Biochemical blood parameters and level of endogenous intoxication in cows suffering from hepatopathies under heat stress

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SUMMARY

Global warming results in increased extreme weather events, including heatwaves, droughts and floods, which exceed plants’ and animals’ tolerance thresholds, thus posing a threat to the economy and agriculture. Under these conditions, heat stress becomes a vital problem for animal husbandry. The paper presents the study results of biochemical parameters and endogenous intoxication in cows suffering from hepatopathies under heat stress. Based on the calculated temperature-humidity index, it was established that during the summer season in the conditions of the Krasnodar Region lowlands, cows are under heat stress. Using the method of paired comparisons, two groups of animals (n = 10) were formed: the first group was a healthy livestock; and the second group consisted of animals suffering from hepatic pathologies. Blood was sampled from all cows at the beginning of the experiment (the first decade of May) and at the end (the last decade of July). Laboratory tests of blood revealed that as the heat stress develops healthy cows show the increase in the protein concentration in blood, and, on the contrary, animals with hepatic pathologies demonstrate the inhibition of protein synthesis. The higher activity of aminotransferases and alkaline phosphatase in the bovine serum in the summer season when compared to the spring season was established. The study of the endogenous intoxication level dynamics in cattle during the development of heat stress, showed that in both groups the concentrations of medium mass molecules (MMM) increased relative to the background data: in the first group (healthy cows) MMM 237 – by 11.8%, MMM 254 – by 14.4%, MMM 280 – by 16.9%; in the second group (cattle with liver pathology) MMM 237 – by 16.9%, MMM 254 – by 20.3%, MMM 280 – by 33%. Thus, under heat stress, the endogenous intoxication in healthy livestock was almost 1.5 times less intense as compared to the animals suffering from hepatopathies.

Keywords: cattle, liver, hepatopathies, biochemical parameters, endogenous intoxication, heat stress

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Состояние биохимического профиля крови и уровня эндоогенной интоксикации у коров с гепатопатиями в условиях теплового стресса

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Интроductory

Human-induced climate change is causing dangerous and widespread disruption in nature and affects the lives of billions of people around the world. According to the data presented in the report of the Intergovernmental Panel on Climate Change, the last seven years have been the warmest on the planet, and each year has been warmer than the previous one on record. Scientists say that in the near future this trend will continue, and by 2030 the Earth's temperature will rise by 1.5 degrees. In addition to warming, all natural systems become imbalanced, leading to a change in the precipitation regime, to temperature anomalies and an increase in the frequency of extreme events such as hurricanes, floods and droughts [1-4].

Climate warming poses a threat to the economy and agriculture, as increased heatwaves, droughts and floods are already exceeding plants' and animals' tolerance thresholds. Under these conditions, heat stress becomes a vital problem for high-yielding dairy production.

Heat stress is the result of an imbalance between metabolic heat production inside the animal body and its dissipation to the surroundings. High air temperature combined with increased or, conversely, very low humidity can lead to the heat stress development. The thermoneutral zone of modern cattle breeds ranges from 4 to 20 °C, and of high-yielding breeds – from 9 to 16 °C. The dairy breeds frequently kept in the Russian commercial farms, such as the Holstein, are most adapted to cold weather conditions, but are sensitive to heat. Heat stress in lactating cows reduces the animals' performance due to a decline in milk yields and milk quality, it affects the health status of the animal and shortens the herd life expectancy, which ultimately leads to serious economic losses in livestock production industry [5-11].

In the conditions of the intensive dairy farming, the desire to maximize the performance of cows without appropriate consideration of their physiological needs leads to metabolic reorientation, functional overload of organs and body systems, and primarily of the liver. According to veterinary reports, in recent years, digestive diseases have been on the top positions among the animal mortality reasons and reach 40% in some farms [12-14]. Since the liver plays a significant role in the chemical thermoregulation of the body, the heat stress, especially in animals with hepatoopathies, can cause serious overall metabolic disorders often leading to death. Milk productivity in these cows is usually not restored to the initial level, such animals are not fertilized, and they are culled [15, 16].

The state of animals under the influence of stress factors is conditioned by the severity of disorders in various organs and body systems, as well as by the degree of hypoxia and endotoxins. Endogenous intoxication syndrome is one of the most common in clinical practice and is observed during a variety of pathologies.

