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FACTORS AFFECTING THE FREEZING POINT OF MILK FROM POLISH HOLSTEIN-FRIESIAN COWS*

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Abstract

The objective of this study was to estimate the influence of lactation number, month of milk sampling, lactation stage and herd size on the freezing point of milk of Polish Holstein-Friesian cows. Data comprised 4,719,787 milk samples from the first seven lactations of 752,770 Polish Holstein-Friesian cows. Milk freezing point (MFP), milk yield, and fat and protein content were analyzed. The mean MFP of milk samples (–0.5326ºC) as well as more than 92% of all milk samples did not exceed the quality limit for the freezing point of cows' raw milk, which, following Polish standards, was taken to be –0.52ºC. The freezing point of milk samples, milk yield, fat and protein content were highly significantly affected by all examined factors: month of sampling, successive lactations, stage of lactation, and herd size. MFP was lowest in milk samples taken from January to March, and highest in samples from November to December. MFP increased with lactation number. Mean MFP decreased with time within lactation, except the first stage (5–35 days in milk). Generally, MFP was highest in small herds (up to 9 cows) and lowest in large herds (more than 150 cows).

Key words: milk freezing point, Polish Holstein-Friesian cows

Milk freezing point (MFP) is an important indicator of milk quality. Depending on the country, mean values of MFP oscillated between –0.521ºC (Netherlands) and –0.539ºC (United Kingdom) (Zagorska and Ciprovica, 2013) whereas in other countries such as Denmark (Bjerg et al., 2005), Estonia (Henno et al., 2008), Czech Republic (Hanus et al., 2010), Romania (Sala et al., 2010) and Poland (Kedzierska-Matysek et al., 2011) MFP averages were between those two values. MFP is used mainly to determine whether water has been added to milk, that is, to verify the processes employed by milk producers. Water can penetrate into milk from milking machines as a necessary addition or can be the result of bad milking practices (Slaghuis, 2001; Hanus et al., 2010).

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The freezing point of milk is determined by the osmolarity of milk, that is, the concentration of water-soluble constituents (Bjerg et al., 2005). Milk typically consists of approximately 87% water and 13% total solids (Kedzierska-Matysek et al., 2011). As milk is more diluted then MFP will approach zero (Zagorska and Ciprovica, 2013). MFP is also affected by other factors which were related to various environments, management practices and breeds. Henno et al. (2008) and Kedzierska-Matysek et al. (2011) reported that breed significantly affected MFP; the results of the latter authors' comparison of four breeds of dairy cows showed the highest milk freezing point for Polish Holstein-Friesian cows (–0.537ºC), and the lowest for Polish Red cows (–0.543ºC). Henno et al. (2008) observed the highest MFP also in indigenous Holsteins (–0.5238ºC), and the lowest in the native Estonian (–0.5368ºC); they concluded that the breed effect on MFP might be due to the effect of breed differences between milk constituents, mainly content of protein. An effect of geographic region on MFP was found by Kedzierska-Matysek et al. (2011). Among the factors that influenced MFP are feed composition (Sala et al., 2010), water intake (Bjerg et al., 2005), and season of the calendar year (Bjerg et al., 2005; Brzozowski and Zdziarski, 2005; Janstova et al., 2006; Henno et al., 2008). Bjerg et al. (2005) and Brzozowski and Zdziarski (2005) observed that MFP increased during summer compared with winter, caused by increased water intake due to increased temperature and sunshine hours, and it was especially pronounced when cows were grazing without access to water. Brzozowski and Zdziarski (2006) and Kedzierska-Matysek et al. (2011) found that MFP fluctuated throughout successive lactations, that is, at different ages. Lactation stage (Brzozowski and Zdziarski, 2006; Henno et al., 2008; Sala et al., 2010; Kedzierska-Matysek et al., 2011) and herd size (Brzozowski and Zdziarski, 2005) were other factors affecting the milk freezing point. Results presented by Brzozowski and Zdziarski (2005) showed that the larger the herd size, the higher the MFP. Differences in MFP between morning and evening milkings have also been reported (Slaghuis, 2001). Some authors indicated a dependence of milk freezing point on daily milk yield (Brzozowski and Zdziarski, 2006; Hanus et al., 2010). Hanus et al. (2010) stated that MFP increased with increasing daily milk yield, whereas Brzozowski and Zdziarski (2006) observed the opposite tendency: MFP lowered with increasing milk yield. MFP is determined to a large degree by the composition of milk. Brouwer (1981) reported that lactose content was responsible for 53.8% of MFP depression. Additionally, Brouwer (1981) observed that MFP depression was related with, for example, potassium (by 12.7%), chloride (by 10.5%), sodium (by 7.2%) and citrates (by 4.3%). Henno et al. (2008) noted that an increase in urea content in milk by 20 mg/L resulted in a decrease in MFP of 0.0004ºC. Milk urea is one indicator of animal nutrition efficiency and, together with milk fat concentration and fat to protein ratio, indicates the energy balance during lactation. Brzozowski and Zdziarski (2006) found that MFP increased together with the increase of somatic cell count, which was the main indicator of mastitis.

