Effect of Dairy Cow Crossbreeding on Selected Performance Traits and Quality of Milk in First Generation Crossbreds

Kamila Puppel, Ewa Bogusz, Marcin Gołębiewski, Teresa Nałęcz-Tarwacka, Beata Kuczyńska, Jan Slósarz, Arkadiusz Budziński, Paweł Solarczyk, Małgorzata Kunowska-Slósarz, and Tomasz Przysucha

Abstract: The main goal of crossbreeding Holstein–Friesian (HF) cows with bulls of other dairy or combined breeds is to improve their performance traits. Thus, the aim of this investigation was to compare the dairy performance traits of first generation crossbreds produced by crossbreeding Polish HF cows with bulls of other breeds (Norman, Norwegian Red, Danish Red, Brown Swiss, Montbeliarde, and Simmental). This was done by analyzing the fatty acid profile and technological quality of the milk from these first generation crossbreds. The investigation showed that crossbreeding greatly influenced the performance parameters and technological quality of the milk of the first generation crossbreds obtained from crossbreeding Polish HF cows with bulls of other dairy or combined breeds. The crossbreed cows characterized by the highest both quantity and quality of milk. Also, the best parameters of milk fat dispersion (that is, the highest values of these parameters) that are useful in the production of hard ripening cheeses and butter were found in the milk of crossbreed cows. Health beneficially, saturated fatty acids level in milk of crossbreeds was by 25.96% lower in crossbreds milk when compared to purebred cows. The most beneficial content of whey proteins was found in the milk of Polish HF and Norwegian Red crosses, where it was 19.04% higher than in pure breed Holsteins. F1 cows tend to express better functional traits than Holstein (PHF) cows. Effect of heterosis was larger and gave better results when there was a greater genetic distance between the animals used for crossbreeding.

Keywords: cattle, heterosis, hybrids, milk

Practical Application: The effects of heterosis are opposite to the effects of inbreeding depression, and it extends the lifespan and use of animals as well as improves their fertility and health. The main goal of crossbreeding Holstein–Friesian cows with bulls of other dairy or combined breeds is to improve their performance traits. F1 cows tend to express better functional traits than Holstein (PHF) cows. Effect of heterosis was larger and gave better results when there was a greater genetic distance between the animals used for crossbreeding.

Introduction

The breeding of cattle aims to improve the genetic ability of animals and ensure that future generations will produce milk in a more efficient way. Cow milk accounts for more than 80% of world milk production, and it is the most universal raw material for processing (Barłowska and others 2011a). Inbreeding is the mating of related animals and results in an increased level of inbreeding depression as well as homozygosity, which increases the risk of the appearance of undesirable effects in the phenotype. These undesirable effects are generally associated with fertility and survival, and can also be noted in production, growth and carcass traits. Selection intensity within a breed is a contributing factor to level of inbreeding (Weigel and Barlass 2003). McParland and others (2007) reported that a faster inbred rate is anticipated if selection for economically valuable traits is based on increased emphasis of the breeders to pedigree information to identify genetically superior animals. According to the newest data, the world population of Holstein–Friesian (HF) cows has become increasingly inbred.

A perfect solution to inbreeding is the use of crossbreeding, which eliminates the typical problems of inbreeding and results in heterosis. The effects of heterosis are opposite to the effects of inbreeding depression, and it extends the lifespan and use of animals as well as improves their fertility and health (Weigel and Barlass 2003; Funk 2006; Heins and others 2012; Buckley and others 2014). For example, El-Tarabany (2015) found that HF × Brown Swiss (BS) hybrids were stronger, less susceptible to metabolic burden and more tolerant to changes in their daily ration compared to purebred HF. According to Dezetter and others (2015), the impact of heterosis is visible in improved efficiency, reproductive measures of cows and in the cytological quality of milk.

The crossbreeding of dairy cattle has become a topic of considerable interest in response to concerns by dairy producers about the declining fertility, health and survival of Holstein cows (Lucy 2001; Kądrowska and Gołębiewski 2013; Buckley and others 2014). The aim of this study was to compare the performance traits and nutritional and technological usefulness of the milk of first generation crossbreds (F1) crossing Polish HF (PHF) cows with bulls of other

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Table 1–Ingredient chemical composition and fatty acid composition of the cow's daily ration.

Composition	Daily diet
Ingredient (kg/d)	
Maize silage	21.0
Grass silage	10.1
Corn silage	2.50
Soybean meal	0.5
Pasture ground chalk	0.1
Vitamin mix[a]	0.13
Salt	0.04
Rapeseed meal	1.0
Magnesium oxide	0.05
Chemical composition (g/kg DM)	
Dry matter (%)	53.5
Ash (% of DM)	4.3
Crude protein (% of DM)	12.5
Acid detergent fiber (% of DM)	30.9
Neutral detergent fiber (% of DM)	42.7
Calcium (% of DM)	0.8
Phosphorus (% of DM)	0.5
Crude fiber (% of DM)	19.36
UFL per kg of DM	1.01
PDI per kg of DM	95.42
Fatty acids mg/g of fatty acids	
Saturated	220.15
Unsaturated	426.1
Oleic (18:1)	129.05
18:1-trans	16.77
Linoleic (18:2. n-6)	222.2
Linolenic (18:3. n-3)	72.16
EPA (20:5. n-3)	0.31
DHA (22:6. n-3)	0.19

[a]Contained (on 1000 g): Ca – 150 g. P – 100 g. Na – 50 g. Mg – 40 g. Zn – 9000 mg. Mn – 7000 mg. Cu – 1000 mg. J – 100 mg. Se – 50 mg. vitamin A – 1200000 j.m. vitamin D3 – 120000 j.m. vitamin E – 5000 mg. vitamin K – 93 mg. vitamin B1– 80 mg. vitamin B6 – 160 mg. vitamin B2 – 110 mg. vitamin B12 – 1 000 mcg.

breeds (Normande, Norwegian Red, Danish Red, Brown Swiss, Montbeliarde, and Simmental).

