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Secretory activity of cattle mammary gland tissues during lactation and duration of the interlactation period

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Changes in the secretory activity of mammary gland during the involution period are designed to create conditions for inexhaustible use of the mammary gland, ensure the normal course of structural and functional regression for the development for the next lactation. For animals in which the interlactation period lasted less than 45 days, the absorption of non-esterified fatty acids in the last week of the involution period was 2.8 times higher than in animals in which the interlactation period lasted at least 55 days, 1.48 times higher than the absorption of acetic acid, 1.24 times higher than β-oxybutyric acid, 1.28 times higher than glucose, 2.82 times the total amount of phospholipids and triacylglycerols. For acetic acid, β-oxybutyric acid and glucose, not only higher absorption rates were observed, but also a tendency to increase the absorption level in the last weeks of lactation in animals with an interlactation period of less than 45 days, indicating metabolic changes in mammary gland tissue during the involution period. The duration of the dry period affects the secretory activity of breast tissue during lactation and the quality of milk produced during this period, which is expressed by lower fat content in the product while reducing the duration of the dry period to less than 45 days and reducing milk fat by 10.42% during the next calving. At the same time, the body weight of newborn calves in animals, whose interlactation period was less than 45 days by 19.3%, was lower than the animals of the control group, i.e. those who were in the interlactation period for at least 55 days, which indicates the negative impact of reducing the duration of the dry period on the body of animals.
Keywords: Absorption; Non-esterified fatty acids; Acetic acid; β-oxybutyric acid; Milk; Animals

Introduction

The biochemistry of the synthesis of milk components and the influence of factors of exogenous and endogenous nature on this process have recently been actively studied in order to develop effective ways to improve the quality of this product. Milk secretion volumes by the mammary glands of cows, as well its qualitative and quantitative composition were subject to manipulation. In order to choose the right way of such correction, it is necessary to understand the biochemical, hormonal way of synthesis of milk components and the possibility of the influence of paratypic factors (housing conditions, feeding) on the process. The biosynthesis of milk components still remains an understudied issue. In particular, this process has not been studied in critical periods of animal life, when it is necessary to provide substances and metabolites of metabolism, changing the direction of nutrient flows to regulate all processes occurring in the body (Górová et al., 2011; Mayasari et al., 2016).

The difficulty of studying this problem is that researchers and practitioners are based in their research on generally accepted postulates and do not take into account the complexity, the one-time course of certain processes under physiological norms. All this indicates the relevance of studies of the secretory function of breast tissue of cows on the verge of critical periods of existence of the organism. However, data on differences in the composition of physicochemical properties of milk, breast, liver, scar, mesh suggest that albumin in the body is synthesized not only in the liver but also in other organs, including the breast. A number of researchers believe that the breast synthesizes from 10% to 20% of albumin (Amos et al., 2014).

The results of the study of the arteriovenous difference in the blood of lactating ruminants proved that the synthesis of milk protein is provided mainly by the adsorption of free amino acids from breast blood plasma. Insufficient intake of amino acids in the breast is compensated by their synthesis in breast cells from glucose, low molecular weight organic acids or by isolating them from tissue proteins (Van Hoeij et al., 2017).

Amino acids that enter the breast from the blood enter its tissues with varying efficiency. The need for amino acids should be determined taking into account the consumption of feed (metabolic energy), the distribution of energy between body tissues, milk, milk composition and lactation. Therefore, the period of lactation must be taken into account, as performance indicators of the previous may affect the next dairy products. This is fully consistent with the results, which indicate a significant effect of the duration of lactation and the dry period on the subsequent secretory activity of breast tissue (Machado et al., 2012; Marey et al., 2016). Undoubtedly, the physiological state of the body of cows is one of the primary factors for milk production, given that the body is simultaneously in a complex fragile homeostatic structure, which aims not only to ensure the secretory function of mammary gland tissue, but also the growth and development of the fetus, providing own anabolic and catabolic

