INFLUENCE OF FAT CONTENT ON QUALITY OF COW'S MILK

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ABSTRACT

The aim of the study was to verify whether changes in the percentage of fat in highly selected cows produce variations in the physical structure of the fat and changes in milk composition. Individual milk was sampled from 50 cows. Fat was evaluated in each individual in order to create two groups of animals with lower and higher percentage. The group with higher fat content showed a significantly larger diameter of the fat globules, less C14:0 and more C16:1. In conclusion the diameter variations observed result in few changes in milk fatty acid composition, thus maintaining a consistent nutritional quality.

Keywords: cow, fatty acids, milk fat globules, milk quality

1. INTRODUCTION

The nutraceutical composition of foods consumed daily is becoming increasingly important. Particular focus has been on consumers belonging to all age groups especially with regard their food lipid intake, since lipids have been implicated in several diseases such as obesity, insulin resistance and atherosclerosis (OLOFSSON *et al*., 2009). For these reasons, the number of studies on the physical and chemical structure of fat in several edible products of animal origin have increased (LE *et al*., 2014, MARTINI *et al*., 2016)
Cow's milk is consumed on a daily basis from childhood, thus it is important to know and monitor the milk lipid changes that occur naturally, or are induced by different farming techniques. It is thus necessary for consumers to drink throughout their life a guaranteed product from a nutritional and nutraceutical point of view. For the dairy industry it is important to assess how changes in the morphometry of the milk fat globules (MFGs) lead to changes in yields, ripening and the nutritional quality of cheeses (MARTINI *et al*., 2016).
Milk lipids are spherical structures and research has shown that the differences related to the diameter and number of MFGs are of interest with regard to the chemical-physical and nutritional properties of milk and lipid digestion and absorption (MARTINI *et al*., 2013).
The secretion of milk fat globule is not yet completely understood. Furthermore it is known that their size and distribution vary as a function of factors such as species, breed, parity, stage of lactation and amount of fat secreted (MARTINI *et al*., 2016). Some authors (WIKING *et al*., 2004 and CAROLL *et al*., 2006) have found positive relationships between daily fat yield and the average diameter of the MFGs. These findings are probably due to the fact that part of the membrane of the mammary epithelial cell is sacrificed to envelop the globule, then larger MFGs may be secreted to reduce the amount of membrane lost by the cell (ARGOV *et al*., 2008). Some authors have hypothesized also that relationships between fat secretion and MFG size are due to the effects on the mammary metabolism of the energy balance and the availability of nutrients (MARTINI *et al*., 2013). Moreover, the effects of energy balance on the metabolism of the mammary gland are not yet fully known (GERMAN, 2011).
The purpose of this study is to check whether small changes in fat secretion in highly selected cows, which have been reared in the same conditions, would result in variations in the physical structure of the fat and in changes in the milk composition.

2. MATERIALS AND METHODS

2.1. Animals and sampling

Milk from the morning milking of 50 pluriparous Friesian cows (151 ± 25 days in milk) was sampled. The cows were reared intensively in a single dairy farm in Tuscany (central Italy). All the cows were fed with the same diet consisting in a mixed ration formulated according to NRC (2001) requirements for dairy cattle and made up of corn silage, corn meal, alfalfa hay, hay ryegrass, soybean meal 44% PG, integral cotton seed, cane molasses, and commercial complementary feed. All individual milk samples were taken during the same day by in-line milk meters (De Laval) and were refrigerated at 4°C before being taken to the laboratory for analysis.

2.2. Milk analysis

All the fresh samples were analysed in duplicate for total solids (TS), fat, protein, casein content according to AOAC methods (2004). Fat extraction was performed using hexane and ethanol, according to Rose-Gottlieb's method modified by SECCHIARI *et al*. (2003).

