

Effect of stage of lactation and production season on fat dispersion in the milk of cows of different breeds

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The aim of the study was to evaluate the effect of the stage of lactation and production season on fat dispersion in the milk of cows of different breeds. A total of 738 milk samples were analysed, including 196 from Polish Black-and-White Holstein-Friesian cows, 168 from Jersey cows, 185 from Polish Red cows, and 189 from White-Backed cows. Milk samples were collected individually from each cow in two production seasons, i.e. spring/summer (May-July) and autumn/winter (December-March). Three stages of lactation were distinguished: ≤ 120 days, 121 to 200 days, and > 200 days. The percentage content of fat and its dispersion (expressed as the mean surface area, circumference and mean diameter of fat globules) were determined. The stage of lactation and the production season (linked to the feeding season) were found to strongly influence the content and dispersion of milk fat. As lactation progressed, the fat content of the milk and its dispersion increased. A significant ($p \leq 0.01$) decrease was noted in the mean diameter and surface area of the fat globules. The fat globules obtained in the initial stage of lactation were the largest (≤ 120 days – $2.90 \mu\text{m}$). The milk produced in the autumn/winter season had higher fat content and the mean size of the fat globules was significantly ($p \leq 0.01$) greater.

KEY WORDS: milk fat / dispersion / lactation / production season / cow breed

Fat is the main component determining the calorific and nutritional value of milk, as well as its suitability for processing. It is the most easily assimilated animal fat, as its digestibility is about 97-99%. Fat is synthesized in the milk-producing cells of the mammary gland. It takes the form of globules coated with a tri-layer phospholipid membrane [12]. The precursors of fat globules are formed in the endoplasmic reticulum, and then transported by the cytosol as small fat droplets covered with a bilayer polar membrane consisting of phospholipids and proteins. During transport to the apical part of the cells,

the fat droplets join with others, thereby increasing in size. During secretion they are coated with another membrane and finally secreted into the milk ducts [17]. When the degree of dispersion is high, they can be absorbed without prior hydrolysis in the digestive tract [13].

Fat globules can be classified into groups according to size: small—diameter under 1 μm (about 80% of all globules), intermediate—from 1 to 7 μm , and large—over 8 μm [17].

Milk fat dispersion significantly affects its digestibility and suitability for processing (particularly production of butter and cheese). Michalski et al. [11] showed that Camembert cheese produced from milk with small fat globules (about 2 μm in diameter) was moister and less compact and had a more elastic texture than cheese made of milk with large fat globules (about 6 μm in diameter). In the case of low-fat cheddar, cheese produced from milk with large fat globules was found to have better texture, palatability and colour than cheese produced from milk with small or medium-sized fat globules.

The size of fat globules also influences the fatty acid profile (smaller globules contain more conjugated linoleic acid). Milk with a high percentage of large fat globules is more susceptible to lipolysis and coalescence during pumping and transport [6]. Fat dispersion is determined by factors such as breed, daily fat yield, and milking frequency [17]. Barłowska and Litwińczuk [2] and Michalski et al. [10] report that the size of milk fat globules also depends on the production season.

The aim of the study was to evaluate the effect of the stage of lactation and production season on the fat dispersion of the milk of different breeds of cows.

Material and methods

The material for the study consisted of milk samples collected from 213 cows of four breeds, including two dairy breeds, i.e. Polish Black-and-White Holstein Friesian (PHF HO; 63 cows) and Jersey (JE; 50 cows), and two native breeds, i.e. Polish Red (RP; 50 cows) and White-Backed (BG; 50 cows). The Jersey and Polish Holstein-Friesian cows were housed in free-stall barns and fed in a TMR (Total Mixed Ration) system, with a feed ration consisting of maize silage, haylage and straw, as well as extraction meals (soybean and rapeseed) and cereal meals. The White-Backed and Polish Red cows were housed in tie-stall barns, and their diet was based on on-farm fodder. They received haylage and hay in the autumn/winter season and pasture forage and hay in the spring/summer period. In both seasons the feed ration was supplemented with on-farm cereal meal. A total of 738 milk samples were evaluated, including 196 from PHF HO, 168 from JE, 185 from RP and 189 from BG. Milk samples with a volume of about 250 ml were collected individually from each cow, from a complete milking procedure during test-day milking (AT4 method), in two production seasons—spring/summer (May-July) and autumn/winter (December-March), for two years. Three stages of lactation were distinguished: ≤ 120 days, 121-200 days, and > 200 days.