obligatory processes and their optimal ratio. So, it becomes clear that exogenous factors become a very important regulatory element of influence on the body, especially in its critical periods, including during involution period (Mayasari et al., 2016).
Milk is secreted by the alveolar epithelial cells of the mammary gland under the action of the neurohypophyseal hormone oxytocin, which causes myoepithelial cells to contract and push the newly synthesized milk into the lumen of the alveoli. Milk secretion directly correlates with blood flow through the mammary gland (Gračner et al., 2015).
The energy requirement of high-yielding cows during lactation peak exceeds the volume of pure energy obtained from the feed. This is a consequence of decreased appetite after childbirth and increased metabolic processes, which, in turn, requires a large number of substrates for the synthesis of milk components. Lack of sufficient nutrients in feed leads to the use of body reserves, because excess fat is mobilized and subjected to lipolytic processes to reduce energy deficiency. Thus, milk synthesis is a rather energy-intensive process (Cant et al., 2002; Greco et al., 2015). The main precursors of blood for milk synthesis are glucose, acetate, β-hydroxybutyrate, triglyceride fatty acids, and amino acids (Górová et al., 2011; Seymora et al., 2005; Sheldon et al., 2014). The two main sources of energy are glucose and acetate. Other compounds are absorbed and used by the mammary gland, but they do not make a significant contribution to the quantitative aspects of the milk composition, although their qualitative aspects are extremely important. From these substrates, the gland synthesizes dairy components such as fat, lactose and protein. Milk proteins are represented by casein (alpha, gamma beta casein), lactoalbumins (alpha and beta), immune globulins and simple proteins – albumins. The sources of these proteins in milk are different: gamma-casein, immune globulins and albumins enter the milk after almost unmodified (or only partially altered) extraction from the blood, casein and lactoalbumins are translation products on the ribosomes of EPR cells of a mammary gland (Kambur et al. 2009).
According to some data, albumins get into milk not only due to their extraction from the blood, but also partial (within 10%) synthesis by mammary gland cells, which changes the traditional idea of the liver as a single organ – the place of albumin synthesis (Santos et al., 2016). Therefore, the prerequisite for changes in the level of proteins that get into milk are surely the energy value of the feed and the physiological state of their body, because both ways of protein penetration into milk – directly or indirectly – are determined by circulation of either proteins in the blood or precursors for their synthesis. The yield of milk protein is of great importance for the dairy industry. The volume and composition of proteins in milk is largely determined by the genetics of the animal. Some authors believe that it is difficult to change this feature through nutrition. However, due to the high need in energy for protein synthesis, the yield of milk protein may be affected by the energy content in the feed, so research is being conducted in this direction (Seeth et al., 2015).
Milk lipids in mammals are produced in the form of milk fat globules. The diversity of synthesized lipids and the complexity of biosynthetic mechanisms in the mammary gland indicate numerous potential roles that ensure proper growth, health and survival of mammalian offspring and, at the same time, reflect the structural determinants resulting from the chemical properties of these compounds (Zhao et al., 2014; Van Hoeij et al., 2017).
Each of the lactation periods (0-100 days after birth – the first period, 101-200 – the second, 201-305 – the third) has its own features of lactogenesis, its patterns of trophic needs of animals, and the maintenance needs. Naturally, the secretory activity of the mammary gland during involution period is gradually deactivated, and the level of absorption of milk precursors from the blood decreases. All these changes are designed to create conditions for the inexhaustible use of the mammary gland, create conditions for natural structural and functional regression for the development for the next lactation. And the duration of the interlactation period is one of the important levers both in terms of restructuring the mammary gland tissue, the development of the pregnant cow, the accumulation of nutrients in its body, and the development of the fetus (Cant et al., 2016; Cheong et al., 2016; Wang et al., 2016).
Thus, it is very important to understand the features of the secretory activity of the mammary gland of cows during involution period and the impact of the duration of the interlactation period on these features. However, there are no studies that would take into account the peculiarities of the synthesis of milk components during the involution period, taking into account the homeostatic stability of the body, which requires provision with passive substances for growth and development during this period.
Therefore, this work intended to study the activity of secretory activity of mammary gland tissue at the last stage of lactation (the 4th, 3rd, 2nd and 1st week before the involution period) depending on the duration of the interlactation period (more than 55, 50-55, 45-49, less than 45 days).

Materials and methods

The research was conducted in the conditions of the state research center of Sumy Institute of Agroindustrial Production, the Department of Anatomy of Normal and Pathological Physiology of Sumy National Agrarian University. In order to study the peculiarities of the secretory process in the mammary gland tissue of cows, we formed four groups of analogue cows of 10 animals each depending on the duration of the interlactation period. Group I (control group) includes animals in the interlactation period of at least 55 days. In cows of Group II (experimental) group, the duration of the interlactation period was from 50 to 55 days, in Group III (experimental group) – from 45 to 49 days. Group IV (experimental group) included cows with an interlactation period of less than 45 days. The animals of experimental groups were provided by nutrients through feeding corn silage, alfalfa hay, weed hay, barley meal, soybean meal, fodder beet, wheat straw in the autumn-winter and winter-spring periods of the year. The cows were selected taking into account the time of their last insemination. Only cows of the third gestation were involved in the experiment. Milking out was consistently reduced 31 days before the beginning of the interlactation period, so that the dry interlactation was at least 55 days, and cows with an interlactation period of 50-55 days were attributed to Group II (experimental group). The cows of Groups III and IV (experimental) were dried off physiologically. As the experimental groups of animals were formed during the involution period 4, 3, 2 and 1 week before the planned

involution period, we studied the use of precursors for the synthesis of milk components by the mammary gland tissue. To do this, we took blood samples in vacuum EDTA tubes (Vacuette, Greiner BioOne, Kremsmunster, Austria) from the caudal artery and milk vein from ten cows of each group. We determined the total content of non-esterified fatty acids (NEFA) (Miksa et al., 2004), the total fraction of phospholipids and triacylglycerols in blood samples by mass spectral analysis in Department 20 of the Institute of Applied Physics of the National Academy of Sciences of Ukraine, as described in (Parchem et al., 2019)., acetic acid (Lester, 1964), β-oxybutyric acid (Williamson et al., 1962), total protein content (Lowry et al., 1951), glucose content was determined photometrically using the Reflotron glucose kit (Roche Diagnostics, Mannheim, Germany) (Hammon et al., 2009).
The fat content in milk was determined Gerber method procedures https://camblab.info/wp/index.php/total-fat-analysis-in-milk-using-the-gerber-method/. The milk of animals of different groups obtained within the same time frame with other studies and the milk of these animals on the 7th day after calving were analyzed. After calving, the weight of newborn calves in each group was determined within 24 hours of birth.
During the experimental studies, we complied with the international requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and the relevant Law of Ukraine "On Protection of Animals from Cruelty" No. 3447-IV of 21.06.2006. The obtained digital material is statistically processed to determine the mean (M), the mean statistical error (m), difference probability (p) between the means of two variation series according to the reliability criterion (t) and according to Student's tables. The difference between the two values was considered probable at $P<0.05$.