Under the regulations of the Polish Ministry of Agriculture and Rural Development, the presence of additional water in raw milk is prohibited, and the freezing point of cows' raw milk cannot be higher than –0.52ºC (MARD, 2004). The objective of this study was to estimate the effects of lactation number, month of milk sampling, lactation stage and herd size on the freezing point of milk of Polish Holstein-Friesian cows.

Material and methods

The data comprised 4,719,787 milk samples collected in 2014 from the first seven lactations of 752,770 Polish Holstein-Friesian cows, and were made available by the Polish Federation of Cattle Breeders and Dairy Farmers. The study analyzed milk freezing point (MFP), milk yield, and fat and protein content in milk. MFP was measured using a MilkoScan™ automatic milk analyzer (FOSS, Hillerod, Denmark).

There were 1,614,834, 1,190,444, 827,726 and 1,086,783 test day (TD) milk samples from 313,919 first, 240,910 second, 170,345 third and 227,718 fourth and later lactations, respectively (Table 1). The cows calved in 20,057 herds in 2013 and 2014 and calvings were distributed regularly during the year from 41,613 in February to 101,362 in August. The average size of herds was 38 cows (standard deviation: 60), with the number of cows per herd being from 1 to 1,409. The data had been collected by three methods: A4 (28% of cows), AT4 (68% of cows) and A8 (4% of cows). A4 is a standard milk recording method in which TD records are collected twice a day (a.m. and p.m. tests) every four weeks. In the AT4 method, TD records are also collected every four weeks but alternately: a.m. or p.m. The A8 method involves performing tests twice a day (a.m. and p.m.) but less frequently: every eight weeks. Daily (24 h) milk yields are estimated using two yields (A4 and A8) or a single yield (AT4) (ICAR, 2014).

The herds were divided into six classes: up to 9 cows, 10–15, 16–25, 26–50, 51–150 and more than 150 cows. Days in milk (DIM) were grouped into 10 lactation stages, defined as 30-day intervals. Four lactation classes were created: first lactations, second lactations, third lactations, and fourth to seventh lactations (4+ class). The following linear model was fitted using the MIXED procedure in SAS (SAS, 2014):

$$Y_{ijklm} = \mu + L_i + H_j + LS_k + M_l + (L \times H)_{ij} + (L \times LS)_{ik} + (L \times M)_{il} + E_{ijklm}$$

where:

Y_{ijklm} – milk freezing point, milk yield, fat content or protein content,
μ – overall mean,
L_i – effect of *i*-th lactation class ($i = 1,\ldots, 4$),
H_j – effect of *j*-th herd size class ($j = 1,\ldots, 6$),
LS_k – effect of *k*-th lactation stage ($k = 1,\ldots, 10$),
M_l – effect of *l*-th month of year ($l = 1,\ldots,12$),
$(L{\times}H)_{ij}$ – effect of interaction between lactation and herd size classes,
$(L{\times}LS)_{ik}$ – effect of interaction between lactation class and lactation stage,
$(L{\times}M)_{il}$ – effect of interaction between lactation class and month of year,
E_{ijklm} – residual effect.

The significance of differences between least square means was determined by the Tukey-Kramer test. The correlations between all analyzed traits (MFP, milk yield, and content of fat and protein), and their significance were calculated using CORR procedure in SAS (SAS, 2014).