Endogenous intoxication is a process caused by the accumulation of endotoxins in abnormally high
concentrations in tissues and biological fluids (endotoxins are the products of natural metabolism (inflammatory mediators, exo- and endotoxins, products of cellular and protein degradation, etc.), which exceed the natural capacity of the neutralization systems and ultimately damage organs and body systems. In this context, the problem of endogenous intoxication syndrome remains one of the most urgent in medicine, which is associated with the important role of endotoxizziness as a link in pathogenesis and a factor determining the severity of the course and outcome of various diseases. One of the main factors of the endotoxizziness progress is the inability of detoxification systems and organs to cope with toxins, due to adaptive mechanism disruption and morphofunctional disorders of the detoxification organs, and primarily of the liver [17–23].

In this regard, research needed to establish the changes in biochemical parameters and endogenous intoxication level in cows with hepatopathies under heat stress is interesting, and the results will allow to develop effective strategies for pharmacological correction for animals exposed to high environmental temperatures.

The aim of the work is to establish changes in the blood biochemical parameters and the level of endogenous intoxication in cows with hepatopathies under heat stress.

MATERIALS AND METHODS

The studies were performed in a Holstein cattle-rearing farm located in the Korenovsky District of the Krasnodar Region.

The period to study biochemical parameters and the level of endogenous intoxication in the body of cows under thermal stress was determined by a retrospective analysis based on the calculated indicators of the temperature-humidity index (THI) in 2018–2020, taking into account the average daily temperature and environment humidity. The index was calculated using the formula: THI = 0.8 × T + (H/100 × (T − 14.4)) + 46.4, where T is the environmental temperature (°C); H – relative humidity (%).

The interpretation of the results was based on the parameters when the THI less than 68 indicates that the cattle are in the comfort zone; from 72 to 79 means the animals are in a moderate heat stress; over 100 – death is possible.

To conduct studies, two groups of cows (10 animals in each group) were formed in May on the principle of the paired comparisons method. The first group involved healthy cattle, the second group consisted of animals with liver pathologies. Animals were selected for the experiment, rated according to their physiological state (2–3 months of lactation), the results of clinical examination, biochemical blood profile, as well as results of the laboratory blood tests.

RESULTS AND DISCUSSION

The period to study biochemical parameters and the level of endogenous intoxication in the body of cows under thermal stress was determined by a retrospective analysis based on the calculated indicators of the temperature-humidity index (THI) in 2018–2020, taking into account the average daily temperature and environment humidity. The index was calculated using the formula: THI = 0.8 × T + (H/100 × (T − 14.4)) + 46.4, where T is the environmental temperature (°C); H – relative humidity (%).

The interpretation of the results was based on the parameters when the THI less than 68 indicates that the cattle are in the comfort zone; from 72 to 79 means the animals experience moderate heat stress; from 80 to 89 means a severe heat stress; more than 90 means an extremely severe heat stress; over 100 – death is possible.

To conduct studies, two groups of cows (10 animals in each group) were formed in May on the principle of the paired comparisons method. The first group involved healthy cattle, the second group consisted of animals with liver pathologies. Animals were selected for the experiment, rated according to their physiological state (2–3 months of lactation), the results of clinical examination, biochemical blood profile, as well as results of the liver ultrasonography.

Animals were handled in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes ETS No. 123 (Strasbourg, March 18, 1986).

To measure the severity of heat stress in cows, the THI for 5 months was calculated (from April to August 2021).

Blood was sampled from all cows at the beginning (the first decade of May) and at the end (the last decade of July) of the experiment.

The clinical examination was carried out according to the generally accepted scheme, paying special attention to the color of the mucous membranes, the coat condition, the number of ruminal contractions, and liver palpation and percussion to establish the area of hepatic dullness, surface and sensitivity.

Ultrasonography was performed using a PS-380V 5.0 mHz veterinary ultrasound scanner (Russia). The echogenicity, structure and sound conductivity of the liver parenchyma were evaluated.

Laboratory blood tests were performed using an automated biochemical analyzer Vitalab Selectra Junior (Vital Scientific B.V., the Netherlands) and reagents from ELITech Clinical Systems (France) and Analyticon biotechnologies AG (Germany). The thymol turbidity test was performed using reagents from CJSC ECOlab (Russia).