Results

The obtained data on the secretory function of the mammary gland tissues of cows depending on the duration of the interlactation period indicate the negative impact of reducing the duration of this factor on animal productivity, because the process of extraction of milk precursors even in the involution period is more intense for animals with the duration of interlactation period of not less than 55 days. Moreover, the shorter the interlactation period, the more pronounced was the extraction. Besides, we observed not only higher rates of absorption of precursors, but also changes in the dynamics of absorption during involution period.
Thus, during the involution period mammary gland tissues in cows of the control group consistently reduced the absorption of non-esterified fatty acids (Table 1).

Table 1. The use of non-esterified fatty acids by the mammary gland during the involution period by animals kept in different interlactation periods, M ± m, n = 40.

Group of animals	lactation/interlactation duration	Number of weeks before involution at the time of sampling	Arterial blood, mmol/L	Venous blood, mmol/L	Arteriovenous difference	
					mmol/L	%
Control group	309/56	4	0.84 ± 0.016	0.42 ± 0.016	0.42 ± 0.015	50.0 ± 1.01
	308/57	3	0.98 ± 0.016	0.56 ± 0.010	0.42 ± 0.015	42.9 ± 1.02
	307/58	2	1.48 ± 0.016	0.96 ± 0.037	0.52 ± 0.016	35.1 ± 0.97
	306/59	1	1.58 ± 0.016	1.18 ± 0.033	0.56 ± 0.016	25.4 ± 1.12
Group II	311/54	4	1.99 ± 0.022	0.95 ± 0.017	1.04 ± 0.032	66.0 ± 1.13
	311/53	3	1.88 ± 0.023	0.84 ± 0.018	1.04 ± 0.021	55.1 ± 1.13*
	312/52	2	1.74 ± 0.033	0.86 ± 0.019	0.88 ± 0.014	49.2 ± 1.16*
	313/51	1	1.67 ± 0.031	0.97 ± 0.010	0.70 ± 0.013	41.8 ± 1.15*
Group III	315/49	4	2.02 ± 0.033	0.70 ± 0.011	1.32 ± 0.014	65.2 ± 1.21*
	316/48	3	1.88 ± 0.034	0.59 ± 0.010	1.29 ± 0.013	68.7 ± 1.12*
	317/47	2	1.76 ± 0.022	0.52 ± 0.012	1.24 ± 0.022	70.4 ± 1.33*
	318/46	1	1.71 ± 0.022	0.48 ± 0.012	1.23 ± 0.022	72.1 ± 1.42*
Group IV	320/44	4	0.75 ± 0.035	0.28 ± 0.016	0.47 ± 0.033	62.7 ± 1.23*
	321/43	3	1.04 ± 0.033	0.36 ± 0.015	0.68 ± 0.011	65.4 ± 1.23*
	322/42	2	1.42 ± 0.034	0.56 ± 0.013	0.86 ± 0.012	60.6 ± 1.31*
	323/41	1	1.64 ± 0.032	0.49 ± 0.012	1.15 ± 0.022	70.0 ± 1.21*

M – arithmetic mean; m – is the arithmetic mean error; n – is the number of goals; *$P<0.05$ compared with the control at the relevant time of sampling.

For non-esterified fatty acids, the arteriovenous difference in the animals of the control group decreased during the involution period by almost 2 times, which indicates their intensive use in the process of energy deposition. The reduction of the interlactation period was accompanied by the preservation of the adsorption activity of non-esterified fatty acids by the mammary gland tissues of cows. Group II preserved higher ability to adsorb during the involution period compared to control values, but still declines during lactation. For groups III and IV, in addition to the fact that the ability to absorb is higher than in other groups, the dynamics indicates an increase in the absorption of non-esterified fatty acids by mammary gland tissues.
This condition, of course, indicates metabolic shifts in the body and metabolic activity of the mammary gland atypical for the involution period.

The study of glucose absorption by breast tissue also found that the most active absorption process at the stage of completion of lactation occurs in animals of group IV, and has a tendency to increase in the last weeks before cessation of lactation (Table 2) and before its completion exceeds 1.28 times the level of animals in the control group.

Table 2. The use of glucose by breast tissue during the end of lactation by animals kept in different dry periods, M ± m, n = 40.

