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Original research

Biochemical abnormality in the Brown Swiss breed cows affected with ketosis in the perinatal period

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Abstract. Ketosis is widespread disturbance among dairy cows, causing significant economic losses and requiring further study of its pathogenesis. This particularly applies to less traditional dairy breeds, including the Brown Swiss. The aim of the study was to investigate the biochemical indicators in the Brown Swiss breed cows of different age at a stage with prevalent ketosis. The research was conducted on a dairy cow herd with a ketosis level exceeding 30%. Blood samples were collected from primiparous cows and cows in their 2nd-3rd lactation 5-7 days before calving (group BC), post calving 2-3 days (group 2-3 PC), and 7-10 days (group 7-10 PC). Biochemical indicators of blood serum aimed at determining the level of lipomobilization, ketone bodies, and liver function were determined using an automatic biochemical analyzer. In the group of primiparous cows 2-3 PC, there was an increase in the content of beta-hydroxybutyrate by 1.83 times, and ketonemia was observed in 40% of cows. The postpartum period in primiparous cows was accompanied by an increase non-esterified fatty acids concentration by 1.75-2 times. The level of AST was the lowest in the serum of gestating cows several days before calving, whereas after calving, an increase was observed. It was discovered that each group had animals with AST levels above 100 U/L. There were 20 % of animals in the 5-7 pre-calving group, and 80% in the 7-10 post-calving group. The level of total bilirubin in the group of primiparous cows 2-3 PC exceeded the level BC by 3.14 times, and in the group 7-10 post-calving by 2.67 times. In the BC group, 20% of cows during their 2nd-3rd lactation were found to have ketonemia, with beta-hydroxybutyrate level 1.43 mmol/L in the serum. The highest level of ketone bodies was detected in cows from the group 2-3 PC, with 60% showing subclinical and 20% showing clinical ketosis. Similarly to primiparous cows, cows in their 2nd-3rd lactation exhibited the development of negative energy balance in the postpartum period. In the group of cows BC, AST activity exceeded 100 U/L in 20% of animals, while in the groups 2-3 PC and 7-10 PC, the number of such animals reached 60%. These changes were accompanied by hyperbilirubinemia, which was observed after calving in 80% of cows in both groups. Therefore, an increase non-esterified fatty acids levels was observed in the postpartum period in primiparous cows and cows in their 2nd-3rd lactation of the Brown Swiss breed. The results indicate the development of lipomobilization syndrome. The most pronounced signs of ketosis development were observed in primiparous cows on days 7-10 after calving, while in multiparous cows, it was detected on days 2-3. The development of negative energy balance led to signs of liver damage characteristic of lipidosis - an increase in total bilirubin levels and, to a lesser extent, AST activity. Biochemical index changes during post-calving period were observed in primiparous cow with more prominent level. However, during late dry period metabolic disorders were detected in multiparous cow in which observed ketonemia.

Keywords: calving; Brown Swiss cattle; lipomobilization; liver lipidosis; hyperbilirubinemia; NEFA; beta-hydroxybutyrate.

Біохімічні порушення у корів швіцької породи з кетозом в перинатальний період

Анотація. Кетоз є одним з найбільш поширених захворювань серед поголів'я молочних корів, що завдає значних економічних збитків та потребує подальшого вивчення патогенезу. Зокрема, це стосується менш традиційних молочних порід худоби, у тому числі, швіцької. Метою роботи було дослідити біохімічні показники у різновікових корів швіцької породи у стаді з поширеним кетозом. Дослідження проведено у стаді молочних корів із рівнем кетозу понад 30 %. Для проведення досліджень проводили відбір проб крові у первісток та корів 2-3 лактації за 5-7 діб до отелення (група BC) та через 2-3 (група 2-3 PC) 7-10 (група 7-10 PC) діб після нього. Визначення біохімічних показників сироватки крові, спрямованих на визначення рівню ліпомобілізації, кетонових тіл та функціонального стану печінки, проводили з використанням автоматичного біохімічного аналізатору. У групі первісток 2-3 PC спостерігалося наростання вмісту бета-гідроксибутирату в 1,83 рази, а кетонемія була у 40 % корів. Післяотельний період у первісток супроводжувався зростанням концентрації неестерифікованих жирних кислот в 1,75-2 рази. У сироватці крові нетелів за кілька днів до отелення рівень АСТ був найнижчим, а після отелення спостерігалося його підвищення. В кожній з груп виявлялися тварини з рівню АСТ понад 100 Од/л – в групі 5-7 BC у 20%, а в групі 7-10 PC – у 80%. Рівень загального білірубіну у групі первісток 2-3 PC перевищував рівень до