Methyl esters of fatty acids (FAME) were obtained after transesterification with sodium methoxide according to Christie (1982). The composition of the milk fatty acids was determined by gas chromatography using a Perkin Elmer Auto System (Perkin Elmer, Norwolk, CT, USA) equipped with a flame ionization detector and a capillary column (30 m × 0.25 mm; film thickness 0.25 mm; FactorFour Varian, Middelburg, the Netherlands). The helium carrier gas flow rate was 1 mL·min^{-1}. The oven temperature program was as follows: level 1, 50°C held for 2 min, level 2, 50 to 180°C at 2°C·min^{-1} then held for 20 min, level 3, 180 to 200°C at 1°C·min^{-1} then held for 15 min, and finally level 4, 200 to 220°C at 1°C·min^{-1} then held for 30 min. The injector and detector temperatures were set at 270 and 300°C, respectively. Individual FAs were identified by comparing their retention times with those of the following authenticated standards: FIM_FAME mix (Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823) and methyl 9(Z), 11(E)-octadecadienoate (Matreya LLC International Dealers & Representatives-Superchrom S.r.l., Via C. Menotti 11, Milan, 20129)
In addition, nonadecanoic acid methyl ester (C19:0 Restek Corporation, 110 Benner Circle, Bellefonte, PA 16823) was used as internal standard to calculate the recovery of the fatty acid methyl esters. The desaturase index was calculated for: cis-9 14:1/14:0, cis-9 16:1/16:0, cis-9 18:1/18:0, following KELSEY *et al*. (2003).

2.3. Morphometric analysis of milk fat globules

A direct method (MARTINI *et al*., 2013) was used to determine the diameter (μm) and the number of fat globules per mL of milk in each fresh sample by a fluorescence microscope (Leica Ortomat Microsystem, Milan, Italy) equipped with a camera (TiEsseLab, Milan, Italy) and TS view 2.0 image software.

2.4. Statistical analysis

The frequency distribution of the total counted and measured MFGs was evaluated and globules were grouped into three size-categories: small globules (SG) with a < 2μm diameter, medium-sized globules (MG) with a diameter from 2 to 5 μm, and large globules (LG) with a > 5 μm diameter.
The variability in the fat secreted from each of the 50 cows was evaluated in order to create two groups of animals (groups A and B) secreting lower fat percentages (less that 32 g kg^{-1}, made up of 16 individuals) and higher (more than 37 g kg^{-1}, made up of 12 individuals) respectively, compared to the average value (34 g kg^{-1}). Individuals whose fat percentage was between 32 and 37 g kg^{-1} were excluded.
The results were analyzed by ANOVA using JMP software (2002)considering the group (A or B) as the fixed factor. Least significance means were compared by *t*-test. Significant differences were considered at $P< 0.05$.

3. RESULTS

Milk yield, chemical and physical characteristics are reported in Table 1.
Milk yield in the two groups of animals was similar. The higher fat content of group B corresponded to a significantly larger average diameter of globules and to increases in the percentages of the large globules. In group B, 22% of the total number of globules was made up of large globules, whereas in group A, the percentage of large globules was only 12%. This confirms the hypothesis of other authors (MARTINI *et al*., 2016) who have linked the positive relationship between the fat secreted and the average diameter of the

MFGs with a balance of the mammary gland cell. In fact, as the secretion of fat is an apocrine mechanism, it involves losses in the membrane, which is sacrificed to envelop the globules. Larger MFGs may therefore be secreted to reduce the amount of membrane lost by the cell per unit volume of fat.

Table 1. Milk yield, quality and characteristics of the fat globules in the milk from two groups of cows.

	Group A (fat<32 g kg^{-1}) (n‡=16)	Group B (fat >37 g kg^{-1}) (n‡=12)
Average daily milk yield (kg^{-1}) †	29.64±1.65	28.58±1.92
Fat (g kg^{-1})	27.9±0.50**	40.5±0.60**
Proteins (g kg^{-1})	30.50±1.00	31.70±1.1
Casein (g $kg^{-1)}$	24.34±0.996	25.08±1.068
Casein/fat ratio	0.90±0.039**	0.62±0.045**
Protein/fat ratio	1.13±0.045**	0.81±0.052**
Dry matter (g kg^{-1})	134.4±5.30	133.9±6.20
Ash (g kg^{-1})	6.9±0.10	6.9±0.10
Number of milk fat globules (n/mL)	$11.82X10^{9}$±9.53	$8.48X10^{9}$±1.10
Milk fat diameter (μm)	2.90±0.18*	3.50±0.21*
SG § (%)	38.15±3.35	30.45±3.89
MG ¶ (%)	49.96±3.44	47.54±4.00
LG ††(%)	11.89±3.13*	21.98±3.64*

* P<0.05; ** P <0.01
†Calculated as kg of milk produced until the day of sampling /days in milk at the day of sampling.
‡ n= number of cows;
§SG=Small globules with a diameter <2 μm.;
¶ MG=Medium globules with a diameter between 2 and 5 μm;
††LG=Large globules with a diameter >5 μm.

