Freezing point and other technological properties of milk of the Polish Coldblood horse breed

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ABSTRACT

Mare milk is a valued and sought-after raw material for the production of innovative dairy products. The high demand, low supply, high price, and lack of accurate characterization of the milk of a given horse breed may provoke its deliberate fraudulent dilution. The aim of this work was to analyze the freezing point against a background of various selected technological parameters of Polish Coldblood mare milk. Research was carried out on multiparous Polish Coldblood mares from 5 to 8 yr of age with live weights between 618 and 851 kg. Their milk was tested on d 1, 3, and 7 postpartum and once a month for the next 9 mo of lactation ($n = 52$). Milk from 13 mares, taken daily in mo 4 of lactation, was collected for the dilution study ($n = 403$). The basic composition of the milk was as follows: solids-not-fat, 84.4 g/kg, fat 15.1 g/kg, lactose 65.3 g/kg, and total protein 24.2 g/kg (made up of casein, 14.6 g/kg, and whey protein, 9.5 g/kg). The dominant amino acids were glutamic acid, proline, leucine, and aspartic acid. The mean freezing point during a 9-mo lactation was -0.557°C (coefficient of variation = -2.787 ; 5th to 95th percentile: -0.563 to -0.550°C). Based on our analysis, we adopted -0.550°C (from 3 to 6 mo) as the reference value for milk from the Polish Coldblood horse. Analysis of the model of changes in freezing point, after dilution, using the coefficient of determination and Akaike's information criterion, showed that a linear model could be recommended ($y = 79.35 + 144.35x$). Addition of 1% water increased the freezing point of Polish Coldblood mare milk by 0.0069°C .

Key words: freezing point, mare milk, technological properties, lactation

INTRODUCTION

In recent years, consumer interest in mare milk (from the domestic horse, *Equus ferus caballus*) has increased because of its chemical composition and nutrient content. Mare milk belongs to the group of albumin milks, and it is characterized by a different casein:whey ratio (1.1:1) from cow milk (4.7:1). In addition, it has a much higher lactose content (6.37%) and a lower fat content (1.21%) than cow milk (Uniacke-Lowe et al., 2010). Mare milk has a high biological activity, which results in part from the presence of immunoglobulins, lactoferrin, and lysozyme, which beneficially affect the human body (Malacarne et al., 2002; Jastrzębska et al., 2017). Clinical studies indicate that mare milk and fermented mare milk products (e.g., kumys) are recommended for feeding children with cow milk allergies and for supporting immunity in the elderly and convalescents (Businco et al., 2000; Salimei and Fantuz, 2012). The consumption of mare milk is traditional in Asia, mainly in Mongolia, Kazakhstan, Kyrgyzstan, and Russia. Currently, mare milk is becoming a sought-after raw material in North American and European countries, as demonstrated by farms operating in the Netherlands, Belgium, Germany, Poland, France, and Italy (Hazeleger and Beumer, 2016; Cosentino et al., 2017). However, it remains a niche raw material and is subject to high volatility in demand. Research (Naert et al., 2013) at farms in Flanders (Belgium) showed large variation in mare milk composition between farms. Sometimes, within-farm variability in composition is greater than between-farm variability, which is a major impediment to the processing of this milk and achieving a standardized composition of products made from it. An additional limitation to the development of processing mare milk is the restricted supply of milk. The average herd in Europe ranges from a dozen to several dozen mares with foals. When determining the average performance from one mare, it is assumed that milk production is proportional to BW, being up to 3 kg per 100 kg of BW. However, Salimei and Fantuz (2012) noted that light horses (average BW of 480 kg) yield

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up to 14 L of milk per day at peak lactation, whereas a heavy horse (880 kg) can yield 22 L/d. According to Onegov and Chirgin (2016), the annual estimated milk yield of the champion mares of the Russian Heavy Draft breed range from 5,000 to 7,185 kg of milk. The lactation period is short and ends with natural weaning at >7 mo of age. Mares are milked from spring to late summer. The volume of the udder itself is also small, on average less than 2 L (Čagalj et al., 2014), ranging from 1.63 to 3.90 L (Onegov and Chirgin, 2016), and labor consumption during milking is also high. These factors cause the price of mare milk to be several times higher than that of cow milk. For many practical reasons (health, functional, technological, and marketing), mare milk might be combined with goat, sheep, and, above all, cow milk (Küçükçetin et al., 2003; Cais-Sokolińska et al., 2016a,b, 2017). The use of mare milk can significantly diversify the range of innovative dairy products reaching the market.

Mare milk is clearer and whiter than cow milk; it has a low fat and protein content, of which only 50 to 55% is casein (Jastrzębska et al., 2017). Therefore, it is more transparent than cow milk and is a delicate shade of blue (similar to diluted cow milk). The addition of water to mare milk in small quantities is therefore not visually noticeable. Thus, its appearance, along with its high demand, low supply, and high price, may provoke the deliberate fraudulent dilution of mare milk. Only detailed analysis of its composition and freezing point can identify fraudulent dilution. Many countries lack standardization documents concerning such milk; there are no specific common standards for mare milk before it is further processed at the dairy plant. There are at least 2 reasons for this: it is a raw material processed on a small scale and the milk often comes from native autochthonous breeds. Additionally, in the scientific and industrial literature, there is no described freezing point for mare milk.