Experimental

The study was conducted in an experimental farm located in Worgule (northern part of Lubelskie Province) in Poland. Animals were cared for following the Minimum Standards for Livestock Maintenance (Regulation of the Minister of Agriculture and Rural Development 2010). Seventy lactating cows (days in milk (DIM) = 150 ± 21 d; parity = 2; milk yield = 22.05 ± 3.34 kg/d) were divided into 7 groups (10 cows per group) according to their genotype: PHF group – Polish HF breed; PHF×NO group – F_1 crossbreds of PHF and Normande cattle; PHF×NRF group – F_1 crossbreds of PHF and Norwegian Red cattle; PHF×RD group – F_1 crossbreds of PHF and Danish Red cattle; PHF×BS group – F_1 crossbreds of PHF and Brown Swiss cattle; PHF×MO group – F_1 crossbreds of PHF and Montbeliarde cattle; PHF×SIM group – F_1 crossbreds of PHF and Simmental cattle.

Cows were housed in individual stalls and fed Total Mi× Ration (TMR). They were fed according their daily requirements. The daily ration was balanced according to the INRA formulation system. The ingredients, chemical composition and fatty acid composition of the cows' daily ration is presented in Table 1. The cows were milked twice a day (between 05.00, 06.00 and 17.00, 18.00) using a milking pipeline.

Sample collection and measurements

Representative feed samples were collected and stored at –20 °C for later chemical analysis. Dried feed samples were analyzed for dry matter (DM), crude fat (ether extract), crude protein, ash and fiber according to guidelines of the Association of Official Analytical Chemists (AOAC 1990).

Milk samples for each group (4 samples from each cow) of cows were collected for analysis 4 times—once a week for 4 consecutive weeks, according to the proportion of morning and afternoon milk yield. The samples were transported at a temperature of 4 °C to the Milk Laboratory of Warsaw Univ. of Life Sciences for further analysis. The gross composition of the milk, that is, fat, protein, casein and lactose content were determined by automated infrared analysis with a MilkoScan FT120 (Foss Electric, Hillerød, Denmark), and the hygienic status of the milk was evaluated through somatic cell count (SCC) recorded on a Somacount 150 (Bentley, Poland).

Whey proteins were determined using an Agilent 1100 Series reverse phase high-performance liquid chromatograph (Agilent Technologies, Waldbronn, Germany) according to the methodology described by Puppel and others (2016). Separations were performed at ambient temperature using a solvent gradient on Jupiter column C18 300A (Phenomenex, Torrance, Calif., U.S.A.). The chromatographic conditions were as follows. Solvent A was acetonitrile (Merck, Darmstadt, Germany), water (Sigma-Aldrich, St Louis, Miss., U.S.A.) and trifluoroacetic acid (Sigma-Aldrich) in a ratio of 50:950:1 (v/v/v). Solvent B was acetonitrile, water and trifluoroacetic acid in a ratio of 950:50:1 (v/v/v). The total run time was 44 min, the flow rate was 1.2 mL/min and the detection wavelength was 220 nm. The injection volume of final solution was 25 μL.

Fatty acid methylation was performed according to transesterification method EN ISO 5509 (2000). Individual fatty acids in crude fat were identified using an Agilent 7890A GC with a flame ionization detector (Agilent, Waldbronn, Germany), HP Chem software and a Varian Select FAME column (100 m length, 0.25 mm diameter, 0.25 μm film thickness; Varian/Agilent Technologies, Waldbronn, Germany). The analysis involved a programmed run with temperature ramps under the conditions and temperatures described by Puppel and others (2016).

Rennet coagulation time (RCT, min), defined as the time from enzyme addition to milk gelation, was measured according to the method described by Jurczak (2003), and the dispersion of milk fat globules (MFG) was analysed according to the method described by Kuczyńska (2011).

Statistical analysis

Data from the 4 milk samples for each group of cows were collected together and analysed as a single factor design. The variance for cow were nested within the genetic group as random error term to the main effect of genetic group. The effect of the genetic group was quantified by running a one-way analysis of variance (ANOVA), using IBM SPSS Statistics 23.0.0.2. (2012). Data were presented as least squares means (LSM) with standard error of the mean (SEM).

Results and Discussion

F1 cows tend to express better functional traits than Holstein (HO) cows (Dechow and others 2007). In our study, crossbreeding significantly affected milk yield and the gross composition of milk (Table 2). PHF×MO cows had the highest milk yield, reaching 27.97 kg, while PHF×NO cows had the lowest—18.93 kg. In Dezetter and others (2015) study, HO × MO and HO × NO crossbred cows with 40% to 59% of HO genes had a 305-d

Table 2–Changes in milk gross composition as affected by the crossbreeding.

