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Study on chemical and fatty acid modifications of cow's milk in relation to fat globules diameter

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RIASSUNTO – Studio delle modificazioni delle caratteristiche chimiche ed acidiche del latte bovino in relazione alle dimensioni dei globuli di grasso. I*n questo studio è stato rilevato come la maggiore presenza nel latte di globuli di grasso con diametro inferiore a 6 μm risulti correlata positivamente alla percentuale di sostanza secca e lipidi, mentre la presenza di globuli con diametro superiore a tale valore favorirebbe una maggiore produzione di latte, una percentuale più elevata di lattosio, sostanza secca magra e ceneri. In relazione al contenuto in acidi grassi, latti con percentuali più elevate di globuli al di sotto del valore di 6μm tenderebbero ad essere più ricchi in acidi grassi insaturi C18:1, C18:2 e CLA, mentre globuli di grasso di dimensioni superiori favorirebbero una prevalenza di acidi grassi saturi (C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C20:0, C21:0, C22:0), oltre che di C20:2 e C18:3ω3. Sulla base di questi risultati il diametro dei globuli di grasso pari a 6μm potrebbe essere considerato come "valore soglia" per la definizione di alcune caratteristiche qualitative del latte bovino.*

Key words: milk fat globules, fatty acid, milk quality, cow.

INTRODUCTION – Studies regarding the range of values for variations in the size of milk fat globules have been conducted over the years by various Authors, based on objective observation and mathematical extrapolation. In 1969, Walstra calculated the existence of a population of globules smaller than 1 μm and identified a population of larger globules, distributed in a "Gaussian" manner and showing a peak at a diameter of around 3-4 micrometers; later Timmen e Patton (1988) separated the cream from the rest of the milk by centrifugation and classified the fat globules into small and large sizes. Other authors (Ruegg and Blanc, 1981; Mehaia, 1995) reported the distribution of milk fat globules from various species, distinguishing globules that had been actually counted and measured, from those which were not visible and were defined as "small". Recently several researchers introduced the technique of milk microfiltration; this allows separation of fat globules with a diameter of approximately 3μm ("small"), from those with a diameter of approximately 6μm, ("large") (Michalski *et al.*, 2003; Briard and Michalski, 2004; Wilking *et al.*, 2004). Our studies on the morphometry of milk fat globules (Cecchi *et al.*, 2003, Martini *et al.*, 2003; Martini *et al.*, 2003a; Scolozzi *et al.*, 2003; Martini *et al.*, 2004; Martini *et al.*, 2004a; Martini *et al.*, 2004b) were based on the distribution of globules that had actually been counted: fat globules from untreated milk were counted, measured and divided into three classes, labelled "small", "medium" and "large", according to the frequency distribution of their diameter size. Up until now studies on the diameter sizes distribution of milk fat globules have not identified an unequivocal size range for defining the classes, rendering it difficult to compare results. This work aimed to verify the existence of a value of the fat globule diameter which could serve as a "threshold value" for various qualitative characteristics of cow's milk, especially for its chemical and fatty acid composition.

MATERIALS AND METHODS – The trial was carried out on 57 Friesian cows, raised in the same herd in the province of Pisa. One sample from each animal was taken during the morning milking. Morphometric analysis of the measured fat globules was carried out following the method of Scolozzi *et al.* (2003). The measured fat globules (73660) were divided by size into nine classes of 1.5μm class widths diameters (from class 1: 1.5 μm< diameter <3 μm to class 9: diameter >13.5 μm). Milk sample were weighed and analyzed for: fat, protein, lactose and dry matter content by infrared analysis (Milkoscan, Italian Foss Electric), ash (A.O.A.C, 1990) non-fat dry matter was calculated to be the difference between dry matter and fat content. Fatty acids from the milk fat were analyzed by gas chromatography as the methyl ester derivates after trans-esterification with sodium methoxide (Christie, 1982); the gas chromatographic apparatus Perkin Elmer Auto System was equipped with a FID detector and a capillary column (OMEGA VAX 320), with helium as carrier gas; peak areas of individual fatty acids were quantified as percent of total fatty acids. The relationships between the nine classes of fat globules and chemical composition and milk fatty acid composition were tested by single pair correlations. Cluster analysis, using the single linkage method, was also applied to all parameters in order to indicate the relationships between milk fat globules and chemical and milk fatty acid composition (STAXS2000, ver.1.0.1998).

RESULTS AND CONCLUSIONS – Table 1 shows the correlations between the milk yield, chemical parameters and each class of fat globules: fat globules having a diameter smaller than 6μm were negatively correlated with values regarding milk yield, percentage of lactose, non fat dry matter (n.f.d.m.) and ash, whereas they correlated positively with the percentage of dry matter and fat. The sign of the correlation changes when fat globules size exceeds this value; this would appear to indicate that this value is a "threshold" for the relationships between values regarding the morphometry of the fat globules and those of the chemical composition of the milk. These observations were confirmed by the application of a Cluster analysis, which identified two groups of parameters in which the milk yield, the percentage of lactose, ash and non fat dry matter were associated with the classes of fat globules having a diameter greater than 6μm, while the percentages of dry matter and total lipids were associated with classes of globules with a diameter less than 6μm.