In addition, as previously observed in sheep (MARTINI *et al*., 2012),subjects with a smaller average diameter (group A) showed a higher number of globules/ml of milk (+39%).
From the point of view of the milk quality, total nitrogen did not differ significantly in the two groups of cows, however lower casein/fat and protein/fat ratios were observed in group B. This may be a limiting factor, especially for cheese-making since protein/fat ratio is a critical characteristics for the cheese yield and for the recoveries of fat and water in the cheese (GUINEE *et al*., 2007). Moreover, some authors have suggested that there is a link between milk protein synthesis and fat (HEID and KEENAN, 2005). CEBO *et al*. (2012) have also reported that genetic polymorphism at the *α*s1-casein (*CSN1S1*) affects both the structure and composition of the MFGs.
On the other hand, the milk of group A showed a lower average diameter of MFGs (P <0.05). It has been observed that milk with smaller sized globules increases cheese yield in Emmental production (MICHALSKI *et al*., 2004), and that in sheep a greater diameter of the MFG leads to a worsening of the rheological parameters, due to negative relations with the content of casein and the casein:fat ratio (MARTINI *et al*., 2016).
Studies on the variability in the average MFG diameter in Friesian cows have shown contrasting results in terms of the relations between diameter and the milk yield and quality. Some authors have reported positive correlations between the diameter and the amount of fat (BRIARD-BION *et al*., 2008), whereas others have not observed any relationship (LOGAN *et al*., 2014).

In addition, the diameter of MFGs has been reported to affect milk fatty acid composition (LOPEZ *et al.,* 2011).
In our study, the changes in the fatty acid profile (Table 2) were limited and concern an increase ($P < 0.05$) in myristic acid in group A (+11% g kg^{-1} of the total fatty acids), and an increase ($P < 0.05$) in palmitoleic acid in group B (+19% g kg^{-1} of the total fatty acids).
This study highlights that, although statistically significant, small changes (0.6 microns) in the diameter of the milk fat globules in homogeneous animals only have a slight effect on the milk fatty acid profile.

Table 2. Fatty acid composition (g kg-1of total fatty acids), classes of milk fatty acids (%) and desaturase indexes in milk from two groups of cows.

	Group A (fat <32.00 g kg^{-1}) (n†=16) g kg^{-1} of the total fatty acids	Group A (fat <32.00 g kg^{-1}) (n†=16) g kg^{-1} of milk	Group B (fat>37.00 g kg^{-1} (n†=12) g kg^{-1} of the total fatty acids	Group B (fat>37.00 g kg^{-1} (n†=12) g kg^{-1} of milk
C4:0	25.88±1.04	0.72±0.029	25.74±0.86	1.04±0.035
C6:0	20.49±0.71	0.57±0.020	19.43±0.85	0.79±0.034
C8:0	12.98±0.48	0.36±0.013	12.2±0.67	0.49±0.027
C10:0	31.07±1.30	0.87±0.036	28.34±0.16	1.15±0.006
C11:0	2.88±1.44	0.08±0.040	2.75±0.17	0.11±0.007
C12:0	36.62±1.45	1.02±0.040	33.34±1.76	1.35±0.071
C13:0	1.36±0.06	0.04±0.002	1.32±0.07	0.05±0.003
C14:0	125.31±3.65*	3.50±0.101*	113.25 ±4.41*	4.59±0.179*
C14:1	13.67±0.40	0.38±0.010	13.19±0.49	0.53±0.020
C15:0	13.59±0.42	0.38±0.012	13.28±0. 51	0.54±0.021
C15:1	3.06±0.15	0.09±0.004	3.26±0.18	0.13±0.007
C16:0	346.77±7.75	9.67±0.216	344.19±9.36	13.94±0.379
C16:1	13.13±0.68*	0.37±0.019*	15.58±0.83*	0.63±0.034*
C17:0	7.81±0.26	0.22±0.007	8.00±0.31	0.32±0.013
C17:1	2.88±0.23	0.08±0.006	3.17±0.28	0.13±0.011
C18:0	89.55±3.65	2.50±0.102	91.84±4.40	3.72±0.178
C18:1 *trans-9*	5.52±0. 27	0.15±0.007	5.52±0.33	0.22±0.013
C18:1 *trans-11*	6.90±0.28	0.19±0.008	7.05±0.34	0.29±0.014
C18:1 cis-9	192.89±9.21	5.38±0.257	203.38±11.12	8.24±0.450
C18:2 *trans*-9,12	2.31±0. 08	0.06±0.002	2.19±0.10	0.09±0.004
C18:2 cis-9,12	35.64±1.90	0.99±0.053	33.53±2.29	1.36±0.093
C18:3n3	3.52±0.15	0.10±0.004	3.60±0.18	0.15±0.007
C18:3 n6	13.06 ±0.11	0.36±0.003	12.03±0.13	0.49±0.005
C20:0	1.17±0.05	0.03±0.001	1.23±0.01	0.05±0.001
CLA *cis*-9, *trans*11	5.34±0.11	0.15±0.003	5.17±0.13	0.21±0.005
C20:1	0.57±0.05	0.02±0.001	0.56±0.06	0.02±0.002
C21:0	0.77±0.01	0.02±0.001	0.86±0.01	0.03±0.001
C20:2	0.22±0.03	0.006±0.001	0.17±0.03	0.01±0.001
C20:3n6	1.17±0. 06	0.03±0.002	1.17±0.07	0.05±0.003
C20:4	0.11±0.04	0.003±0.001	0.14±0.05	0.01±0.002
C20:3 n3	0.25±0.03	0.007±0.001	0.21±0.03	0.01±0.001
C22:0	0.73±0.06	0.02±0.002	0.70±0.07	0.03±0.003
C22:1	1.61±0.11	0.04±0.003	1.42±0.13	0.06±0.005