Component		Breed						
		PHF	PHF×NO	PHF×NRF	PHF×RD	PHF×BS	PHF×MO	PHF×SIM
Milk yield (kg/d)	LSM	23.25^{ABC}	18.93^{ADEFGH}	25.44^{DIjKl}	20.69^{BEILMN}	23.13^{FjŁO}	27.97^{CGKMOU}	23.63^{HlNU}
	SEM	7.018	4.388	7.713	6.513	4.997	3.944	4.517
Protein (%)	LSM	3.19^{AB}	3.59^{ACDEFG}	3.40^{BCHIJK}	3.19^{DHl}	3.23^{EIŁ}	3.02^{FJłLM}	3.22^{GKM}
	SEM	0.431	0.581	0.155	0.283	0.276	0.24	0.323
Fat (%)	LSM	3.90^{ABCd}	4.77^{AEFGH}	3.91^{EiJk}	4.21^{BFilkŁm}	4.79^{CJlNO}	3.61^{dGkŁNu}	3.91^{HmOu}
	SEM	0.8	0.165	0.636	0.805	0.686	0.632	1.559
Lactose (%)	LSM	4.49	4.5	4.26	4.42	4.48	4.47	4.42
	SEM	0.245	0.284	0.275	0.186	0.11	0.118	0.11
Protein/Fat	LSM	0.83^{ABC}	0.97^{ADEF}	0.69^{BDGHI}	0.78^{EGJK}	0.90^{HJŁŁ}	0.78^{EILM}	0.63^{CFKŁM}
	SEM	0.047	0.104	0.074	0.147	0.056	0.104	0.12
Casein (%)	LSM	2.55^{A}	2.89^{ABCDEF}	2.6^{BG}	2.54^{CHI}	2.63^{DHJ}	2.40^{EGIJK}	2.56^{FK}
	SEM	0.338	0.346	0.158	0.205	0.208	0.148	0.187
Casein number	LSM	0.8	0.81	0.78	0.8	0.82	0.8	0.8
	SEM	0.034	0.032	0.03	0.023	0.012	0.018	0.031
% Casein in protein	LSM	79.98	80.83	77.59	79.68	81.50	79.66	79.69
	SEM	3.363	3.176	3.037	2.285	1.227	1.752	3.388
Urea (mg/L)	LSM	276.73^{ABCDEF}	336.33^{AGH}	258.80^{BGIJKL}	318.47^{CIŁ}	232.63^{DHJŁMN}	317.08^{EKM}	323.57^{FLN}
	SEM	84.832	62.268	38.141	86.028	55.795	60.397	98.63
SCC (tys/mL)	LSM	192.73	84.67	73	172.82	197.13	136.85	158.29
	SEM	197.267	102.919	36.38	164.526	161.269	127.515	147.074

PHF group consisted cows belonged to Polish Holstein–Friesian breed (PHF); PHF×NO group– F_1 crossbreeds between PHF and Normande cattle; PHF×NRF group– F_1 crossbreeds between PHF and Norwegian Red cattle; PHF×RD group– F_1 crossbreeds between PHF and Danish Red cattle; PHF×BS group– F_1 crossbreeds between PHF and Brown Swiss cattle; PHF×MO group– F_1 crossbreeds between PHF and Montbeliarde cattle; PHF×SIM group– F_1 crossbreeds between PHF and Simmental cattle.
Means in the same rows marked with the same letters differ significantly at: lowercase- $P \leq 0.05$; capitals $P \leq 0.01$.
SEM, standard error of mean; LSM, least square of mean.

milk yield of respectively 859 and 1411 kg, which was lower than purebred HO cows. In a study using HO × Jersey (J) first-cross cows, Coffey and others (2016) found that the yield of milk solids in crossbreds exceeded their respective parental average performance. Additionally, Maki-Tanila (2007) found that the effect of heterosis was larger and gave better results when there was a greater genetic distance between the animals used for crossbreeding. Our results are in agreement with Swalve and others (2008), who reported no difference in milk yield for BS × HO and HO cows. Additionally, Gojam and others (2017) reported, that lactation milk yield obtained F_1FB (Boran heifers × WWS sires) reflects progressive trend of milk production from 1st to 5th parity (1874.65 ± 67.7 to 2582.69 ± 111.2 kg).

Our results showed that PHF×NO, PHF×NRF, PHF×BS, and PHF×SIM cows had higher concentrations of protein and fat in milk compared with the PHF group. Stocco and others (2017) reported that compared with HF cows, BS cows had a slightly lower productive potential, which was compensated for by greater milk fat and protein contents (fat was 0.27 percentage units higher and protein was 0.28 units higher in BS), and, in particular, by much more favorable milk properties. On the other hand, Dechow and others (2007) showed that BS×HF hybrids had a higher milk yield with a higher content of fat and protein compared with HF cows. This was also confirmed by Swalve and others (2008) and Van Raden and Sanders (2003). However, Ezra and others (2016) showed no significant differences in the fat content of milk from HF and HF×NRF cows.