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отелення в 3,14 рази, а в групі 7-10 PC у 2,67 рази. У сироватці крові корів 2-3 лактації групи 5-7 BC виявлено 20 % тварин з кетонемією (рівень бета-гідроксибутирату 1,43 ммоль/л). Найвищий рівень кетонових тіл було встановлено у корів групи 2-3 PC, серед яких 60 % мали субклінічний, а 20 % – клінічний кетоз. Аналогічно до первісток, у корів 2-3 лактації у післяотельний період спостерігався розвиток негативного енергетичного балансу. У групі корів BC активність АСТ у 20 % тварин перевищувала значення 100 Од/л, а в групах 2-3 PC та 7-10 PC кількість таких тварин досягала 60 %. Такі зміни супроводжувалися білірубінемією. Вона виявлялася після отелення у 80 % корів обох груп. Отже, у первісток та корів 2-3 лактації швіцької породи у післяотельний період спостерігалося підвищення рівню неестерифікованих жирних кислот, що вказує на розвиток ліпомобілізаційного синдрому. Найбільш виражені ознаки розвитку кетозу спостерігалися у первісток на 7-10 добу після отелення, тоді як у корів – на 2-3 день. Розвиток негативного енергетичного балансу призводив до ознак ураження печінки, характерних для ліпідозу – підвищення рівню загального білірубіну та, менш виражено, активності АСТ. Зміни біохімічних показників в післяотельний період були вираженішими у первісток, однак, у багатоотельних корів були виявлені метаболічні розлади з проявом кетонемії наприкінці сухостійного періоду.

Ключові слова: отелення; швіцька худоба; ліпомобілізація; ліпідоз печінки; білірубінемія; НЕЖК; бета-гідроксибутират.

Introduction

Dairy cows exhibit a high frequency of various pathological conditions during the transition period (Daros et al., 2022). The transition period is the stage of a cow's life during which metabolic processes, digestive and reproductive functions undergo changes, determining the health status during calving and in the first weeks thereafter (Deniz et al., 2020). Cows experience significant energy requirements due to fetal intrauterine development and colostrum synthesis during the last 2-4 weeks of pregnancy. This is accompanied by reduced dry matter intake and leads to the development of negative energy balance (NEB) (Bezerra et al., 2014). Decreased nutrient intake and NEB stimulate fat mobilization in the form of non-esterified fatty acids (NEFA) with accumulation of beta-hydroxybutyric acid (BHB) in the blood (Pérez-Báez et al., 2019). Although these changes are adaptive processes in high-producing cows, disruption can lead to metabolic disorders, resulting in clinical and subclinical ketosis (Mekuriaw, 2023; Wankhade et al., 2017). Ketosis is a metabolic disorder in dairy cows characterized by high levels of ketone bodies (BHB, acetoacetate, and acetone) in the blood, urine, and milk at the onset of lactation (Zhang & Ametaj, 2020).

The researches have analyzed for 541 commercial dairy farms in 12 countries across South and Central America, Africa, Asia, Eastern Europe, Australia, and New Zealand where the prevalence of subclinical ketosis showed about 24.1% with ranging from 8.3% to 40.1% (Brunner et al., 2019). The group of cows with subclinical ketosis had significantly higher average lactation productivity (7076 kg compared to 6409 kg in healthy cows), and about 55.6% of affected cows experienced other postpartum period pathologies (Vince et al., 2017).