C20:5	0.24±0.03	0.007±0.001	0.22±0.04	0.01±0.002
C23:0	0.34±0.02	0.01±0.001	0.33±0.03	0.01±0.001
C22:2	0.34±0.03	0.01±0.001	0.42±0.04	0.02±0.002
C24:0	0.43±0.03	0.01±0.001	0.44±0.04	0.02±0.002
C24:1	0.19±0.06	0.005±0.002	0.30±0.06	0.01±0.002
C22:5	0.77±0.04	0.02±0.001	0.74±0.05	0.03±0.002
C22:6	0.94±0.21	0.03±0.006	0.55±0.25	0.02±0.010
	Group A (fat <32.00 g kg^{-1}) (n†=16) % of the total fatty acids	**Group A (fat <32.00 g kg^{-1}) (n†=16) g kg^{-1} of milk**	**Group B (fat>37.00 g kg^{-1} (n†=12) % of the total fatty acids**	**Group B (fat>37.00 g kg-1 (n†=12) g kg-1 of milk**
Short chain FA‡ (≤C10)	9.04±0.28	2.52±0.078	8.57±0.34	3.47±0.014
Medium chain FA (≥C11≤C17)	55.66±1.14	15.53±0.032	55.13±1.37	22.33±0.055
Long chain FA (≥C18)	35.29±1.28	9.85±0.036	36.30±1.54	14.70±0.062
Saturated FA	70.73±1.11	19.73±0.031	69.72±1.34	28.24±0.054
Monounsaturated FA	24.04±1.07	6.71±0.030	25.34±1.21	10.26±0.049
Polyunsaturated FA	5.18±0.22	1.45±0.006	4.89±0.49	1.98±0.020
	Group A (fat <32.00 g kg^{-1}) (n†=16)		**Group B (fat>37.00 g kg^{-1} (n†=12)**	
Unsaturated /Saturated FA ratio§	0.42±0.03		0.44±0.03	
n3/n6 ratio¶	0.06±0.005		0.07±0.006	
Desaturase C14 index††	0.10±0.003		0.11±0.004	
Desaturase C16 index††	0.04±0.002		0.04±0.003	
Desaturase C18 index††	0.32±0.01		0.31±0.01	

* P<0.05

†n= number of cows; ‡ FA: Fatty Acids; § Unsaturated /Saturated FA ratio: :∑unsaturated fatty acids/:∑saturated fatty acids; ¶n3/n6 ratio:∑n3 fatty acids /∑n6 fatty acids; †† Desaturase C14 index: [C14:1]/[C14:1+C14:0].; Desaturase C16 index: [C16:1]/[C16:1+C16:0]; Desaturase C18 index: [C18:1c9]/[C18:1cis 9+C18:0]

4. CONCLUSIONS

In high-selected dairy breeds significant variations in fat secreted influence the morphometry of the fat globules, casein:fat and protein:fat ratios. However, the range of variation observed in the diameter does not lead to significant changes in the fatty acid composition. Thus maintaining a consistent nutritional quality of bovine dairy milk.

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