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Relationship Between Globulins in the Late Dry Period with Biochemical Parameters, Fertility and Culling of Cows within 90 Days after Calving

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Abstract. Diseases of the transit period in cows and their culling after calving attract considerable attention. Therefore, early diagnosis and prediction of pathological processes during the transition period in cows using biochemical markers stays relevant. The purpose of this study was to find the relationship of globulin levels in the late interlactation period with biochemical parameters and the level of fertilisation and culling of cows within 90 days after calving. The blood serum of cows of the main herd was investigated 10-15 days before calving, based on which the animals were conditionally divided into three groups (with signs of hypogammaglobulinemia (less than 30 g/l)), as well as with normal and elevated (more than 40 g/l) globulin levels. Apart from indicators of protein-nitrogen metabolism, enzymatic activity was also found in the blood serum and the state of carbohydrate-lipid, mineral, and vitamin metabolism was assessed using biochemical, chromatographic, and spectrophotometric research methods. It was found that cows with hypoglobulinemia showed substantially lower activity of asparagine transaminase, as well as differences in carbohydrate-lipid metabolism (high glucose levels and reduced concentration of high-density lipoproteins). Furthermore, these cows were found to have a decrease in the content of magnesium, as well as copper. It was discovered that within 90 days after calving, the highest level of culling was observed among cows with reduced globulin levels, and the lowest – in animals with normoglobulinemia. Therefore, a direct relationship between the level of globulins in the blood serum 10-15 days before calving with the reproductive capacity and the level of culling of cows was proved. This allows promptly diagnosing and predicting the development of post-partum pathology and reproductive disorders. The results obtained can be used by practical specialists and scientists to plan and investigate the effectiveness of preventive measures to improve the safety of cows and their reproductive capacity in the post-calving period

Keywords: interlactation period, post-partum pathology, globulins, biochemical parameters, mineral metabolism, reproductive ability

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INTRODUCTION

In dairy cows, transition diseases lead to considerable economic losses in the dairy industry. These losses include the cost of treatment and premature culling of cows. Early diagnosis or prediction of transition pathologies allows developing measures for their prevention, which reduces the incidence rate and reduces economic losses in the dairy industry (Venjakob *et al.*, 2021; Hailemariam *et al.*, 2014).

In the conditions of industrial cattle breeding and high milk productivity, the duration of the reproductive life of cows does not exceed 2.5-3 lactations. One of the reasons for this is a violation of reproductive function, caused by the wide spread of diseases of the reproductive system in the prenatal, birth, and post-partum periods (Yazlik *et al.*, 2020; Sewalem *et al.*, 2008). The rate of culling cows from the herd on dairy farms during the year is about 21.0-38.2% (Pinedo *et al.*, 2010; Stojic *et al.*, 2013). According to Diniso & Jaja (2021), the most common causes of this are reproductive issues, namely infertility (7.9%), reproductive disorders (89.9%) and dystocia (1%). At the same time, successful fertilisation of cows in the post-partum period is crucial for further reproductive efficiency and profitability in modern dairy cattle breeding (Sammad *et al.*, 2022).

Many post-partum diseases of cows are based on a violation of metabolic processes during the transition period, which determine the further condition of animals in the post-natal period, their reproductive ability and the level of culling of animals. The development of energy and protein deficiencies leads to a decrease in the activity of polymorphonuclear neutrophils (Yazlik *et al.*, 2020), as well as the release of inflammatory cytokines (tumour necrosis factor α and interleukin-6), which lead to systemic inflammation and insulin resistance (Wankhade *et al.*, 2017). Systemic inflammation in the early post-partum period is negatively associated with the ovulatory status of the first dominant follicle after calving (Cheong, 2017).