Group of animals	lactation/interlactation period	Number of weeks before involution at the time of sampling	Arterial blood, mmol/L	Venous blood, mmol/L	Arteriovenous difference	
					mmol/L	%
Control group	309/56	4	1.91 ± 0.02	1.77 ± 0.01	0.14 ± 0.002	7.33 ± 0.31
	308/57	3	1.92 ± 0.02	1.78 ± 0.02	0.14 ± 0.002	7.29 ± 0.32
	307/58	2	1.88 ± 0.03	1.75 ± 0.02	0.13 ± 0.002	6.91 ± 0.42
	306/59	1	1.83 ± 0.02	1.71 ± 0.01	0.12 ± 0.001	6.56 ± 0.41
Group II	311/54	4	1.90 ± 0.02	1.77 ± 0.02	0.13 ± 0.001	7.37 ± 0.21
	311/53	3	1.90 ± 0.02	1.76 ± 0.01	0.14 ± 0.001	7.36 ± 0.29
	312/52	2	1.93 ± 0.02	1.79 ± 0.02	0.14 ± 0.002	7.25 ± 0.31
	313/51	1	1.82 ± 0.02	1.68 ± 0.02	0.14 ± 0.002	7.14 ± 0.32
Group III	315/49	4	1.78 ± 0.02	1.65 ± 0.01	0.13 ± 0.002	7.30 ± 0.22
	316/48	3	1.84 ± 0.01	1.71 ± 0.01	0.13 ± 0.001	7.07 ± 0.23
	317/47	2	1.78 ± 0.01	1.65 ± 0.01	0.13 ± 0.002	7.30 ± 0.24
	318/46	1	1.81 ± 0.02	1.67 ± 0.02	0.14 ± 0.002	7.73 ± 0.25*
Group IV	320/44	4	1.90 ± 0.02	1.76 ± 0.02	0.14 ± 0.002	7.37 ± 0.26
	321/43	3	1.84 ± 0.02	1.70 ± 0.02	0.14 ± 0.002	7.61 ± 0.21
	322/42	2	1.80 ± 0.02	1.65 ± 0.02	0.15 ± 0.003	8.33 ± 0.22*
	323/41	1	1.79 ± 0.02	1.64 ± 0.02	0.15 ± 0.003	8.38 ± 0.31*

Note: M – arithmetic mean; m – is the arithmetic mean error; n – is the number of goals; *P<0.05 the differences are significant compared to the control group of animals in the corresponding sampling period.

Quite an unexpected result due to the fact that we did not perform additional parenteral or oral glucose supplementation. Therefore, this feature is caused by a decrease in the duration of the dry season.
It was also found that mammary gland tissues in cows of the control group consistently reduced the absorption of acetic acid and β-oxybutyric acid during the involution period (Figures 1 and 2).

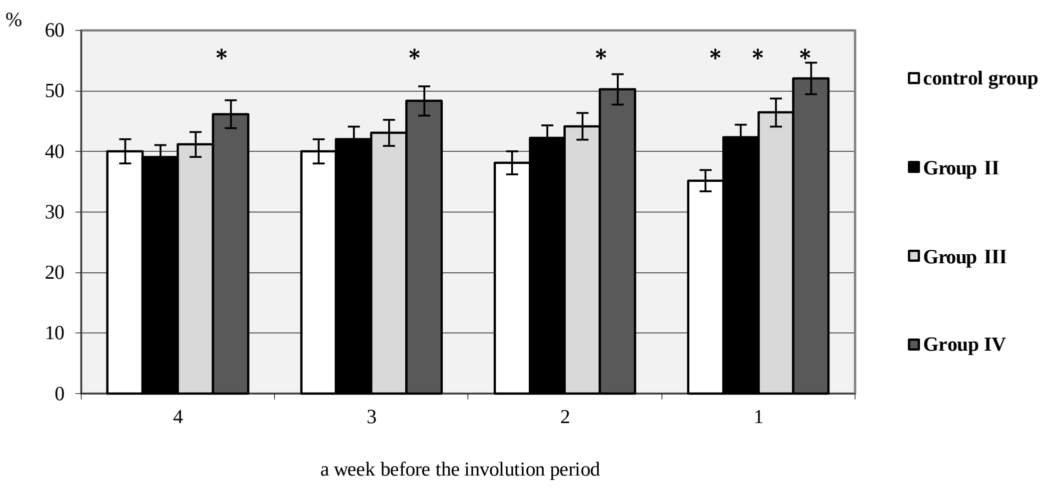

Fig. 1. Arteriovenous difference (%) of the use of acetic acid by the mammary gland of cows during involution period.

In cows of the second experimental group, the dynamics of the use of precursors for the synthesis of milk components by the mammary gland tissues of cows differs from this process in animals of the control group. The reduction of the interlactation period is accompanied by the preservation of the adsorption activity of the precursors by the mammary gland tissues of cows for the synthesis of secretion. The absorption capacity of the breast tissue of animals was found in relation to acetic acid 1.12 times and β-oxybutyric acid 1.18 times (P<0.05) higher than in control group cows in the last week of lactation (Figures 1 and 2). In cows of the third experimental group, the absorbing activity of the mammary gland during lactation corresponded to almost the indicators of the 6-7th month of lactation, as we found intensive use of breast tissue of cows acetic and β-oxybutyric acid, respectively, 1.32 and 1.44 times higher than in animals of the control group.
In cows of the fourth experimental group, the secretory function of the mammary gland tissues was also found at a high level. By the end of involution period, they absorbed all metabolites probably more than the mammary glands of the control group cows. Thus, they absorbed acetic and β-oxybutyric acid 1.48 and 1.24 times more than the mammary gland tissues of the control group cows (P<0.05).