The overall economic losses from ketosis in dairy cattle are significant. However, systematic analysis of publications has shown large variations in their assessment across different farms depending on the country, herd productivity, milk prices, cow and feed costs, and veterinary services. As a result, the reported costs per case range from 19 to 812 euros (Cainzos et al., 2022). According to other data, in the USA, the total cost of one case of ketosis per year varies from 129 to 289 US dollars (Liang et al., 2017), while in Europe, it ranges from 130 to 257 euros (Lei & Simões, 2021).

Health problems that arise during the transition period in dairy cattle are extremely important as they can contribute to the development of various metabolic diseases with significant disruptions in reproductive function (Kraevskiy et al., 2022; Youssef & El-Ashker, 2017). Alterations in colostrum composition due to metabolic disorders lead to decreased colostrum quality and decreased viability of newborn calves (Masiuk et al., 2019; Kaskous & Fadlelmoula, 2015). Subclinical ketosis is considered a triggering mechanism for metabolic disorders such as metritis, mastitis, clinical ketosis, and displaced abomasum. Specifically, cows with subclinical ketosis had 1.5, 9.5, and 5.0 times higher odds of developing metritis, clinical ketosis, and displaced abomasum, respectively, with the likelihood of lameness being 1.8 times greater (Suthat et al., 2013).

Some researchers indicate that during the prepartum period, there are various changes in blood parameters, particularly, AST activity and NEFA levels, which can be used as risk indicators for predicting the occurrence of subclinical ketosis in cows after calving (Wang et al., 2021). Biochemical markers one week after calving had high prognostic value for the development of displaced abomasum (with BHB concentration above 1000 µmol/L, the odds were 13.6 times higher) and for the development of clinical ketosis (with serum NEFA above 1.0 mmol/L the odds were 6.3 times higher, and 4.7 times higher with BHB concentration above 1200 µmol/L) (Seifi et al., 2011).

However, the majority of publications concerning the biochemical changes during ketosis development in herds was predominantly focused on studies conducted on the Holstein breed (Ha et al., 2022; Marutsova, 2023). Data regarding the metabolic processes in herds with a high prevalence of ketosis among the Brown Swiss cattle require further investigation.

The aim of the study was to estimate the changes in biochemical parameters in heifers and cows of the Brown Swiss breed with prevalent ketosis.

Materials and methods

Study design. The research was conducted in a Brown Swiss dairy herd with cows weighing 570-620 kg. At the time of the study, the daily yield for first-lactation cows was 25.6 kg, and for cows in the main herd, it was 37.6 kg during the first month of lactation. The feeding regimes for animals in the dry and postpartum periods, calculated on a dry matter (DM) basis, are provided in Table 1.

Additionally, animals in both groups had free access to a salt lick. The concentrate feeds for animals in both groups included grain crops, high-protein ingredients, as well as mineral-vitamin premixes and balancing additives (Table 2).

High level of cow culling from the herd was observed in farming cows at 22 days after calving where primiparous cows accounting for 32%. Ketonemia was detected in more than 30% of the cow herd in seventh day after calving while the detection ketonemia performed with using Blood Ketone Meter.

Serum blood biochemistry. Blood samples were collected from heifers and cows 5-7 days before calving (group BC), 2-3 days post-calving (group 2-3 PC) and 7-10 days post-calving (group 7-10 PC) in order to establish the mechanisms of metabolic disorders. There were 5 cows in each group. After obtaining serum, biochemical analysis was performed using the Miura-200 automatic biochemical analyzer (Italy) with ready-made reagent kits from High Technology (USA), PZ Cormay (Poland), Spinreact, BioSystems (Spain), and Dialab (Austria).