During the transit period, there are substantial changes in the functioning of organs and the hormonal status of the animal body (Kucuk Baykan & Özcan, 2019). Cows' appetite worsens, and the amount of feed eaten does not compensate for the rapidly increasing cost of milk production. Animals compensate for this lack by mobilising the reserves of fats, proteins, micro- and macronutrients of their body. It is no coincidence that it is during this period that dystrophy and cirrhosis of the liver, emaciation (cachexia), rumen acidosis, ketosis, inflammation of the reproductive organs and, as a result, culling and loss of animals take place (Grummer, 1995). The situation is aggravated by the development of hypocalcaemia and the intensification of protein metabolism in the post-partum period, which has adverse consequences for the health and reproductive ability of dairy cows (Sammad *et al.*, 2022).

Violations of metabolic processes occurring in the body are primarily accompanied by changes in several biochemical parameters. In this regard, the search for individual proteins, metabolites, enzymes, and hormones that would have diagnostic and prognostic significance continues. Andjelić *et al.* (2022) show that higher serum calcium concentrations after calving are associated with higher levels of progesterone, glucose, and triglycerides. In turn, subclinical hypocalcaemia leads to the development of metritis, prolongation of the service, period and a decrease in the size of the follicle (Paiano *et al.*, 2020).

Blood serum proteins are also related to the physiological condition of cows. As shown by Stravsky *et al.* (2020), the albumin-globulin ratio can be used as a prognostic factor to identify cows prone to uterine subinvolution. Globulin concentrations decrease 5 weeks before calving and increase after calving, and their change during calving is substantially related to the frequency of fertilisation (Rowlands *et al.*, 1980).

The purpose of this study was to find the level of culling and fertilisation capacity of cows within 90 days after calving, depending on the level of globulins and other biochemical parameters in the blood serum in the late interlactation period.

MATERIALS AND METHODS

Selection of animals and organisation of the experiment. The experimental part of the studies was performed on 37 dairy cows of 4-7 years of age with an average annual productivity of 7,200 kg of milk on one of the commercial farms of the Astarta-Kyiv agro-industrial holding. Biochemical studies were performed 10-15 days before calving. According to the results of the study, cows were conditionally divided into three groups. The first group (5 animals) included cows with a low globulin level of 25.6±0.8 g/l (in all animals – up to 30 g/l). The second (control) group (10 cows) was formed from animals with an average globulin content of 35.3±0.9 g/l (within 30-40 g/l), which corresponded to the level of conditionally normal indicators, and the third group included 22 cows with a globulin content of 49.9±1.5 g/l (within 40-65 g/l). Thus, according to the level of globulins, the second group was assigned to conditionally healthy animals (the first group included cows with hypogammaglobunemia, and the third group included cows with hypergammaglobunemia).

The general condition of the cows was monitored for 90 days after calving. Therewith, the number of culled cows was recorded due to obstetric pathology, mastitis, rennet displacement, and diseases of the distal extremities. Furthermore, fertilisation and infertility rates were identified in animals of each group.

Sampling. Blood for biochemical studies was taken 10-15 days before calving from the tail vein before feeding the animals. Selected blood samples were used to obtain a serum, which was frozen at -20°C and transported and stored in this state until the time of research. Biochemical studies were carried out under

the conditions of the laboratory of clinical biochemistry of the Research Centre for Biosafety and Environmental Control of Agricultural Resources of the Dnipro State Agrarian and Economic University.

Biochemical studies. The blood serum samples were examined for the content of total protein (with biuret reagent), albumin (with bromocresol green), urea (enzymatically according to the Berthelot reaction), creatinine (by the rate of formation of the creatinine-picrate complex in the Jaffe reaction), glucose (glucose oxidase method), total calcium (with arsenazo III), inorganic phosphorus (with molybdenum reagent), potassium (by turbidimetric method with sodium tetraphenylborate), iron (by reaction with ferrozine). An automatic biochemical analyser Miura-200 (Italy) was used for analysis with ready-made sets of reagents produced by Spinreakt (Spain), Dialab (Austria), Cormay (Poland), and HTI (USA). High-density lipoproteins were determined according to Warnick & Albers (1978).