Results

The daily average of milk yield was 26 kg milk, and 4.096% fat and 3.336% protein content. The mean freezing point of the milk samples was –0.5326ºC.

Table 1 presents the least square means (LSMEAN) and standard errors (SE) of the analyzed traits by lactation. Means of MFP varied among lactations but within each group of lactations they did not exceed the Polish quality limit, –0.52ºC. Average MFP was lowest in the first lactation (LSMEAN=–0.5347ºC) and highest in fourth and later (4+) lactations (LSMEAN=–0.5296ºC). Milk samples with MFP higher than the limit (–0.52ºC) were more frequent in later lactations (12.8%) than in first lactations (about 4.2%), with less than 8% of all records. The first, second, third and 4+ lactations showed highly significant differences (P<0.001) for each trait: MFP, milk yield, and fat and protein content in milk (Table 1).

Table 2 presents the correlations between MFP and the other traits (milk yield, and fat and protein concentrations). All correlations were low, negative but highly significant (P<0.001). The correlations between MFP and milk yield were lowest (from –0.021 to –0.067), especially in second lactations. The correlations of MFP with fat content were higher than with milk yield but lower than with protein content and they increased a little with lactation number (from –0.144 in first to –0.168 in 4+ lactations). MFP and protein content showed the highest correlations (from –0.191 to –0.271), which were strongest in first lactations and weakest in 4+ lactations.

Table 3 gives the LSMEAN and standard errors of MFP, milk yield, and percentages of fat and protein obtained in each month of the year. LSMEAN of MFP was lowest in January and March (–0.5352ºC) and highest in December (–0.5281ºC). The percentages of samples with MFP exceeding the quality limit (–0.52ºC) followed the similar pattern: lowest (3.61%) in March and highest (14%) in December. In most cases the differences in mean MFP between months were highly significant (P<0.001); the only exceptions were two pairs of months: January–March and May–June. Highly significant between-month differences (P<0.001) in milk yield, fat and protein content were also observed. For MFP, milk yield, fat and protein content, highly significant interactions (P<0.001) between lactation class and month of the year were observed. It meant that simultaneous influence of both lactation class and month of the year was not additive. A similar trend for seasonal variation of milk freezing point was found when lactations were analyzed separately (Figure 1). MFP was lowest for milk sampled from January to March, and highest at the end of the year. The highest and the most marked difference in MFP was between March and April (about 0.0041ºC). Within each month, MFP increased with lactation number. The difference between lactation class 4+ and first lactation varied in consecutive months of the year from 0.0046ºC in July to 0.0057ºC in November.

Table 1. Number of lactations, milk samples, and least square means (LSMEAN) with standard errors (SE) for milk freezing point (MFP), milk yield, fat content and protein content, by lactation

Lactation no.	Number of		MFP (°C)		No. of samples with MFP>–0.52°C (%)	Milk yield (kg)		Fat content (%)		Protein content (%)	
	lactations	milk samples	LSMEAN	SE		LSMEAN	SE	LSMEAN	SE	LSMEAN	SE
1	313,919	1,614,834	–0.5347 A	0.000010	4.19	22.18 A	0.0081	4.078 A	0.0009	3.281 A	0.00035
2	240,910	1,190,444	–0.5324 B	0.000011	6.64	24.63 B	0.0088	4.138 B	0.0010	3.368 B	0.00038
3	170,345	827,726	–0.5310 C	0.000012	8.98	25.47 C	0.0098	4.166 C	0.0011	3.337 C	0.00043
4+	227,718	1,086,783	–0.5296 D	0.000011	12.81	24.37 D	0.0087	4.199 D	0.0010	3.334 D	0.00038

A, B, C, D – values within the same column marked by different letters differ highly significantly (P<0.001).

Table 2. Correlations[1)] of milk freezing point (MFP) and milk yield (MY), fat content (F%) and protein content (P%), by lactation

Lactation no.	MFP – MY	MFP – F%	MFP – P%
1	–0.049	–0.144	–0.271
2	–0.021	–0.152	–0.254
3	–0.040	–0.162	–0.219
4+	–0.067	–0.168	–0.191
Total	–0.026	–0.137	–0.222

[1)] All correlations highly significant (P<0.001).

