Scientific Annals of Polish Society of Animal Production - Vol. 7 (2011), No 3, 57-65

The influence of cow breed and feeding system on the dispersion state of milk fat and content of cholesterol

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The aim of the present study was to determine the influence of the cow breed and feeding system on the fat content in milk, state of its dispersion and potential association of these parameters with the cholesterol concentration in milk. The research included 510 milk samples obtained from cows of 6 breeds, namely: Polish Holstein-Friesian of Black-White variety (PHF--HO) – 152 and Red-White variety (PHF-RW) – 59; Simmental (SM) – 83, Jersey (JE) – 45; Polish Red (RP) – 67 and White-backed (BG) – 104. Two feeding systems were distinguished within PHF-HO, PHF-RW and SM breeds, namely traditional and TMR. Jersey cows were fed exclusively with TMR system, whereas RP and BG were fed traditionally. Investigated parameters of milk included concentrations of fat and cholesterol and proportion of milk fat globules (MFG) in certain average diameters (i.e. <6 µm; 7-10 µm; >10 µm). It was determined that cow breed had a significant ($p \leq 0.01$) influence on the cholesterol content in milk. Milk of JE cows was characterized by the highest content of fat (5.41%) and simultaneously the lowest content of cholesterol (14.21 mg/100 ml of milk). The highest content of cholesterol was observed in milk from cows of breeds PHF-RW (24.04 mg/100 ml of milk) and PHF-HO (21.90 mg/100 ml of milk). Milk from PHF-HO cows was characterized by the highest proportion of small MFG (60.30%), conversely to milk from JE cows (45.27%). Positive correlations were determined between the content of fat and proportion of large MFGs ($r=0.21^{***}$) and between the content of cholesterol and proportion of small MFGs ($r=0.27^{***}$), whereas a negative correlation was found between the content of cholesterol and proportion of large MFGs ($r=-0.23^{***}$). Milk collected from animals fed with TMR system contained significantly ($p \leq 0.01$) more fat (by 0.16%) and cholesterol (by 2.18 mg/100 ml of milk) and was additionally characterized by a higher proportion of small MFGs (by 9.74%) and lower share of medium MFGs (by 10.21%).

KEY WORDS: cow breed / milk fat / cholesterol / milk fat globules

Fat in milk is present in form of globules with an average diameter ranging from 0.1 to 15 μm [19]. Milk fat globules (MFG) consist of a triglyceride core coated with a natural biological membrane. Milk fat globule membrane (MFGM) comprises elements typical of any biological membrane, including cholesterol, enzymes, glycoproteins and glycolipids [9]. Månsson [15] reported that lipids account for 30% of MFGM; including phospholipids (25%), cerebrosides (3%) and cholesterol (2%). Remaining 70% are built by proteins.

The size (diameter) of milk fat globules has a crucial impact on the digestibility of milk fat and its nutritional value. High dispersion state has a positive influence on the fat availability due to the fact that lipolytic enzymes have facilitated access to the small MFGs [1]. Moreover, the size of MFGs has a significant effect on the fatty acid profile. Small MFGs (1.5 μm) contain higher proportion of short-chain fatty acids (SCFA; C4:0 – C8:0) and concomitantly less oleic (C18:1 cis-9) and linoleic (C18:2 n-6) acids as compared with large MFGs (7.3 μm) [4]. Due to the fact that cholesterol is located in the MFGM, it is assumed that the dispersion state of milk fat and the content of cholesterol may be associated. Faquant et al. [9] confirmed this relation to some extent.

Additionally, the average diameter of milk fat globules has a remarkable contribution to the technological suitability of milk, i.a. for manufacture of cheese and butter. Superior quality parameters of soft cheeses are obtained from milk with a dominant proportion of small MFGs (they bind water more easily, which results in a desirable softer consistency of the final product). Conversely, milk with a dominant share of large MFGs is more suitable for the manufacture of hard cheeses. Larger fat globules bind less water, thus the obtained cheese is more dense and firm; additionally, the process of proteolysis occurring during the maturation is more rapid [16, 17, 18]. Furthermore, milk with a higher share of large MFG is also more suitable in case of butter production. Such fat globules contain twice less membrane material; therefore, the concentration of cholesterol in the final product is lower. Butter manufactured from such fat tends to be more yellow, with a softer consistency (higher proportion of unsaturated fatty acids) and exhibits higher spreadability [10].