Sodium levels were found by colorimetric reaction with magnesium-uranyl acetate (Human, Germany), and magnesium – by colour reaction with calmagite (HTI, USA) on a semi-automatic biochemical analyser Humalazer 3000 (Human, Germany).

The activity of transamination enzymes (ALT and AST) was found kinetically using a set of Spinreakt reagents (Spain), alkaline phosphatase – by the rate of 4-nitrophenol formation (Cormay, Poland). The carotene concentration was found spectrophotometrically (Ulab-2, China) after precipitation of serum proteins with ethyl alcohol and extraction of carotene with hexane.

The concentration of vitamins A and E was found using high-performance liquid chromatography on an Agilent Technologies 1260 Infinity liquid chromatograph, column C18 with spectrophotometric detection (Yefimov & Sofonova, 2014). Reagents manufactured by Honeywell (USA) and Carlo Erba (Italy) were used to manufacture the components of the solvent system.

The concentration of copper, zinc, and manganese in blood serum samples was found by Atomic Absorption Spectrophotometry with flame atomisation on a Selmi FCM 115 spectrophotometer (Ukraine).

The globulin content and protein coefficient were found by calculation.

Statistical analysis. In each group of cows, statistical processing of all the biochemical parameters under study was performed. The resulting digital material was processed using variational statistics methods using SPSS Data editor 17.0 version according to the Tukey Test with Bonferroni correction.

RESULTS AND DISCUSSION

When analysing the results of biochemical studies, it was found that the total protein in the peripheral blood serum of cows at the end of the interlactation period depended mainly on the concentration of globulins, since the level of albumins practically did not change.

With a low level of globulins (the first group), the level of total protein in the blood serum was also 10.5% lower compared to animals with their physiological values (the second group), and 1.4 times lower compared to animals of the third group (Table 1).

Table 1*. Biochemical parameters of blood serum of cows with different levels of globulins (M±m)*

Indicators	Group 1	Group 2	Group 3
Total protein, g/l	57.4±2.542	67.9±2.2**	82.1±2.1***
Albumins, g/l	31.8±2.3	32.6±2.1	32.3±1.0
Globulins, g/l	25.6±0.8	35.3±0.9***	49.9±1.5***
Protein coefficient, units	1.24±0.09	0.93±0.06*	0.65±0.02***
Creatinine, μmol/l	110.8±9.5	113.0±5.0	119.6±4.3
AST, IU/l	55.6±6.8	104.2±12.2**	91.2±7.8**
ALT, IU/l	19.4±1.9	17.6±1.9	19.7±1.5
Alkaline phosphatase, IU/l	96.5±8.0	109.0±12.2	101.3±6.2
Glucose, mmol/l	2.68±0.11	2.30±0.06**	2.30±0.05**
High-density lipoproteins, mg/100 ml	773.2±95.1	1,088.1±105.9*	1,073.0±76.2*

Note: ** – p≤0.05; ** – p≤0.01; ** – p≤0.001 – relative to animals of the first group*
Source: *compiled by the authors*

Accordingly, the protein coefficient was highest in cows of the first group and amounted to 1.24±0.09, in the second group it was lower by 33.3% (p≤0.02), and in the third – by 1.9 times (p≤0.001).

Apart from the above data on the indicators of total protein, globulins and albumins, certain differences in the activity of transamination enzymes were noted. The activity of AST, which is an indicator enzyme for the liver in cattle, in animals of the first group was almost 2 times lower compared to the second and third groups (p≤0.01) and by 64.0% (p≤0.01), respectively. Indicators of carbohydrate-lipid metabolism in cows of the first group were

described by a high level of glucose and, at the same time, a low content of lipoproteins. The concentration of glucose in the blood of cows of the first group was 1.17 times higher (p≤0.01), and lipoproteins – 1.4 times (P≤0.05) lower than in animals of the second and third groups. Considering the importance of indicators and the nature of mineral and vitamin metabolism in the pathogenesis of various pathologies of the transit period, the level of basic macro- and microelements, as well as vitamins A, E and carotene was investigated (Table 2).