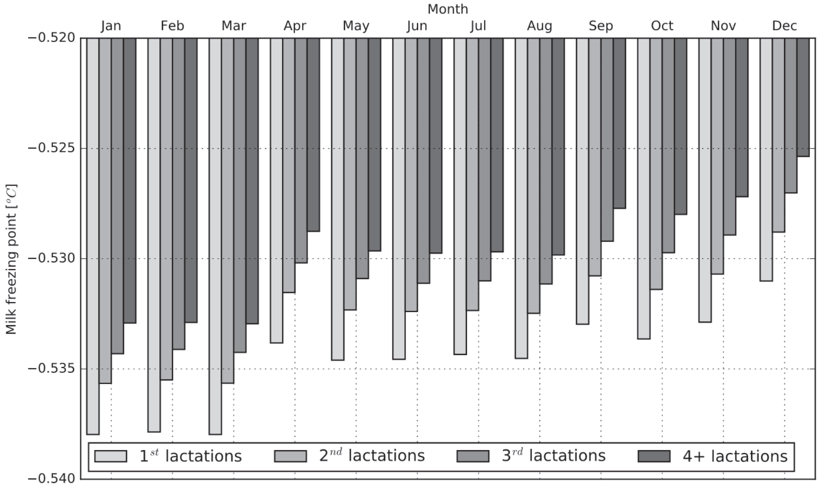

Figure 1. Effect of month of year on milk freezing point (LSMEAN, 0.00002180≤SE≤0.00004148)

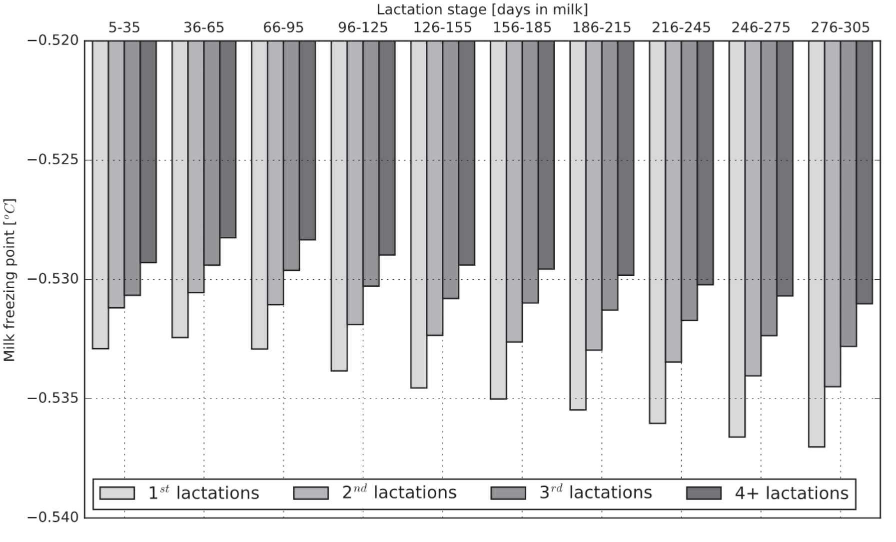

Figure 2. Effect of lactation stage on milk freezing point (LSMEAN, 0.00002187≤SE≤0.00003946)

Table 3. Least square means (LSMEAN) with standard errors (SE) for milk freezing point (MFP), milk yield, fat content and protein content, by month of year