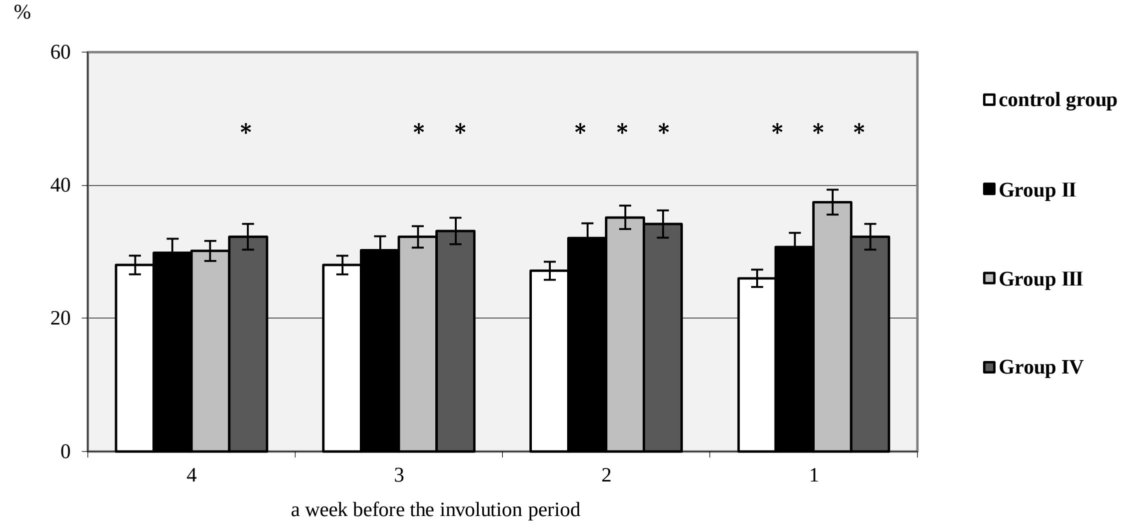

Fig. 2. Arteriovenous difference (%) in the use of β-oxybutyric acid by the mammary gland of cows during involution period.

Determination of the difference in the total content of phospholipids and triacylglycerols revealed that the tendency of changes in these compounds expresses a gradual decrease in their absorption by the mammary gland, but does not repeat the established picture of increased absorption by the end of lactation for other precursors for milk synthesis. There was a gradual decrease in the level of fat absorption by the mammary gland in control group animals (Figure 3) and experimental groups, but their indicators remained significantly higher relative to the control group animals (Figure 3).

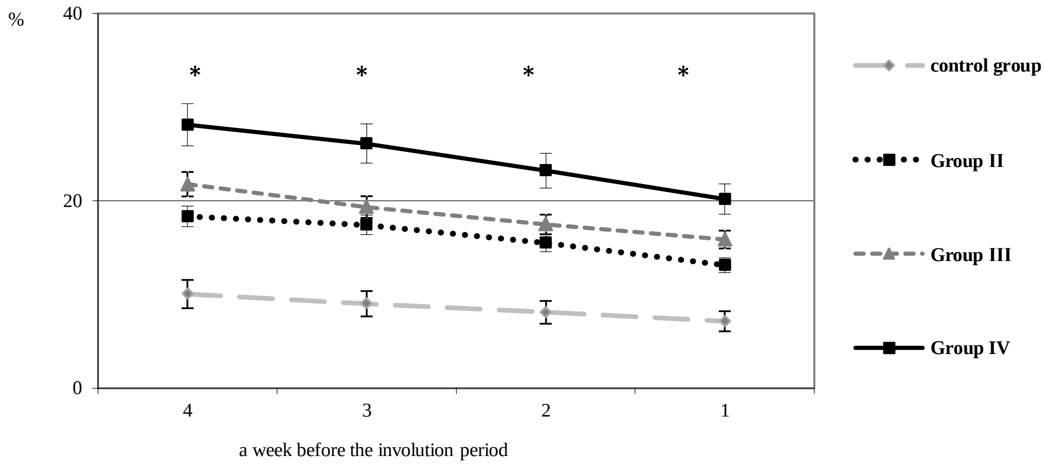

Fig. 3. Dynamics of changes in the arteriovenous difference in the content of the total amount of phospholipids and triacylglycerols in the blood of animals kept in different interlactation periods.

Thus, both metabolites of lipid metabolism and precursors for milk synthesis are more intensively absorbed by the mammary gland of cows of III and IV experimental groups, i.e. those who were in the interlactation period less than 50 and less than 45 days. Given the more active processes of absorption of milk precursors by the breast, it seems likely that the quality of milk, in particular its fat content, will be higher in these circumstances. However, this assumption was not confirmed when checking the relative fat content in milk obtained at 4, 3, 2 and 1 week before the cessation of lactation in representatives of groups III and IV (Fig. 4).

In general, for all groups there was a tendency to gradually reduce the fat content of milk at the stages of research, and this value was highest in animals of the control group. For animals of group IV milk fat content was reduced. The study of milk quality of these animals after calving showed a decrease in fat content of milk of animals of group IV relative to control by 10.42%, which indicates a negative impact of reducing the duration of the dry period on the body of animals (Fig. 5). The obtained results were expressed negatively on the body weight of newborn calves (Table 3), whose weight was lower by 1.16 and 1.24 times relative to the control group. Thus, the weight of newborn calves was 27.3 ± 1.12 in control group, 26.07 ± 1.13 in Group II, 23.53 ± 0.5 in Group III, and 22.02 ± 0.6 in Group IV (n=40).

Thus, the reduction in the duration of the interlactation period is accompanied by increased absorption activity of the mammary gland, and results in the birth of calves with lower body weight. The level of metabolic changes is inversely proportional to the duration of the interlactation period.

