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Comparison of fat and protein fractions of milk constituents in Montbeliarde and Polish Holstein-Friesian cows from one farm in Poland

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Abstract

The aim of this study was to determine the genetic effect of cow breed on the yield and quality of cow's milk. On the selected dairy farm, cows of two different breeds Montbeliarde and Polish Holstein-Friesian between 45th-100th days of lactation, were managed under the same environmental conditions. Milk samples were collected twice (in May and June 2008) from 97 (n = 51 and 46 for Montbeliarde and Polish Holstein-Friesian, respectively) cows. Montbeliarde cow's milk was characterized by significantly lower milk yield and somatic cell count. Higher level of C18: 2 *cis*-9 *trans*-11 and almost 2-fold C20:5 n-3 ($P \leq 0.01$) was also recorded. However, Polish Holstein-Friesian cow's milk had a better composition of protein fraction (higher concentration of lactoferrin, α-lactoalbumin and β-lactoglobulin) and higher concentrations of vitamins D and E. Moreover, milk of Polish Holstein-Friesian breed was characterized by more favorable fat fraction with significantly lower concentrations of saturated fatty acids (C14:0, C18:0) as well as lower atherogenic and thrombogenic indexes and higher concentration of C18:2 *trans*-10 *cis*-12, C20:3 n-6 and C 22:6 n-3. This study was the first one comparing biological quality of Polish Holstein-Friesian and Montbeliarde milk under the same environmental conditions in Polish production systems.

Cattle, somatic cell count, fatty acid, whey proteins, vitamins soluble in fat

Composition of cow's milk is very important for the dairy industry. It is also fundamental for its nutritive value as well as processability. Quality and quantity of milk depends upon genetic and environmental factors. Considering the environmental ones, nutrition is the most important determinant of milk chemical composition, followed by production system, health status, as well as the effect of herd and labour (Collomb et al. 2002; Nałęcz-Tarwacka 2006). Within genetic factors, the cow's breed seems to have substantial influence on milk compounds (D'Hour et al. 1995; Verider et al. 1995; Dillion et al. 2003; Soyeurt et al. 2006). Studies in Poland have reported genetic variation of protein and fat percentages, and protein and fat yields (Gołębiewski and Brzozowski 2008; Gołębiewski et al. 2011). Polish Holstein-Friesian is the most popular as well as the most productive dairy breed in Poland (over 94% of the whole population). French Montbeliarde cows were imported to Poland in 1995. They were characterized by moderate production, good functional traits (health, longevity, fertility, temperament) as well as outstanding quality of their milk, which is valuable substrate for cheese industry (Coulon et al. 1996). Productive and non-productive traits of French cows has made them a more popular breed, resulting in a rapid increase in the number of farms specialized in Montbeliarde breeding (Gołębiewski and Brzozowski 2008).

The aim of this study was to find the genetic effect of cattle breed (Montbeliarde and Polish Holstein-Friesian) on biological quality of milk, health-benefit value and processability, and to compare the chemical composition of lipid and protein fractions in milk in the two above mentioned cattle breeds.

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Materials and Methods

Animals and diets

The experiment was conducted on a farm located in the south-west part of Poland in the Barycz River valley. On the farm, dairy cows of two different breeds were kept –Montbeliarde (n = 200 cows) and Polish Holstein-Friesian (n = 180 cows). Fifty one Montbeliarde and 46 Polish Holstein-Friesian lactating primiparous sows between the 45th -100th days of lactation were used in the experiment. The cows of both breeds were kept under the same environmental conditions on the farm in a free stall system, and were fed a total mix ratio. Daily cow's rations were balanced according to the Institut National de la Recherche Agronomique (INRA) feeding system. Deficiency of protein in the ration was supplemented by adding soya and rape seed meal (Table 1). All cows were milked twice a day in "side by side" parlour - Baumantic. Samples of milk were collected automatically twice in May and June in 2008 from all cows. Combined milk from morning and evening milking was placed in sterile bottles, preserved with Mlekostat CC. After milking, samples were immediately submitted to the Cattle Breeding Division (Milk Testing Laboratory of Warsaw University of Life Sciences) for analysis.