These issues prompted us to analyze the freezing point against the composition and selected parameters of Polish Coldblood mare milk. The Polish Coldblood has the largest population of all Polish breeds and those from elsewhere in Eastern Europe. They are also bred for slaughter and used in agritourism and organic farms. The population of Polish Coldblood horses in Poland increased from 15,000 in 2002 to 26,800 in 2012, according to Porter et al. (2016). According to the Polish Horse Breeders Association, there were 273,510 horses in the country in 2016, including 137,003 from all "cold-blooded" breeds (<http://pzhk.pl/hodowla/poglowie-koni-polsce/>).

A preliminary description of the chemical composition, physicochemical parameters, and whey protein

profile in the late-lactation milk of Polish Coldblood mares was presented by Markiewicz-Kęszycka et al. (2013). Selected parameters of the milk of this breed from mares in the fourth month of lactation have been described in our previous studies (Cais-Sokolińska et al., 2016a,b). In the current study, other compositional (including AA profile) and technological parameters have been studied, mainly the freezing point. The scope of changes in freezing point of Polish Coldblood mare milk were examined in cases of intentional dilution. We devised a mathematically calculated function that describes the relationship between the amount of water added and the freezing point of the milk. We hypothesized that we could describe a function concerning the relationship between the freezing point and adulteration as a result of diluting mare milk and determine the correlation between the freezing point and changes in milk color. The rationale for the selection of these methods to assess the processing value of this milk is the fact that they are quick, simple, and require small milk volumes to be measured, so they can be used to assess the suitability of milk for processing.

MATERIALS AND METHODS

Raw Milk Sample

Our research was carried out on multiparous Polish Coldblood mares reared on an equine dairy farm in the Wielkopolska region (western Poland). The mares were from 5 to 8 yr of age with BW between 618 and 851 kg. All horses were managed in a similar way. The mares were fed (1) crushed oats (6 kg/d) with the following average chemical composition: DM, 86.6%, CP, 2.2%, crude fiber, 11.9%, crude fat, 1.6%, and ash, 2.6%; (2) wheat meal (1.5 kg/d): DM, 86.5%, CP, 14.2%, crude fiber, 9.3%, crude fat, 2.7%, and ash, 2.8%; and (3) hay (5 kg/d): DM, 92.1%, CP, 7.2%, crude fiber, 38.9%, crude fat, 0.5%, and ash, 6.4%. The mares had free access to pasture, the botanical composition of which was 70% grasses (mainly perennial ryegrass), 20% legumes, and 10% others, including herbs, and to water and salt-mineral blocks.

The mares were foaled in the same period (January and February). Their milk was tested on d 1, 3, and 7 postpartum and once a month for the next 9 mo of lactation (until September and October). The mares were mechanically milked from both halves of the udder once a day in the evening after 3 h of physical separation from their foals. The mares, together with their foals, spent most of the day in the pasture, which was the source of their consumed roughage. Individual milk samples from 13 mares were collected according to

ISO 707 (ISO-IDF, 2008) for testing. The research was carried out from 2014 to 2017 ($n = 52$ samples).

Milk from 13 mares, taken daily in mo 4 of lactation, was collected for the dilution study ($n = 403$). This time of lactation was chosen because the literature indicates that full stabilization of the chemical composition, density, and titratable acidity of the milk—and the stabilization of its freezing point—had occurred at this time (Mariani et al., 2001). Second, by that time, foals had started to eat forage and bulk food, with the mare milk supplementing these food sources.

Compositional and Physicochemical Analysis

Compositional and acidity analyses were carried out using standard methods (AOAC International, 1995) under the conditions described by Cais-Sokolińska et al. (2016a,b). The total protein content was calculated as the total amount of N $\times$ 6.38, according to official method 991.20 (AOAC International, 1995). An appropriately calibrated Lactostar analyzer (Funke-Dr.N.Gerber Labortechnik GmbH, Berlin, Germany) was used for current monitoring of milk composition (ISO-IDF, 2013). The analyzer used thermal measurement effects and impedance/turbidity sensory technology with 4 different optical wavelengths. Gross energy was calculated with the following coefficients: 8.97 for fat, 5.25 for protein, and 4.10 for lactose (Doreau et al., 1986). The pH was measured using a CP-411 pH-meter (Elmetron, Zabrze, Poland) equipped with an ESAGP-301W combination electrode (Eurosensor, Gliwice, Poland). Titratable acidity values were expressed in Soxhlet-Henkel degrees ($1^\circ\text{SH} = 0.0225$ lactic acid %). The absolute values of the dynamic viscosity of the samples before fermentation were recorded using a Höppler KF10 viscometer from RheoTec Messtechnik GmbH (Ottendorf, Germany). A CC-401 conductometer coupled with an EC-60 sensor and a CT2B-121 temperature sensor was used (Elmetron). The hygienic quality of the raw milk was assessed by determining the SCC and total bacterial count in bulk milk using an IBC-m instrument (Bentley Instruments, Chaska, MN). The content of macroelements was determined using an SP-2900 atomic absorption spectrophotometer (Pye Unicam Ltd., Cambridge, UK) with background correction and a flame system, using appropriate cathode lamps and dry mineralization of samples at a temperature of up to 450°C . The AA content was determined using ultra-high performance liquid chromatography (UPLC) after acidic initial hydrolysis. The AA derivatization process was carried out (AccQ-Tag Ultra 186003836, Waters, Milford, MA) according to

