Metabolism in calves two weeks before diarrhea at the age of 19-52 days of life

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Abstract. The article presents data on the study of metabolism in calves two weeks before diarrhea at the age of 19-52 days of life. For this purpose, three groups of conditionally healthy animals of the Holstein breed were formed at the age of 19, 30, 52 days of life. To assess the metabolic status of young cattle, biochemical and general clinical blood tests were carried out. In the course of a laboratory blood test in young cattle, two weeks before disease manifestation, disorders of mineral and protein metabolism, a decrease in liver function were established. At the same time, with the increase in the age of the calves, the imbalance in protein metabolism was aggravated against the background of a change in diet and mineral metabolism improved as a result of the maturation of the digestive system.

1. Introduction
The formation of young animals in the first two months of life is a complex period rich in stressful factors, implying complex processes of adaptation to external environmental conditions, which negatively affect the level of animal’s metabolism and health in general [1, 2]. The aggravating factor is the immaturity of the immune system of the calves, the destruction of colostral immunoglobulins [3], starting from seven days of life, and the transition period in feeding at an older age.

Diseases of young cattle, occurring with diarrheal syndrome, is one of the main problems that worsen the health of the herd, which entail significant economic damage due to the complex etiology of the disease, a high percentage of morbidity and mortality in calves, as well as the significant cost of treatment measures [4, 5].

Such diseases have various negative consequences: anemic conditions, pneumonia, disorders of the cardiovascular system and metabolism [6, 7], which in turn leads to a lag in the growth and development of animals, significantly reducing the genetic potential of calves.

The imbalance of metabolic processes in calves in most cases is caused by errors in feeding and management of pregnant cows, as well as calved cows and young animals [8]. Metabolic disorders accompany the development of any disease and are not always clinically expressed, which greatly complicates the process of diagnosis and therapy. Therefore, a very informative factor in assessing the general condition of the animal, as well as the metabolic status, is a laboratory blood test. Thus, a number of authors noted that any impact, especially on a young organism, entails shifts in the quantitative and qualitative characteristics of blood [9, 10].
One of the effective methods of combating diseases is the prevention of the development of pathology [11, 12]. At the same time, the study of the level of metabolism fully contributes to the development of effective preventive measures.

Thus, one of the fundamental factors in maintaining the health of the herd is a properly organized preventive work, supported by monitoring studies of the blood of calves, focused on preventing diseases of young cattle accompanied by diarrhea.

The aim is to study the metabolism of calves two weeks before diarrhea at the age of 19-52 days of life.

2. Methods and materials

The studies were carried out on young Holstein cattle in the conditions of the Amur region. We studied the level of metabolism in calves, for this, three groups of conditionally healthy animals were formed: Group 1 (G1) - calves at the age of 19.0±0.44 days of life; Group 2 (G2) - calves at the age of 30.0±0.87 days of life; Group 3 (G3) - calves at the age of 52.0±2.41 days of life. The level of metabolic processes in calves was assessed according to the results of a biochemical blood test using a ‘StatFax 1904 + R’ biochemical photometer (Awareness Technology Inc., USA, 2012) and ‘Vital’ reagents (Manufactured by ‘Vital Development’, Russia, 2019). Determined: total protein; albumen; globulins; urea; glucose; calcium (Ca); phosphorus (P); magnesium (Mg); triglycerides; cholesterol; bilirubin; ALT (alanine aminotransferase); AST (aspartate aminotransferase). Calcium to phosphorus (Ca:P) ratios and protein index (albumin / globulins) were calculated. Studies were carried out to determine the general clinical parameters of blood: the number of erythrocytes and leukocytes, the level of hemoglobin (Hb) and color index (CI), also a differential count of leukocytes was carried out, followed by the calculation of the nuclear index of Dashtayants G D (NI = monocytes + young + stab neutrophils / segmented neutrophils) [13]. Blood sampling was carried out in the morning before feeding. For biochemical research, blood serum was used, and for clinical analysis, whole stabilized blood was used by introducing heparin into a test tube at the rate of 2-3 drops of a 1% solution per 10 ml of blood. The results obtained in the course of laboratory blood tests in the animals under study were compared with the generally accepted values, taking into account their age, indicated in the literature sources [14-15]. The observation period for the studied animals was 14 days from the moment of blood sampling.