Month	Number of milk samples	MFP (ºC)		No. of samples with MFP> –0.52ºC (%)	Milk yield (kg)		Fat content (%)		Protein content (%)	
		LSMEAN	SE		LSMEAN	SE	LSMEAN	SE	LSMEAN	SE
January	415,910	–0.5352 A	0.000015	3.92	24.07 A	0.0128	4.275 A	0.0014	3.373 A	0.00056
February	417,670	–0.5351 B	0.000015	4.02	24.37 B	0.0127	4.267 B	0.0014	3.369 B	0.00056
March	422,176	–0.5352 A	0.000015	3.61	24.58 C	0.0127	4.208 C	0.0014	3.346 C	0.00055
April	418,270	–0.5311 C	0.000015	8.86	24.81 D	0.0127	4.168 D	0.0014	3.313 D	0.00055
May	416,755	–0.5319 D	0.000015	6.02	25.40 E	0.0127	4.036 E	0.0014	3.280 E	0.00056
June	404,941	–0.5319 D	0.000016	6.63	25.25 F	0.0129	3.979 F	0.0014	3.254 F	0.00056
July	363,428	–0.5318 E	0.000016	6.60	24.90 D	0.0135	3.944 G	0.0015	3.213 G	0.00059
August	255,921	–0.5320 F	0.000019	6.65	23.77 G	0.0157	3.950 H	0.0017	3.244 H	0.00068
September	405,492	–0.5302 G	0.000015	9.66	23.46 H	0.0129	4.079 H	0.0014	3.343 I	0.00056
October	414,911	–0.5307 H	0.000015	10.33	22.90 I	0.0127	4.207 C	0.0014	3.402 J	0.00056
November	418,279	–0.5300 I	0.000015	11.63	22.95 I	0.0127	4.293 I	0.0014	3.423 K	0.00055
December	366,034	–0.5281 J	0.000016	14.00	23.46 H	0.0134	4.340 J	0.0015	3.400 J	0.00058

A, B, C, ... K – values within the same column marked by different letters differ highly significantly ($P<0.001$).

Table 4. Least square means (LSMEAN) with standard errors (SE) for milk freezing point (MFP), milk yield, fat content and protein content, by lactation stage

Lactation stage (DIM)	Number of milk samples	MFP (ºC)		No. of samples with MFP> 0.52ºC (%)	Milk yield (kg)		Fat content (%)		Protein content (%)	
		LSMEAN	SE		LSMEAN	SE	LSMEAN	SE	LSMEAN	SE
5–35	543,008	–0.5309 A	0.000014	8.95	28.47 A	0.0114	4.380 A	0.0013	3.188 A	0.00050
36–65	510,207	–0.5301 B	0.000014	9.74	29.48 B	0.0117	3.870B	0.0013	2.981 B	0.00051
66–95	505,450	–0.5304 C	0.000014	9.33	28.05 C	0.0118	3.851 C	0.0013	3.084 C	0.00051
96–125	493,795	–0.5312 D	0.000014	8.22	26.39 D	0.0119	3.923 D	0.0013	3.199 D	0.00052
126–155	483,593	–0.5318 E	0.000014	7.42	24.87 E	0.0120	4.018 E	0.0013	3.293 E	0.00052
156–185	471,560	–0.5321 F	0.000015	7.00	23.46 F	0.0121	4.121 F	0.0013	3.369 F	0.00053
186–215	459,959	–0.5324 G	0.000015	6.47	22.20 G	0.0122	4.202 G	0.0013	3.439 G	0.00053
216–245	449,065	–0.5329 H	0.000015	6.21	20.90 H	0.0124	4.227 H	0.0014	3.508 H	0.00054
246–275	426,718	–0.5335 I	0.000015	5.96	19.52 I	0.0126	4.364 I	0.0014	3.583 I	0.00055
276–305	376,432	–0.5339 J	0.000016	5.90	18.28 J	0.0133	4.449 J	0.0015	3.656 J	0.00058

A, B, C, ... J – values within the same column marked by different letters differ highly significantly (P<0.001).

Table 5. Least square means (LSMEAN) with standard errors (SE) for milk freezing point (MFP), milk yield, fat content and protein content, by herd size

Herd size (no. of cows)	Number of		MFP (ºC)		No. of samples with MFP> – 0.52ºC (%)	Milk yield (kg)		Fat content (%)		Protein content (%)	
	herds	milk samples	LSMEAN	SE		LSMEAN	SE	LSMEAN	SE	LSMEAN	SE
1–9	1,899	56,958	–0.5311 A	0.000038	13.27	19.26 A	0.0312	4.232 A	0.0034	3.279 A	0.00136
10–15	2,752	210,830	–0.5312 AB	0.000020	11.13	20.58 B	0.0162	4.244 A	0.0018	3.281 A	0.00071
16–25	5,188	660,067	–0.5313 B	0.000011	10.52	22.13 C	0.0092	4.208 B	0.0010	3.304 B	0.00040
26–50	6,955	1,545,509	–0.5318 C	0.000007	8.67	24.11 D	0.0061	4.160 C	0.0007	3.341 C	0.00026
51–150	2,818	1,332,085	–0.5325 D	0.000008	6.35	27.00 E	0.0065	4.093 D	0.0007	3.391 D	0.00029
>150	445	914,338	–0.5338 E	0.000010	4.51	31.88 F	0.0079	3.935 E	0.0009	3.384 E	0.00034

A, B, C, D, E, F – values within the same column marked by different letters differ highly significantly (P<0.001).