In our study, the lowest percentage content of protein and fat were found in the milk of PHF×MO cows (Table 2). Our results are in agreement with Heins and Hansen (2012) who reported similar results, with higher protein content in NO × HO compared with pure HO cows. Our results are, however, inconsistent with findings of other authors. For instance, Blöttner and others (2011a) reported no significant differences for milk, protein, and fat yields over the first 3 lactations for pure HO and BS×HO cows. Walsh and others (2008) reported no differences in properties of milk between HO, MO × HO and NO × HO cows throughout the whole lactation period, except for fat yield, which was lower for MO × HO crossbreds than HO cows. A different conclusion was made by Heins and others (2006, 2010), who found a higher content of fat and protein in the milk of MO×HF crosses compared with HF cows.

The highest percentage content of lactose was found in the milk of PHF×NO cows. In turn, the lowest content of lactose was found in the milk of PHF×NRF cows. Similar results were reported by Malchiodi and others (2014a, 2014b) who found that crossbreds produced milk with a lower lactose content, but with a higher content of protein and fat compared to HF cows.

The lowest SCC was found in the milk of PHF×NRF crosses. These results are consistent with data reported by other authors, for example, Pawlak (2013). The highest SCC was noted in PHF×BS cows. Van Raden and Sanders (2003) demonstrated the effect of heterosis on SCC in the following breeds: HO, Guernsey (G) and J, and their crosses. In addition, Begley and others (2009) showed an improvement in udder health in NRF×HF crosses. An opposite conclusion was drawn from the study by Blöttner and others (2011b) who recorded no significant differences in SCC between HF and HF×BS cows. Ezra and others (2016) also found no differences in SCC between HF and HF×NRF cows. In addition, Heins and Hansen (2012) concluded that SCC in HF×NO crosses was similar to SCC in HF cows, and that it was significantly lower in the crosses of HF×MO and HF with bulls of the Scandinavian breeds. Heins and others (2010) found that HO × MO crosses had a SCC that was on average 0.29 lower than HO cows.

The highest percentage content of casein in total protein was found in the milk of PHF×BS crosses—over 81%. Percentage content of casein in the protein of the milk of the PHF cows was 79.98%. Malchiodi and others (2014a, 2014b) noted a tendency for high casein content in total protein in outbred crosses.

Table 3–Changes in the concentration of functional fatty acids of milk fat as affected by the crossbreeding.

Component		Breed						
		PHF	PHF×NO	PHF×NRF	PHF×RD	PHF×BS	PHF×MO	PHF×SIM
C4:0	LSM	2.09^{abcd}	2.74^{aefGHI}	2.22^{eJKL}	2.09^{flmn}	1.71^{bGJł}	1.66^{cHKm}	1.64^{dILm}
	SEM	0.575	0.777	0.397	0.68	0.925	0.973	0.793
C18:1 *trans*-11	LSM	1.61^{aBCDEF}	1.79^{aGHIJK}	6.42^{BGLŁMNO}	3.90^{CHŁUPR}	2.51^{DIMUS}	2.10^{EJNPT}	4.64^{FKORST}
	SEM	0.51	0.245	0.55	0.644	0.473	0.969	0.171
C18:1 -9	LSM	22.45^{ABACDef}	18.20^{AGHIJ}	18.2^{0BKLŁM}	23.88^{CGKN}	20.15^{DHLNOU}	23.21^{eIŁO}	23.57^{fJMU}
	SEM	1.624	1.297	1.973	1.598	1.022	1.245	1.712
C18:2 n-6	LSM	1.54^{AbCDE}	0.90^{AFGHIJ}	1.95^{bkLŁ}	1.63^{CGkmn}	1.22^{DHLmO}	2.02^{EInOU}	1.51^{JŁU}
	SEM	0.263	0.265	0.266	0.242	0.233	0.256	0.253
C18:2 *cis*-9 *trans*-11	LSM	0.44^{abcd}	0.44^{efghi}	0.55^{aeJK}	0.50^{bfŁŁ}	0.32^{cgJLM}	0.51^{dhMN}	0.37^{iKŁŁN}
	SEM	0.12	0.074	0.164	0.125	0.156	0.161	0.13
C18:3 n-3	LSM	0.24^{A}	0.24^{B}	0.59^{ABCDEF}	0.25^{C}	0.17^{D}	0.26^{E}	0.19^{F}
	SEM	0.011	0.048	0.101	0.034	0.102	0.051	0.062
C20:4 n-6	LSM	0.09^{A}	0.06^{B}	0.13^{ABCDEF}	0.06^{C}	0.08^{D}	0.08^{E}	0.09^{F}
	SEM	0.006	0.003	0.003	0.003	0.003	0.003	0.002
C20:5 n-3	LSM	0.05 ^{A}	0.05 ^{B}	0.12 ^{ABCDEF}	0.04^{C}	0.04^{D}	0.05^{E}	0.05^{F}
	SEM	0.002	0.002	0.003	0.001	0.001	0.002	0.003
C22:5 n-3	LSM	0.07^{A}	0.06^{B}	0.09 ^{ABCDEF}	0.05^{C}	0.05^{D}	0.07^{E}	0.05^{F}
	SEM	0.004	0.003	0.003	0.002	0.001	0.003	0.001
C22:6 n-3	LSM	0.01^{A}	0.01^{B}	0.03 ^{ABCDEF}	0.01^{C}	0.01^{D}	0.01^{E}	0.01^{F}
	SEM	0.007	0.007	0.002	0.001	0.005	0.006	0.005

Means in the same rows marked with the same letters differ significantly at: lowercase- $P \leq 0.05$; capitals $P \leq 0.01$. SEM, standard error of mean; LSM, least square of mean.