the manufacturer's protocol, using an AccQ-Tag Ultra amino acid standard (WAT088122, Waters). The column used for separation was an AccQ-Tag Ultra Column 2.1×100 mm (186003837, Waters). The analysis used a self-developed phase gradient, with detection by a photodiode array detector at a wavelength of 260 nm. Further analyses were performed using the LabSolutions program (Shimadzu, Tokyo, Japan).

A milk sample was placed in an optical glass cuvette (2/96G/10, Starna Scientific Company Ltd., Ilford, UK) with dimensions of $28 \text{ mm} \times 16 \text{ mm}$, height of 40 mm, and volume of 7.2 mL. The measurement was performed with a D65 light source, whose continuous spectrum in the visible range is similar to that of natural light, and a 10° observation angle using an SP-60 camera (X-Rite, Grandville, MI) equipped with a spherical geometry (diffusive), and a measurement chamber with a DRS-811 ceramic insert. The camera was calibrated based on the black and white SP-62-162 reference standards (X-Rite).

The color was analyzed based on CIELAB L (lightness), a^* (−green/+red color) and b^* (−blue/+yellow color; Gaiani et al., 2011). The whiteness index (**WI**) was calculated using Eq. [1] (Vargas et al., 2008):

$$\text{WI} = 100 - [(100 - L)^2 + a^{*2} + b^{*2}]^{0.5}. \quad [1]$$

Freezing Point

The freezing point of the milk was determined according to the ISO 5764 (ISO-IDF, 2009) standard method using an Advanced Model 4D3 cryoscope with a 3LH700 thermistor probe (Advanced Instruments Inc., Norwood, MA). The calibration process was carried out using milk calibration standards -408°C and 600°C and Lactrol 530 Reference Solution -512°C for use with Advanced Instruments cryoscopes (Advanced Instruments Inc.). The milk was poured into 2-mL sample tubes (DZ 3LA823, Advanced Instruments Inc.).

Mathematical Modeling of Freezing Point as a Function of Dilution

Both linear and nonlinear regression models were used to model the changes of freezing point with respect to the dilution of mare milk. The analysis was performed based on 403 observations obtained by the measurement of the freezing point for different dilutions in the range 0.2 to 99.8% with steps of 0.2%. The shape of the points cloud suggested that 3 regression lines, linear, quadratic, and exponential, could be applied

Table 1. Gross composition (g/kg unless otherwise noted) and physicochemical characteristics of milk of Polish Coldblood mares throughout 9 mo of lactation¹

Characteristic	1 d	3 d	7 d	30 d	60 d	90 d
SNF	150.2 ± 0.2 ^f	98.1 ± 0.2 ^e	93.5 ± 0.2 ^d	93.8 ± 0.2 ^d	87.9 ± 0.2 ^b	84.7 ± 0.2 ^a
Fat	31.3 ± 0.1 ^f	20.7 ± 0.2 ^e	15.8 ± 0.3 ^d	15.2 ± 0.1 ^c	15.0 ± 0.2 ^c	15.1 ± 0.1 ^c
Total protein ²	97.8 ± 0.3 ^h	38.7 ± 0.1 ^g	28.4 ± 0.3 ^f	25.2 ± 0.4 ^e	24.0 ± 0.2 ^d	24.1 ± 0.1 ^d
Casein ²	72.1 ± 0.5 ⁱ	19.9 ± 0.2 ^h	14.2 ± 0.2 ^g	12.6 ± 0.1 ^f	14.5 ± 0.4 ^d	14.5 ± 0.1 ^d
Whey protein ²	25.8 ± 0.1 ^f	18.7 ± 0.1 ^e	14.1 ± 0.2 ^d	12.4 ± 0.1 ^c	9.4 ± 0.1 ^b	9.6 ± 0.2 ^b
Casein:whey	2.8:1	1.1:1	1.0:1	1.0:1	1.5:1	1.5:1
Lactose	37.1 ± 0.5 ^a	57.2 ± 0.3 ^b	60.5 ± 0.1 ^c	64.0 ± 0.3 ^d	65.4 ± 0.1 ^e	65.5 ± 0.3 ^e
Ash	8.8 ± 0.2 ^f	7.6 ± 0.4 ^e	6.3 ± 0.2 ^d	5.7 ± 0.1 ^c	5.6 ± 0.2 ^{bc}	5.5 ± 0.1 ^{bc}
pH	6.45 ± 0.04 ^a	6.57 ± 0.02 ^b	6.87 ± 0.03 ^c	6.91 ± 0.02 ^{cd}	6.91 ± 0.01 ^{cd}	6.93 ± 0.02 ^d
Acidity (°SH ³)	25.44 ± 0.14 ^f	9.71 ± 0.23 ^e	6.52 ± 0.12 ^d	4.12 ± 0.10 ^c	2.84 ± 0.08 ^b	2.66 ± 0.02 ^a
Freezing point (°C)	-0.574 ± 0.002 ^a	-0.581 ± 0.001 ^a	-0.573 ± 0.004 ^a	-0.564 ± 0.003 ^b	-0.556 ± 0.003 ^c	-0.550 ± 0.002 ^c
Density ⁴ (kg/m ³)	1,047 ± 1 ^b	1,047 ± 1 ^b	1,039 ± 3 ^a	1,037 ± 2 ^a	1,036 ± 1 ^a	1,037 ± 3 ^a