***Table 2**. Indicators of mineral and vitamin metabolism in cows in the interlactation period with different levels of globulins (M±m)*

Indicators	Group 1	Group 2	Group 3
Total calcium, mmol/l	2.40±0.14	2.13±0.16	2.25±0.10
Inorganic phosphorus, mmol/l	1.54±0.15	1.86±0.16	2.04±0.10*
Glucose, mmol/l	0.92±0.02	2.02±0.66	1.53±0.31°
Potassium, mmol/l	5.02±0.78	4.53±0.62	4.98±0.40
Glucose, mmol/l	137.6±8.3	136.9±2.9	137.2±3.2
Creatinine, μmol/l	28.2±2.3	25.1±1.7	23.3±1.6
Copper, μg/100 ml	55.4±4.2	74.3±3.5**	82.5±3.0***
Zinc, μg/100 ml	76.0±4.5	76.5±7.3	83.8±4.7
Manganese, μg/100 ml	2.4±1.2	1.6±0.2	1.8±0.2
Vitamin A, μg/100 ml	18.3±0.6	19.1±2.6	19.3±1.0
Vitamin E, μg/ml	2.2±0.4	2.9±0.5	3.1±0.3°
Carotene, μg/100 ml	306.2±11.6	261.2±15.4*	287.3±10.9

***Note:** °p≤0.1; * – p≤0.05; ** – p≤0.01; ** – p≤0.001 – relative to animals of the first group*
***Source:** compiled by the authors*

Considering the indicators of mineral metabolism, one should pay attention to the level of calcium, phosphorus, and its ratio in the blood serum of cows with various levels of globulins. The total calcium content in animals of separate groups did not significantly differ. At the same time, animals of the second and third groups showed an increase in the level of inorganic phosphorus, which was significant in cows with a globulin content of more than 40 g/l. A violation of mineral metabolism is indicated by a tendency to reduce the level of magnesium in the blood of cows of the first group. Copper levels were 1.3 (p≤0.01) and 1.5 (p≤0.001) times lower than in animals of the second and third groups, respectively.

The content of other macro- and microelements in the blood serum of cows of all groups did not differ. The frequency of culling and the results of the diagnostic stage of obstetric and gynaecological medical examination in all groups of cows during 2-3 months of lactation are presented in Table 3.

***Table 3**. Results of obstetric and gynaecological medical examination of cows within 90 days after calving*

Indicators	First group		Second group		Third group	
	n	%	n	%	n	%
Culled	4	80.0	1	10.0	5	22.7
Pregnant	0	0	7	70.0	5	22.7
Barren	1	20.0	2	20.0	12	54.6

***Source:** compiled by the authors*

Within 2-3 months after calving, 4 cows (80%) were culled from the herd in the first group, which is significantly more than in the second group – 1 cow (10%) and third group – 5 cows (22.7%). The reasons for culling were obstetric and gynaecological pathology (development of chronic mastitis and/or displacement of the rennet), as well as diseases of the distal extremities. At the same time, the metabolic status of cows in the second and third groups was affected during the post-partum period and reproductive function during the experiment. The fertilisation rate of cows of different groups within 3 months after calving also differed. In cows of the second group, it was 70.0%, which is 47.3% higher than in the third group, and the number of infertile cows was 34.6% higher in the third group.