The percentage content of fat was determined in the milk samples (with a Bentley Infra-red Milk Analyzer), as well as its dispersion (expressed as the mean surface area, circumference and mean diameter of fat globules). The analysis was performed with a microscope at 1,000x magnification, on slides stained with Sudan III (in three fields of view), using Motic Images Plus 2.0 software. The somatic cell count was determined (with a Bentley Somacount 150 apparatus) to eliminate samples from cows with diseased mammary glands (over 400,000 SCC per ml). Data on the daily milk yield of the cows in the months when samples were collected were obtained from documentation of milk performance evaluations (RW-2 reports) conducted by the Polish Federation of Cattle Breeders and Dairy Farmers.

Statistical computations were performed in StatSoft Inc. STATISTICA software ver. 6. The analysis was based on a General Linear Model (GLM)—an ANOVA procedure for factorial designs with interaction. Parameters of the fat fraction of the milk were evaluated using two statistical models, taking into account the breed of cattle and production season, and the breed of cattle and stage of lactation. As there was no significant interaction between production season and stage of lactation, an abbreviated model was used. Significance of differences between means was determined by Tukey's test at $p \leq 0.05$ and $p \leq 0.01$.

Results and discussion

The data contained in Table 1 indicate that the stage of lactation affected the amount of milk produced, the fat concentration, and its dispersion. A greater decline in productivity as lactation progressed was observed in the native breeds, particularly Polish Red—by as much as 41%. In the dairy breeds this decrease ranged from 24% in the Jersey breed to 27% in the Polish Black-and-White Holstein-Friesians. The fat content in the milk increased as lactation advanced, but the differences were not confirmed statistically. The tendencies observed are confirmed in studies by other authors [1, 3, 4, 16]. Litwińczuk et al. [5] found that cows of native breeds, i.e. Polish Red and White-Backed, had much poorer lactation persistency, i.e. in the final third of lactation they produced only 55.8% as much milk as in the first third. Much more favourable lactation persistency (66%) was observed in Simmental cows, and the best (73.4%) in the cows of the reference group, i.e. Polish Holstein-Friesians raised in an intensive system.

As lactation progressed a significant ($p \leq 0.01$) decrease was observed in the mean diameter and surface area of fat globules in the milk of all breeds analysed. There was also a significant ($p \leq 0.01$) decrease in the mean circumference of the fat globule membranes in the field of view (except in the milk of the White-Backed cows, where the differences were not confirmed statistically). The fat globules of the milk obtained in the initial stage of lactation were the largest (≤ 120 days – 2.90 μm). The results confirm the theory presented by Singh [14], according to whom the decrease observed in the mean diameter of the fat globules as lactation progresses are explained by the changing level of substrates for production of fat globules. In the initial stage of lactation (from about 180 days), the content