To determine the level of endogenous intoxication, integral biological tests are used, among which an important place is given to the determination of medium mass molecules (MMM) in biological fluids, characterized by one common property – a molecular weight of 300 to 5,000 Da. MMM content in serum was determined using the screening method of N. I. Gabrielyan and V. I. Lipatova [24] at three wavelengths: λ = 237 nm (MMM 237), λ = 254 nm (MMM 254) and λ = 280 nm (MMM 280). To measure the optical density in the ultraviolet region of the spectrum, an Ecoview UV-1100 spectrophotometer (Shanghai Mapada Instruments Co., Ltd, China) was used.

The obtained digital data were processed by variational methods, where significance using Student’s t-test and the significance the differences between the groups were determined.
During the study, biochemical tests were performed and cows demonstrating abnormal biochemical blood profile results were selected into the group of animals with liver pathology (Table 3). Thus, the selected animals had a low level of total protein (76.50 ± 2.48 g/L) and urea (2.98 ± 0.07 mmol/L), which indicates a decrease in the hepatic protein synthesis. A positive thymol turbidity test (with a degree of turbidity from + to the maximum value ++++) made it possible to diagnose the inflammatory process in the liver, including in the hepatic parenchyma. The cytolytic syndrome, manifested by the destruction of hepatocyte membranes and the release into the bloodstream of transamination enzymes – transaminases (alanine aminotransferase – ALAT and aspartate aminotransferase – ASAT), was confirmed by the fact that moderate hyperenzymemia was detected in sick cows, the difference in comparison with the healthy cow values was 27.2% for ALAT and 31.3% for ASAT. The activity of alkaline phosphatase (ALP) exceeded the normal parameters and exceeded the enzyme level in healthy cows by 20.2%, which may suggest cholestasis. Hypoglycemia was found in sick animals with a difference of 16.9% in glucose concentration if compared with healthy cows.

The level of endogenous intoxication in cows with liver pathology was higher relative to healthy animals; the difference in MMM 237 was 20%, MMM 254 – 25.1% and MMM 280 – 21%.

The development of heat stress in cows changed the biochemical blood parameters. Thus, in healthy livestock, the amount of total protein in serum increased by 6%. Most likely, the increase in the protein concentration in the blood is the result of the general dehydration of the cows’ body under hyperthermia conditions, which leads to blood thickening and increase in total protein in it. It is also possible that in the process of adaptation of animals to high environmental temperatures, an increase in the total protein level in the blood is mediated by corticosterone, which regulates the physico-chemical mechanisms of maintaining blood volume during dehydration due to osmotic pressure. In case of the body overheating, there is probably a disruption in the ureagenesis in the liver, since in the group of healthy cows, despite the increase in total protein, the content of urea in the serum decreased by 26.2% ($p \leq 0.01$) as compared to the baseline values. In animals with hepatopathology under heat stress, further
Table 3
Dynamics of biochemical parameters and endogenous intoxication markers in bovine blood under heat stress (M ± m, n = 10)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 healthy animals</th>
<th>Group 2 animals with hepatic pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>start of the study</td>
<td>end of the study</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>82.60 ± 3.12</td>
<td>87.90 ± 2.26</td>
</tr>
<tr>
<td>Urea, mmol/L</td>
<td>5.11 ± 0.15</td>
<td>4.05 ± 0.09**</td>
</tr>
<tr>
<td>Thymol turbidity test, CU</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ALAT, Unit/L</td>
<td>29.80 ± 2.13</td>
<td>34.90 ± 0.96*</td>
</tr>
<tr>
<td>ASAT, Unit/L</td>
<td>90.40 ± 5.46</td>
<td>93.70 ± 4.51</td>
</tr>
<tr>
<td>ALP, Unit/L</td>
<td>129.10 ± 3.15</td>
<td>141.80 ± 3.16*</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>2.56 ± 0.12</td>
<td>2.47 ± 0.14</td>
</tr>
<tr>
<td>MMM 237, CU</td>
<td>0.638 ± 0.033</td>
<td>0.723 ± 0.021*</td>
</tr>
<tr>
<td>MMM 254, CU</td>
<td>0.195 ± 0.020</td>
<td>0.229 ± 0.013*</td>
</tr>
<tr>
<td>MMM 280, CU</td>
<td>0.181 ± 0.012</td>
<td>0.218 ± 0.029</td>
</tr>
</tbody>
</table>

Differences are significant: *p ≤ 0.05, **p ≤ 0.01 as compared to the background data.

inhibition of protein synthesis was observed with a significant (p ≤ 0.05) decrease in total protein by 7.8% and urea by 12.9% in the serum.