Studies have shown that before calving, the majority of cows (73%) had globulin levels higher or lower than animals with conditionally normal values (Brscic

et al., 2015; Alberghina *et al.*, 2011; Kahn *et al.*, 2010). It is known that among the protein globulin fractions, gamma globulins predominate, which provide the function of immune protection of animals (Elshahawy & Abdullaziz, 2017; Bertoni *et al.*, 2008). Therefore, the low concentration of these proteins in cows of the first group has a low level of their nonspecific resistance. At the same time, elevated globulin levels in cows of the third group may be due to increased protein levels during the acute phase of inflammation. As noted by Tothova *et al.* (2016), they belong to the α-fraction, and their level increases when there is a focus of inflammation or a systemic inflammatory response in the body. That is, the noted changes may indicate the activation of the immune system and the activation of inflammatory processes before calving, including in the liver. This is indicated by the research of Bertoni *et al.* (2008), who established the dependence of the level of α-globulins in the first weeks after childbirth and the frequency of violations of reproductive ability in cows.

In animals with reference globulin levels, the concentration of total protein and albumins also did not exceed normal values. Notably, the concentration of albumins did not differ between animals of different groups. According to Spaans *et al.* (2022), the globulin concentration decreases 7-14 days before calving, but the albumin content remains relatively stable.

Changes in the content of globulins in the blood serum are probably closely related to general disorders of protein metabolism. Cows with low levels of globulin protein fractions also had low levels of total protein. On the one hand, this may be caused by the high intensity of the use of the total pool of amino acids in the mother's blood for protein synthesis in the foetal body. At the same time, this may indicate the use of immunoglobulins G for colostrum formation during the transient period (Conneely *et al.*, 2013).

Ingvartsen (2006) indicates that AST levels in healthy cows range from 78 to 132 IU/l. Its increase can be observed at the end of the interlactation period, since uterine tissues have a fairly high activity of this enzyme (Sattler & Fürll, 2004). Presumably, a decrease in AST activity in cows against the background of a decrease in globulin levels may indicate a decrease in the number of functionally active hepatocytes due to the previous cytolytic syndrome and replacement of parenchymal cells with connective tissue. Thus, the hepatocyte resource of the liver is depleted with damage to hepatocytes because of autointoxication with decay products and xenobiotics (Djoković *et al.*, 2013).

Changes in carbohydrate-lipid metabolism indicate that cows of the second and third groups had a lower glucose level against the background of an increase in the concentration of high-density lipoproteins in their blood serum. Such biochemical changes are inherent in the development of a negative energy balance, which begins to develop in highly productive cows at the end of the transient period (Ha *et al.*, 2022). At the same time, blood glucose levels in cows with low globulin levels were higher than in animals of the second and third groups. The content of lipoproteins, on the contrary, was lower. Such dynamics of indicators can be explained by a lower intensity of colostrum formation and, accordingly, lower requirements for metabolic energy for its production.

Analysing the reduced copper content, Kaya *et al.* (2016) indicates that 95% of the copper in serum or plasma is part of the protein ceruloplasmin, which is synthesised in the liver. Therefore, it can be assumed that it is functional liver failure that causes a decrease in copper concentration. Nobili *et al.* (2013) noted a decrease in ceruloplasmin activity in children with non-alcoholic fatty hepatodystrophy. Such changes lead both to a decrease in neutrophil activity in cows and to a decrease in the level of antioxidant protection, which depends on the level of ceruloplasmin (Hernández *et al.*, 2022).

The obtained research results coincide with the data of Rowlands *et al.* (1980) who indicate that pre-calving globulin concentrations are related to subsequent reproductive performance of cows. Thus, at the end of the interlactation period, changes in metabolic processes and the development of immunodeficiency in cows with hypoglobulinemia are noted. Among these animals, 80% were subsequently culled within three months of calving. Apparently, cows with weakened immunity, especially after calving, are more prone to many diseases of the transient period, which is also indicated by Zhelavskyi *et al.* (2020). As a result of culling up to 28.0% in the first 100 days after calving (Kucuk Baykan & Özcan, 2019). Therewith, the reasons for culling of animals from the herd are different – from ketosis to rennet displacement, mastitis, limb pathology, which coincides with the results obtained.