The lowest casein content in total protein in our study was noted in PHF×NRF cows—77.59%. The relationship between casein content and milk coagulation properties has been investigated by Politis and Ng-Kwai-Hang (1988) and Tyrisevä and others (2003, 2004), who reported a positive association between these traits: an increase of casein content is associated with an increase in the strength of coagulation. Additionally, Islam and others (2014) reported, that proportion of casein to whey protein was lower in Holstein cross milk and this milk was found higher in β-lactoglobulin.

The content of selected functional fatty acids is presented in Table 3. Our study showed no significant differences in the content of butyric acid (C4:0) between the milk of purebred cows and crosses. The highest content of this acid was found in PHF×NO crosses (2.74 g/100 g fat). The lowest content of this acid was found in PHF×SIM crosses—1.64 g/100 g fat. Palladino and others (2010) demonstrated that the genotype of cows had no effect on the level of short-chain fatty acids (C4 to C14), which reflects results in our investigation.

Our study determined that genotype affects the content of mono unsaturated fatty acids (MUFA) in milk fat. The highest content of trans-vaccenic acid (TVA) in milk fat was found in PHF×NRF crosses (6.42 g/100 g fat), whereas the lowest content in PHF×NO cows (0.79 g/100 g fat). The content of TVA in PHF cows was 1.61 g/100 g fat. The highest content of oleic acid (C18:1 *cis*-9; OA) was found in the milk of PHF×RD cows—23.88 g/100 g fat, whereas OA was at a level of 22.45 g/100 g fat in PHF cows. A lower content of OA than in PHF cows was reported for PHF×NO and PHF×BS cows. Ferris and others (2011) also found a higher content of MUFAs in pure HF than in J×HF crosses.

The highest content of conjugated linoleic acid (CLA) was found in the milk of PHF×NRF crosses (0.55 g/100 g fat), and the lowest in PHF×BS crosses—0.32 g/100 g fat. Lock and Bauman (2004) found that SIM×HF crosses had a higher level of CLA in milk compared to the purebred HF cows, despite being kept under the same environmental conditions. A study conducted by Sasanti (2014) also found a higher concentration of CLA in the milk of SIM×J crosses compared to that of purebred J cows.

Our study found that the content of linoleic acid (C18:2 n-6; LA) in the milk fat of PHF×MO crosses (2.02 g/100 g fat) was 30% higher than in PHF cows. In turn, the content of linolenic acid (C18:3 n-3; LNA) was higher by 145% in PHF×NRF (0.59 g/100 g fat) compared to PHF cows. Sasanti (2014) also reported a higher content of LA and LNA in the milk fat of crosses (SIM×J), compared to the values obtained for purebred cows. The highest contents of C20:4 n-6, C22:5 n-3 and C22:6 n-3 acids were found in milk from PHF×NRF cows. To summarize, the most beneficial (that is, the highest) concentrations of MUFAs and polyunsaturated fatty acids (PUFA) were found in the milk of the crosses of PHF and red breeds (NRF and RD).

A high content of saturated fatty acids (SFAs) is a typical trait of cow milk fat. The acids with this configuration constitute over 60% of the total fatty acids in cow milk fat (Puppel 2011). The contents of individual fatty acids are presented in Table 4. The results show that genotype significantly affects the contents of C12:0, C14:0, and C16:0 in milk fat. The lowest contents of lauric, myristic, and palmitic acids were found in the milk fat of PHF×RD crosses (2.11, 7.63, and 32.66 g/100 g fat, respectively). White and others (2001) showed that breed significantly affects levels of fatty acids with 6 to 14 carbon atoms in a molecule, and this was reflected in our investigation. Similar effects were also noted by Palladino and others (2010), who found a significantly higher content of C16:0 in the milk fat of pure J cows and of J×HF crosses, compared to purebred HF cows. Kuczyńska and others (2012) evaluated the fatty acid composition of pure PHF and pure MO cows in the same environmental conditions. They reported a higher content of C4:0, C14:0, C16:0, and C18:0 in the milk of MO cows, but differences were significant only for the content of C14:0 and C18:0. Heins and others (2006) evaluated the milk yield and milk quality of MO × H compared with purebred H. The authors reported that milk fat was higher for purebred H compared to the crosses.

Table 4–Changes in the concentration of selected saturated fatty acid as affected by the crossbreeding.

Component		Breed						
		PHF	PHF×NO	PHF×NRF	PHF×RD	PHF×BS	PHF×MO	PHF×SIM
C12:0	LSM	2.71^{Abc}	2.64^{DEfgh}	3.69^{ADIjkL}	2.11^{bEI}	3.02^{fj}	3.05^{gk}	2.17^{chL}
	SEM	0.985	0.647	0.129	0.36	0.32	0.055	0.414
C14:0	LSM	10.03^{AbCDE}	8.27^{AFgh}	11.47^{bFIJK}	$7.63C^{gi}$	9.4^{Dj}	10.23^{h}	8.32^{Ek}
	SEM	1.477	1.39	1.303	1.161	1.883	1.722	1.83
C16:0	LSM	32.92^{A}	30.63^{B}	32.66^{C}	26.51^{ABCDEF}	31.90^{D}	30.82^{E}	30.40^{F}
	SEM	2.359	2.805	2.821	2.812	2.596	2.644	2.5

Means in the same rows marked with the same letters differ significantly at: lowercase- $P \leq 0.05$; capitals $P \leq 0.01$. SEM, standard error of mean; LSM, least square of mean.

