) and Czech Fleckviehs ($r=-0.5$).

Conclusions

Freezing point of milk for multiparous cows was higher than for primiparous cows. MFP decreased with increasing daily milk yield, protein, urea, lactose content and dry matter content whereas it slightly increased with increasing fat content and somatic cell count.

Almost no change in MFP was observed in case of two SCC classes. Desired tendency, i.e. decreasing MFP was observed when protein, urea, lactose and dry matter content increased. The change of MFP was similar (about 0.0036 °C) in case of first and last protein and urea classes, whereas the difference in MFP (0.0132 °C) was almost four times greater when lactose content increased from <4.75% to >5%. Results of this paper shows that deterioration MFP (increase) is related with reduction lactose content in milk, which is indicator of energy status, so it might be worth to use the MFP as an additional factor together with lactose content in determination of metabolic energy deficit in dairy cows.

Acknowledgments

The data were made available by the Polish Federation of Cattle Breeders and Dairy Farmers.

This research was financed by the Ministry of Science and Higher Education of the Republic of Poland (funds for statutory activity, DS 3254).

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