All the obtained digital material was statistically processed by the method of variation statistics for the reliability of differences in the compared indicators relative to the previous age (group) using the Student's test, adopted in biology and zootechnics, using the Microsoft Excel 7 software package (StatSoft, USA) and is presented as the arithmetic mean (M) and its error (m).

3. Results and discussion

As a result of the study to assess the metabolic status and general condition of calves at the age of 19 days (G1), no deviations in the general condition were recorded. At the same time, from the side of the blood picture, the following were established: low values of the protein index (1.0±0.02), calcium (1.9±0.12 mmol/l), phosphorus (3.2±0.18 mmol/l), magnesium (0.7±0.05 mmol/l) and the wrong ratio of calcium to phosphorus (0.6±0.01:1), going beyond the lower limits of the norm by 9%, 27%, 29%, 46% and 65%, respectively (table 1).

In the course of hematologic study an increased number of erythrocytes (10.3±0.14 10¹²/l), low values of segmented (13.2±1.48%) and stab (1.8±0.25%) neutrophils, absence of eosinophils and high nuclear index (0.82±0.12%), as well as increased values of lymphocytes (77.4±1.59%) and monocytes (7.6±0.16%) were noted, which is very likely a response of the studied animals for planned anti-epizootic measures, namely vaccination against salmonellosis (formol - aluminuos vaccine against salmonellosis of calves, manufactured by FGUP (Federal State Unitary Enterprise) Armavir biofactory, Russia). The nuclear index (0.82±0.12) and bilirubin (13.8±4.15 μmol/l) exceeded the reference limit, this fact indicates an age-related imperfection of the bilirubin conjugation system.

On the 14th day of observation of the studied animals of the first group, clinical signs of dysfunction of the digestive system were registered, manifested by diarrhea in 40% of cases.
Table 1. Calf blood counts two weeks before diarrhea, M±m.

| Indicators                        | Group 1       | Group 2       | Group 3       |
|-----------------------------------|---------------|---------------|---------------|
| Total protein, g/l                | 58.1±4.19     | 67.6±0.63 *   | 68.5±5.17     |
| Albumin, %                        | 49.7±0.47     | 41.7±0.85 *** | 25.1±1.46 *** |
| Globulins, %                      | 50.3±0.47     | 58.3±0.86 *** | 74.9±1.46 *** |
| Protein index                     | 1.0±0.02      | 0.7±0.03 ***  | 0.3±0.02 ***  |
| Urea, mmol /l                     | 3.5±0.47      | 4.1±0.08      | 1.8±0.04 ***  |
| Glucose, mmol /l                  | 4.0±0.57      | 2.9±0.04      | 3.3±0.75      |
| Calcium, mmol /l                  | 1.9±0.12      | 2.8±0.05 ***  | 2.2±0.10 ***  |
| Phosphorus, mmol /l               | 3.2±0.18      | 3.8±0.06 *    | 2.7±0.48 *    |
| Calcium / Phosphorus              | 0.6±0.01:1   | 0.7±0.01:1 ***| 1.0±0.14:1 *  |
| Magnesium, mmol /l                | 0.7±0.05      | 0.9±0.05 *    | 1.8±0.32 *    |
| Triglycerides, mmol /l            | 0.13±0.015    | 0.30±0.030 ***| 0.20±0.044    |
| Cholesterol, mmol /l              | 1.9±0.13      | 1.2±0.05 ***  | 1.6±0.13 **   |
| Bilirubin, µmol /l                | 13.8±4.15     | 5.0±0.58 *    | 9.1±1.53 *    |
| AST, U/l                          | 72.2±4.43     | 31.5±0.47 *** | 55.9±5.25 *** |
| ALT, U/l                          | 17.6±1.33     | 12.3±0.09 *** | 12.0±1.64     |
| Erythrocytes, 10^{12} /l          | 10.3±0.14     | 8.2±0.08 ***  | 7.7±0.64      |
| Hemoglobin, g/l                   | 114.5±1.61    | 110.8±0.61 *  | 91.2±7.48 *   |
| Color index                       | 0.7±0.01      | 0.7±0.00      | 0.6±0.02 *    |
| Leucocytes, 10^9 /l               | 12.4±0.83     | 9.7±0.09 **   | 7.3±0.21 ***  |
| Basophils,%                       | 0             | 0             | 0             |
| Eosinophils,%                     | 0             | 2.0±0.29      | 0.8±0.32 *    |
| Myelocytes,%                      | 0             | 0             | 0             |
| Young,%                           | 0             | 0             | 0             |
| Stool neutrophils,%               | 1.8±0.25      | 1.8±0.25      | 1.2±0.21      |
| Segmented neutrophils,%           | 13.2±1.48     | 25.2±0.83 *** | 28.0±2.77     |
| Lymphocytes,%                     | 77.4±1.59     | 68.4±0.49 *** | 68.5±2.87     |
| Monocytes,%                       | 7.6±0.16      | 2.6±0.16 ***  | 1.5±0.22 ***  |
| Nuclear index G.D.                | 0.82±0.12     | 0.18±0.013 ***| 0.09±0.013 ***|