CONCLUSIONS

Given that the limits of globulin levels did not overlap between the groups of animals under study, it became possible to identify pathognomic indicators of metabolic disorders and use them to diagnose and predict the complicated course of the transient period, which leads to premature culling of animals. These pathological processes are accompanied by an increased or reduced content of globulins, due to the pathological condition of the liver and/or other organs that can be detected even before calving.

In the blood serum of cows with reduced globulin levels, AST activity decreases, glucose levels are increased, as well as lower concentrations of copper and lipoproteins, which indicates damage to the liver and other organs. As a result, their culling from the herd in the first 90 days after calving reaches 80%. At the same time, an increased level of blood globulins in the pre-pregnancy period is associated with an increased level of infertility, which reaches 54.6%. This

may indicate the development of a general inflammatory syndrome due to pathological processes in the organs of the reproductive system. In the blood serum of cows with reduced globulin levels, glucose was 1.17 times higher, while lipoproteins were 1.4 times lower than in the second and third groups. Cows with hypogamaglobulinemia develop mineral metabolism disorders, specifically a decrease in the level of magnesium and copper in the blood. Therefore, the level of globulins in the blood serum of cows in the late interlactation period can be used as a prognostic indicator of the complicated course of the post-calving period. Globulin levels below 30 g/l 10-14 days before calving should be considered the greatest risk for the culling of cows. Exceeding the indicator over 40 g/l in the future significantly increases the frequency of reproductive function disorders.

Further studies may be aimed at evaluating the effectiveness of therapeutic and preventive measures in animals with detected violations of globulin levels in the late interlactation period.

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Взаємозв'язок рівня глобулінів у пізній сухостійний період з рівнем запліднення та вибуття корів протягом 90 діб після отелення

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Анотація. Захворювання транзитного періоду у корів та їх вибуття після отелення привертають значну увагу. Тому рання діагностика та прогнозування патологічних процесів у перехідний період у корів із використанням біохімічних маркерів залишається актуальною. Метою роботи було визначити взаємозв'язок рівня глобулінів у пізній сухостійний період з біохімічними показниками та рівнем запліднення та вибуття корів протягом 90 діб після отелення. Було проведено дослідження сироватки крові корів основного стада за 10-15 діб до родів, на підставі чого умовно розділено тварин на три групи (з ознаками гіпогамаглобунемії (менше 30 г/л), а також з нормальним та підвищеним (понад 40 г/л) рівнем глобулінів. Крім показників білково-азотистого метаболізму, в сироватці крові також визначали ензиматичну активність та оцінювали стан вуглеводно-ліпідного, мінерального і вітамінного обмінів з використанням біохімічних, хроматографічних та спектрофотометричних методів досліджень. Було встановлено, що у корів з проявом гіпоглобулінемії спостерігалася суттєво нижча активність аспарагінової трансамінази, а також відмінності показників вуглеводно-ліпідного обміну (вищий рівень глюкози та знижена концентрація ліпопротеїдів високої щільності). Крім того, у цих корів було виявлено зниження вмісту магнію, а також міді. Досліджено, що впродовж 90 діб після отелення спостерігався найвищий рівень вибуття серед корів зі зниженим рівнем глобулінів, а найнижчий – у тварин з нормоглобулінемією. Отже, доведено безпосередній зв'язок між рівнем глобулінів у сироватці крові за 10–15 діб до отелення з репродуктивною здатністю та рівнем вибуття корів. Це дає змогу проводити ранню діагностику та прогнозування розвитку післяродової патології та порушення репродуктивної функції. Одержані результати можуть бути використані практичними фахівцями та науковцями для планування та вивчення ефективності профілактичних заходів з підвищення збереженості корів та їх відтворювальної здатності у післяотельний період

Ключові слова: сухостійний період, післяродова патологія, глобуліни, біохімічні показники, мінеральний обмін, репродуктивна здатність