Table 5–Changes in the concentration of the family of fatty acids of milk fat as affected by the crossbreeding.

Parametr		Breed						
		PHF	PHF×NO	PHF×NRF	PHF×RD	PHF×BS	PHF×MO	PHF×SIM
SCFA	LSM	9.38^{a}	11.54^{abCDEF}	10.64^{bGhiJK}	9.08^{CGHL}	9.83^{DI}	9.18^{EJ}	8.33^{FKL}
	SEM	0.474	1.154	0.935	0.524	0.707	0.559	0.79
MCFA	LSM	47.97	49.02	46.79	47.23	49.41	45.72	46.00
	SEM	1.178	2.867	2.322	1.302	1.756	1.388	1.963
LCFA	LSM	42.61^{A}	40.55^{ABCDE}	45.24^{BF}	43.69^{CG}	40.89^{FGHI}	45.08^{DH}	45.63^{EI}
	SEM	1.45	3.53	2.859	1.603	2.162	1.71	2.417
PUFA n-3	LSM	0.12^{A}	0.11^{B}	0.19^{ABCDEF}	0.10^{C}	0.10^{D}	0.12^{E}	0.11^{F}
	SEM	0.01	0.025	0.02	0.011	0.015	0.012	0.017
MUFA	LSM	26.72^{ABCDE}	24.90^{AFGHIJ}	28.93^{BFK}	29.69^{CGL}	$26.49^{HKLŁM}$	$28.54^{DIŁ}$	28.85^{EJM}
	SEM	1.142	2.779	2.252	1.262	1.702	1.346	1.903
PUFA	LSM	3.30^{aBCD}	2.83^{aEfGh}	4.16^{BEIJk}	3.39^{fl}	2.68^{CJ}	$4.0l^{DG}$	3.21^{hk}
	SEM	0.131	0.318	0.258	0.144	0.195	0.154	0.218

MUFA (monounsaturated fatty acid) = Σ $C_{10:1}$. $C_{12:1}$. $C_{14:1}$. $C_{15:1}$. $C_{16:1}$. $C_{17:1}$. TVA. $C_{18:1}$. $C_{20:1}$; PUFA (polyunsaturated fatty acid) = Σ LA. GLA. DGLA. CLA. LNA. AA. EPA. DPA. DHA; SCFA (short chain fatty acid) = Σ $C_{4:0}$. $C_{6:0}$. $C_{8:0}$. C $_{10:0}$; LCFA (long chain fatty acid) = Σ $C_{18:0}$. TVA. OA. LA. GLA. LNA. CLA. $C_{20:1}$. $C_{20:3n3}$. $C_{20:4n6}$. DGLA. EPA. DPA. DHA; MCFA (*middle chain fatty acid*) = Σ $C_{12:0}$. $C_{12:1}$. C $_{14:0}$. C $_{14:1}$. $C_{15:0}$. $C_{15:1}$. $C_{16:0}$. $C_{16:1}$. $C_{17:0}$. $C_{17:1}$. Means in the same rows marked with the same letters differ significantly at: lowercase- $P \leq 0.05$; capitals $P \leq 0.01$. SEM, standard error of mean; LSM, least square of mean.

The contents of individual fatty acids are summarized in Table 5. Our study noted a significant effect of genotype on the contents of particular families of fatty acids in milk fat. Short-chain fatty acids (SCFAs) are an excellent, fast-released source of energy and have anti-inflammatory properties. As reported by Kuczyńska (2011), these anti-inflammatory properties inhibit the activity of inflammatory mediators in intestinal epithelium, namely NFκB macrophage activation. For this reason, an increased concentration of SCFAs significantly improves the nutritional value of milk.

Polyunsaturated fatty acids (PUFAs) are not synthesized in the human body due to the lack of enzymes that enable the formation of double bonds at the 6th and 3rd atom of carbon in the chain from the side of the methyl group—CH_3. PUFAs therefore need to be provided to the body through diet. Due to this, a high content of PUFAs in milk fat is of great significance from the nutritional perspective. Our study found that genotype significantly affects the level of PUFAs in the milk fat of the cows. The highest concentration of PUFA n-3 was noted in PHF×NRF crosses—0.19 g/100 g fat, and this was higher than the value found in PHF cows by 58%. Kelly and others (1998) and Schroeder and others (2005) also reported that breed significantly affected the level of PUFAs in milk fat.

Based on the above results, it can be concluded that the most beneficial (that is, the highest) concentrations of SCFA and PUFA n-3 were found in the milk of PHF×NRF crosses.

Whey proteins are used in cheese production. After their thermal desaturation in milk, casein integrates and coagulates with whey proteins, meaning that they can be better used in cheese making technology (Oldfield and others 2000). In addition, according to Cichon (1979), a higher content of whey proteins in rennet cheeses contributes to their higher nutritional value.