Note: Relative to the previous group at p<0.05 (*); p<0.01 (**); p<0.01 (**). On the 14th day of observation of the studied animals of the first group, clinical signs of dysfunction of the digestive system were registered, manifested by diarrhea in 40% of cases. Thus, despite the outwardly satisfactory general condition of 19-day-old calves at the beginning of the study, there were changes in the blood picture, indicating an imbalance of mineral metabolism, the presence of a sluggish inflammatory process in the body and insufficient resistance of the young calves, which was confirmed by a high percentage of calves morbidity on 14th day of the study. As a result of the conducted biochemical and clinical studies of the blood of young cattle of the second group at the age of 30 days, deviations from the normative values of some indicators were established, thus an increased level of total protein (67.6±0.63 g/l) by 7% was recorded against the background of low protein index (0.7±0.03), the discrepancy of which was 46%, reduced values of triglycerides (0.30±0.030 mmol/l), cholesterol (1.2±0.05 mmol/l) and AST (31.5±0.47 U/l), exceeding the norm by 33%, 8% and 30%, respectively. Also macronutrients such as phosphorus (3.8±0.06 mmol/l), magnesium (0.9±0.05 mmol/l) and the ratio of calcium to phosphorus (0.7±0.01:1), were out of physiological values and below the generally accepted values by 15%, 25% and 59%, respectively. It should be noted that the reduced levels of the protein index and AST may indicate an
insufficient protein-forming function of the liver, which is consistent with the data of Koryakina L P and Borisova N I [16]. Clinical studies of the blood of calves of the second group showed increased values of leukocytes (9.7±0.09 10^9/l), lymphocytes (68.4±0.49%), a reduced percentage of stab neutrophils (1.8±0.25%), as well as a reduced level of eosinophils (2.5±0.29%) and an increased nuclear index (0.18±0.017). An increase in NI against the background of the normative value of bilirubin and a satisfactory general condition indicates an insufficiently formed system of bilirubin binding, which is often found in young animals at the age of 30 days. It should be noted that at the time of blood sampling, there were no abnormalities in the general condition of the animals studied, and two weeks later 60% of the calves showed signs of diarrhea.

Thus, in the course of the study to assess the general condition and metabolic rate, it was found that the calves of the second group, against the background of an outwardly satisfactory general condition, had deviations from the side of protein and mineral metabolism, an insufficient level of digestion processes, protein-forming liver function, the presence of a latent inflammatory process and insufficient body resistance, which was largely confirmed by the morbidity rate of calves on the 14th day of observation.