	120 d	150 d	180 d	210 d	240 d	270 d
SNF	84.0 ± 0.1 ^a	84.5 ± 0.3 ^a	84.3 ± 0.2 ^a	84.5 ± 0.4 ^a	85.5 ± 0.2 ^a	86.9 ± 0.1 ^c
Fat	15.1 ± 0.1 ^c	15.2 ± 0.2 ^c	15.1 ± 0.1 ^c	12.1 ± 0.1 ^b	7.9 ± 0.1 ^a	7.6 ± 0.1 ^a
Total protein	24.4 ± 0.1 ^d	24.1 ± 0.2 ^d	24.2 ± 0.5 ^d	17.6 ± 0.4 ^c	15.6 ± 0.3 ^b	15.5 ± 0.3 ^a
Casein	14.9 ± 0.2 ^d	14.6 ± 0.2 ^d	14.5 ± 0.1 ^d	13.7 ± 0.4 ^c	12.2 ± 0.1 ^b	11.6 ± 0.1 ^a
Whey protein	9.6 ± 0.2 ^b	9.4 ± 0.1 ^b	9.6 ± 0.1 ^b	3.8 ± 0.1 ^a	3.5 ± 0.3 ^a	3.7 ± 0.3 ^a
Casein:whey	1.5:1	1.5:1	1.5:1	3.6:1	3.5:1	3.1:1
Lactose	65.2 ± 0.3 ^e	65.3 ± 0.4 ^e	65.0 ± 0.1 ^e	65.0 ± 0.3 ^e	68.4 ± 0.2 ^g	66.6 ± 0.2 ^f
Ash	5.4 ± 0.3 ^{bc}	5.5 ± 0.2 ^{cb}	5.5 ± 0.2 ^{cb}	5.5 ± 0.1 ^{cb}	5.1 ± 0.2 ^b	4.4 ± 0.4 ^a
pH	6.93 ± 0.01 ^{cd}	6.90 ± 0.02 ^d	9.93 ± 0.01 ^d	6.94 ± 0.01 ^d	6.92 ± 0.01 ^{cd}	6.92 ± 0.02 ^d
Acidity (°SH)	2.64 ± 0.02 ^a	2.64 ± 0.03 ^a	2.61 ± 0.05 ^a	2.61 ± 0.02 ^a	2.62 ± 0.04 ^a	2.63 ± 0.04 ^a
Freezing point (°C)	-0.550 ± 0.002 ^c	-0.551 ± 0.003 ^c	-0.550 ± 0.001 ^c	-0.550 ± 0.001 ^c	-0.550 ± 0.003 ^c	-0.521 ± 0.003 ^d
Density (kg/m ³)	1,037 ± 2 ^a	1,037 ± 2 ^a	1,037 ± 4 ^a	1,037 ± 4 ^a	1,037 ± 2 ^a	1,035 ± 2 ^a

^{a-f}Values within a row (from 1 to 270 d) with different lowercase superscripts indicate statistically significant differences at $\alpha = 0.05$.

¹Values represent mean ± SD (n = 52).

²Total protein = (total N - NPN) × 6.38; casein = (total N - noncasein N - NPN) × 6.38; whey protein = (noncasein N - NPN) × 6.38.

³°SH = degrees Soxhlet-Henkel.

⁴Measured at 20°C.

Statistical Evaluation

To verify the statistical hypotheses, a level of significance of $\alpha = 0.05$ was adopted. An ANOVA was carried out, whereas for multiple post hoc comparisons, a Tukey HSD test was used. The statistical calculations were carried out using Statistica data analysis software, (version 10; StatSoft, Tulsa, OK). The coefficient of determination (R^2) and Akaike's information criterion (AIC) were used in analyzing the milk dilution model, using R Core Team software (Project for Statistical Computing, Vienna, Austria).