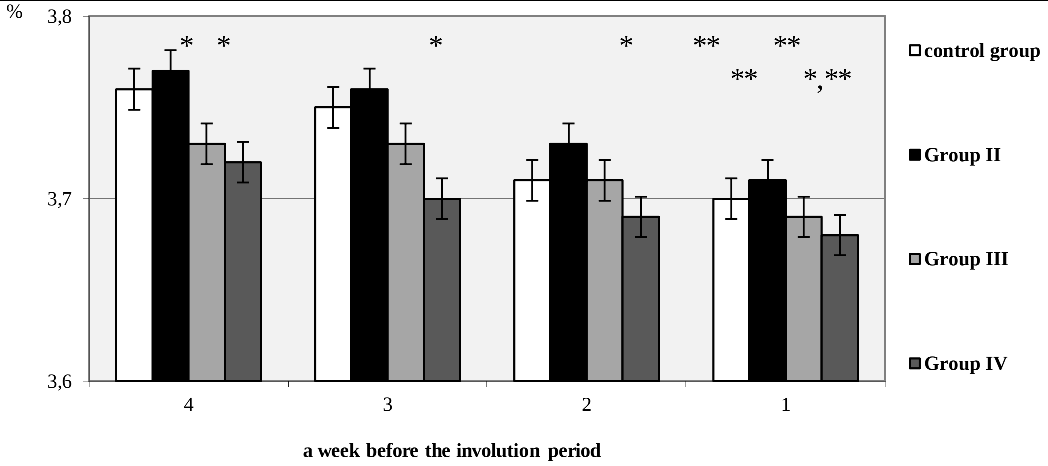

Fig. 4. The fat content in the milk of animals kept under different modes of dryness.

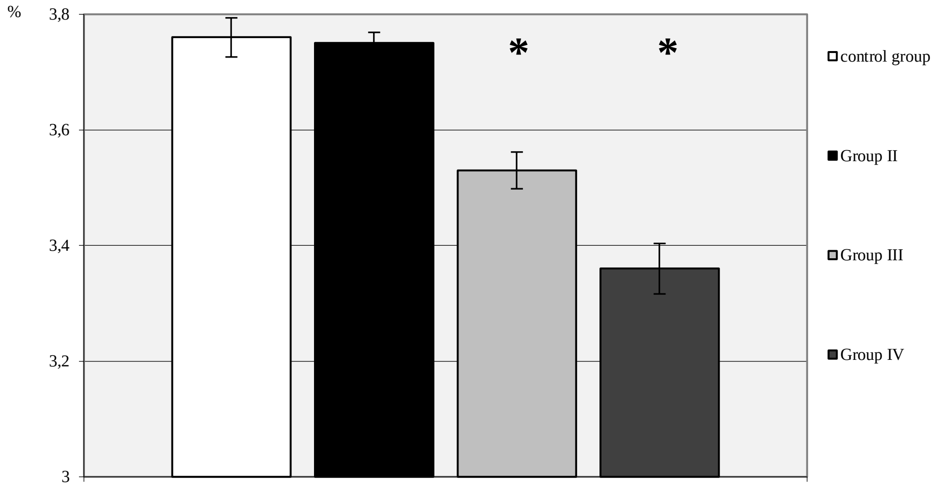

Fig. 5. The fat content in the milk of animals kept under different modes of dryness after calving.

Discussion

The obtained results indicate a negative effect of reducing the duration of the interlactation period on the condition of the body of cows in the last stage of lactation. The result of reducing the interlactation period to less than 45 days was the birth of calves with a body weight 1.24 times lower than calves born to mothers who were in the interlactation period of at least 55 days. This fact can be provoked by the lower energy balance in the body of cows with a lower duration of the interlactation period. Besides, reduced lactation in cows with a shorter interlactation period poses challenges in terms of further feeding of calves (Accorsi et al., 2002; Hammon et al., 2009).

Biochemical changes in the activity of mammary gland tissues during involution period, as one of the critical physiological moments in the life of a mature cow, reveal the specifics of metabolic changes and their dependence on the duration of the interlactation period. Thus, it was found that the intensity of metabolic activity of the breast in relation to the absorption of milk precursors is inversely proportional to the duration of the interlactation period. The involution process is normally characterized by a decrease in the level of lipid fractions in the blood, and it is believed that the decrease in the intensity of lipid absorption by lactic acid during lactation directly correlates with the level of milk productivity. Fluctuations in the content of total lipids in the blood depend on the physiological state of the body, the level of albumin, which act as transporters of these hydrophobic compounds, the time after meals, etc. (Chew et al., 1981; Ribeiro et al., 2016). Although a decrease in absorption was typical for all groups closer to the end of the lactation period, the level of arteriovenous difference was, however, the highest in animals of Group 4 (Figure 3). More intensive absorption of these compounds by the mammary gland may indicate a longer adaptation of the mammary gland tissues to physiological changes and, therefore, may cause various pathogenic processes in the udder. In addition, more intensive use of lipids by the mammary gland can adversely affect the condition of the fetus, due to the energy-unbalanced distribution of triacylglycerols and phospholipids. Weight loss in newborn calves is one of the possible consequences of the established increased adsorption of lipids by the mammary gland.