In young cattle with an average age of 52 (G3) days on the first day of the study, low values of albumins (25.1±1.46%), protein index (0.3±0.02) and urea (1.8±0.04 mmol/l) by 34%, 77%, 50%, respectively were noted. Along with that, the indicators of mineral metabolism did not correspond to the generally accepted physiological values, thus the levels of calcium, phosphorus, Ca/P were below normal by 15%, 52% and 41%, respectively, while magnesium (1.8 ± 0.32 mmol/l) exceeded the norm by 38%. As a result of the study of the morphological composition of the blood, low values of hemoglobin (91.2±7.48 g/l) and color index (0.6±0.02) were found, which were outside the normal range by 19% and 14%, respectively; the number of leukocytes (7.3±0.21 10^9/l) by 21%; stab neutrophils (1.2±0.21%) by 40%; the percentage of eosinophils (0.8±0.32%) by 73%; monocytes (1.5±0.22%) by 25% against the background of a slightly increased percentage of lymphocytes (68.5±2.87%). There was also a slight increase in bilirubin (9.1±1.53 μmol/l), while the nuclear index (0.09±0.013) corresponded to generally accepted norms, which indicates the final stage of the formation of the bilirubin conjugation system.

It should be noted that at the beginning of the research, no deviations from the general condition were found in calves of the third group, while after two weeks the morbidity of calves with full-blown diarrheal syndrome was 50%.

Thus, the studies conducted in the third group showed that 52-days-olds were characterized by changes in some blood parameters, indicating an imbalance of protein metabolism towards oppression, insufficiency of protein and urea-forming functions of the liver, impairment of mineral metabolism [17], a reduced level of saturation of erythrocytes with hemoglobin and low resistance of the body, manifested by a high percentage of morbidity in young animals.

As a result of studying of the blood parameters of young cattle in the age aspect, a decrease in the albumin level in the order of calf growth was found, so by the age of 30 days this indicator decreased by 16% (p<0.001), then by the age of 52 days by 40% more (p<0.001). The same consistency was noted on the part of the protein index, which in calves of the second group was lower by 30% (p<0.001) relative to animals of the first group, and in calves of the third group the same indicator was lower by 57% (p<0.001) relative to the previous group (figure 1). This fact is possibly associated with a transitional period in the diet of young animals and / or with a decrease in the protein-synthesizing function of the liver [18], which is indicated by a significant decrease in transferase levels relative to 19-days-old calves: AST by 56% (second group, p<0.001) and by 23% (third group, p<0.05); ALT - by 30% (second group, p<0.05) and by 32% (third group, p< 0.05). It should be noted that the values of AST and ALT in two groups (the first and third groups) did not exceed the generally accepted regulatory limits, which indicates the absence of hepatocyte cytolysis and cholestasis (figure 2) [19].
Cholesterol and bilirubin levels were wavy. So the values of these indicators were the smallest in calves of 30 days old and were lower by 37% (p<0.001) and 64% (p<0.05) relative to the first group. In calves of 52 days old, these indicators were higher than those in calves of 30 days old by 33% (p<0.01) and by 86% (p<0.05), but there was noted an increase in these indicators by 52 days of life by 33% (p<0.01) and 82% (p<0.05) relative to 30-day-old calves. Triglycerides were higher in older calves, so their greatest value fell on the age of 30 days and their value exceeded 2.3 times (p<0.001) than of 19-day-old calves, then with age increasing this indicator decreased by 33%. Apparently, this is associated with the intensification of lipid metabolism during the growth and development of the studied animals, as well as to the possible increase in glycolysis, which is consistent with the results of the studies of Koryakina L P and Borisova N I (2016).

The level of calcium was higher in older calves, so in the second group, calcium was higher than in the first group by 47% (p<0.01). In the third group, this indicator is reliably lower by 21% (p<0.001), but remained higher than the same indicator in calves of the first group by 16%. Phosphorus is the highest in animals of 30 days of life (3.8 ± 0.06 mmol/l), which is reliably higher than the indicators of 19-day-old calves by 19% (p<0.05); in young animals at the age of 52 days of life, it was reliably lower relative to the same of the previous group by 29% (p<0.05). The calcium-phosphorus ratio increased in proportion to age, so in 30-day-old calves the ratio was reliably higher by 17% (p<0.001) relative to 19-day-old calves, then in 52-day-old animals this indicator exceeded the same in the previous group by 43% (p<0.05). The level of magnesium with the age of calves also increased, so in animals of the second
group it was higher relative to the first group by 29% \((p<0.05)\) and in the third group – 2.0 times \((p<0.05)\) relative to the second group (figure 1).