RESULTS

Main Components of Milk and its Physicochemical Properties

The composition and technological properties of mare milk from the Polish Coldblood breed throughout the lactation period are given in Table 1. With the elapse of lactation time, the following parameters reached a stable point in lactation sooner: density, pH, and fat content. The parameters that took the longest to stabilize were solids-not-fat and acidity. The mean freezing point over the entire lactation period was $-0.557 \pm 0.015^\circ\text{C}$ (mean $\pm$ SD; CV = -2.787 ; 5th to 95th percentile: -0.563 to -0.550°C). Lactation time did not affect the freezing point of milk during the period from 60 to 240 d ($P > 0.05$). However, we could statistically determine the stabilization period for the ingredient contents and parameters tested. No significant difference was found for any parameter in milk from 3 to 6 mo ($P > 0.05$). We considered composition and physicochemical parameters to be typical in this period of lactation, thus we present data for this lactation period in Table 2.

In addition, we showed how the AA profile changed in milk throughout lactation (Table 3). The AA content in milk in the individual study periods varied from 939.5 to 975.6 g/kg of protein. During the 9 mo of lactation, the dominant AA were glutamic acid, proline, leucine, and aspartic acid. Those found in the smallest amounts were cysteine, glycine, methionine, and histidine. The highest variability during lactation was found for methionine, phenylalanine, leucine, and lysine. The most stable in terms of quantity (i.e., the most homogeneous AA) during lactation was cysteine; from 1 to 9 mo of lactation, the content of cysteine was 13.05 g/kg ($P > 0.05$). Using the relative distribution of data around the mean value, we showed that the AA could be divided into 3 groups according to the coefficient of variation (CV) over the whole lactation. The lowest CV values

(3.10–5.80) were for valine, leucine, histidine, glutamic acid, and serine. The highest CV values (18.86–27.01) were for threonine, tyrosine, arginine, proline, cysteine, and alanine, which mostly belong to the group of non-essential AA.

Freezing Point of Milk and Using it to Determine the Function of the Dilution

We measured the freezing point of milk in mid lactation; it was found to be -0.550°C (Table 2), which we accepted as a reference value for milk of this breed. Analysis of the model of changes in freezing point with respect to the dilution of mare milk showed that the goodness-of-fit criteria assessed by the R^2 value and AIC were similar. The fit of the regression lines, their equations, and R^2 coefficients are presented in Figure 1. The t -tests for the coefficients of the 3 lines showed very high significance (P -value = 0.000 in all cases).

Table 2. Composition and technological properties of milk of Polish Coldblood mares in mid lactation (3–6 mo)¹

Parameter	Value
SNF (g/kg)	84.4 $\pm$ 2.4
Fat (g/kg)	15.1 $\pm$ 0.7
Total N ² (g/kg)	3.80 $\pm$ 0.02
NPN ² (g/kg)	0.02 $\pm$ 0.01
Noncasein N ² (g/kg)	1.48 $\pm$ 0.01
Total protein ² (g/kg)	24.2 $\pm$ 1.1
Casein ² (g/kg)	14.6 $\pm$ 0.6
Whey protein ² (g/kg)	9.5 $\pm$ 0.1
Lactose (g/kg)	65.3 $\pm$ 2.3
Ash (g/kg)	5.5 $\pm$ 0.1
Total gross energy (GE) (kcal/kg)	530.9
GE fat/total GE (%)	25.5
GE protein/total GE (%)	24.1
GE lactose/total GE (%)	50.4
Ca (mg/L)	870 $\pm$ 2
Na (mg/L)	349 $\pm$ 7
K (mg/L)	927 $\pm$ 1
Mg (mg/L)	108 $\pm$ 2
P (mg/L)	883 $\pm$ 4
pH	6.93 $\pm$ 0.14
Acidity ($^\circ\text{SH}^3$)	2.65 $\pm$ 0.2
Freezing point ($^\circ\text{C}$)	-0.550 ± 0.002
Density at 20 $^\circ\text{C}$ (kg/m ³)	1,037 $\pm$ 4
Color: lightness (L)	63.31
Color: green-red (a*)	-2.34
Color: blue-yellow (b*)	-4.04
Color: whiteness index	65.55
Viscosity (mPa·s)	3.05 $\pm$ 0.6
Conductivity (Ω^{-3}/cm)	3.948 $\pm$ 0.074
Water activity	0.9904 $\pm$ 0.0063
SCC ($\times 10^3$ cells/mL)	27.2 $\pm$ 2.1
Total bacterial count ($\times 10^3$ cells/mL)	9.6 $\pm$ 0.9

¹Values represent mean $\pm$ SD (n = 208).

²Total protein = (total N – NPN) $\times$ 6.38; casein = (total N – noncasein N – NPN) $\times$ 6.38; whey protein = (noncasein N – NPN) $\times$ 6.38.

³SH = degrees Soxhlet-Henkel.

The smallest value of AIC, 2,690.43, was obtained for the quadratic model; however, the AIC values for the other lines were similar: 2,778.22 for the exponential model and 2,804.40 for the linear model.