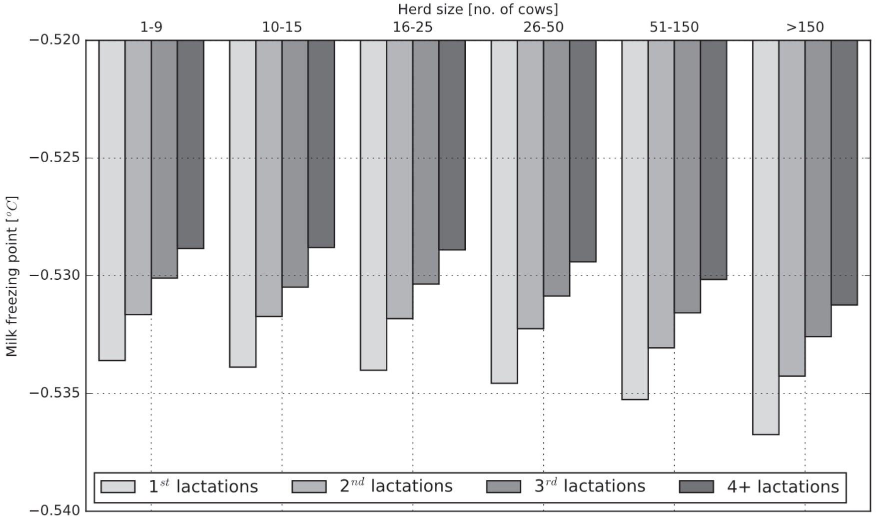

Figure 3. Effect of herd size on milk freezing point (LSMEAN, 0.00001263≤SE≤0.00010245)

Table 4 shows how MFP changed during a lactation. As the lactation progressed, the freezing point of milk decreased, together with the percentage of milk samples with high MFP (exceeding –0.52ºC), starting at 36 DIM (second lactation stage). This decrease matches the changes in mean milk yield. The trend in concentrations of fat and protein is the reverse: they increased in consecutive stages of lactation, starting with the third stage (more than 65 DIM). During that time both fat and protein content increased by more than 0.5 kg. There were highly significant differences ($P<0.001$) between lactation stages for MFP as well as for milk yield, and percentages of fat and protein. For all analyzed traits, highly significant interactions ($P<0.001$) between lactation class and lactation stage were observed. Figure 2 presents the effect of lactation stage on MFP in consecutive lactation classes. In each group of lactations, MFP slowly decreased from the second stage (36 to 65 DIM) to the end of lactation. The decline in value was greatest in the first lactation (0.0046ºC) and the lowest in 4+ lactations (0.0028ºC).

Table 5 illustrates how MFP changed with increasing herd size. LSMEAN of MFP was highest (–0.5311ºC) in herds with few cows (1–9 cows) and lowest (–0.5338ºC) in the biggest herds (more than 150 cows). The number of milk samples with high MFP (>–0.52ºC) decreased with increasing herd size. Highly significant differences ($P<0.001$) between herd size classes were found for all analyzed traits except for one: fat percent in small herds (1–15 cows). For MFP, milk yield, and fat and protein content, highly significant interactions ($P<0.001$) between lactation class and herd size were observed. It meant that the effect of herd size on MFP was different in different lactation classes and vice versa. The milk freezing point also decreased with increasing herd size in consecutive lactations (Figure 3). The changes in MFP through different herd sizes in second and later lactations were similar in shape to those in first lactations. Generally, both in small and large herds the later the lactation the higher the MFP.