The established differences in the absorption of non-esterified fatty acids by the mammary gland, depending on the duration of the interlactation period, actually indicate the diverse processes that take place at the end of lactation in these circumstances. It is known that the fraction of non-esterified fatty acids is represented by more than 20 types of monounsaturated and polyunsaturated fatty acids, fluctuations in blood levels within the physiological norm may be due to feeding, sampling time, and so on. Due to the fact that there is a gradual decrease in the absorption of non-esterified fatty acids from the blood in animals of the control group, the decrease in the need for absorption of non-esterified fatty acids by the mammary gland is due to the involution period. However, the level of absorption of non-esterified fatty acids by the mammary gland increases in animals of Groups III and IV. An increase in the level of non-esterified fatty acids is characteristic of a negative energy balance, which provokes the use of the body's internal reserves, i.e. lipolysis of lipids, their decomposition into non-esterified fatty acids and glycerol. Non-esterified fatty acids are usually mobilized for the synthesis of lactose if the intake of glucose in the body is insufficient. Given that the increase was recorded during the involution period, we observe an imbalance between the extraction of non-esterified fatty acids and the need for the mammary gland cells to continue milk synthesis. On the other hand, structural rearrangements that may begin in the mammary gland during lactation require plastic material for cell rearrangement. One way or another, the reduction in the duration of the interlactation period is accompanied by changes in the dynamics of absorption of non-esterified fatty acids relative to animals with the longer interlactation period.

As we know, milk yield positively correlates with the concentration of butyrate ($r^2 = 0.47$) and propionate ($r^2 = 0.23$) (Seymoura et al., 2005). and, at the same time, positively correlates with dry matter consumption. This statement is consistent with the data obtained in our studies for animals of the control group with the interlactation period of at least 55 days, and with a gradual decrease in the extraction of β-oxybutyric acid from the blood during the involution period. At the same time, the longer interlactation period compared to other groups ends with the birth of calves with higher body weight. Thus, the duration of the interlactation period of 55 days is a positive factor in terms of starting point for postnatal development of calves, because their physical inclinations are higher, as well as the volume of milk produced. The results obtained in other studies show that the functioning of the secretory function of the mammary gland and, consequently, milk production depend on the sex of the fetus, which in turn causes not only "sex dependence" of the volume of milk produced, but is closely related to the weight of newborns, because bulls are usually more massive. In addition, when calves with larger body weight are born milk production by the mammary gland is higher, which may be due to higher concentrations of estrogen and placental lactogens during gestation (Chew et al., 1981). On the other hand, data from other studies lead to the opposite conclusion: (Swali & Wathes, 2006) found that calves with lower birth weight were associated with higher milk production during gestation.

Probably, such essentially opposite results can be explained by different formulation of the experiment and interpreted from the point of view of the duration of the interlactation period in cows, because in our studies the weight of newborns was maximum at 55 days.

Reducing the duration of the dry period is accompanied by the preservation of high activity of breast tissue. Thus, the adsorbing activity of the breast during the end of lactation of animals of groups III and IV remains at a high level for acetic and β-oxybutyric acid, and also tends to increase. The data obtained are an alarming signal, primarily in terms of breast recovery after previous lactation. It is known that the end of lactation is accompanied by involution of the breast and histological changes that affect the ability to synthesize and secrete milk components. Therefore, for the full functioning of the breast during the next lactation, physiological, naturally occurring intervals are necessary, which would allow to complete the complex of these changes. In addition, it is believed that the dry period can cure existing breast infections, but the wrong approach can lead to the risk of new infections (Seeth et al., 2015).

Reduced duration of the interlactation period is accompanied by the preservation of high activity of the mammary gland tissues. Thus, the adsorbing activity of the mammary gland during the involution period of animals of Groups III and IV remains high for acetic and β-oxybutyric acid, and also tends to increase. The data obtained are an alarming signal, primarily in terms of mammary gland recovery after previous lactation. As we know, the involution period is accompanied by involution of the mammary gland and histological changes that affect the ability to synthesize and secrete milk components. Therefore, physiological naturally occurring intervals are necessary for the full functioning of the mammary gland during the next lactation, which would allow to complete the complex of these changes. In addition, it is believed that the interlactation period can cure existing mammary gland infections, but the wrong approach can lead to the risk of new infections (Seeth et al., 2015).

Thus, in addition to the negative consequences in the form of weight loss of newborn calves we have identified, the reduction of the interlactation period really threatens a higher probability of mammary gland infection, which increases the likelihood of a negative effect of the interlactation period reduction. The mammary gland is one of the few tissues that undergoes repeated cycles of structural and functional development and regression. Mammary gland regression is characterized by massive apoptosis and loss of the mammary gland alveolar epithelial cells (Accorsi et al., 2002; Seeth et al., 2015; Jeong et al., 2017). Epithelial regeneration is a controlled and clearly termed process under the influence of growth factors. The need for rapid regeneration of epithelial cells in animals with a shorter interlactation period as in other processes that require immediate construction/restoration of the capillary network (such as tumours) may well cause the formation of epithelium, the diameter of the pores of which will allow substances not intended for milk production to penetrate from the blood into the mammary gland cells or to extract the necessary substances more than required during current and/or next lactation. After all, it is known that during involution the mammary gland loses of integrity of the epithelium, and various substances get from the blood into the lumen of the mammary gland. Histological and morphological changes continue during the involution of the steady state and the period of neolactogenesis. Thus, the composition of the secretion formed by the mammary epithelium changes markedly (Seeth et al., 2015). Since in our experiment we observe the most intensive use of breast metabolites of lipid metabolism, non-esterified fatty acids, acetic and β-oxybutyrate as the interlactation period decreases, we should examine the condition of blood vessels in the mammary gland in these circumstances. After all, as we know, the volume of milk, in particular, depends on some properties of blood vessels. However, greater milk vein internal diameter surface 50 days following the second calving was correlated with higher milk production during the second lactation (Gračner et al., 2015).

