



## Impact of Heat Stress on Productivity and Health in Holstein Dairy Cows

Toktar Bexeitov <sup>1</sup>, Erzhan Akhazhanov <sup>1,\*</sup>, Kairulla Akhazhanov <sup>1</sup>, Nursultan Kainidenov <sup>1</sup>, Gulnara Jaxybayeva <sup>1</sup>, Maxim Syrovatskiy <sup>2</sup> and Sergei Beketov <sup>3</sup>

<sup>1</sup>Research Institute of Agroinnovation and Biotechnology NAO Toraigyrov University, Pavlodar, 140000, Kazakhstan

<sup>2</sup>K.I. Skryabin Moscow State Academy of Veterinary Medicine and Biotechnology - MVA, Moscow, 109472, Russia

<sup>3</sup>Federal State Budgetary Institution of Science Institute of General Genetics named after N.I. Vavilov, Russian Academy of Sciences, Moscow, 119991, Russia

\*Corresponding author: [erakhazhanov@mymail.academy](mailto:erakhazhanov@mymail.academy)

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### ABSTRACT

Heat stress, one of the prominent problems in dairy farming, poses a significant threat to dairy production, particularly for high-yielding breeds like Holstein in continental climates such as Kazakhstan. This study comprehensively assessed the impact of heat stress on 90 lactating Holstein cows in Kazakhstan, stratified by stress resistance (high, medium, low) based on baseline blood cortisol levels. Behavioral, physiological, and productivity parameters were also evaluated during a thermoneutral period (May, 16-21°C average daytime temperature, and 65% humidity) and a heat stress period (June, 22-35°C average daytime temperature, and 60% humidity). Under heat stress, all groups exhibited reduced feed intake (ranging from 2.1 to 3.4kg/day,  $P<0.01$ ) and milk yield (ranging from 2.18 to 2.26L,  $P<0.01$ ). Cows with high stress resistance were the most sensitive physiologically, showing significant decreases in blood calcium (2.16 vs. 2.10mmol/L,  $P<0.01$ ), phosphorus (2.34 vs. 2.04mmol/L,  $P<0.01$ ), and mean corpuscular hemoglobin (14.73 vs. 14.27pg,  $P<0.05$ ). Despite a greater physiological response, the high-resistance group maintained the highest absolute milk production (27.13L vs. 24.87L and 23.13L in the medium and low groups, respectively). The results indicate that blood cortisol is an effective biomarker for stratification and that selecting for high stress-resistant cows, despite their acute physiological sensitivity, is a promising strategy to mitigate production losses in dairy herds exposed to heat stress.

**Keywords:** Holstein cows, Heat stress, Cortisol, Stress tolerance, Biomarkers, Milk productivity.

### INTRODUCTION

Currently, one of the problems of dairy cattle breeding is heat stress occurring in summer (Dobson et al. 2001; Buryakov et al. 2016). At the same time, the rapid onset of global warming significantly aggravates and exacerbates this problem (Zhao et al. 2019). This is clearly seen in Kazakhstan, where from 1941 to 2012, for each ten-year period, the average annual air temperature increased by 0.38°C in the West Kazakhstan Oblast, by 0.23-0.25°C in the South Kazakhstan, East Kazakhstan, Almaty and Mangistau Oblasts, and by 0.27-0.31°C in other regions (Zhylgeldiyeva et al. 2025). At the same time, absolute maximum air temperatures can reach 42°C in the northern regions, 48°C in the central regions, 46°C in the west, 45°C in the east and up to 51°C in the south of the country (Salnikov et al. 2014). Globally the heat stress problem has posed a threat to food security and economic balance. A study by Thornton et al. (2022) on the global loss estimate

of dairy production due to heat stress displayed that heat stress is projected to cost \$39.94 billion (95% CI 34.39-45.49 billion) per year by the end of the century for a high greenhouse-gas emission scenario (SSP 5-8.5), accounting for 9.8% of the value of cattle meat and milk output in 2005. Under a low emission scenario (SSP 1-2.6), annual production losses were \$14.89 billion (12.62-16.95 billion), or 3.7 percent of the 2005 value. In all scenarios, losses in most tropical regions were expected to be far greater than in temperate regions. They further suggested the adoption of heat tolerant cow species.