Discussion

The literature reports various values found for the freezing point of milk. In the present research the mean milk freezing point for Polish Holstein-Friesian cows was –0.5326ºC, not exceeding the Polish quality limit for MFP (–0.52ºC; MARD, 2004). Kedzierska-Matysek et al. (2011) calculated a slightly lower mean value for the MFP (–0.537ºC) for Polish Holstein-Friesian Black and White cows. Our results are consistent with those of Sala et al. (2010), who analyzed MFP in Romanian Red Spotted and Romanian Black and Red Spotted cows; they obtained average MFP of –0.532ºC. Brzozowski and Zdziarski (2005; 2006) also reported similar mean MFP (–0.5331ºC). Our mean MFP was lower than results obtained by Slaghuis (2001) (–0.5209ºC), Bjerg et al. (2005) (–0.5249ºC), Henno et al. (2007) (–0.5257ºC) and Hanus et al. (2009) (–0.5221ºC). Slaghuis (2001) and Hanus et al. (2009) used bulk milk samples. Slaghuis (2001) observed an increase of 0.0026ºC in MFP over a decade, between the 1980s and 1990s. He suggested that an increase of MFP over time might be connected with changes in milking methods (from hand to machine), methods of storing milk (from can to bulk tank) and steady increase of production level. Changes in feed management as well as changes in milk equipment and cleaning methods might also contribute to changes in MFP (Slaghuis, 2001).

In our study the effect of lactation number on MFP was highly significant: MFP increased with lactation number. This might be partly explained by the fact that generally milk yield increased with lactation number, so the amount of water in milk in later lactations would be expected to be higher. At the same time, small changes of the concentration of water-soluble constituents in milk were observed, so the milk would freeze faster. Kedzierska-Matysek et al. (2011) showed that the MFP increased with lactation number. They found the lowest MFP in first lactations (–0.539ºC), slightly higher in sixth lactations (–0.535ºC), and highest for very old cows, that is, in eleventh lactations (–0.525ºC), and concluded that cow age and production level were associated with an increase of MFP. These results were consistent with ours, however we used least square means. Also, daily milk yield and fat and protein content in milk were significantly affected by successive lactation (Kedzierska-Matysek et al., 2011). Fluctuations in MFP dependent on age of cows were also found by Brzozowski and Zdziarski (2006). They also observed a significant effect of successive lactations on the protein content of milk. An effect of cow age on milk freezing point was reported by Sala et al. (2010) in the Romanian population as well.

Our study indicates a highly significant effect of the month of milk sampling on the freezing point of milk, with MFP nearing the mean during summer. The effect found for season of milk sampling on MFP varies among studies. Bjerg et al. (2005) and Henno et al. (2008) reported that MFP was higher in summer than in winter, and suggested that changes in temperature and diet were mainly responsible for the seasonal effect on MFP. According to Bjerg et al. (2005), the increase of MFP during summer may result from larger water intake due to high temperature and more hours of sunshine in that season. Janstova et al. (2007) found that the freezing point of goat milk was lowest in summer, and explained that organism dehydration during hot summer days could reduce MFP. Brzozowski and Zdziarski (2005) reported that MFP was higher in the autumn-winter period and lower in the pasture season. Additionally, they found significant effect of month of year on fat and protein content in milk. Kedzierska-Matysek et al. (2011) did not find a significant effect of season on the MFP of Polish cows. They found that the milk obtained in the autumn-winter season had a more favourable chemical composition, including the concentrations of fat and protein, as compared to the milk obtained in spring-summer. Additionally, daily milk yield differed significantly between those two seasons (Kedzierska-Matysek et al., 2011).

The results of our study showed the highest least square means of MFP in December (–0.5281ºC) and the lowest in January and March (–0.5352ºC). Furthermore, only 3.61–3.92% of milk samples from January and March exceeded the quality limit for MFP (–0.52ºC); there were three times more such samples in December (14%). According to the literature, depression of the milk freezing point was often related to an increase in protein and solids content as well as to a decrease in the lactose percent in milk (Brouwer, 1981; Brzozowski and Zdziarski, 2006; Kedzierska-Matysek et al., 2011). The relatively big difference between MFP occurring in adjacent months of the winter season (December and January) could be partly explained by the fact that in the milk samples analyzed in this study the LSMEAN of protein content was lower in January (3.373%) than in December (3.397%). At the same time, lactose content was the highest in January and March (4.82–4.83%) and lowest in December (4.76%), in agreement with the conclusions of the cited papers.