Table 1
Milk fat dispersion in cows of each breed by stage of lactation

Breed	Stage of lactation (days)	Number of milk samples	Daily milk yield (kg)		Fat (%)	Surface area of fat globules in field of view (μm^2)		Circumference of fat globules in field of view (μm)		Mean diameter of fat globules (μm)	
			\bar{x}	SD		\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
PHF HO	≤120	67	27.81	6.93	4.10	0.72	6.70 ^B	1.66	8.31 ^{Bb}	1.03	2.65 ^{Bb}
	121-200	56	26.00	6.44	4.29	0.55	5.39 ^A	1.14	7.56 ^{Aa}	0.89	2.41 ^{Aa}
	>200	73	20.30	5.41	4.75	0.71	4.71 ^A	1.62	6.98 ^A	1.11	2.22 ^A
JE	≤120	55	24.35	6.29	4.87	1.09	10.21 ^B	5.11	10.10 ^B	2.66	3.21 ^B
	121-200	55	21.55	3.96	5.07	0.71	7.84 ^A	3.36	8.85 ^A	1.95	2.82 ^A
	>200	58	18.34	2.96	5.45	0.74	6.84 ^A	2.55	8.36 ^A	1.58	2.66 ^A
RP	≤120	78	16.40	3.87	4.15	0.83	8.84 ^B	3.50	9.44 ^B	1.84	3.01 ^B
	121-200	38	12.85	2.86	4.60	0.56	8.45 ^{Bb}	3.60	9.29 ^{AB}	1.84	2.96 ^{AB}
	>200	69	9.56	4.35	4.55	0.71	7.20 ^{Aa}	2.20	8.69 ^A	1.40	2.77 ^A
BG	≤120	71	15.49	7.43	3.82	0.95	6.86 ^{Bb}	3.01	8.74	2.52	2.78 ^{Bb}
	121-200	44	12.32	7.03	4.05	0.57	5.61 ^{Aa}	3.03	7.92	2.45	2.52 ^{Aa}
	>200	74	10.91	5.37	4.46	0.71	4.75 ^A	2.85	7.23	2.07	2.32 ^A
Total	≤120	271	20.87 ^B	8.08	4.21 ^A	0.98	8.09 ^C	3.88	9.11 ^C	2.20	2.90 ^C
	121-200	193	20.23 ^B	7.80	4.51 ^B	0.71	6.74 ^B	3.20	8.35 ^B	1.95	2.66 ^B
	>200	274	15.33 ^A	6.56	4.75 ^C	0.82	5.80 ^A	2.60	7.77 ^A	1.73	2.48 ^A
Influence of the factor	breed		***		***		***		***		***
	stage of lactation		***		***		***		***		***
	breed x stage of lactation		*		*		*		*		*

PHF HO – Polish Black-and-White Holstein-Friesian; JE – Jersey; RP – Polish Red; BG – White-Backed
A, B, C – differences significant at $p \leq 0.01$; a, b – differences significant at $p \leq 0.05$; ** at $p \leq 0.01$, *** at $p \leq 0.001$

of the five main classes of phospholipids (phosphatidylethanolamine, phosphatidylcholine, phosphatidylinositol, phosphatidylserine and sphingomyelin) is relatively constant. After that a gradual decrease in their concentrations is observed in the milk, and is most pronounced in the final two months of lactation. The smallest fat globules are also observed at this time. Mesilati-Stahy and Argov-Argaman [9], in order to evaluate the effect of stage of lactation on milk fat dispersion, estimated the ratio of the quantity of lipids forming the globule membrane to the quantity of components of its core. They observed that in the case of small globules, this ratio was highest on about day 150 of lactation. In the case of large fat globules, the highest ratio was noted on about the 100th day of lactation. Similar relationships were observed by Wiking et al. [17], who reported that the mean fat globule diameter is correlated with daily fat production. In the case of increased fat production, synthesis of membrane material is reduced, resulting in secretion of fat in the form of large globules, as observed in the initial stage of lactation.

A simultaneous effect of breed and stage of lactation was found on the amount of milk produced, its fat content, and the mean diameter, circumference and surface area of fat globules ($p \leq 0.05$).

The chemical composition and physicochemical properties of milk are determined by both genetic and environmental factors. The most important non-genetic factor affecting the chemical composition and nutritional value of milk is diet, which in traditional farming conditions is strongly linked to the production season.

Table 2 presents the results of two-way analysis of variance taking into account the effect of breed and production season on the milk parameters analysed. In the cows from high-yielding breeds (PHF HO and JE), in which the same TMR system was used year round, the production season had no significant effect on daily yield. In the Jersey cows higher ($p \leq 0.001$) fat content was noted in the milk from the autumn/winter season. In the case of the native breeds, in which traditional (seasonal) feeding was used, daily yield was higher in the spring/summer season, when the cows were pastured. The milk obtained in the autumn/winter season was found to have higher fat content, with significantly ($p \leq 0.01$) lower dispersion. The fat globules in the milk obtained in this season (irrespective of the breed of cow) were significantly larger. Evaluation of the simultaneous effect of cow breed and production season showed significant interactions for daily yield ($p \leq 0.05$), fat content ($p \leq 0.01$), mean diameter of fat globules, their circumference, and their surface area ($p \leq 0.001$).