Prolonged exposure of animals to high temperatures causes deterioration of their condition and reduction of general resistance, cows become more susceptible to mastitis, metritis and other infectious diseases. Under the influence of stress, cattle undergo a number of physiological changes i.e., slowing down or strengthening of intestinal peristalsis, partial or complete loss of appetite, tachycardia, arrhythmia, muscle tension, muscle tremors,

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increased body temperature, dilated pupils, rapid breathing and heartbeat, urination and defecation (Giannone et al. 2023; Țogoe and Mincă 2024).

Increased ambient temperature, along with humidity and daylight hours, introduces an imbalance in the functional activity of the hypothalamic-pituitary-gonadal axis, which affects the development of follicles, corpus luteum, oocytes and embryos and, as a consequence, contributes to reduced fertility and reduced hunting behaviour (Garcia-Ispuerto et al. 2006; Heikkilä et al. 2012; De Rensisa et al. 2017; Grelet et al. 2022). Oliveira et al. (2025) also reported in their study that despite the relative reduction in milk production and reproductive capacity, there has been a decrease in the heat stress threshold of Holstein dairy cows' due to genetic improvement. They further highlighted the application of physiological indicators such as respiratory rate as a more efficient indicator since milk production reduction was not instantaneous.

Gupta et al. (2022) also reported prenatal heat stress had profound and lasting consequences on an offspring's immunity. Heat stress exposure in late gestation increased the incidence of diseases like pneumonia and diarrhea in calves. An impaired transfer of immunoglobulin (IgG) from the dam's colostrum led to reduced passive immunity.

Heat-stressed calves' exhibit altered immune profiles, including higher platelets and acute phase proteins but lower lymphocytes and reduced expression of critical immune markers like TNF- $\alpha$ . Similar immune suppression is observed in other species like humans and pigs. Postnatally, heat stress also directly challenges the immune systems of pre-weaned calves and growing heifers, reducing IgG concentrations and altering immune cell function. In lactating and dry cows, heat stress increases somatic cell counts and the incidence of diseases like mastitis, metritis, and retained placenta. It also directly suppresses immune cell function, such as reducing lymphocyte proliferation and neutrophil activity. In the rumen of cows subjected to heat stress, a decrease in acetate concentration and an increase in lactic acid levels are observed. This causes a decrease in pH and available energy, leads to damage and increased permeability of rumen walls, impaired pre-gastric functions and creates conditions for the development of subacute rumen acidosis (SARA) (Khafipour et al. 2009; Kleen et al. 2013).

Against the background of metabolic transformations, the profile of the rumen microbiome changes, which is characterized by an increase in the number of *Streptococcus*, *Enterobacteriaceae*, *Ruminobacter*, *Treponema*, *Bacteroidaceae* and heat-resistant cellulolytic bacteria with a simultaneous decrease in the number of actinic and acetobacteria (Kim et al. 2022).

Under the influence of high temperatures in ruminants, feed intake decreases. This leads to a decrease in metabolic heat production, disturbance of energy balance and reduction of milk productivity of dairy cows (Mukhanina et al. 2024; Besteiro et al. 2025; Rauw et al. 2025). Laporta et al. (2024) highlighted on their study that heat stress to pregnant dairy cows can cause dramatic changes in the resulting offspring. Recent molecular studies showed that in a close study of intergenerational effects of heat stress, the performance of animals in F1 and F3 generations were affected by their ancestor's exposure of heat stress. This phenomenon may be as a result of long term exposure and

has raised numerous concerns for maternal heat stress.

It is imperative to understand that highly productive dairy breeds are especially sensitive to heat stress (Worku et al. 2023). This is important because Kazakhstan imports a lot of Holstein cows; as of the beginning of 2024, 30% of all the dairy cattle in the Republic are Holsteins and that number is expected to rise (Mussayeva et al. 2022). Based on these evidences, the importance of researching the mechanism of heat stress, causative factors and ways of preventing and controlling cannot be over emphasized.

However, despite the importance of the problem, studies on the effect of heat stress on Holstein cows in Kazakhstan have not been conducted so far (Dairy News Today 2023) and this is despite the fact that in conditions of sharply continental climate prevailing in the territory of the republic, under the influence of high temperature and intense solar radiation in imported highly productive cows, negative consequences of heat stress are most often noted. In this regard, the aim of our work was to study the effect of heat stress on behavioral changes, indicators of general and biochemical blood analysis, chemical composition of feces, and milk productivity of Holstein cows kept on one of the dairy farms in the Republic of Kazakhstan.