Table 1. Chemical composition of the cows' diet

Chemical composition	Dry matter (%)
Maize silage	45.62
Alfalfa silage	8.49
Grass silage	2.89
Vitamin and mineral premix	0.2
Sodium chloride	0.1
Sodium bicarbonate	0.7
Limestone	0.3
Concentrate (commercial)	41.7
Crude protein (%)	15.82
Ether extract (%)	1.63
Ash (%)	3.56
Crude fibre (%)	9.32
ADF (%)	21.5
NDF (%)	36.2
UPM/kg DM	0.93

ADF - acid detergent fibre, NDF - neutral detergent fibre, UPM- unit of milk production on kg dry matter, DM - dry matter

Sampling, measurements, and analysis

Basic variables of milk, i.e. fat, protein, casein and lactose content were examined on Milkoscan FT–120. Evaluation of hygienic status of the milk was based on somatic cell count on Somacount–150 (Bentley, Poland).

Extraction of fat was performed according to Röse-Gottlieb procedure (AOAC 1990) at a room temperature. Fatty acid methylation was performed according to the transesterification method by EN ISO 5509:2000. The profile of fatty acids was examined on Hewlett Packard 6890 GC with HP Chem software, a flame-ionization detector FID and CP-Sil 88 capillary column (100 m length, 0.25 mm in diameter and 0.25 μm thick), split 1:40. The separation was performed at pre-programmed temperature: 130 °C for 1 min; 130-170 °C at 6.5 °C/min; 170-215 °C at 2.75 °C/min; 215 °C for 12 min, 215-230 °C at 20 °C/min and 230 °C for 3 min. Other parameters were: carrier gas (He) pressure – constant, injector temperature 240 °C, and detector temperature 300 °C. The flow rate of the carrier gas was equal to 25 cm/s.

Atherogenic (AI) and thrombogenic indices (TI) were calculated using equations described by Pikul et al. (2008).

Whey proteins were examined on HPLC–Agilent 1100 series with Zorbax 300SB-C18 columns (4.6 × 250 mm) and Supelguard with particle size 5 μm and pore size 300 Å. Conditions for linear gradient elution were following: 0%–32% B for 10 min, 32%–52% B for 25 min, 52%–80% B for 3 min, flow rate of mobile phase 1.2 ml/min and ambient temperature of separation. Chromatographic separation was performed using the following eluents: A – 0.1% TFA in the solution, acetonitrile:water (5:95), B – 0.1% TFA in the acetonitrile:water (95:5). Whey proteins compounds were identified using UV detector at 214 nm wavelength.

Analysis of vitamins A (α-retinol), D_3 (cholecalciferol), E (α-tocopherol), K (fitochinon) was done by a method described by Puppel et al. (2011). Separations were performed at ambient temperature using 4.6 × 150 mm and 5 μm Zorbax Eclipse XDB C8 column. Solvent flow rate was maintained at 1.2 ml/min in a linear gradient of 90/10 (vol./vol.) methanol to water. Eluate from the column was monitored at 280 nm by Agilent UV detector.

The main technological value of milk was characterised by casein ratio, according to application of the standards of the Center of Chemical Patterns in Italy and Foss, and also, by the casein number and casein index (Schopen et al. 2009).

Representative total mixed ration samples were pooled and stored at –20 C until analyzed. Samples were analyzed at the Cattle Breeding Division (Poland) for dry matter, ash, crude protein, ether extract and crude fibre. The chemical composition of the total mixed ration was calculated from the chemical composition of individual ingredients of the diet.

Statistical analysis

Results were processed by the SPSS (SPSS 14.0 PL) procedure, using ANOVA with single effect of the breed ($P \leq 0.05$). Only interactions between factors whose influence was significant ($P \leq 0.01$ or $P \leq 0.05$) were considered in the study.

Results

The basic milk components and milk yield of Polish Montbeliarde and Polish Holstein-Friesian cows are presented in Table 2. Polish Holstein-Friesian cows were characterised by 24% higher milk yield than Montbeliarde breed ($P \leq 0.01$). Statistical analysis of somatic cell count (SCC) confirmed significant difference between both breeds. There was also significant difference ($P \leq 0.01$) between both breeds when analysing the casein index of milk.

