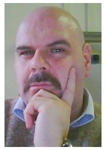
Milk fat globule as a nano/microscale release device: effect of breed



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INTRODUCTION

A great body of evidence has been reported for the nutritional/functional properties of milk depending on its microstructure (Dewettinck K. 2008); while many researchers faced with the supramolecular characteristics of proteins, mainly caseins, fat globules have often been considered as milk component with lesser importance. In the last decade, however, the structure of milk fat globule in milk has raised its importance, since the membrane of globules (a phospholipid/protein trilayer) are paramount for the metabolic destiny of triglycerides (the main globule component) and the membrane proteins, not only for their molecular characteristics, but also for their size (Michalski M.-C. 2005), so the milk fat globule was accounted of a micro/nanoscale device (Argov N. 2008).

In fact, the complex, heterogeneous protein/membrane interaction in the fat globule could be studied as a natural fat or protein release device, with unique properties in terms of gut interactions and vehiculation modalities.

Milk fat globule size has been widely analyzed in commercial milk as well as in widespread cow breeds, but there is a lack of information about niche breeds. In particular, several autochtonous endangered bovine breeds in Italy are nowadays submitted to recovery also in order to characterize and increase the value of local dairy products. In this perspective, the aim of the present work was to evaluate possible differences between the “standard” cow in our region (i.e. Holstein Friesian) and four local breeds.

MATERIAL AND METHODS

Milk samples from five bovine breeds -namely Friesian as a control, and four typical Italian breeds, were collected manually in the morning hours; considered breeds were Holstein Friesian (n=70), Cabannina (n=41), Rendena (n=34), and Varzese (n=57). Milk samples were forwarded to the laboratory and submitted to a complete granulometry screening. Samples were previously treated with pH 7-buffered NaEDTA as casein micelle disruptor, as recommended by Lopez (2005), and then processed by a laser-scatter granulometer (Coulter Counter LS230). In order to assess the globule membrane stability to NaEDTA, possibly due to membrane-casein interactions, four measurements per sample were performed with increasing NaEDTA amounts; therefore, 0, 1.25, 2.5 and 17.5 mL of NaEDTA were put into the measurement cell prior to size determination. On the globules were determined the surface-weighted diameter (ds), the volume-weighted diameter (dv), the specific surface area (SSA), the 10th, 50th and the 90th percentile of diameter (d10, d50 and d90, respectively) and the span (adimensional value of dispersion, calculated as (d90-d10)/d50).

Raw data were processed with an analysis of covariance (ANCOVA) by la general linear model (GLM procedure) by SAS 9.1 (SAS Institute); breed and EDTA amount were considered as fixed factors and day of lactation as covariate. For multiple comparisons of breeds, the least significant difference test for multiple comparison was applied.

RESULTS AND DISCUSSION

The dimensional parameters for the fat globules in the different breeds appear somewhat variable; these differences are statistically confirmed by the ANCOVA procedure (table 1).

	d_s	d_v	SSA
Breed	0.001	<0.001	0.0025
EDTA	n.s.	n.s.	n.s.
Lactation day	<0.0001	<0.001	<0.001

Table 1: P-values for the ANCOVA analysis in the dimensional parameters for milk fat globules. n.s.- not significant.

In particular, for all parameters, a significant effect of breed was observed (at least $p<0.0025$), and so for the day of lactation ($p<0.0001$); the effect of NaEDTA was negligible, underlining a great stability of all breeds to a casein micelle disruptor.

Parameter	Holstein	Cabannina	Rendena	Varzese
d_s(µm)	$2.5±0.28^a$	$2.3±0.21^b$	$2.5±0.36^a$	$2.5±0.20^a$
d_v (µm)	$3.3±0.67^a$	$2.9±0.32^b$	$3.0±0.22^c$	$3.1±0.47^c$
SSA (cm^2/ml)	24532^a	26087^b	24876^a	24657^c
d10 (µm)	$1.5±0.13^a$	$1.4±0.10^b$	$1.5±0.08^a$	$1.5±0.17^a$
d50 (µm)	$2.9±0.38^a$	$2.7±0.31^b$	$2.9±0.31^a$	$2.8±0.27^c$
d90 (µm)	$5.4±1.87^a$	$4.8±0.59^b$	$4.9±0.43^b$	$5.2±1.50^{ab}$
Span	$1.31±0.41^a$	$1.3±0.10^{ab}$	$1.2±0.09^b$	$1.3±0.50^a$

Table 2: dimensional parameters for the milk fat globules in the four breeds: data are reported as mean±sd. Different superscripts indicate a p<0.05 difference.

The differences in dimensional parameters between breeds are reported in Table 2: multiple comparisons put in evidence an actual discrimination between Holstein (as a widely-selected breed) and the other breeds. The reported differences are calculated after adjusting for the effect of lactation day. Holstein breed shows the higher fat globule diameter, and the difference are particularly evident when d_v are considered; the Rendena and Cabannina had the smallest globules with respect to the other two breeds; noteworthy was the difference in SSA: Holstein and Rendena have the lesser area, and Cabannina the largest, with a mean difference of about 1500 cm^2• ml^{-1} with respect to Holstein.

The difference between breeds are particularly evident when percentiles are examined, in particular d90: Holstein cows reach the 5.4 µm diameter, whilst the Cabannina and Rendena breeds did not reach the mean diameter of 5 µm. Varzese cows are in a mean position.

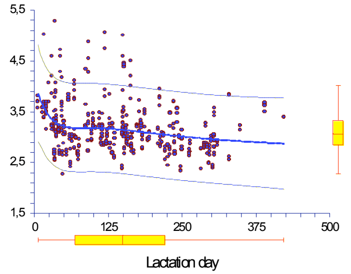

Figure 1: Polynomial ratio trend for d_v in the total of the subject. The 95% limits are reported.

By considering the whole number of samples, the relation between day of lactation and dv, taken into account as the most representative parameter well-fitted a third-degree polynomial ratio curve (figure 1), showing a steep globule diameter reduction at the starting phase of lactation and a subsequent diameter stabilization in the advanced lactation.

CONCLUSIONS

In this work, a first wide survey on the dimensional distribution of milk fat globules in different cow breeds and different lactation stages, focusing on local breeds. The results are interesting under different points of view, since they cover physiological, functional and possibly commercial aspects.

Milk is a highly complex system, in which fat is more than a mere nutritional component: the presence of a phospholipid/protein membrane and the variability in their size are the structural keystone to fat absorption and metabolism. The data presented show significant differences among breeds, and a fast reduction in the first lactation phase. All these characteristics could reflect different needs in newborn physiology during growth. The complex structure of the fat globule can therefore serve as a microscale/nanoscale vector for triglycerides, complex lipids and proteins. The interactions among these components associated with the size distribution, changing during lactation, can define the fat globule as a dynamic release device, adapting to the newborn needs; in the next future, these features could be more deeply studied in order to design further membrane modifications with the aim to vehiculate and target proteins or other membrane-linked molecules.

Moreover, breed differences in fat globule dimensions could underline deeper changes in whole milk characteristics: milk and dairy products texture sensory characteristics and evolution depend on protein and fat globule interactions: the process and the dimensional characteristics of milk fat can direct several characteristics towards certain properties: therefore, the differences between "standard" breeds and local strains could explain possible typical characters in local dairy products.

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