Table 1 – Feeding ration content for dry and newly calved cows

The ration ingredient, kg	Dry cows and heifers (21 days before calving)		Newly calved cows (up to 30 days of lactation)	
	kg	kg DM	kg	kg DM
Barley straw	1.0	0.92	5.7	5.24
Alfalfa silage	5.1	1.23	9.58	2.30
Corn silage	10.15	3.45	16.74	5.52
Molasses	0.1	0.07	0.64	0.46
Concentrate feed for dry cows	2.93	2.64	-	-
Concentrate feed for newly calved cows	-	-	8.28	7.45
Meadow hay	0.56	0.50	-	-
Water	6.5	-	5.0	-
Planned daily consumption of feed mixture	29.86	12.37	40.01	16.15

The following parameters were determined in the blood serum: total protein (using the biuret method), albumin (with bromocresol green), globulins, and A/G ratio (calculated), urea (enzymatic by the Berthelot reaction), creatinine (kinetically using the Jaffe reaction), glucose (using the glucose oxidase method), total bilirubin (via reaction with 3,5-dichlorophenyl diazonium), total calcium (by reaction with arsenazo III), inorganic phosphorus (with ammonium molybdate), and magnesium (with calmagite). Among the serum enzymes, the activity of AST and ALT was determined (kinetically based on the Warburg optical test), alkaline phosphatase (kinetically via formation of 4-nitrophenol), and GGT (kinetically with L-γ-glutamyl-3-carboxy-4-nitroanilide).

The level of lipomobilization was determined by the concentration of NEFA using the Trinder reaction with acyl-CoA synthetase and acyl-CoA oxidase. The intensity of ketogenesis was assessed by the level of beta-hydroxybutyrate (enzymatically with hydroxybutyrate dehydrogenase).

The obtained data were statistically processed using the specialized software Statistica 6 (StatSoft Inc, USA). The reliability of differences between cows post-calving and animals pre-calving was assessed using the Student's t-test. The homogeneity of data within groups was evaluated using the coefficient of variation (Cv) and the range of values (lim). Data presentation includes the following notations: M - mean; m - standard error of the mean (SEM). The changes were considered statistically significant at * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the group BC.

Table 2 – Composition of concentrate feeds for dry and freshly calved cows (g per kg)

Feed ingredient	Dry cows and heifers	Newly calved cows
Wheat	174	149
Cehavit ProFeed	-	123
Chalk	-	17
Table salt	-	12
Corn	-	273
Cowfit premix for lactating cows	-	23
Insorb mycotoxin binder	1	1
Cehave Mineral-Buffer Complex	-	24
Dried corn distillers grains, CP not less than 35%	-	120
Cehavit Dry Supreme premix	56	-
Soybean meal, CP not less than 43%	769	240
Protected fat PALMAC TM 80-16	-	18

Results

The content of ketone bodies was assessed to explain the presence ketosis and its rate in the herd. A low level of ketone bodies was observed in the serum of heifers 5-7 days before calving (Table 3).

Within 2-3 days after calving, a significant increase in ketone body content was observed with levels rising by 1.83 times ($p<0.01$). It is important to note that this indicator exhibited relatively high variability (Cv 28.55%). This variability was even more pronounced in the group 7-10 PC (Cv 95.98%). In primiparous cows during this post-calving period, the fluctuation range varied from 0.30 to 3.41 mmol/L, with 40% of animals exceeding the level of 1.0-1.2 mmol/L.

In groups 2-3 PC and 7-10 PC, there was an increase in the concentration of NEFA in the serum of primiparous cows compared to the pre-calving period, by 1.75 and 2 times respectively ($p<0.01$).

There was a decrease in the concentration of albumins by 16.8% ($p<0.05$) in the group of primiparous cows 2-3 PC. However, a slight recovery of this indicator was observed on days 7-10 of lactation. Among the indicators of nitrogen metabolism, a decrease in the content of creatinine after calving was found, which was 22.5% ($p<0.05$) lower in the group 7-10 PC.