Table 2. Basic composition of milk of Montbeliarde and Polish Holstein-Friesian cattle breeds

Trait	Montbeliarde		Polish Holstein-Friesian	
	LSM	SE	LSM	SE
Daily milk yield (kg)	27.20**	0.704	33.80**	0.842
Fat (%)	4.12	0.195	3.80	0.233
Protein (%)	3.77	0.064	3.79	0.076
Casein (%)	3.03	0.056	3.05	0.067
Lactose (%)	4.90	0.079	4.82	0.095
SCC ($\times 10^3$/cm^3)	357**	45.76	564**	54.78
Casein number	0.797	0.007	0.796	0.008
Casein index	94.22**	0.244	93.01**	0.262

SCC - somatic cell count, Casein number - casein/protein, Casein index -∑casein/(∑casein+∑whey)x100, LSM - least square of mean, SE - standard error, $^{**}P \leq 0.01$

Table 3. Composition of protein fraction of milk of Montbeliarde and Polish Holstein-Friesian cattle breeds (g/l)

Compound	Montbeliarde		Polish Holstein-Friesian	
	LSM	SE	LSM	SE
Lactoferrin	0.347	0.021	0.395	0.025
α-lactoalbumin	1.743	0.073	1.840	0.087
β-lactoglobulin	3.081**	0.141	4.099**	0.168

LSM - least square of mean, SE - standard error, $^{**}P \leq 0.01$

In the analysed protein fraction of Polish Holstein-Friesian milk, higher content of Lf, A-LA and B-LG ($P \leq 0.05$) was found compared to Montbeliarde cows (Table 3). Lactoferrin content was similar in milk of both breeds, fluctuating from 0.347 g/l for Montbeliarde and 0.395 g/l for Polish Holstein-Friesian.

Fatty acid composition in milk of both breeds is presented in Table 4. We found a higher content of C4:0 butyric (BA), C14:0 (myristic acid), C16:0 (palmitic acid, PA) and C18:0 (stearic acid, SO) in the milk of French breed, but differences were significant only for C14:0 ($P \leq 0.01$) and C18:0 ($P \leq 0.05$). The concentration of the unsaturated fatty acids was higher in milk of Polish Holstein-Friesian cows (Table 5). The content of C18:2 *trans*-10, *cis*-12 was higher in Polish Holstein-Friesian milk ($P \leq 0.01$). Also the concentration of docosahexaenoic acid (DHA) was significantly ($P \leq 0.05$) higher in milk of this breed. In contrast, the content of conjugated linoleic acid (CLA) C18:2 *cis*-9, *trans*-11 ($P \leq 0.01$) and DHA ($P \leq 0.05$) was higher in Montbeliarde milk. The differences in activity of Δ^9 desaturase enzyme in milk were significant only for proportion C16:1/C16:0 at $P \leq 0.01$.

Concentrations of fat-soluble vitamins in milk are presented in Table 6. Non-significant differences between both breeds were found. The content of vitamin A and K was higher in Montbeliarde milk.

Table 4. Selected fatty acid composition of milk of Montbeliarde and Polish Holstein-Friesian cattle breeds (g/100 g of fat)

Fatty acid	Montbeliarde		Polish Holstein-Friesian	
	LSM	SE	LSM	SE
C 4:0	2.69	0.094	2.62	0.086
C 14:0	11.08**	0.225	10.30**	0.290
C 16:0	28.50	0.502	28.40	0.663
C 16:1	0.923**	0.054	1.33**	0.076
C 18:0	13.16*	0.589	11.00*	0.845
C 18:1 *trans*-11	2.57	0.166	2.29	0.209
C 18:1 *cis*-9	22.71	0.823	22.68	1.047
C 18:2 n-6	2.11	0.056	2.20	0.064
C 18:3 n-6	0.186	0.027	0.163	0.007
C 18:3 n-3	0.325	0.016	0.328	0.018
C18:2 *cis*-9, *trans*-11	0.872**	0.025	0.593**	0.072
C 18:2 *trans*-10, *cis*-12	0.084**	0.007	0.126**	0.016
C 20:3 n-6	0.047*	0.004	0.069*	0.009
C 20:4 n-6	0.168	0.009	0.227	0.041
C 20:5 n-3	0.160*	0.032	0.070*	0.007
C 22:5 n-3	0.045	0.005	0.043	0.006
C 22:6 n-3	0.037*	0.004	0.043*	0.008