Cholesterol is a lipid substance, which serves numerous major functions in the human body. It is a substantial structural element of cell membranes; therefore, deficiency in its concentration results in the impairment of the membrane functions. Moreover, cholesterol participates in the synthesis of bile lipids, steroid hormones (including, estrogen and androgen) and vitamin D [5]. Cholesterol is an endogenous substance; hence additional dietary intake is reflected in the increased concentration of cholesterol in blood, which subsequently raises the risk of occurrence of cardio-vascular diseases associated with atherosclerosis. According to dietary guidelines of WHO/FAO [8] daily intake of cholesterol should not exceed 300 mg. Nutritionists recommend to limit the intake of dairy products rich in fat as they are traditionally considered to be one of the main sources of cholesterol in the human diet [26, 27]. However, Parodi [20] claims that such products should not be omitted in the diet as they contain a variety of biologically active substances with anticancerogenic activity, including CLA, sphingomyelin, butyric acid, eter lipids, β-carotene, and vitamins A and D [21].

Jensen [13] reported that the concentration of cholesterol in bovine milk ranges from 10 to 20 mg/100 ml of milk, whereas Viturro et al. [27] declared that the values are in a range of 10 to 30 mg/100 ml. Precht [23] determined that concentration of cholesterol in milk fat is at the level of 204.4 to 382.5 mg/100 g of fat.

The concentration of cholesterol of milk is determined by genetic factors as well as environmental factors, predominantly the cow feeding system [23, 25].

The aim of the present study was to determine the influence of cow breed and feeding system on the content of fat in milk, its dispersion state and potentially to associate those parameters with concentration of cholesterol.

Material and methods

The research material consisted of 510 milk samples collected from cows of 6 breeds, namely Polish Holstein-Friesian of Black-White variety (PHF-HO) – 152 and Red-White variety (PHF-RW) – 59, Simmental (SM) – 83, Jersey (JE) – 45, Polish Red (RP) – 67 and White-backed (BG) – 104. Two different feeding systems, namely traditional (with pasture grazing in the summer season) and TMR, were distinguished within following breeds: PHF-HO, PHF-RW and SM, whereas JE cows were fed exclusively with TMR system and RP and BG cows were fed with traditional system.

TMR feeding ration (constant composition of fodder throughout year) consisted mainly of maize silage and hay silage. Traditionally fed cows of PHF-HO and PHF-RW breeds received pasture delivered to the barn and maize silage. The feeding rate in lactation period was completed with industrial breeder mash. SM cows during the summer season were grazing on the pasture and additionally received hay silage or hay. Their feeding in the winter season was based on the haylage and maize silage. RP cows in the summer season were also grazing on the pasture and additionally received hay, whereas haylage or hay were the basis of winter season feeding. BG cows in the summer season were mainly grazing on the pasture and additionally received hay; winter feeding consisted mainly of hay and fodder beets or potatoes. The complementing feeding rate of SM, RP and BG cows comprised crushed cereal meal produced on the farm.

Milk samples were collected twice a year, namely in summer (VI-VII) and winter (XI--II) production season, from cows between 30th and 240th day of lactation. Milk samples were obtained during routine Milk Recording procedures. The examinations involved samples collected from animals with a healthy udder, namely with somatic cell count not exceeding 400,000 SC in 1 ml of milk.

Investigation of individual milk samples included: the content of fat with Infrared Milk Analyzer manufactured by Bentley, the proportion of MFGs in certain average diameter (i.e. <6µm, 7-10µm i >10µm) under microscope in 1000x magnitude in specimens dyed with Sudan III and the concentration of cholesterol according to methodology developed by Institute of Animal Science in Balice with own modifications.

The obtained results were analyzed statistically using StatSoft Inc. STATISTICA v. 6 software by one- and two-way ANOVA; means and standard deviations were given for individual analyzed traits, the significance of differences between means was estimated by Fisher's LSD test at p level =0.05 and p=0.01. The evaluation of the feeding system impact involved exclusively milk collected from 3 following breeds of cows: PHF-HO, PHF-RW and SM and it was analyzed with the two-way ANOVA with interaction. Pearson's correlation coefficients were also calculated; the significance of the obtained correlations was determined at the level of p=0.05 (*); p=0.01(**) and p=0.001(***).

Results and discussion

The data in Table 1 indicate on the significantly ($p \leq 0.01$) highest concentration of fat in milk from Jersey cows (5.41%). The content of fat in milk from other breeds was lower by approximately 1% and ranged from 3.98% in White-Backed breed to 4.25% in Polish Red breed. According to data collected by PFHBiPM for year 2010 [22], the active population of Jersey breed in Poland produced 5426 kg of milk with an average content of fat 5.26% and protein 3.81%.