More intensive absorption of the studied substances during the short-term interlactation period may also be due to factors of another nature – stress. It is possible that the hormonal adjustment of the body against the background of prolonged lactation may be one of the stressors for cows. In response to stress, the body responds by changing the activity of antioxidant enzymes and changes in the activity of certain hormones, including adiponectin, which protects the mammary glands of cattle from stress reactions of the endoplasmic reticulum by increasing mammary epithelial cells (Jeong et al., 2017).

The higher uptake of mammary epithelial cells, the ability of mammary glands to produce milk is determined by the number of cells that secrete milk, and the level of their activity (Amos et al., 2014; Marey et al., 2016). In this case, in relation to the mammary gland and its ability to lactate, we can apply the concept of mammary gland plasticity, i.e. the ability of the gland to adapt to various factors. The main mechanisms contributing to this phenomenon are changes in the activity and number of mammary epithelial cells. Factors such as changes in milking frequency, hormonal manipulations, reproductive status, growth hormone treatment, changes in feed levels and, as correlated with our study, the duration of the interlactation period and the specifics of nutrition modulate milk production along with changes in milk cell activity. Besides, the plasticity of mammary gland tissues is influenced by lactation stage, health status, and genetic factors (Machado et al., 2012; Sheldon et al., 2014).

Thus, the reduction in the duration of the interlactation period may be accompanied by a complex of successive changes in the mammary gland at the end of lactation, which will affect the physiological, biochemical, and structural aspects of this tissue.

One of the criteria for the condition of the breast is the content of non-esterified fatty acids in the blood (Miksa et al., 2004). In healthy animals (under a balanced diet and energy-sufficient state), non-esterified fatty acids mainly occur due to the breakdown of triglycerides that enter the diet through chylomicrons (lipoprotein lipase releases non-esterified fatty acids from chylomicron residues). Increased plasma concentrations of non-esterified fatty acids are associated with a negative energy balance. Besides, several studies have shown a positive relationship between increased plasma esterified fatty acid concentrations during the last 2-3 weeks of pregnancy and the development of postpartum diseases such as ketosis, fatty liver, displaced metritis (Miksa et al., 2004). In our studies, there was an absorption of non-esterified fatty acids from the outflowing blood of the breast, which was inverse to the duration of the interlactation period. This functional activity of the mammary gland tissues of cows of the fourth experimental group reduced energy deposition in the body of cows, and further reflected in the results of subsequent gestation. While the high levels of non-esterified fatty acids are evidence of a negative energy balance, increased consumption of non-esterified fatty acids during lactation is a negative criterion for energy deposition in cows and threatens negative consequences for the fetus and future lactation.

The obtained results eloquently testify to the importance of the correct approach when choosing the terms of interlactation for cows and the impact of such a choice on the condition of animals at the involution stage. The stage of cessation of milk production is one of the key periods in preparation for the next lactation, in terms of the impact on the mammary gland plasticity, which depends on the condition of the animals during the next lactation and fetal development. The interlactation period is necessary for the recovery of the mammary gland of cattle after previous lactation. The decrease in the duration of this period is accompanied by increased adsorption activity of the mammary gland and results in the birth of calves with lower body weight. The level of activity of metabolic changes is inversely proportional to the duration of the interlactation period. The activity of the processes of absorption of milk precursors is most pronounced of all groups in animals with the interlactation period of less than 45 days.

Thus, in animals in which the dry period lasted less than 45 days, the activity of milk precursor absorption processes is most pronounced of all groups, and the negative effects on the weight of newborn calves and the fat content of milk they will receive are more pronounced.

Conclusion

The duration of the dry period affects the secretory activity of the mammary gland tissues during the involution period. The decrease in the duration of the interlactation period is accompanied by increased absorption capacity of the mammary gland tissues, and this dependence is inversely proportional. For animals in which the interlactation period lasted less than 45 days, the absorption of non-esterified fatty acids in the last week of the involution period was 2.8 times higher than in control group animals, 1.48 – absorption of acetic acid, 1.24 – β-oxybutyric, 1.28 – glucose, 2.82 times the total amount of phospholipids and triacylglycerols. Thus, the activity of the mammary gland tissues was probably higher in animals whose interlactation period did not exceed 45 days. The duration of the dry period affects the secretory activity of breast tissue during lactation and the quality of milk produced during this period, which is expressed by lower fat content in the product while reducing the duration of the dry period to less than 45 days and reducing milk fat by 10.42% during the next calving. At the same time, the body weight of newborn calves in animals of Group IV by 19.3% was lower than the animals of the control group, i.e. those who were in the interlactation period for at least 55 days. For acetic acid, β-oxybutyric acid and glucose, not only higher absorption rates were observed, but also a tendency to increased level of absorption in the last weeks of lactation of Group IV animals, indicating metabolic changes in the mammary gland tissues at the involution stage.

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