Changes in milk yield, fat and protein mass fractions in mares’ milk within 24 hours

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Abstract. During the day protein mass fraction and fat mass fraction in mares’ milk changes in different ways. The variability of fat content in mare’s milk reaches 60% within 24 hours. Several experiments, including the milking of mares continuously during the day, were conducted to explain the nature of this phenomenon. Differences in the variability of fat and protein contents in the mares’ milk during the day and various way of secretion of the milk components from udder’s secretory cells were explained by the authors in this scientific article. The protein is secreted due to merocrine secretion, and fat secretion mainly occurs due to apocrine secretion. Reduction of fat content in the mares’ milk is caused by the significant increase of milk yield in the early morning, when secretion of synthesized fat slows down. When milk yield is reduced, the secretion of stored fat is increased, which also increases the fat content in milk. It was found that in the morning milk yield of mares is higher than at night by 16.39%. It was also found that during the day protein mass fraction is higher than it is at night by 5.24%. The fat content in the mares’ milk during the day is higher than it is at night by 12.24%. The ratio of daily milk yield and fat content was amounted to 0.22, of daily milk yield and protein mass fraction was + 0.44, and of fat mass fraction and protein mass fraction was + 0.47.

1. Introduction
Mares differ from females of other farm animals in the fact that their udder is small, so foals get their mouth on the udder very often, and milking is carried out every 2-3 hours. During the study of mares’ milk producing ability it was found that within 24-hour fat mass fraction (FMF) in mares’ milk varies approximately by 55-60%, which is not characteristic of other animal species. According to our data, the minimum fat content in mares’ milk is observed at 5-6 am during the first morning milking, and then fat content of milk increases within six hours, reaches a maximum level at twelve o’clock in the afternoon, and after that decreases and stays stable until the end of milking (до 21.00) [1].

For example, in the studies of G. A. Averyanov changes in fat content on mares’ milk during the day were the following: the first milking – 1.03 %, the second milking – 1.68 %, the 3rd milking – 1.73 %, the 4th milking – 1.82 % [2]. I. A. Saygin described a similar situation in his work: the first milking – 0.85 %, the second milking – 1.80 %, the 3rd milking – 2.11 %, the 4th milking – 1.65 % [3]. In the surveys of V. S. Yavorskiy the changes in fat content in mares’ milk were also similar: the first milking – 0.99 %, the second milking – 1.75 %, the 3rd milking – 1.85 %, the 4th milking – 1.68 %, the 5th milking – 1.69 %, the 6th milking – 1.65 %, the 7th milking – 1.69 %, the 8th milking – 1.73 % [4]. Consequently, depending on the fact during which time of a day the milk samples were obtained, fat
mass fraction in mares' milk could vary significantly from 0.85% to 2.11%. Therefore, results of many researchers on fat mass fraction in mares' milk of the same breed can vary considerably very often.

There was no acceptable explanation for this steady decline, and then dramatic increase in fat mass fraction in the mares' milk of mares in the works' of authors listed above. For example, V. S Yavorsky explained this phenomenon in the following way: "The residual milk in the udder from the previous milking is rich in fat. In the subsequent milking, the residual milk, poured out into the udder's central cavity by newly formed milk, to some extent affects the fat content of milk.

The foal before weaning from the mother thoroughly sucks it out and by the first milking there is no residual milk in the udder." [4]. When milking mares, the foals are taken away from the mares at 4.00 am and reunited with the mares only at 9.00 pm. That is in the morning there was no residual milk in the mares’ udder, so fat mass fraction in the morning milk was minimal. But this fact does not explain the increase in the mass fraction of fat, and then its decline in subsequent milking, as mares were without foals all this time.

H. Dusembin in his studies found a similar dependence in cows. During the first milking at five o'clock in the morning, the mass fraction of fat in cows' milk was the lowest - 3.5-5.0%; then the cows were milked every two hours, until 8.00 pm. During three milkings or about six hours the fat content of cows' milk gradually increased and after six hours reached a maximum level of 5.3-8.0%; after that the butterfat percentage decreased and remained approximately at the same level till the end of the day [4].