The AST level was the lowest in the serum of heifers in the group BC, but it increased after calving. At the same time, there were no statistical changes of the parameter, which can be explained by its significant oscillation (Cv 25.16-43.98%). Animals in each group exhibited AST activity levels of 100 U/L or higher, with one observed before calving, three in the group 2-3 PC, and four in the group 7-10 PC.

The level of total bilirubin in primiparous cows during the perinatal period showed significant changes. Specifically, in the group 2-3 PC, it exceeded the level of the group BC by 3.14 times ($p<0.001$), and it was greater by 2.67 times ($p<0.001$) in the group 7-10 PC. At the same time, the activity of GGT had significantly smaller changes in the postpartum period, exceeding the values of heifers by 19.1% ($p<0.1$) and 41.2% ($p<0.05$) on days 2-3 and 7-10, respectively.

Table 3 – Biochemical parameters of serum blood primiparous cows in perinatal period

Parameter	Animal groups					
	BC		2-3 PC		7-10 PC	
	M ± m	lim	M ± m	lim	M ± m	lim
Total protein, g/L	51.2 ± 2.48	45-60	47.0 ± 1.79	42-52	52.0 ± 3.07	47-64
Albumin, g/L	32.2 ± 0.86	30-35	26.80 ± 1.23*	21-28	28.8 ± 1.39	24-32
Globulins, g/L	19.0 ± 1.90	13-25	20.2 ± 0.66	18-22	23.2 ± 2.87	17-34
A/G ratio	1.76 ± 0.19	1.4-2.5	1.36 ± 0.11	1.0-1.6	1.30 ± 0.18	0.9-1.9
Urea, mmol/L	5.12 ± 0.65	3.3-6.6	5.82 ± 0.58	4.0-6.8	5.06 ± 0.54	3.6-6.9
Creatinine, μmol/l	106.8 ± 1.91	101-113	94.4 ± 6.61	76-108	82.8 ± 4.93**	64-92
AST, U/L	91.4 ± 17.98	66-162	117.6 ± 20.04	82-188	120.4 ± 13.54	88-165
ALT, U/L	11.4 ± 1.21	8-14	9.4 ± 1.91	7-17	13.6 ± 1.75	8-19
Alkaline phosphatase, U/L	110.3 ± 10.6	81.7-143.8	68.0 ± 5.0**	54.4-85.2	75.6 ± 9.5*	57.5-112.0
Glucose, mmol/L	2.22 ± 0.07	2.0-2.4	2.22 ± 0.06	2.0-2.3	2.14 ± 0.05	2.0-2.3
Total calcium, mmol/L	1.92 ± 0.04	1.8-2.0	1.88 ± 0.06	1.8-2.1	1.88 ± 0.06	1.8-2.1
Phosphorus inorganic, mmol/L	1.62 ± 0.06	1.5-1.8	1.42 ± 0.09	1.1-1.6	1.52 ± 0.08	1.3-1.7
Magnesium, mmol/L	1.12 ± 0.04	1.0-1.2	1.06 ± 0.02	1.0-1.1	1.08 ± 0.04	1.0-1.2
Total bilirubin, μmol/l	4.06 ± 0.92	1.7-6.1	12.76 ± 1.39***	10.5-18.2	10.82 ± 0.94***	8.6-13.2
GGT, U/L	13.60 ± 0.98	13-17	16.20 ± 0.58	15-18	19.20 ± 0.93*	14-24
BHB, mmol/L	0.47 ± 0.03	0.43-0.59	0.86 ± 0.11**	0.48-1.15	1.30 ± 0.56	0.30-3.41
NEFA, mmol/L	0.16 ± 0.02	0.13-0.22	0.28 ± 0.02**	0.24-0.36	0.32 ± 0.03**	0.25-0.43

Note: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared to the group BC

The levels of BHB and NEFA also exhibited the highest Cv values in the serum of the cows in their second and third lactations. Specifically, in the group of cows BC, the Cv of BHB was 67.25%, primarily due to an increase in the indicator in one of the animals to 1.43 mmol/L (see table 4).

