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Investigation of Casein Micelle Particle Size Distribution in Raw Milk of Estonian Holstein Dairy Cows

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Abstract. The particle size of milk influences its microstructure and defines many properties of dairy products such as colloidal stability, texture etc. Differences in particle size can significantly affect milk processing especially when membrane technology is used.
Aim of this investigation was to estimate casein micelle size in the raw milk of Estonian Holstein dairy cows and its variability concerning individual animals.
Milk samples were collected during 12 months with the interval of 25–35 days. DLS analyses were performed using a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, Malvern, UK).
Average mode of casein micelle particles size in raw milk of 44 cows was 171.13 nm with the variation range 70.1 nm and its distribution resembled a normal one. Casein micelles size mode of individual cows varied in a wide range from 148.5 (with variation range 18.2) to 194.1 (with variation range 27.6) nm which may be caused by differences in physiological and health status, stage of lactation and other factors concerning milk production.

Key words: casein micelle, dynamic light scattering, particle size, particle size distribution.

INTRODUCTION

Particle size (PS) and particle size distribution (PSD) gives valuable information about colloidal systems, among others milk and dairy products also (Beliciu & Moraru, 2009). Commonly used techniques for the analysis of PS and PSD are dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), scanning electron microscopy (SEM), size exclusion chromatography (SEC), cell electrophoresis, analytical ultracentrifugation (AUC) etc. Various analytical methodologies may give different results (Anema et al., 2005; Thu Tran Le et al., 2008; Dejan, 2010; Raza et al., 2011). Of the mentioned techniques, DLS is the most user-friendly and it gives relatively accurate and consistent results of protein samples which can be obtained in short period of time (Vasco et al., 2010). Main challenges to estimate casein micelle (CM) PSD by DLS, is the fact that measurements need to be performed with considerably pure solution and at low concentration of sample (Alexander & Dalgleish, 2006). For reliable estimation of CM PSD, 10 to 1 µg milk must be diluted in one millilitre of solution (Beliciu & Moraru, 2009). Casein micelle consists of four different protein fractions (α_{S1}-, α_{S2}-, β-, κ-casein). Each CM is covered with a layer of water molecules which affects the hydrodynamic diameter of micelle.

In former studies CM PSD had been mainly estimated in raw bulk milk, skimmed milk, reconstituted skimmed milk, lactose-free milk and pasteurized milk (Martin et al., 2007; Tran Le et al., 2008; Liu et al., 2013).

There is few published research which deal with CM in raw milk of individual cows, for example Bijl et al. (2014) investigated how milk chemical composition influenced casein micelle size of individual cows and de Kruif & Huppertz (2012) investigated how lactation stage affects CM PSD. The aim of current study was to approve suitability of dynamic light scattering (DLS) method for estimation of casein micelle PSD in raw milk and investigation of its variability of individual Estonian Holstein dairy cows during one year period.

MATERIALS AND METHODS

Raw milk samples of 44 Estonian Holstein dairy cows were collected at the Experimental Farm of Estonian University of Life Sciences (EMÜ) from January 2013 to December 2013, with the interval of 25 to 35 days. After milking samples were cooled down and stored at 5°C, all analyses where made in the same day.

For stable results, samples were diluted at refrigerator temperature (5°C) just before the measurement using RPMI 1640 (PAA Laboratories GmbH, Pasching, Austria) as diluting media to concentration 1µg/ml, and filtered before measurement using a 0.45 µm, Ø 15mm Premium Syringe Filters (Agilent Technologies, Santa Clara, California). This procedure was necessary to remove larger particles such as fat, dust etc. 1500 µl of each sample was inserted into a single-use disposable sizing cuvette DTS0012 (Sarstedt REF 67.754, Sarstedt AG&Co, Nümbrecht, Germany). DLS analyses were performed using a Zetasizer Nano ZS analyzer (Fig. 1). The particle size estimations were made at fixed 173° backscattered angle using the default ‘protein analysis mode’ with automatic duration and four consequent measurements from a sample without delay. Automatic attenuation selection was switched off and number six was inserted as value for attenuator.