H. Dusembin noted that during the day in the cows "... in most cases the dynamics of fat, casein and lactose has the same character, slightly varying during different types of rations" [4]. It can only be added that the fat content in the cow milk varied during these six hours, as well as in mares’ milk, that is, the maximum limit of diurnal variability of fat mass fraction in milk was the same 60%, as well as in mares.

But calves do not suck cows’ milk at night. Consequently, this phenomenon does not depend on united keeping animals with animal yield, and in any case, it is common for the females of hoofed animals, if not for all mammals. Consequently, our research task was to establish the physiological mechanism of this phenomenon.

2. Experimental research

Studies were conducted in the breeding koumiss complex of CJSC SF "Semenovskiy" of the Mari El Republic from 1986 to 2013 and on the farm of LLC "BelKoumissProm" of the Republic of Belarus in 2014-2015. The milk of Russian and Lithuanian heavy draft mares was examined.

We examined the composition of the mares' milk taken for analysis from each of the eight milking, from 6.00 am to 8.00 pm. Analyses were taken both from a group of mares in general, and from individual mares of different breeds, different ages in summer and in winter, in spring and in autumn. The milk was analyzed in different months of lactation, from the second to the eighth months.

Milk samples were taken in accordance with NSS 26809-85. Fat mass fraction (FMF) in milk was determined in accordance with NSS 5867-90 - Milk and dairy products, and protein mass fraction (PMF) in mares' milk was determined in accordance with NSS 23327-98 - Milk and dairy products.

It turned out that regardless of the lactation period, the mares’ breed and their age the lowest FMF in the mares' milk was observed at 6 am, and the highest FMF was observed at 10-12 hours of the day. The biggest difference between the minimum and maximum of FMF in mares' milk - 1.32% - was observed in the first three months of lactation. In subsequent periods of lactation, this difference decreased and it was from 0.58% to 0.94% of FMF in milk.

In order to check changes in milk yield, FMF and PMF in the mares' milk for a full day, without foals' influence on these processes, we transferred the group of mares on the 5th-6th month of lactation for round-the-clock milking. To do this, we took away the foals from these mares and added night milking to daytime milking. Mares had no contact with the foals 24 hours a day, as they were milked constantly, and there was no need in the presence of the foals at night with the mares.
The experiment was conducted in a week after the beginning of round-the-clock milking of a group of mares, when the animals were accustomed to the new milking regime. The experiment was carried out for two adjacent days, the average results of the experiment are presented in the table 1.

Table 1. Changes in milk producing ability of mares within 24 hours (n=10).

| Indicators          | Start Time of Mares Milking, hour | On average per day |
|---------------------|-----------------------------------|-------------------|
| 20:00               | 22:00                             | 24:00             |
| FMF, %              | 1.85                              | 1.86              |
| PMF, %              | 2.06                              | 1.95              |
| Milk Yield, kg      | 8.25                              | 6.40              |
| 1 % Fat, kg         | 15.26                             | 11.91             |
| Protein, kg         | 17.00                             | 12.48             |
| Milk Fat, g         | 152.6                             | 119.1             |
| Protein, g          | 170.0                             | 124.8             |

According to the table data it can be stated that at 4-6 o'clock in the morning the yield of mares increased, and at the same time fat mass fraction synchronously decreased, and then within three milkings it increased higher than 2.0%.

3. Results and considerations

We can bindingly claim that change of fat mass fraction by early afternoon did not depend on foals, feeding, and operating personnel. Apparently, increase in milk yield of animals in the early morning was caused by the change of night and day. In the morning, yield increases in all mammals having daily life. In the studies of V. A. Afanasyev and A. A Nikishov it was shown that correlation of milk yield of animals with the sun activity is + 0.98 [5]. This fact is confirmed by the research of A. G. Taranenko: "The higher the level of milk production, the shorter the latent period of the lactation reflex. It is not the same during the day. So, in the morning milking the latent period is shorter than in the day or evening milking..." [6].

At the same time, mass fraction of protein in milk was changing insignificantly: in the daytime it was slightly above 2.1%, and at night - below 2.1%. In our opinion, differences in daily variations of fat and protein mass fractions in mares milk lie in different types of milk components secretion inside the milk alveoles in mares during lactopoiesis. Thus, secretion of protein in the mammary gland proceeds according to the merrocrine type [7]. Protein granules pass through the smallest pores of the plasmolemma of the secretory cells apical part, without violating the integrity of the cell membrane. According to Kurosami et al., secretion of fat mainly occurs due to the apocrine type with a violation of the integrity of the secretory cell membrane [8]. In this case, the apical part of the secretory cell is
protruded inside the milk alveoles, the apical plasmolemma flows around the fat drop and comes off with it.