It was stated that cow breed has a significant ($p \leq 0.01$) impact on the concentration of cholesterol. Milk of Jersey cows was characterized by the highest content of fat and concomitantly the lowest concentration of cholesterol (14.21 mg/100 ml of milk). Among the other analyzed cow breeds, the highest content of fat and the lowest content of cholesterol – 17.58 mg/100 ml of milk were determined for RP cows. The highest content of cholesterol was in the milk from cows of Polish Holstein-Friesian breed of Red-White (24.04 mg/100 ml of milk) and Black-White (21.90 mg/100 ml) varieties (Table 1). Grega et al. [11] also reported the impact of cow breed on the cholesterol content in milk. However, Šterna i Jameljanovs [24] claim that the concentration of cholesterol increases along with the increasing fat content. The present study did not confirm such relation. Moreover, it was determined (Table 3) that contents of fat and cholesterol were negatively correlated ($r = -0.09^{*}$).

The data in Table 1 also indicated on a significant ($p \leq 0.01$) influence of cow breed on the dispersion state of milk fat. The largest proportion of small MFGs was characteristic of milk from cows of Polish Holstein-Friesian breed of Black-White variety (60.30%), whereas this proportion for Jersey breeds was the lowest (45.27%). In regard to the proportion of large fat globules (over 10 μm), the highest share was observed in milk of Jersey (20.96%) and Polish Red cows (18.46%). Large MFG proportion in other analyzed breeds was in a range of 9.22% in White-backed to 9.90% in Simmental. Higher share of large fat globules in milk of Jersey cows as compared with Holstein-Friesian breed is also confirmed by other authors [2, 3, 6, 13]. Czerniewicz et al. [7] determined that the average diameter of fat globules in Holstein-Friesian milk was 6.19 μm, while in Jersey milk it was 7.68 μm. Wiking et al. [29] proposed a hypothesis that increased production of fat reduces the synthesis of membrane material, which subsequently imposes the production of bigger MFG, which are characterized by a lesser surface area of MFGM as compared to smaller fat globules. This thesis is also reflected in the results of the present study. As it was determined, the fat content and proportion of large MFG were positively correlated ($r = 0.21^{***}$), while the fat content and proportion of medium MFG were negatively correlated ($r = -0.10^{*}$) – Table 3. Furthermore, Couvreur et al. [6] also indicated on the relationship between fat content and size of MFGs.

It was stated that the dispersion state of milk fat and cholesterol content are related. Milk with a higher dispersion state of fat, i.e. milk from cows of PHF-HO and PHF-RW breeds was characterized by a higher cholesterol content (Table 1). It was also confirmed by studies of Faquant et al. [9]. According to these authors, MFGM of small fat globules (i.e. with a diameter of 3.2 ±0.3 μm) contained more cholesterol as compared with large fat globules (with a diameter of 6.3 ±0.1 μm). Briard et al. [4] reported that small MFGs are characterized by greater MFGM surface per unit of fat as compared to large MFGs. It does confirm the thesis

Table 1
The content of cholesterol and proportion of MFG in certain average diameters in bovine milk

Breed	Feeding system	n		Fat content (%)	Cholesterol content (mg/100 ml)	MFG proportion (%)		
						small (<6 µm)	medium (7-10 µm)	large (>10 µm)
PHF-HO	traditional	47	$\bar{x}$	4.09	20.75	48.15	40.57	10.04
			SD	0.52	6.66	16.74	12.16	5.24
	TMR	105	$\bar{x}$	4.24	22.42	65.7	24.39	9.70
			SD	0.73	7.05	15.98	13.52	6.13
	Average	**152**	$\bar{x}$	**4.20^{B}**	**21.90^{aC}**	**60.30^{C}**	**29.39^{aA}**	**9.80^{A}**
			SD	**0.67**	**6.95**	**18.10**	**15.07**	**5.68**
PHF-RW	traditional	25	$\bar{x}$	4.01	22.42	58.62	36.67	8.71
			SD	0.70	6.31	11.35	9.31	5.35
	TMR	34	$\bar{x}$	4.23	25.23	54.24	35.99	9.68
			SD	0.39	5.65	18.15	15.33	6.59
	Average	**59**	$\bar{x}$	**4.15^{AB}**	**24.04^{bC}**	**56.09^{BC}**	**34.58^{B}**	**9.27^{A}**
			SD	**0.55**	**6.05**	**15.67**	**13.12**	**6.01**
SM	traditional	53	$\bar{x}$	4.05	19.51	53.79	37.46	8.96
			SD	0.43	6.59	15.64	12.94	6.54
	TMR	30	$\bar{x}$	4.11	22.09	58.30	29.16	12.55
			SD	0.33	3.21	5.92	5.03	4.08
	Average	**83**	$\bar{x}$	**4.07^{AB}**	**19.66^{bB}**	**54.98^{B}**	**35.27^{B}**	**9.90^{A}**
			SD	**0.40**	**5.87**	**13.85**	**11.94**	**6.17**
JE	TMR	**45**	$\bar{x}$	**5.41^{C}**	**14.21^{A}**	**45.27^{A}**	**33.55^{B}**	**20.96^{B}**
			SD	**0.78**	**5.20**	**10.81**	**11.00**	**11.21**
RP	traditional	**67**	$\bar{x}$	**4.25^{B}**	**17.58^{B}**	**46.46^{A}**	**35.27^{B}**	**18.46^{B}**
			SD	**0.82**	**7.42**	**14.95**	**12.63**	**9.63**
BG	traditional	**104**	$\bar{x}$	**3.98^{A}**	**19.28^{B}**	**57.61^{BC}**	**32.98^{aB}**	**9.22^{A}**
			SD	**0.65**	**7.74**	**11.99**	**10.59**	**6.83**