LSM - least square of mean, SE - standard error, *$P \leq 0.05$, **$P \leq 0.01$

Table 5. Sums of the selected fatty acids and indicators influencing activity of Δ^9 – desaturase (g/100g of fat) in milk of Montbeliarde and Polish Holstein-Friesian cattle breeds

Fatty acid	Montbeliarde		Polish Holstein-Friesian	
	LSM	SE	LSM	SE
SFA	62.60*	0.721	61.96 *	1.011
PUFA	3.83	0.093	3.85	0.133
UFA	35.34	0.761	36.04	1.092
UFA/SFA	0.564	0.015	0.575	0.031
PUFA/SFA	0.061*	0.002	0.062*	0.003
n-6/n-3	4.42	0.192	5.55	0.139
AI	2.21*	0.006	2.08*	0.010
TI	2.95*	0.195	2.56*	0.124
CLA/TVA	0.330	0.013	0.258	0.025
C 14:1/C 14:0	0.066	0.006	0.075	0.005
C 16:1/C 16:0	0.032**	0.002	0.047**	0.003
C18:1/C 18:0	1.73	0.006	2.06	0.009

SFA - saturated fatty acids, PUFA - polyunsaturated fatty acids, UFA - unsaturated fatty acids, AI - atherogenic index, TI - thrombogenic index, CLA - conjugated linoleic acid C18:2 *cis*-9, *trans*-11, TVA - *trans* vaccenic acid, LSM - least square of mean, SE - standard error, *$P \leq 0.05$, **$P \leq 0.01$

Discussion

In Poland, Gołębiewski and Brzozowski (2008) found a higher content of protein and a lower content of fat in milk of Montbeliarde compared to Polish Holstein-Friesian breed. Similar results were obtained in France (Coulon et al. 1998). Also Walsh et al. (2008) confirmed the cow's genetic influence on the chemical composition of milk. According

Table 6. Vitamins soluble in fat of milk of Montbeliarde and Polish Holstein-Friesian cattle breeds (mg/l)

Vitamins	Montbeliarde		Polish Holstein-Friesian	
	LSM	SE	LSM	SE
A	0.915	0.080	0.779	0.106
D	0.351	0.040	0.461	0.054
E	1.355	0.150	1.582	0.198
K	0.021	0.003	0.017	0.004

LSM - least square of mean, SE - standard error, $*P \leq 0.05$, $**P \leq 0.01$

to French studies, Montbeliarde cows revealed a lower proportion of mastitis incidence compared to French Holstein-Friesians. Lower susceptibility to mastitis significantly reduces the cost of veterinary treatment and increases the price of better quality milk (Detilleux 2002; Barnouin et al. 2005). The authors stated that Somatic Cell Count of the milk of Polish Montbeliarde corresponded to similar results obtained in France.

Casein number is the most important constituent for the cheesemaker, as only this fraction precipitates during the cheesemaking process. Calculated by Coulon et al. (1998), the proportion of casein in cow's milk true protein for French Prime Holstein and Montbeliarde amounted to 0.811 and 0.817, respectively. According to Lawless et al. (1999), Montbeliarde cow's milk has a much higher content of polyunsaturated fatty acids, especially of conjugated linoleic acid what was confirmed in our study. The contents of vitamins E and D were slightly higher in Polish Holstein-Friesian milk, confirming the genetic effect of breed on that trait.

In Polish production systems, the yield and quality of Montbeliarde milk are significantly different from Polish Holstein-Friesian milk while keeping the animals in the same environment. We proved that the breed of cows has a significant effect on the contents of C14:0, C18:0, CLA, EPA, DHA, B-LG and SCC. Milk from Polish Holstein-Friesian cows had higher concentrations of fat soluble vitamins D and E. Moreover, milk of the Polish breed was characterized by more favourable fat fraction: significantly lower concentration of saturated fatty acids (C14:0, C18:0) as well as lower atherogenic and thrombogenic indexes.

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