The highest level of ketone bodies was observed in the cows of the group 2-3 PC. 60% of the animals had BHB levels above 1.2 mmol/L, whereas such animals were not found in the group 7-10 PC. In 40% of cows in this group, the beta-hydroxybutyrate content exceeded 1.0 mmol/L. The maximum value of the indicator in the group 2-3 PC was 3.51 mmol/L. The average value for the group exceeded by 2.8 times ($p<0.05$) and 2.9 times ($p<0.1$) in cows of groups BC and 7-10 PC, respectively. Similarly to primiparous cows, an increase in NEFA levels was observed in cows at the time of the 2nd-3rd lactation during the postpartum period, which was significantly higher by 1.67 times ($p<0.05$) compared to dry cows by 7-10 days post-calving.

The level of creatinine in the serum of cows during the postpartum period gradually decreased. The lowest values were observed in the group of cows 7-10 PC, which was 25.9% lower ($p<0.05$) than in the group BC. The concentration of urea had the highest values in the group of cows 2-3 PC, increasing by 31.3% ($p<0.05$). At the same time, this parameter was 7 mmol/L or higher in 40% of the animals.

The increase in the dynamics of changes in the concentration of total bilirubin during the postpartum period was observed. However, no significant changes were established due to considerable Cv values (35.4%-50.7%). It should be noted that the level of bilirubin exceeded 10 μmol/L in 20% of the animals in the BC group. However, the percentage of such animals in both groups reached 80% after calving. At the same time, the activity of GGT did not show a similar pattern, and its average values did not differ significantly across the studied groups.

The highest levels of AST activity were found in cows from the 2-3 PC group. However, the wide variable range in AST activity for all groups did not allow us to confirm the significant changes in this index. It is also worth noting that AST levels exceeded 100 U/L in 20% of the animals in the BC group, while the percentage of such animals reached 60% in the 2-3 PC and 7-10 PC groups. However, 20% of the animals exhibited an increase in enzyme activity levels above 200 U/L in the 7-10 PC group.

The level of inorganic phosphorus decreased by 21.7% ($p<0.05$) at the first 2-3 days after calving. However, inorganic phosphorus level recovering was observed during 7-10 days of lactation. No changes in the concentration of total calcium were detected.

Discussion

Ketosis is a common disease in dairy herds worldwide (Brunner et al., 2019). Exceeding the concentration of beta-hydroxybutyrate in serum above 1.0-1.2-1.4 mmol/L has been proposed as a criterion for diagnosing subclinical ketosis (Satoła & Bauer, 2021; Krempaský et al., 2014). Some publications suggest that due to the negative impact on the health and productivity of cows, the threshold value should be considered as 0.9 mmol/L (Jansen et al., 2021).

BHB levels in the blood of heifers ranged from 0.43 to 0.59 mmol/L 5-7 days before calving. According to Wang et al. (2021), exceeding the level of 0.43 mmol/L for BHB and 0.27 mmol/L for NEFA before calving indicates a high risk of developing subclinical ketosis in the postpartum period. In our study, 20% of heifers had BHB levels above 1.0 mmol/L within 2-3 days after calving, and this number increased to 40% within 7-10 days. 20% of cows had butyrate levels exceeding 1.0 mmol/L before the 2nd-3rd calving, which indicated subclinical ketosis. Clinical ketosis is characterized by BHB levels above 3.0 mmol/L (Vallejo-Timarán et al., 2020). Therefore, 20% of cows in the 2-3 PC group and 20% of heifers in the 7-10 PC group had BHB levels typical of acute ketosis.