In our study, MFP fluctuated during lactation. In the first stage (5–35 DIM) it increased slightly and then slowly decreased until the last stage (276–305 DIM). The depression of milk freezing point was associated with an increase of milk yield. Fluctuations in MFP during lactation have been observed by many authors (Brzozowski and Zdziarski, 2006; Henno et al., 2008; Sala et al., 2010; Kedzierska-Matysek et al., 2011). Henno et al. (2008) noted a significant effect of month of lactation on MFP, daily milk yield and protein content in milk. They reported that MFP was highest in the second and third months of lactation and lowest in the last month of lactation, with a difference of 0.0032ºC; MFP was highest in months when protein content was lowest. Higher MFP related to lower protein content was also observed by Kedzierska-Matysek et al. (2011). Sala et al. (2010) showed that MFP decreased until the third month of lactation (from –0.5279ºC to –0.5339ºC), then increased to the maximum in the fourth month (–0.5276ºC), and decreasing slightly until the end of lactation afterwards. Kedzierska-Matysek et al. (2011) found that MFP, milk yield and fat and protein content in milk were significantly affected by the lactation stage. They defined three stages of lactation and showed that MFP changed from –0.536ºC in the first 100 days of lactation (first stage) to –0.539ºC in the last 100 days of lactation (third stage). They concluded that the depression of MFP was associated with an increase of protein and total solids content, which changed during lactation. In our study the increase in protein percent and the decrease in MFP were also observed (r=–0.222). A similar relationship was noted by Brzozowski and Zdziarski (2005), Henno et al. (2008), Sala et al. (2010) and Kedzierska-Matysek et al. (2011). Sala et al. (2010) showed that a 0.1% decrease of protein percentage resulted in an increase of MFP by about 0.002% for cows up to 6 years old, and by 0.01% for older cows. Brzozowski and Zdziarski (2005) found that decreasing MFP was associated with an increase in protein (r=–0.165) and fat (r=–0.113) content.

The results of our study showed that MFP decreased with increasing herd size. This finding may be associated in part with the milking method. In small herds, where hand milking was preferred, there was less chance of water penetration, whereas in big herds, where milking was often automated, dilution of milk might happen and it could be partly caused by using milking machines (Brzozowski and Zdziarski, 2005; Hanus et al., 2010). Another reason could be the composition of milk i.e. amount of lactose and protein in milk (Brouwer, 1981; Brzozowski and Zdziarski, 2005). According to Brouwer (1981) lactose content has been responsible for 53.8% of MFP depression. It means that the influence of lactose content on MFP depression is higher when lactose percentage in milk is higher. It was in agreement with the results of our previous research in which the influence of milk composition on the freezing point of first-lactation milk of Polish Holstein-Friesian cows was examined (Otwinowska-Mindur and Ptak, 2016). The effects of all examined milk components (fat, protein, urea and lactose content, somatic cell count) on the MFP were highly significant. On average, the MFP was highest for lower lactose content, i.e. –0.5286ºC when lactose content was up to 4.75% and –0.5405ºC when lactose content was over 5.00%. In the data we analyzed, lactose content was highest in milk from the biggest herds (4.84%) and lowest in milk from the smallest herds (4.71%), so it could be the explanation for decreasing of MFP with increasing herd size. Brzozowski and Zdziarski (2005) reported the opposite trend: MFP increased together with the increase of herd size. They also confirmed that herd size significantly affected the fat and protein content of milk.

The mean freezing point of milk of Polish Holstein-Friesian cows, found to be –0.5326ºC, did not exceed the quality limit for the freezing point of cows' raw milk. More than 92% of the milk samples met Polish standards (MARD, 2004). The freezing point of milk of Polish Holstein-Friesians was significantly affected by all analyzed factors: month of year, lactation number, stage of lactation and herd size. MFP was lowest for milk samples taken from January to March, and highest for samples from November to December. It increased with lactation number. Mean MFP decreased with increasing days in milk, except in the first lactation stage (5–35 DIM). Generally, the freezing point of milk was highest in small herds (up to 9 cows) and lowest in large herds (more than 150 cows).

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