Considering that milk dilution with a small amount of water is difficult to observe, the fitness of regression on the initial interval of dilution is especially important. To examine this, we calculated quadratic deviations between the predicted and observed values for a range of dilutions from 0 to 7%. The mean of the quadratic deviation was 4.355 (SD: 6.219) for the linear model and 2.434 (SD: 2.709) for the quadratic model. Thus, in this case too, the parabolic curve fitted the data better.

The dilution of milk significantly affected WI ($P < 0.05$). The directional coefficient of the linear fit between WI and freezing point was -0.019 with a very high R^2 (0.926; Figure 2). The measurement of the CIE $L^*a^*b^*$ coordinates reflected the amount of water

added to the milk and could be a useful tool for quickly assessing milk dilution.

DISCUSSION

The basic composition and density of Polish Coldblood milk were similar to those described by Pieszka et al. (2011). The current study and that of Pieszka et al. (2011) confirmed that Coldblood milk is characterized by a lower content of fat than, for example, milk from Hucul mares or Polish horse mares, which results from the higher milk production of the Coldblood breed. The composition and nutritive value of milk can vary according to the lactational stage, age, parity, and nutrition (Cosentino et al., 2017).

In terms of AA content, similar results were obtained by Mazhitova et al. (2015) and Mazhitova and Kulmyrzaev (2016). They researched milk from mares

Table 3. Amino acid profile (g/kg of protein) of milk of Polish Coldblood mares throughout 9 mo of lactation¹

Amino acid	1 d	3 d	7 d	30 d	60 d	90 d
Lysine	82.1 ± 0.1 ^h	80.4 ± 0.1 ^g	68.3 ± 0.1 ^f	68.7 ± 0.1 ^f	62.1 ± 0.1 ^{bc}	60.1 ± 0.1 ^a
Threonine	59.6 ± 0.2 ^g	58.4 ± 0.1 ^f	36.8 ± 0.1 ^e	33.2 ± 0.1 ^b	30.8 ± 0.1 ^a	34.9 ± 0.1 ^{cd}
Valine	50.7 ± 0.3 ^c	52.6 ± 0.2 ^f	54.7 ± 0.2 ^g	52.3 ± 0.1 ^{ef}	51.6 ± 0.1 ^{de}	50.6 ± 0.1 ^c
Methionine	23.5 ± 0.1 ^c	24.1 ± 0.1 ^d	25.2 ± 0.1 ^f	22.2 ± 0.1 ^b	25.7 ± 0.1 ^g	24.4 ± 0.1 ^e
Isoleucine	51.1 ± 0.1 ^f	51.0 ± 0.1 ^f	43.5 ± 0.1 ^e	41.9 ± 0.1 ^d	41.9 ± 0.1 ^d	40.5 ± 0.5 ^c
Leucine	91.6 ± 0.1 ^h	89.7 ± 0.1 ^g	87.0 ± 0.1 ^e	89.0 ± 0.1 ^f	86.6 ± 0.1 ^e	84.2 ± 0.1 ^d
Phenylalanine	40.2 ± 0.1 ^a	41.4 ± 0.1 ^b	43.8 ± 0.1 ^e	42.6 ± 0.1 ^{cd}	42.4 ± 0.1 ^c	42.8 ± 0.1 ^d
Histidine	23.2 ± 0.1 ^a	27.8 ± 0.1 ^{cd}	28.5 ± 0.1 ^{def}	28.2 ± 0.1 ^{ef}	27.2 ± 0.1 ^{bcde}	27.4 ± 0.1 ^{bcde}
Aspartic acid	97.0 ± 0.1 ^f	96.4 ± 0.1 ^f	81.9 ± 0.1 ^e	80.8 ± 0.1 ^{de}	83.1 ± 0.1 ^e	77.5 ± 0.1 ^{bc}
Glutamic acid	188.7 ± 0.1 ^{ab}	190.3 ± 0.1 ^b	198.8 ± 0.1 ^d	216.9 ± 0.1 ^g	206.9 ± 0.1 ^f	203.1 ± 0.1 ^e
Glycine	15.5 ± 0.1 ^c	17.5 ± 0.1 ^d	18.0 ± 0.1 ^d	17.1 ± 0.1 ^d	14.2 ± 0.1 ^{ab}	14.5 ± 0.1 ^{bc}
Serine	44.0 ± 0.1 ^a	45.6 ± 0.1 ^{ab}	46.1 ± 0.1 ^{ab}	46.0 ± 0.1 ^{ab}	47.3 ± 0.2 ^{bc}	47.2 ± 0.1 ^{bc}
Tyrosine	29.4 ± 0.1 ^a	37.6 ± 0.1 ^b	41.4 ± 0.1 ^c	42.7 ± 0.1 ^c	41.8 ± 0.1 ^c	42.8 ± 0.1 ^c
Arginine	23.7 ± 0.1 ^a	33.9 ± 0.1 ^b	57.4 ± 0.1 ^d	61.5 ± 0.1 ^d	59.9 ± 0.1 ^d	61.8 ± 0.1 ^d
Proline	63.8 ± 0.4 ^a	65.7 ± 0.2 ^a	68.5 ± 0.4 ^a	73.5 ± 0.4 ^b	89.3 ± 0.1 ^c	84.0 ± 0.1 ^c
Cysteine	19.3 ± 0.1 ^b	18.4 ± 0.1 ^b	19.4 ± 0.1 ^b	13.4 ± 0.1 ^a	13.4 ± 0.2 ^a	13.8 ± 0.1 ^a
Alanine	46.5 ± 0.2 ^e	44.8 ± 0.1 ^{de}	40.8 ± 0.2 ^d	34.2 ± 0.1 ^c	33.2 ± 0.1 ^{bc}	29.9 ± 0.4 ^{abc}