Higher aminotransferase activity was established in the serum of cows in the summer season as compared to the spring season. Moreover, the ALAT activity in healthy animals tended to upper reference values and amounted to (34.90 ± 0.96) Unit/L, which is 14.6% higher than the initial data (at p ≤ 0.05). In cows of Group 2, this value was higher – (40.80 ± 1.53) Unit/L (the difference with the start of the experiment was 7.1%). Increased ALAT activity in the serum is considered as an indicator of the hepatocyte destruction and, possibly, in cows with liver dystrophy, a significant proportion of hepatocytes had already been destroyed, which caused a less pronounced increase in the enzyme in these animals. ASAT concentration in healthy cows practically did not change, and in sick animals increased by 10.5%.

The increase in the ALP activity in all experimental animals was consistent and amounted to 8.9% in Group 1 and 11.5% in Group 2. The increase in the ALP activity can be caused not only by the influence of heat stress, but also by increased activity of both placenta-like (a marker of the fetoplacental system normal functioning) and bone-specific (fetal bone matrix maturation and mineralization) alkaline phosphatase. Since all cows selected for the experiment had increased pregnancy periods, hyperenzymemia in this case was of a physiological and adaptive nature due to changes occurring in the body of a pregnant animal.

In all animals the glucose concentration in the serum tended to decrease with the most pronounced changes revealed in Group 2 (difference with the baseline data is 6.8%). The possible pathophysiological mechanism of these changes is conditioned by the inverse relationship between the level of cortisol and glucose, which leads to depletion of glucose and glycogen stores in the liver, as well as a change in the insulin apparatus functions when exposed to stress factors.

The study of the dynamics of the endogenous intoxication level in cattle during the development of heat stress, showed that in both groups the concentrations of middle molecules increased relative to the baseline data: in Group 1 (healthy cattle) MMM 237 – by 11.8%, MMM 254 – by 14.4%, MMM 280 – by 16.9%; in Group 2 (cattle with liver pathology) MMM 237 – by 16.9%, MMM 254 – by 20.3%, MMM 280 – by 33.0%. In general, the obtained results confirm the data that long-term exposure to factors which compromise the homeostasis brings the organism to a lower reactivity. Under stress conditions, the organism is challenged by the task to maintain normal homeostasis and optimize it, but with prolonged exposure excessive waste products of normal or impaired metabolism accumulate in the tissues and biological fluids of the body, which cause the development of endogenous intoxication and increased MMM levels in the blood.

CONCLUSION

Thus, the data obtained indicate that in the summer season, in the conditions of the Krasnodar Region lowlands, cattle are constantly in a state of heat stress. With the development of heat stress in healthy cows, the protein concentration in blood increases, which is facilitated by the general dehydration of the animal organism by hyperthermia, leading to blood thickening and an increase in the total protein in it. Cows with hepatopathy under heat stress, on the contrary, demonstrated the inhibition of protein metabolism, which suggest an insufficient protein-synthesis liver function. The higher activity of aminotransferases and alkaline phosphatase in the bovine serum in the summer season when compared to the spring season was established. Changes in the dynamics
of values characterizing the development of endogenous intoxication syndrome under heat stress in dairy cattle were revealed. The intensity of the increase in the concentration of medium mass molecules in the serum of healthy livestock was almost 1.5 times lower as compared to the animals with hepatopathy. Perhaps this is explained by the fact that cows with a diseased liver suffer from insufficient adaptive and compensation body responses and with prolonged stress, there is a significant increase in integral endotoxocosis markers, which serve as an additional cause of various body system malfunctioning. The results obtained can serve as a justification for the development of an effective strategy for pharmacological correction of heat stress in cattle.

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