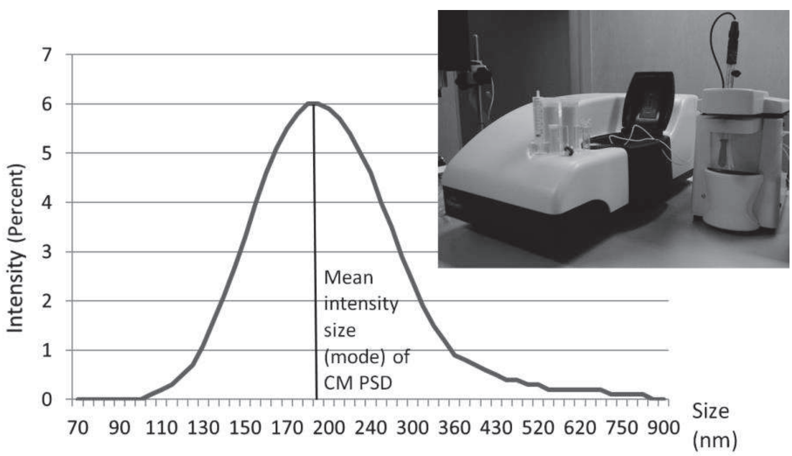

Figure 1. Typical example of CM PSD and Malvern Zetasizer Nano ZS analyser.

Measurement temperature was chosen 20°C, because this is a temperature commonly used in DLS measurements studies (Beliciu & Moraru, 2009). Data collection and first elaboration of these was carried out by Zetasizer software 7.01. The mode of PSD (mean intensity size of hydrodynamic diameter), average of total PSD (harmonic intensity of averaged particle diameter or Z-Average diameter) and all other data were exported to Microsoft Excel for further analyses. Typical example of CM PSD and measurement equipment are presented in Fig. 2.

RESULTS AND DISCUSSION

From obtained data only mean intensity of different variables of size was used. This index corresponds to the mode of CM PSD curve and represents the most essential information in it. Average of CM PSD modes of 328 samples was 171.13 nm with variation range 70.1 nm and it had standard deviation (SD) of 14.06. Histogram of these modes resembles normal distribution and covers the range 135–210 nm (Fig. 2).

More than half of the modes (58.5%) covered the range 155–175 nm. Tails of this modes distribution were represented by four (1.2%) samples in range from 135–140 nm and six (1.5%) samples in the range 200–210 nm. Former studies about CM PSD in bulk (and treated) milk by different methods showed up quite the same variability in average mode of CM PSD: 150–200 nm (Table 1). Some variation in results can be explained by different measurement methodologies used for preparation of probes and by differences in milk itself (bulk milk of different production system, cows breed etc.).

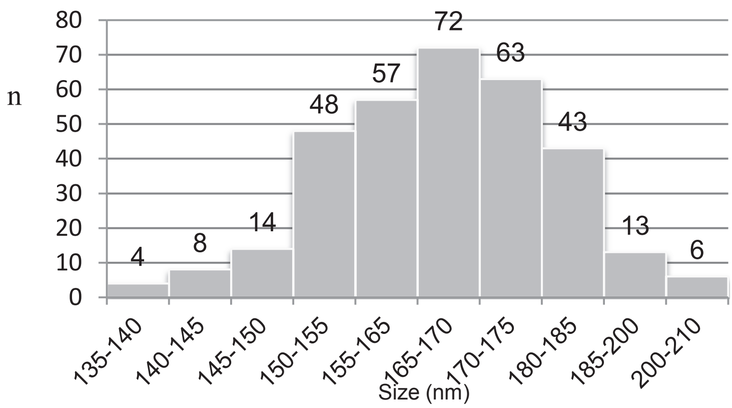

Figure 2. Histogram of CM PSD modes in raw milk probes of Estonian Holstein dairy cows.