The number of erythrocytes is reliably lower in calves at the age of 30 days (by 20% at \(p<0.001\)) relative to 19-day-old calves, at the age of 52 days their number was even lower by 6% (figure 3). A significant decrease in the level of hemoglobin is proportional to the increase in age, so in 30-day-old calves hemoglobin was 3% lower, and in 52-day-old calves this indicator had lower values - by 18% \((p<0.05)\) relative to the previous age. The color indicator was at the same level in calves at the age of 19 and 30 days, and in 52-day-old calves this indicator was reliably lower by 14% \((p<0.05)\).

![Figure 3](image-url)

**Figure 3.** General clinical blood parameters of calves at the age of 19-52 days of life: (a) basic hematological indicators; (b) results of differentiated leukocyte counting.

The number of leukocytes in young cattle decreased with age by 22% (second group, \(p<0.01\)) - 25% (third group, \(p<0.001\)) relative to the previous group. The percentage of segmented neutrophils by 30
days of calf life increased by 91% (p < 0.001) and increased by 11% by 52 days of life. Lymphocytes, on the contrary, were higher in animals of the first group by 13% relative to the same indicator in the second (p < 0.001) and third groups (p < 0.05). Monocytes decreased with age by 66% (p < 0.001) by 30 days of life, and by 52 days it was even lower by 42% (p < 0.001). The nuclear index also decreased relative to the increase in age, so in the second group this indicator was lower by 78% (p < 0.001), and in the second – by 50% (p < 0.01) relative to the previous group.

Thus, on the basis of studying the blood parameters of calves in the age aspect, it was found that protein metabolism was suppressed with an increase in the age of calves against the background of a transitional period in feeding [20] and/or a decreased protein-synthesizing function of the liver without damage to the parenchyma and stagnation phenomena. Some activation of mineral metabolism with the age of the studied animals was noted, this fact indicates an improvement in the processes of calcium absorption in the small bowel, which is indirect confirmation of the maturation of the digestive system, which should reach its peak by the age of 6 months. Also, deterioration in hemoglobin and color index was recorded, which indicates the development of anemia with age of calves and may be the result of a lack of cobalt, cyanocobalamin, amino acids and other substances necessary for hematopoiesis in feed, which is very typical for the Amur region.

4. Conclusions
The results of studies to assess the general condition and metabolic rate in calves two weeks before diarrhea showed that the predisposing factors for the development of pathology are: violations of mineral metabolism (manifested by a decrease in the calcium-phosphorus ratio by 41-65% and the level of magnesium by 25-46% in calves at the age of 19, 30 days of life, as well as a very significant increase in this indicator in 52-day-old calves by 38%); decreased protein-synthesizing function of the liver (decreased protein index by 53-77% and albumin by 50% against the background of a reduced level of transferases). Considering the data obtained in the age aspect, it should be noted that with the increase in the age of the studied animals, the protein imbalance worsened against the background of the transition from the milk type of feeding to the adult diet. At the same time, in calves with protein metabolism indicators close to the normative values, the morbidity rate was lower by 10-20%, relative to those with a low level of this type of metabolism. This means that protein deficiency and inhibition of protein synthesis in the liver (hypoproteinemia against the background of a decrease in the level of aminotransferases) reduces the body's ability to withstand unfavorable factors of the external and internal environment, which is largely manifested by a high percentage of diseases of various origins in such animals. Mineral metabolism, on the contrary, tended to recover, which is associated with the improvement of absorption processes in the small bowel as a result of the maturation of the digestive system. The activation of the liver conjugating system also occurred in proportion to the increase in the age of the calves.

The results of our research can be fully used as a prognostic material, on the basis of which it seems possible to develop an effective algorithm of measures to prevent pathologies accompanied by diarrhea.

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