a, b, c, A, B, C – differences between breeds; A, B, C – significant at $p \leq 0.01$; a, b, c – significant at $p \leq 0.05$

that a higher share of small fat globules is associated with a relatively greater area surface of membranes, where cholesterol is present. Consequently, it contributes to the increased cholesterol concentration in milk. Significant positive correlation between the content of cholesterol and proportion of small MFGs (r=0.27***) and negative correlation for large MFGs proportions (r=–0.23***) were observed in the present study – Table 3.

Table 2

The content of cholesterol in milk and proportion of MFG in certain average diameters in regard to the cow feeding system

Specification			Feeding system	
			traditional	TMR
n			125	169
Fat content (%)		$\bar{x}$	4.06**	4.22**
		SD	0.53	0.63
Cholesterol content (mg/100 ml)		$\bar{x}$	20.56**	22.74**
		SD	6.60	6.56
MFG proportion (%)				
	small (<6 μm)	$\bar{x}$	52.63**	62.37**
		SD	15.71	16.32
	medium (7-10 μm)	$\bar{x}$	37.67**	27.46**
		SD	12.25	13.99
	large (>10 μm)	$\bar{x}$	9.31	10.04
		SD	7.91	6.59

**Differences between feeding systems significant at p≤0.01

Table 3

Correlation coefficients for analyzed traits of milk fat and cholesterol content

Traits	Cholesterol content (mg/100 ml)	Proportion of small MFG (%)	Proportion of medium MFG (%)	Proportion of large MFG (%)
Fat content (%)	–0.09*	–0.04	–0.10*	0.21***
Cholesterol content (mg/100ml)		0.27***	–0.17***	–0.23***
Proportion of small MFG (%)			–0.82***	–0.57***
Proportion of medium MFG (%)				0.01

*Values significant at p≤0.05; **significant at p≤0.01; ***significant at p≤0.001

It was determined that cow feeding system (traditional and TMR) significantly ($p \leq 0.01$) affects the content of fat, cholesterol, and the proportion of small and medium MFGs (Table 2). Milk collected from animals fed with TMR system contained more fat (by approximately 0.16%) and cholesterol (by approximately 2.18 mg/100 ml) and had a higher proportion of small MFGs (by approximately 9.74%) and lower proportion of medium MFGs (by approximately 10.21%) – Table 2. White et al. [28] observed an increase of contents of fat (by 0.10%) and lactose (by 0.20%) in milk of Holstein cows fed with TMR as compared to cows grazing on the pasture. Following differences were reported for Jersey cows: content of fat higher by 0.42%, protein – 0.19% and lactose – 0.07%. Lopez et al. [14] indicated that the cow diet is considered to modify also the dispersion state of milk fat.

The evaluation of simultaneous impact of two analyzed factors, i.e. cow breed and feeding system revealed that cow breed had a significant ($p \leq 0.01$) influence on cholesterol content and proportion of small and medium MFGs. Additionally, significant ($p \leq 0.01$) interactions between these two factors were determined for the proportion of small and medium MFGs.

In conclusion it may be stated that the cow breed and feeding system (traditional and TMR) are important factors, which determine the content and dispersion state of milk fat and cholesterol concentration in milk. The obtained results imply that the cholesterol concentration in milk is influenced to a greater extent by the dispersion state of milk fat than its total content.

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