Studies of Beliciu & Moraru (2009) and Liu et al. (2010) are in agreement with results of our investigation. They found that CM PSD average in treated bulk skim milk varies from 176.3 to 178.8 nm. This result is only somewhat bigger than average mode of CM PSD in our study. Overall accordance of above mentioned studies with our investigation suggests that DLS method is suitable for estimation of CM PSD in raw milk of cows too.

To investigate the role of individual animals on mode of CM PSD, all data were rearranged into groups by cow number. Of all animals under this study (n = 39) milk of six cows has been analysed 3–5 times and milk of 33 cows 6–11 times during the total investigation period.

Table 1. Summary of former studies about the CM size in milk

Author	Method	Mode of CM size, nm
Tran et al., 2008	NTA	192
Martin et al., 2007	Cell electrophoresis	185
Tran et al., 2008	DLS	186
Liu et al., 2010	DLS at 20°C	177
	DLS at 40°C	200
Beliciu and Moraru, 2009	DLS at 20°C	176
	DLS at 50°C	194
De Kruif and Huppertz, 2012	DLS	154–230
Raza Hussain et al., 2011	DLS (solvent NaCl)	150

Differences in number of analyses were caused by changes in lactation stage mainly.Individual dairy cows' average mode of CM PSD in descending order is presented in Fig. 3 and numerical values of modes and their variations are given in Table 2.

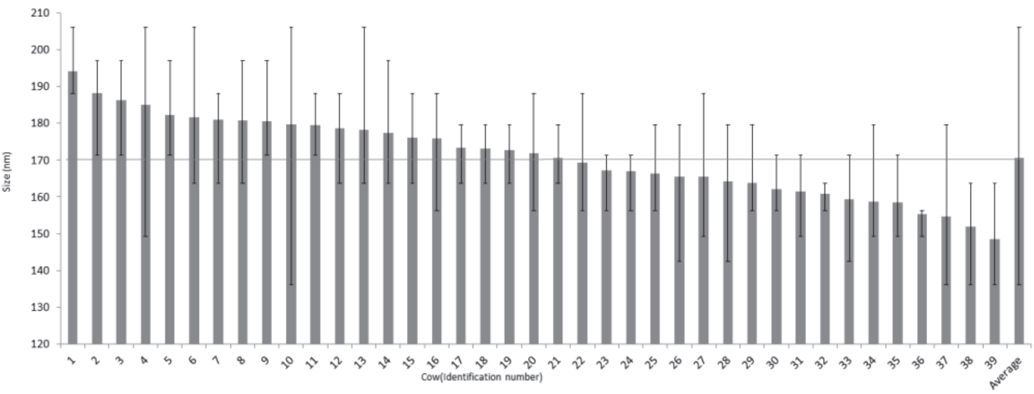

Figure 3. Individual dairy cows average mode of CM PSD in descending order.

Average mode of CM PSD in this study varied in range from 148.5 nm to 194.06 nm (group average of 39 cows was 170.95 nm, with variation range of 27.40 nm and SD of 19.4). This data is similar to the results obtained in analyses about the total herd (44 cows/328 samples).

Largest average mode of CM PSD in this dataset was 194.06 nm, with 9.1 nm variation and the smallest one had PSD of 148.5 nm with 27.6 nm variation. In Fig. 4 casein micelle PSD variability of five individual cows are presented. Cow No. 1 and cow No. 39 average mode of CM PSD have extreme values (min and max), three other cows (10, 18, 32) have average mode closer to mean of the herd. Average mode value of CM PSD and its variation of different cows seem to be independent from each other. It was confirmed by statistical analyses – they showed up only a very slight positive correlation (0.195). Also increasing number of samples does not make this relation better. For example, by 10 cows of 11 which had 10 CM PSD estimations each, variation mean value (19.4 nm) of the mode was exceeded. The only reasonable explanation to that may be found in cows' individuality.