Table 4 – Biochemical parameters of serum blood cows in their 2nd-3rd lactation

Parameter	Animal groups					
	BC		2-3 PC		7-10 PC	
	M ± m	lim	M ± m	lim	M ± m	lim
Total protein, g/L	57.2 ± 3.60	45-65	53.4 ± 2.38	47-60	56.6 ± 2.23	49-60
Albumin, g/L	33.4 ± 1.03	31-37	32.0 ± 2.24	25-37	31.6 ± 0.81	29-34
Globulins, g/L	23.8 ± 3.15	13-32	21.4 ± 0.60	20-23	25.0 ± 2.28	18-31
A/G ratio	1.52 ± 0.26	1.0-2.5	1.50 ± 0.11	1.1-1.8	1.30 ± 0.14	0.9-1.7
Urea, mmol/L	4.54 ± 0.16	4.0-4.9	5.96 ± 0.59*	4.3-7.3	5.48 ± 0.59	3.7-7.4
Creatinine, µmol/l	97.4 ± 8.43	85-129	85.8 ± 12.62	61-129	72.2 ± 5.89*	53-77
AST, U/L	93.0 ± 24.32	62-189	118.2 ± 19.73	77-191	102.4 ± 7.43	79-123
ALT, U/L	13.4 ± 2.82	7-22	8.8 ± 2.82	2-16	17.8 ± 6.05	2-36
Alkaline phosphatase, U/L	63.34 ± 12.37	46.7-112.5	61.02 ± 3.56	52.6-72.0	92.24 ± 35.56	41.9-232.2
Glucose, mmol/L	2.16 ± 0.07	2.0-2.4	2.08 ± 0.04	2.0-2.2	2.06 ± 0.02	2.0-2.1
Total calcium, mmol/L	1.96 ± 0.09	1.8-2.3	1.94 ± 0.05	1.8-2.1	2.04 ± 0.05	1.9-2.2
Phosphorus inorganic, mmol/L	1.38 ± 0.04	1.3-1.6	1.08 ± 0.12*	0.8-1.5	1.30 ± 0.10	1.0-1.5
Magnesium, mmol/L	0.98 ± 0.04	0.9-1.1	1.14 ± 0.07	0.9-1.2	1.04 ± 0.05	1.0-1.2
Total bilirubin, µmol/l	8.04 ± 1.82	4.2-14.8	13.48 ± 2.77	8.1-23.8	12.24 ± 1.94	7.5-18.4
GGT, U/L	17.4 ± 1.69	13-23	15.2 ± 1.83	10-21	19.2 ± 0.8	18-22
BHB, mmol/L	0.69 ± 0.21	0.29-1.43	1.93 ± 0.48*	0.87-3.51	0.66 ± 0.16	0.30-1.03
NEFA, mmol/L	0.27 ± 0.04	0.22-0.42	0.36 ± 0.04	0.23-0.45	0.45 ± 0.02*	0.40-0.53

Note: * $p < 0.05$ compared to the group BC

While most researchers focus on determining BHB levels in the postpartum period, studying biochemical changes during the prepartum period allows to understand better the pathogenesis of ketosis (Zhang & Ametaj, 2020). It has been shown that the supply of nutrients to the fetus and mammary gland tissues has a high degree of metabolic priority during late pregnancy (Hayirli, 2006). Perhaps, this leads to the development of a lipomobilization syndrome in certain cows during the prepartum period before the 2nd-3rd lactation according to our study. A threshold for lipomobilization was taken as NEFA concentration of 0.3 mmol/L (Ospina et al., 2010). Accordingly, in heifers, its manifestation began to be registered on days 2-3 after calving (in 20% of animals), intensifying by days 7-10 (in 40% of heifers). Conversely, in cows, the increase in NEFA content was observed in 20% of animals in the 5-7 PC group and reached 100% in the 7-10 PC group. Thus, a negative energy balance developed, creating conditions for the development of hepatic lipidosis and ketosis (Andjelić et al., 2022; Batista et al., 2020).