It should be noted that fullness of the udder with milk and increase of pressure inside the udder influence a little merocrine protein secretion. Note that secretion of protein in the mekrinovomu type has little effect on the filling of the udder with milk and the increase in intra-venous pressure. Milk proteins are placed in the udder in the form of colloidal solution and make particles ranging in size from 5 to 100 nm. Due to this fact, they are equally distributed throughout the udder [6, p. 65]. According to our research, protein content in consecutive portions of a one-time milk yield always remains constant [8].

Milk fat is contained in milk in the form of small balls with a diameter of 0.1 to 10 microns. It is not dissolved in milk plasm and is not osmotically active [3]. Fat globules that are larger than protein particles do not interact with the milk liquid part. When the udder is filled with milk, these globules are aggregated and stay in alveolar cavities and openings of milk ducts and strokes [6]. That is why the amount of fat gradually increases in consecutive portions of a one-time milk yield. The lowest fat content is in cisternal milk - from 0.1% to 0.8%, in alveolar milk fat mass fraction varies from 1.2% to 2.2%, and the highest fat content is in the last portions of a one-time milk yield and vestigial milk, that is 2.4% -2.6% [1]. In addition, high pressure inside the udder slows down fat extrusion from secretory cells into the udder when the its milk tanks are filled.

That is why release of fat drops from the secretory cells into the milk alveoles of the mares’ udder slows down in case of the morning sharp increase of milk yield (figure 1).

The figure clearly shows that as soon as mares yield starts to increase, fat mass fraction in milk starts to decrease synchronously. As soon as milk yield began to decrease, fat mass fraction in mares milk began to increase simultaneously. At the same time, protein mass fraction in milk did not react to the change in milk yield. If we calculate the amount of 1% milk by fat and protein, we get a very interesting picture (figure 2).

![Figure 1](image1.png) **Figure 1.** Daily variations of milk yield and composition of mares’ milk.

![Figure 2](image2.png) **Figure 2.** Daily variations of milk yield and amount of milk with fat and protein content of 1%.

Changes in the amount of 1% milk by protein completely coincide with changes in milk yield of mares. In general, the line of the high and law points of the amount of 1% milk by fat coincides with the line of milk yield by the character, but its base is twice as wide, and its increase in fat mass fraction begins later than the increase in milk yield by two milkings or by four hours. The 2 figure clearly shows that the amount of milk, and especially the amount of basic fat milk (1.5%) is distributed unevenly during the day.

In addition to the theoretical conclusions, practical conclusions can be made on the basis of experiment results.

4. Conclusion

Firstly, the total amount of milk yield for six "daytime" milking was 51.85 kg of milk, and for six "night" milking was 44.55 kg of milk. The difference was reliable (P≤0.01) and amounted to 7.3 kg or
16.39%. Probably, this factor should be taken into account, and it is necessary to make adjustments to the calculation of daily milk productivity in mares. Now the amount of milk from mares during daytime milking, is recounted according to Saygin’s formula for daily productivity, assuming that the level of milk production in mares is the same both in the day time and at night. Taking into account our experiments, the results of calculations must be multiplied by coefficient of 0.918.

Secondly, the composition of the mares’ milk in the day time and at night differs a bit. On average, fat mass fraction in mares’ milk per day was 1.96%, protein mass fraction was 2.10%. In “daily” milk, the figures were slightly higher: FMF was 2.08% and PMF was 2.15%. In “night” milk the figures were 1.84% and 2.04% correspondingly. Differences between milk that comes for processing and the milk that is given for foals at night were 0.11% (P≤0.01) of protein and 0.24% (P≤0.001) of protein in absolute units or 5.24% of protein and 12.24% of fat relating to the average indicators.

Correlation coefficients on experiment results’ bases were the following: between the daily milk yield and fat mass fraction - 0.22, between daily milk yield and protein mass fraction + 0.44, between fat mass fraction and protein mass fraction + 0.47.

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