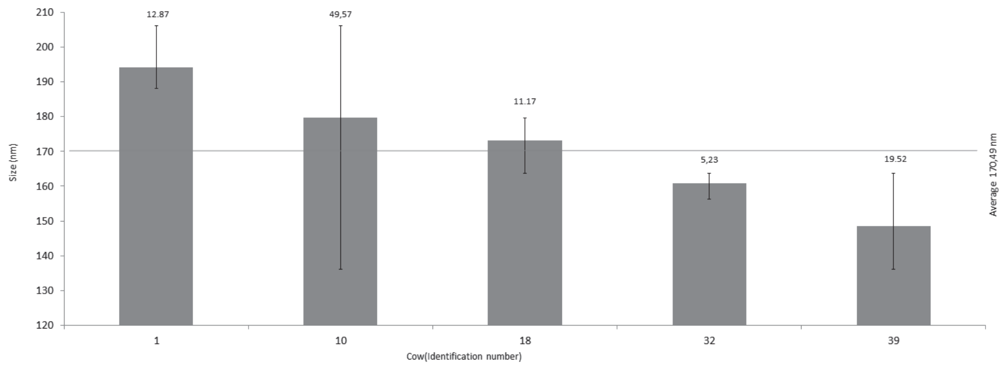

Figure 4. Casein micelle PSD variability of individual cows 1, 10, 18, 32 and 39.

Table 2. Average modes of CM PSD in milk of individual cows in descending order, their variations and standard deviations

Cow identi-fication number	Mode of CM PSD	Variation range of CM PSD	SD of CM PSD	Count of samples	Cow identi-fication number	Mode of CM PSD	Variation range of CM PSD	SD of CM PSD	Count of samples
1	194.1	18.2	12.9	3	**21**	170.6	15.8	11.2	9
2	188.3	25.5	18.0	6	**22**	169.3	31.7	22.4	10
3	186.2	25.5	18.0	9	**23**	167.2	15.1	10.7	9
4	184.9	57.0	40.3	9	**24**	167.0	15.1	10.7	7
5	182.2	25.5	18.0	10	**25**	166.4	23.2	16.4	9
6	181.7	42.5	30.1	10	**26**	165.6	37.0	26.2	10
7	180.9	24.3	17.2	7	**27**	165.4	38.8	27.4	11
8	180.8	33.2	23.5	9	**28**	164.3	37.0	26.2	7
9	180.5	25.5	18.0	10	**29**	163.8	23.2	16.4	9
10	179.7	70.1	49.6	9	**30**	162.1	15.1	10.7	9
11	179.6	16.6	11.7	6	**31**	161.4	22.2	15.7	3
12	178.7	24.3	17.2	9	**32**	160.7	7.4	5.2	5
13	178.2	42.5	30.1	10	**33**	159.5	28.9	20.4	10
14	177.3	33.2	23.5	10	**34**	158.7	30.3	21.4	10
15	176.0	24.3	17.2	9	**35**	158.6	22.2	15.7	10
16	175.8	31.7	22.4	8	**36**	155.4	7.1	5.0	8
17	173.2	15.8	11.2	9	**37**	154.6	43.4	30.7	10
18	173.1	15.8	11.2	5	**38**	152.0	27.6	19.5	3
19	172.7	15.8	11.2	7	**39**	148.5	27.6	195	7
20	171.9	31.7	22.4	3	**Average**	170.9	27.4	19.4	8.1

Also the absence of tight correlation between CM PSD average mode and its variation refers to influence of certain factors connected to cows' individuality (changes in physiological status, disease incidences, stages of lactation, etc). All these aspects should be topics of further investigations.

CONCLUSION

For the first time, studies of casein micelle PSD variability in milk samples of individual Estonian Holstein dairy cows have been carried out by DLS measurements during one year period. The main results of this study can be summarized as follows: 1) Average mean intensity (mode) of CM PSD in raw milk of Estonian Holstein dairy cows was 171.13 nm and its variation (range 135–210 nm) resembled statistically normal distribution. 2) Weak correlation between CM PSD average mode and its variation in milk samples of individual cows may refer to the possible influence of cows' physiological status, disease incidences and stages of lactation etc. which will be studied in further research.

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