The level of AST activity is directly correlated with the content of triglycerides in liver cells (Batista et al., 2022; Mohsin et al., 2022). AST activity may indicate hepatic lipidosis according to some researches. For moderate hepatic lipidosis, enzyme activity slightly exceeds 100 U/L, and for severe lipidosis, it exceeds 125 U/L according to Elshafey et al. (2023). Similar data are provided by other researchers (Mostafavi et al., 2013). Reference values for AST activity are up to 100 U/L (Meyer & Harvey, 2004), although most researchers cite much lower values in clinically healthy cows (Stojević et al., 2005; İssi et al., 2016). We observed increased enzyme activity above 100 U/L in 20% of the heifers before calving, and subsequently, the number of such animals increased to 80% in the 7-10 PC group of primiparous cows. Similar dynamics of AST were observed in cows as 20% of animals before calving had elevated AST activity, and subsequently, the number of such animals increased to 60%. These changes may indicate the development of hepatic lipidosis due to lipomobilization and ketosis. To some extent, this is confirmed by the increase in urea content in cows, which is observed with lipid deposition in the liver (Singh et al., 2020).

AST is an enzyme of the acute phase of pathology, mainly found in mitochondria, and is released into the blood during liver damage (Stojević et al., 2005). However, we noted high intra-group variability in this indicator. This may somewhat reduce its diagnostic value, considering the relatively short half-life of AST in the blood (Russell et al., 2007). Similar views are also held by some authors (Ahmadi et al., 2016).

The level of albumin remained relatively stable, slightly decreasing in the first days after calving. It is more likely that inflammatory processes influenced the concentration of albumin rather than liver dysfunction (Batista et al., 2022). Calving is accompanied by the development of oxidative stress (Yehia et al., 2020), which leads to increased cell apoptosis (Baydas et al., 2007), and disruption of the functional capabilities of various organs.

The presence of bilirubinemia can be considered indicative and likely associated with the development of hepatic lipidosis (Vlizlo et al., 2021; Bombik et al., 2020). Particularly pronounced changes were observed in primiparous cows during the postpartum period. However, no significant increase in bilirubin concentration was observed in cows after calving. The reference values for bilirubin levels are up to 9 µmol/L (McSherry et al., 1984; Cozzi et al., 2011). 20% of the indicators exceeded these values in the group of cows during the prepartum period, and the number increased to 80% by 2-3 days after calving. These changes are likely a result of impaired liver function, which is most pronounced in the postpartum period in primiparous and multiparous cows.

Thus, the identified biochemical changes indicate the development of signs of liver lipidosis and subclinical and clinical ketosis in cows in the early postpartum period. The main reasons for this could be the abrupt transition from a dry cow ration to a lactating cow ration. It is known that the diet usually changes in the pre-fresh transition period and then after calving. This high-energy diet with low fiber content provides adaptation of rumen microorganisms to the new feed (Grummer et al., 2004). However, there may be other factors, such as reduced dry matter intake due to heat stress, as the studies were conducted in the hot summer period (Sammad et al., 2020). The obtained data expand the understanding of the pathogenesis of ketosis, particularly in cows of the Brown Swiss breed, and require further study in the context of the effects of technological, climatic, and other factors.

Conclusion

An increase in the concentration of BHB was observed in the Brown Swiss primiparous cows during the postpartum period, indicating the development of lipomobilization syndrome. The most pronounced signs of ketosis were observed on days 7-10 after calving. The proportion of animals with subclinical ketosis was 20% on days 2-3 and 7-10 of the postpartum period, while the group 7-10 PC had 20% of animals with clinical ketosis. Negative energy balance led to signs of liver impairment such as an increase in the level of total bilirubin and, to a lesser extent, AST activity, which is typical of lipidosis.

Ketonemia was detected in the serum of 20% of cows before the 2nd-3rd lactation 5-7 days pre-calving, indicating the possibility of ketosis development in the prepartum period. The maximum number of cows showing signs of subclinical (60%) and clinical (20%) ketosis was observed on days 2-3 after calving. General changes in biochemical parameters in multiparous cows were similar to those in primiparous cows, but the dynamics during the transition period were less pronounced. This is attributed to the development of metabolic disorders at the end of the dry period, lipomobilization syndrome and pathological changes in the liver even before calving.

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