The effect of crossbreeding on the concentration of whey proteins is presented in Table 6. Significant differences in whey protein concentration between the crosses can be seen. The highest concentrations of lysozyme, lactoferrin (Lf), α-lactalbumin (A-LA), and β-lactoglobulin (B-LG) were found in PHF×NRF crosses: 10.46 μg/L, 0.7 g/L, 1.52 g/L, and 4.95 g/L, respectively. The milk of the PHF×NRF crosses had the lowest concentrations of the respective proteins: 7.27 μg/L, 0.49 g/L, 1.06 g/L, and 3.45 g/L. Kuczyńska and others (2012) compared purebred PHF with MO cows, and found that concentrations of whey proteins (Lf, A-LA and B-LG) were higher in PHF milk. Król and others (2011a, b) also noted a highly significant effect of genotype on changes in the concentration of whey proteins in milk. Our investigation demonstrated a similar dependency between PHF and PHF×SIM to that obtained by Król and others (2011a) regarding the concentration of whey proteins in milk and rennet whey for the SIM and PHF breeds. To sum up, the most beneficial (that is, the highest) concentration of whey proteins were obtained in the PHF×NRF crosses.

The effect of crossbreeding on the technological usability of milk is presented in Table 7. The genotype of the cows had a significant effect on changes in the dry matter content of milk. The highest dry matter content was found in PHF×NO crosses (13.92 g/100 g milk), and the lowest in PHF×MO crosses (12.15 g/100 g milk). Similar tendencies were demonstrated in level of fat-free dry matter (8.91 g/100 g for PHF×NO and 8.33 g/100 g for PHF×MO).

Table 6–Changes in the concentration of whey protein fraction of milk as affected by the crossbreeding.

		Breed						
Component		**PHF**	**PHF×NO**	**PHF×NRF**	**PHF×RD**	**PHF×BS**	**PHF×MO**	**PHF×SIM**
Lysozyme (μg/L)	LSM	8.78^{aB}	9.61^{acdefg}	$10.46B^{chijk}$	8.94^{dh}	8.22^{ei}	8.47^{fj}	8.98^{gk}
	SEM	0.11	0.228	0.457	0.524	0.089	0.378	0.16
Lactoferrin (g/L)	LSM	0.59^{A}	0.64^{B}	0.70^{ABCDEF}	0.60^{C}	0.55^{D}	0.56^{E}	0.60^{F}
	SEM	0.013	0.021	0.027	0.0102	0.033	0.032	0.044
α-Lactoalbumin (g/L)	LSM	1.28^{a}	1.40^{abcd}	1.52^{befgh}	1.30^{e}	1.20^{cf}	1.23^{dg}	1.31^{h}
	SEM	0.0293	0.047	0.021	0.020	0.015	0.020	0.031
β-Lactoglobulin (g/L)	LSM	4.16^{aBC}	4.55^{ade}	4.95^{BdfGhi}	4.23^{fj}	3.89^{CGj}	4.01^{eh}	4.25^{i}
	SEM	0.452	0.529	0.69	0.722	0.516	0.652	0.223

Means in the same rows marked with the same letters differ significantly at: lowercase- $P \leq 0.05$; capitals $P \leq 0.01$. SEM, standard error of mean; LSM, least square of mean.

Table 7–Changes in milk quality usefulness parameters as affected by the crossbreeding.

		Breed						
Component		**PHF**	**PHF×NO**	**PHF×NRF**	**PHF×RD**	**PHF×BS**	**PHF×MO**	**PHF×SIM**
Density (g/cm^3)	LSM	1.03	1.03	1.03	1.03	1.03	1.03	1.03
	SEM	0.001	0.001	0.002	0.002	0.001	0.002	0.003
TS (g/100g)	LSM	12.59	13.92	12.65	12.87	13.52	12.15	12.61
	SEM	0.67	0.518	0.61	0.873	0.652	0.645	0.460
SNF (g/100g)	LSM	8.52	8.91	8.49	8.5	8.62	8.33	8.56
	SEM	0.464	0.389	0.382	0.313	0.302	0.178	0.265
Acidity (°SH)	LSM	7.23	8.62	7.93	7.32	7.15	6.96	7.20
	SEM	1.075	1.028	0.643	1.235	0.825	0.599	0.884
Citric acid (g/100g)	LSM	0.16	0.14	0.13	0.17	0.17	0.15	0.18
	SEM	0.03	0.017	0.018	0.023	0.016	0.022	0.031
FFA (mmol/100g of fat)	LSM	0.10	0.06	0.11	0.09	0.01	0.12	0.09
	SEM	0.054	0.068	0.045	0.045	0.054	0.061	0.026
FPD (°C)	LSM	0.53	0.55	0.51	0.52	0.53	0.51	0.52
	SEM	0.030	0.018	0.028	0.021	0.047	0.017	0.032
Renet coagulation time (RCT) (min)	LSM	15.51^{ABCdEf}	14.19^{AGHiJ}	19.8^{BGKLLM}	11.49^{BHKNOU}	14.19^{dLNpR}	$13.05^{EiŁOpS}$	16.35^{fJMURS}
	SEM	0.240	0.240	0.440	0.380	0.540	0.420	0.300

Means in the same rows marked with the same letters differ significantly at: lowercase- $P \leq 0.05$; capitals $P \leq 0.01$. SEM, standard error of mean; LSM, least square of mean.