Table. 1. Correlation between milk yield (1), chemical parameters (%) and classes of fat globules.

	CL1	CL2	CL3	CL4	CL5	CL6	CL7	CL8	CL9
	1.5-3 μm	3-4.5 μm	4.5-6 μm	6-7.5 μm	7.5-9 μm	9-10.5 μm	10.5-12 μm	12-13.5 μm	>13.5 μm
Milk yield	-0.383**	-0.412**	-0.335*	0.355**	0.442**	0.383**	0.409**	0.489**	0.349**
dry matter	0.222	0.319*	0.216	0.192	-0.322*	-0.330*	-0.234	-0.294*	-0.200
protein	0.033	0.056	0.015	0.024	-0.013	-0.021	0.032	0.044	-0.018
fat	0.379**	0.469**	0.306*	0.260*	-0.494**	-0.488**	-0.413**	-0.510**	-0.333*
lactose	-0.270*	-0.327*	-0.159	0.093	0.307*	0.312*	0.346**	0.450**	0.321*
n.f.d.m.	-0.300*	-0.269*	-0.163	0.110	0.321*	0.285*	0.350**	0.427**	0.249
ash	-0.306*	-0.251*	-0.138	0.166	0.348**	0.218	0.311*	0.262*	0.171

$^{**}P\leq0.01$; $^{*}P\leq0.05$.

Table 2 shows the correlations between the classes of globules and milk fatty acids: as in the case of the chemical parameters, an inversion of the sign of the correlations is observed in correspondence with a globule size of 6 μm. Fatty acids showing negative correlations with fat globules smaller than 6 μm, and positive correlations with ones larger than 6μm are: C6:0, C8:0, C10:0, C12:0, C14:0, C16:0, C18:3ω3, C20:0, C20:2, C21:0, C22:0. Milk samples with a higher percentage of fat globules smaller than 6 μm in diameter presented positive correlations with unsaturated fatty acids C18:1, C18:2 e CLA (table 2). This trend was confirmed by Cluster analysis, which identified the presence of two groups of fatty acids, associated with fat globule sizes either greater than or less than 6μm. As a result, milk with a high percentage of fat globules having a diameter smaller than 6μm would present higher percentages of unsaturated fatty acids such as oleic, linoleic and conjugated linoleic acid, while the presence of larger globules indicates a prevalence of saturated fatty acids, in addition to α-linoleic acid and C20:2. In light of these considerations, it is possible to conclude that a fat globule diameter size corresponding to 6μm may be considered a "threshold value", regarding relationships between the morphometric values of the globules and the chemical and fatty acid quality of cow's milk. Therefore, it may be justified to define two classes of globules associated with milk of different qualities, starting from values less than and greater than this diameter. Further research is needed to confirm the relationships between quality milk and fat globule diameter size.

Table 2. Correlation between fatty acid (%) and classes of fat globules.

	CL1 1.5-3 μm	CL2 3-4.5 μm	CL3 4.5-6 μm	CL4 6-7.5 μm	CL5 7.5-9 μm	CL6 9-10.5 μm	CL7 10.5-12 μm	CL8 12-13.5 μm	CL9 >13.5 μm
C6:0	-0.307*	-0.016	-0.046	0.108	0.168	0.120	0.209	0.224	0.183
C8:0	-0.38*	-0.077	-0.134	0.142	0.278*	0.188	0.383*	0.360**	0.292*
C10:0	-0.192	-0.246	-0.304*	0.046	0.357**	0.197	0.452**	0.419**	0.360**
C12:0	-0.143	-0.232	-0.325*	0.132	0.313*	0.145	0.323*	0.352**	0.228
C14:0	-0.095	-0.239	-0.248	0.076	0.192	0.044	0.235	0.227	0.175
C16:0	-0.053	-0.324*	-0.216	0.033	0.273*	0.158	0.183	0.177	0.114
C18:0	-0.158	-0.188	-0.058	0.149	0.084	0.002	0.0284	0.104	0.078
C18:1	0.552**	0.161	0.140	-0.417**	-0.414**	-0.273*	-0.282*	-0.345**	-0.198
C18:2	0.110	0.390**	0.233	-0.117	-0.296*	-0.181	-0.228	-0.223	-0.173
C18:3ω3	-0.416**	-0.352**	-0.02	0.238	0.407**	0.337*	0.229	0.429**	0.371**
Total CLA	0.391**	0.247	0.015	-0.124	-0.329*	-0.214	-0.332*	-0.431**	-0.401**
C20:0	-0.546**	-0.438**	-0.192	0.383**	0.579**	0.514**	0.352**	0.460**	0.360**
C20:2	-0.356**	-0.201	-0.116	0.315*	0.338*	0.261*	0.143	0.178	0.167
C21:0	-0.300*	-0.357**	-0.077	0.144	0.400**	0.209	0.223	0.401**	0.447**
C22:0	-0.363**	-0.478**	-0.248	0.245	0.566**	0.455**	0.278*	0.421**	0.413**

**P≤0.01; *P≤0.05

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