	120 d	150 d	180 d	210 d	240 d	270 d
Lysine	61.6 ± 0.1 ^b	62.5 ± 0.1 ^c	63.3 ± 0.1 ^d	64.2 ± 0.1 ^e	64.1 ± 0.1 ^e	64.0 ± 0.1 ^e
Threonine	34.5 ± 0.1 ^c	34.5 ± 0.1 ^c	35.2 ± 0.1 ^d	31.2 ± 0.1 ^a	31.0 ± 0.1 ^a	31.5 ± 0.1 ^a
Valine	48.2 ± 0.1 ^a	49.2 ± 0.1 ^b	49.5 ± 0.1 ^b	51.0 ± 0.1 ^{cd}	50.6 ± 0.1 ^c	51.0 ± 0.1 ^c
Methionine	21.5 ± 0.1 ^a	27.4 ± 0.1 ⁱ	27.2 ± 0.1 ^h	27.1 ± 0.1 ^h	27.7 ± 0.1 ^j	26.9 ± 0.1 ^h
Isoleucine	39.2 ± 0.2 ^{ab}	38.9 ± 0.1 ^a	39.1 ± 0.1 ^a	40.1 ± 0.1 ^{bc}	39.0 ± 0.1 ^{ab}	39.7 ± 0.1 ^{abc}
Leucine	80.6 ± 0.1 ^c	78.8 ± 0.1 ^{ab}	78.6 ± 0.1 ^a	78.9 ± 0.4 ^{ab}	79.9 ± 0.2 ^{bc}	79.7 ± 0.1 ^{abc}
Phenylalanine	47.4 ± 0.1 ^f	48.2 ± 0.1 ^g	47.6 ± 0.1 ^f	48.6 ± 0.1 ^{hi}	48.7 ± 0.1 ⁱ	48.0 ± 0.1 ^{gh}
Histidine	26.4 ± 0.3 ^{bc}	26.5 ± 0.2 ^b	27.5 ± 0.1 ^{bcd}	28.9 ± 0.1 ^f	27.2 ± 0.1 ^{bcde}	28.7 ± 0.1 ^{ef}
Aspartic acid	79.3 ± 0.1 ^{cd}	76.5 ± 0.1 ^{abc}	75.2 ± 0.3 ^{ab}	74.2 ± 0.1 ^{ab}	74.6 ± 0.1 ^{ab}	73.3 ± 0.1 ^a
Glutamic acid	193.9 ± 0.1 ^c	190.2 ± 0.1 ^{bc}	188.2 ± 0.1 ^{ab}	186.1 ± 0.1 ^a	190.7 ± 0.1 ^{ab}	189.0 ± 0.1 ^{ab}
Glycine	14.4 ± 0.1 ^{abc}	14.2 ± 0.1 ^{abc}	14.3 ± 0.1 ^{abc}	13.2 ± 0.1 ^{ab}	13.6 ± 0.1 ^{ab}	13.3 ± 0.1 ^a
Serine	46.7 ± 0.3 ^b	47.2 ± 0.1 ^{bc}	45.8 ± 0.1 ^b	48.2 ± 0.1 ^{bc}	49.0 ± 0.1 ^c	48.9 ± 0.1 ^c
Tyrosine	58.1 ± 0.1 ^d	57.8 ± 0.1 ^d	57.8 ± 0.1 ^d	59.9 ± 0.1 ^d	58.6 ± 0.1 ^d	58.3 ± 0.1 ^d
Arginine	45.7 ± 0.1 ^c	47.6 ± 0.1 ^c	48.5 ± 0.1 ^c	48.1 ± 0.1 ^c	43.1 ± 0.1 ^c	47.6 ± 0.1 ^c
Proline	109.4 ± 0.1 ^d	110.4 ± 0.2 ^d	110.5 ± 0.1 ^d	107.0 ± 0.1 ^d	108.4 ± 0.1 ^d	110.1 ± 0.1 ^d
Cysteine	13.5 ± 0.1 ^a	12.7 ± 0.4 ^a	12.8 ± 0.3 ^a	12.6 ± 0.2 ^a	12.9 ± 0.1 ^a	12.4 ± 0.1 ^a
Alanine	30.3 ± 0.1 ^{ab}	28.4 ± 0.3 ^a	27.8 ± 0.3 ^a	28.6 ± 0.1 ^a	30.0 ± 0.2 ^b	29.2 ± 0.1 ^a

^{a-j}Values within a row (from 1 to 270 d) with different lowercase superscripts indicate statistically significant differences at $\alpha = 0.05$.