Milk coagulation properties are affected by different factors such as milk quality, breed, season, herd (Toffanin and others 2012), as well as stage of lactation and parity of the cow (Penasa and others 2014). Rennet Coagulation Time (RCT) has a significant effect on cheese quality and, therefore, is of great economic significance (Johnson and others 2001). Regarding the effect of season of sampling, Barłowska and others (2012) and Chládek and others (2011) reported the shortest RCT in the summer. The effect of breed regarding milk coagulation properties was reported by several authors (Macheboeuf and others 1993; Ikonen and others 2004; Penasa and others 2014), and their results confirmed those of our study. Crossbreeding also significantly affected RCT in our investigation (Table 7). Jõudu and others (2008) reported that an increase of milk protein and casein contents reduced RCT, and our results reflect this. The longest coagulation time was found in the milk of PHF×NRF crosses (19.60 min). A similar result was noted by Malchiodi and others (2014a, 2014b) for crosses of HF with the Swedish Red breed. In addition, De Marchi and others (2007) stated that the low acidity of milk extended the duration of coagulation.

Table 8 shows the effect of breed on the size and distribution of MFG. Our study revealed statistically significant differences between mean diameter of MFG medium MFG in PHF cows and the other breeds. The average diameter of MFG ranged from 8.71 to 11.15 μm, and was larger in all crosses compared to the purebred PHF (Table 8). The greatest diameter of MFG was noted for PHF×BS (11.15 μm), and the most similar to that of PHF cows was found for PHF×SIM (9.44 μm). The highest percentage content of small MFG (<3 μm; SFG) was found in pure PHF cows (5%), and the lowest in PHF×NO and PHF×RD crosses (2%). The content of medium-sized MFG (from 3 to 6 μm) was the lowest in PHF×NRF and PHF×BS crosses and the highest in PHF and PHF×NO cows. The highest percentage content of MFG was found in the milk of PHF cows as well as PHF×NO crosses (51%). The highest content of SFG being found in the milk of PHF cows was also demonstrated by Barłowska and others (2011b). The same investigation also found a lower percentage content of SFG and, thus, a higher content of large MFG in the milk of SIM cows compared to PHF cows, which is consistent with results obtained in our study. In addition, the above study found a lower percentage content of SFG in the milk of Polish Red cows compared to PHF cows, which is comparable with results from our investigation regarding PHF×NRF and PHF×NO crosses. In our study, the percentage content of large MFG (with diameters above 6 μm) was the lowest in the milk of purebred PHF (43%). The strongest milk fat dispersion was observed in the milk of PHF cows (3.15×10^9).

Considering the above results, it may be concluded that the best parameters of milk fat dispersion (that is, the highest values of these parameters) that are useful in the production of hard ripening cheeses and butter were found in the milk of PHF×NO and PHF×NRF crosses following by PHF×RD and PHF×BS cows. The best raw material for the consumption and production of fermented drinks turned out to be milk of PHF cows.

Table 8–Changes in dispersion of milk fat as affected by the crossbreeding.

		Breed						
Component		**PHF**	**PHF×NO**	**PHF×NRF**	**PHF×RD**	**PHF×BS**	**PHF×MO**	**PHF×SIM**
Average diameter of the milk fat globule (μm)	LSM	8.71^{ABCDEF}	10.02^{AghI}	10.85^{Bj}	$9.77C^{gjk}$	11.15^{DhkL}	10.26^{E}	9.44^{FIL}
	SEM	0.231	0.274	0.229	0.218	0.213	0.240	0.214
SFG (%)	LSM	5^{AB}	2^{Acdef}	4^{cg}	2^{Bghij}	4^{dh}	4^{ei}	4^{fj}
	SEM	0.30	0.30	0.21	0.25	0.25	0.22	0.25
MFG (%)	LSM	51^{ABC}	51^{DEF}	45^{ADgH}	46^{BEi}	45^{CF}	49^{g}	50^{Hi}
	SEM	0.24	0.36	0.49	0.41	0.71	0.29	0.28
LFG (%)	LSM	44^{ABC}	47^{def}	51^{AdgH}	52^{BeIJ}	51^{Bfkl}	47^{gIk}	46^{HJl}
	SEM	0.66	0.47	0.20	0.39	0.26	0.48	0.32
Dyspersion ($\times 10^9$)	LSM	2.08^{ABC}	1.495^{ADefG}	2.29^{DHI}	1.58^{BHJK}	1.98eJ	1.62^{CfIL}	2.12^{GKL}
	SEM	0.33	0.36	0.38	0.39	0.21	0.29	0.36

SFG, small milk fat globules (<3 μm); MFG, medium milk fat globules (3 to 6 μm); LFG, large milk fat globules (>6 μm). Means in the same rows marked with the same letters differ significantly at: lowercase- $P \leq 0.05$; capitals $P \leq 0.01$. SEM, standard error of mean; LSM, least square of mean.

Conclusion

F1 cows tend to express better functional traits than Holstein (PHF) cows. Effect of heterosis was larger and gave better results when there was a greater genetic distance between the animals used for crossbreeding.

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