The correlations noted correspond with results obtained by other authors. Barłowska and Litwińczuk [2], analysing the effect of production season on milk fat dispersion, found that the percentage of large fat globules (diameter over 10 μm) was nearly twice as high in milk obtained in the winter as in milk from the summer (11.93% vs 6.09%). Logan et al. [6], in an analysis of milk fat parameters in a herd of Holstein-Friesians, reported larger fat globules in milk obtained in the autumn.

Ménard et al. [8] report that the size of fat globules also depends on the intensity of fat secretion; larger globules in buffalo milk (average diameter 5 μm) as compared to cow milk (3.5 μm) were associated with higher fat yield (65-80 vs 40 g/kg, respectively). The size of milk fat globules is directly linked to the physiology of the lactating female. Secre-

Table 2
Milk fat dispersion in cows of each breed by production season

Breed	Production season	Number of milk samples	Daily milk yield (kg)	Fat (%)	Surface area of fat globules in field of view (μm^2)		Circumference of fat globules in field of view (μm)		Mean diameter of fat globules (μm)	
					\bar{x}	SD	\bar{x}	SD	\bar{x}	SD
PHF HO	spring/summer	54	23.24	4.32	4.91 ^A	2.11	6.94 ^A	1.37	2.21 ^A	0.44
	autumn/winter	142	24.43	4.42	5.85 ^B	1.49	7.87 ^B	0.96	2.50 ^B	0.31
JE	spring/summer	78	22.07	5.54	4.82 ^A	2.87	7.77 ^A	1.69	2.47 ^A	0.54
	autumn/winter	90	20.83	3.80	5.39 ^B	4.03	10.23 ^B	1.97	3.26 ^B	0.63
RP	spring/summer	74	14.53 ^B	4.56	4.33	2.55	8.45 ^A	1.47	2.69 ^A	0.46
	autumn/winter	111	10.95 ^A	4.80	4.45	3.42	9.57 ^B	1.74	3.05 ^B	0.55
BG	spring/summer	133	13.78	7.43	4.00	2.61	7.37 ^A	2.50	2.35 ^A	0.79
	autumn/winter	56	12.97	6.84	4.12	2.38	9.38 ^B	1.40	2.99 ^B	0.45
Total	spring/summer	339	17.38	7.20	4.39 ^A	3.09	7.62 ^A	1.93	2.43 ^A	0.64
	autumn/winter	399	18.29	8.44	4.62 ^B	3.41	9.08 ^B	1.79	2.89 ^B	0.57
Influence of the factor	breed		***	***	***	***	***	***	***	***
	season		**	**	***	***	***	***	***	***
	breed x season		*	**	***	***	***	***	***	***

PHF HO – Polish Black-and-White Holstein-Friesian; JE – Jersey; RP – Polish Red; BG – White-Backed
A, B – differences significant at $p \leq 0.01$; influence of the factor: *at $p \leq 0.05$, **at $p \leq 0.01$, ***at $p \leq 0.001$

tion of small fat globules is a biological process requiring production of a large quantity of material for the construction of the membrane covering the surface of the fat globules, and thus high activity of synthesis of milk-producing cells is beneficial. Therefore the milk-producing cells of females secreting fat in the form of large globules have potentially lower metabolic activity as compared to females secreting fat in the form of small globules [7].

To sum up, the stage of lactation significantly differentiates the content and dispersion of milk fat. As lactation progresses the fat content increased and the mean size of fat globules successively ($p \leq 0.01$) decreased. The production season significantly affected mainly fat dispersion. Irrespective of the breed of cow and the feeding system it was significantly lower ($p \leq 0.01$) in the autumn/winter. Thus we can conclude that milk obtained in the initial stage of lactation and in the spring/summer is a more digestible food product, but milk from the final period of lactation and from the autumn/winter is a better raw material for production of milk and hard cheeses.