¹Values represent mean ± SD (n = 52).

that foaled between February and April, showing that during 4 mo of lactation (from May to August), the dominant AA in milk was glutamic acid, followed by leucine, lysine, and aspartic acid. Amino acid content is one of several characteristics of milk as a raw material for processing with a certain nutritional and biological value. There are no literature data on the effect of AA on freezing point in milk overall.

Pagliarini et al. (1993) reported the freezing point of mare milk to be -0.554°C . Čagalj et al. (2014) measured the freezing point of Croatian Coldblood mare milk, and the mean over 11 mo was -0.5318°C , being -0.5423°C in the first month of lactation and -0.5221°C in the second month. The same authors reported lactose content (60.5 and 62.7 g/kg, respectively) and TS content (122.9 and 105.1 g/kg, respectively) at the same time

points in lactation. Martuzzi et al. (2004) examined milk from Italian Saddle and Haflinger mares. They did not find any strong influence of breed on mean freezing point. The freezing point of Italian Saddle mare milk ranged from -0.529 to -0.533°C , with a mean of -0.531°C . The freezing point in Haflinger mares ranged from -0.531 to -0.522°C , with an average value of -0.527°C . The lactose content in Italian Saddle and Haflinger mare milks was 64.0 and 69.2 g/kg, respectively. Martuzzi et al. (2004) showed an interesting dependence of freezing point on the content of mineral salts: freezing point decreased when mineral salts increased, and this was dependent on the lactation period. In the milk from Italian Saddle mares, freezing point decreased from -0.529°C in early lactation (3 to 30 d postpartum) to -0.533°C in late lactation (after

Figure 1. The relationship between freezing point and dilution of mare milk of the Polish Coldblood horse was tested using 403 random fresh milk samples. Analysis for correlation was performed using 3 regression lines: linear, quadratic, and exponential.

180 d). In contrast, in milk from Haflinger mares, the situation was reversed: freezing point was lower in early than in late lactation (going from -0.531 to -0.522°C). Mariani et al. (2001) studied milk from the Haflinger breed in the first 6 mo of lactation. They showed that from d 4 after delivery until d 60 of lactation, the freezing point changed from -0.536 to -0.520°C ; that is, tending toward the freezing point of water. Then, stabilization took place up to 6 mo later. The average freezing point for Haflinger mare milk was -0.524°C (Mariani et al., 2001), 0.003°C higher than that found by Martuzzi et al. (2004).

With regard to examples of other mammal species, it has been shown that the freezing point of milk depends on factors related to variations in environment, management, and breed (Zagorska and Ciprovica, 2013).

However, the most important factor affecting the freezing point of milk is water addition. The addition of 1% water increases the freezing point of cow milk by 0.006°C , bringing it closer to zero (Navrátilová et al., 2006). The freezing point also depends on the biochemical composition of the soluble phase; lactose represents 55% of the total variability, 25% is due to salts, especially Cl^{-} , and the last 20% is represented by Ca^{2+} , K^{+} , Mg^{2+} , lactate, citrate, and phosphate (Raynal-Ljutovac et al., 2005). The presence of such solutes depresses the freezing point below 0°C . Lactose lowered the freezing point of cow milk the most, by 0.296°C , and chloride with Na^{+} and K^{+} cations lowered it by 0.119°C (Navrátilová et al., 2006). Casein decreased the freezing point by 0.000001°C and whey proteins by 0.000407°C (Kessler, 1984).

Figure 2. Linearity correlations between freezing point (y-axis) and the spectrophotometric determination of whiteness index of milk (x-axis). The solid black line indicates the regression line and the dotted lines indicate the 95% CI. The oval indicates the unique prediction.

CONCLUSIONS

We present typical composition and technological properties of mare milk of the Polish Coldblood horse breed. These values are characteristic for milk mares throughout mid lactation (from 3 to 6 mo). During this period, the freezing point of Polish Coldblood mare milk was found to be -0.550°C . We identified a high correlation between freezing point and milk color expressed as whiteness index, which could be a quick tool for assessing and confirming milk dilution. The model best fitting the relationship between the level of milk dilution (y) and freezing point (x) was quadratic (highest R^2 coefficient and lowest AIC value). However, the differences in R^2 for all 3 estimated models were close to 1%. From a practical point of view, it would be useful to assess this relation in the simplest way, providing clear interpretations in real situations. Therefore, the use of a linear model ($y = 79.35 + 144.35x$) is also recommended. The estimated linear regression coefficient allows us to state that a 1% addition of water (i.e., an increase in milk dilution) will cause an increase in freezing point temperature of 0.0069°C .

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