REFERENCES

1. ANTKOWIAK I., PYTLEWSKI J., KUCERA J., 2003 – Effect of some selected factors on somatic cell concentration in milk of Jersey cows. *Acta Scientiarum Polonorum Zootechnica* 2, 2, 9-18.
2. BARŁOWSKA J., LITWIŃCZUK Z., 2006 – Technological usefulness of milk from two local breeds maintained in the regions with great grassland share. *Archiv Tierzucht Dummerstorf* 49, special issue, 207-213.
3. BOHDANOWICZ-ZAZULA M., NOWOPOLSKA-SZCZYGLEWSKA A., SYNOWIEC M., PAWELSKA M., 2003 – Zmienność składu i parametrów technologicznych mleka krów żywionych w systemie TMR w zależności od pory roku, okresu laktacji i poziomu wydajności. *Zeszyty Naukowe Przeglądu Hodowlanego* 69, 197-204.
4. GARDZINA-MYTAR E., WĘGLARZ A., FELEŃCZAK A., ORMIAN M., MAKULSKA J., 2007 – Wydajność i skład mleka krów rasy polskiej czerwonej utrzymywanych w stadzie zachowawczym i doskonałym. *Roczniki Naukowe Zootechniki* 34, 2, 3-10.
5. LITWIŃCZUK Z., BARŁOWSKA J., MATWIJCZUK A., SŁOMIANY J., 2016 – Changes in milk yield and quality during lactation in Polish Red and White-Backed cows included in the genetic resources conservation programme in comparison with the Simmental breed. *Annals of Animal Science* 16 (3), 871-887.
6. LOGAN A., AULDIST M., GREENWOOD J., DAY L., 2014 – Natural variation of bovine milk fat globule size within a herd. *Journal of Dairy Science* 97, 4072-4082.
7. LOPEZ CH., 2011 – Milk fat globules enveloped by their biological membrane: Unique colloidal assemblies with a specific composition and structure. *Current Opinion in Colloid & Interface Science* 16, 391-404.
8. MÉNARD O., AHMAD S., ROUSSEAU F., BRIARD-BION V., GAUCHERON F., LOPEZ C., 2010 – Buffalo vs cow milk fat globules: size distribution, zeta-potential, compositions in total fatty acids and in polar lipids from the milk fat globule membrane. *Food Chemistry* 120, 544-551.

9. MESILATI-STAHY R., ARGOV-ARGAMAN N., 2014 – The relationship between size and lipid composition of the bovine milk fat globule is modulated by lactation stage. *Food Chemistry* 145, 562-570.
10. MICHALSKI M.C., CAMIER B., BRIARD V., LECONTE N., GASSI J.Y., GOUDE-DRANCHE H., MICHEL F., FAUQUANT J., 2004 – The size of native milk fat globules affects physic-chemical and functional properties of Emmental cheese. *Le lait* 84, 342-358.
11. MICHALSKI M.C., GASSI J.Y., FAMELARD M.H., LECONTE N., CAMIER B., MICHEL F., BRIARD V., 2003 – The size of native milk fat globules affects physic-chemical and sensory properties of Camembert cheese. *Le lait* 83, 131-143.
12. PISANU S., MAROGNA G., PAGNOZZIA D., PICCININI M., LEO G., TANCAA., ROGGIO A.M., ROGGIO T., UZZAU S., ADDIS M.F., 2013 – Characterization of size and composition of milk fat globules from Sarda and Saanen dairy goats. *Small Ruminant Research* 109, 141-151.
13. REKLEWSKA B., BERNATOWICZ E., 2002 – Bioaktywne składniki frakcji tłuszczowej mleka. *Przegląd Hodowlany* 11, 1-5.
14. SINGH H., 2006 – The milk fat globule membrane – A biophysical system for food applications. *Current Opinion in Colloid & Interface Science* 11, 154-163.
15. STATSOFT INC., 2003 – STATISTICA (data analysis software system), ver. 6. www.statsoft.com.
16. SUMMER A., FORMAGGIONI P., MALACARNE M., SANDRI S., MARIANI P., 2003 – Composition, Acidity and Rennet-coagulation Properties of Early- and Late-lactation Milks from Italian Friesian Cows. *Veterinary Research Communications* 27, Suppl. 1, 269-272.
17. WIKING L., STAGSTED J., BJORCK L., NIELSEN J.H., 2004 – Milk fat globule size is affected by fat production in dairy cows. *International Dairy Journal* 14, 909-913.