## MATERIALS AND METHODS

The work was carried out in NAO "Toraigyrov University" on the basis of breeding farm "Pobeda" LLP (Republic of Kazakhstan, Pavlodar region, Shcherbakti district, Orlovka village).

Studies were conducted on Holstein breed of cattle imported from Germany. Full-aged dairy cows of 3 lactations (90 animals in total) were selected for the experiment. Distribution of animals by types of stress resistance was carried out according to the "Method of determining the boundaries of types of stress resistance of dairy cows" (2024). The level of cortisol hormone in the blood was the evaluation criterion when cows were divided into groups. In total three groups were formed. The evaluation criterion was the level of cortisol hormone in blood:  $M < 3m$  (36goals) - high degree of stress resistance,  $M \pm 3m$  (27goals) - medium degree of stress resistance,  $M > 3m$  (27goals) - low degree of stress resistance (Where  $M$ =mean value and  $m$ =Standard error of mean value).

All experimental cows were fed according to the method of total mixed ration (TMR - total mixed ration). In the first phase of the experiment (May), animals of all groups were kept on the farm for 20 days at a moderate daytime temperature of 16-21°C and an average humidity of 65% (Normal conditions). In the following month (June), 20 days at 22-35°C and average humidity 60% (change in THI from 69 to 87).

Both before and during stress exposure, daily assessment of the feed table (visual analysis and accounting of feed residues), cud level, the proportion of lying animals and milk productivity of experimental animals were carried out. Physico-chemical analysis of milk quality was carried out twice a week based on the results of morning and evening milkings. At the end of each stage of the experiment, blood samples were taken from the tail vein of the cows to control clinical and biochemical hematological parameters.

The content of cortisol hormone in blood was determined by enzyme immunoassay on microplates using

the test kit 'K210 Cortisol- ELISA' (Russia) (Novgorodova 2024). The clinical and biochemical blood tests (WBC, LYM, MID, GRA, LYM, MID, GRA, RBC, HGB, MCHC, MCH, MCV, RDW, HCT, PLT, MPV, PDW, PCT, P-LCR, total protein, ALP, ALT, AST, creatinine, BUN, calcium, magnesium, phosphorus) were carried out on an automatic hematological analyzer High Technology MICROSS-20Vet (USA). Physico-chemical composition of milk was determined using the instrumentation of the laboratory of the Research Institute of Agroinnovation and Biotechnology of NAO "Toraigrov University". In particular, the fat content (%) in milk was determined by Gerber method (volumetric method); SOMO (%) - by calculation method; milk density ( $^{\circ}$ A) on lactodensimeter; protein content (%) - by Kjeldahl method (Beketov et al. 2022). Daily milk productivity was estimated based on the results of morning and evening milkings. The chemical composition of dairy cow feces (moisture, crude protein, starch, ADF, aNDF, crude ash, calcium, phosphorus, magnesium, potassium) were analyzed in an independent feed laboratory KZ NOVA LAB associated with Cumberland Valley Analytical Services (USA).

#### Data Analysis

Sample data were analyzed with preliminary determination of Normality of distribution by the Kolmogorov-Smirnov and Shapiro-Wilk criteria. In case of failure to fulfil this provision, the nonparametric Mann-Whitney U-test (M-W U-test) was used, otherwise - Student's t-statistic (Triolo et al. 2018). The standard error was determined as an indicator of variability of the mean value of the trait. Grouping of primary data and biometric calculations were performed using Microsoft Excel and STATISTICA programs.

## RESULTS AND DISCUSSION

According to the results of the conducted experiment, it can be seen that the effect of heat stress influenced the feed intake of the tested animals. At the same time, cows of all experimental groups showed a decrease in feed intake. Thus, under heat stress, cows with a high degree of stress resistance showed a decrease in feed intake of approximately 2.2kg/day, those with medium resistance decreased by 2.1kg/day and those with low resistance by 3.4kg/day (Table 1). This result doesn't align with the studies of Chen et al. (2023) who concluded that heat stress does not affect fat concentrations or feed efficiency. This contradiction may be as a result of differences that exist between various cow species. On the other hand, our study aligns with Correa-Calderón et al. (2022) who highlighted that subsequent modification of eating and water in-take

was observed in Holstein cows during heat stress.

Already starting from the moderate temperature-humidity index THI 69 and as it approached THI 72, cows started to show behavioral signs of heat stress: animals started to stand longer than usual, trying to cool down with the cooler airflow inside the room. Accordingly, under the influence of heat stress, the number of lying cows in the groups with high, medium and low stress tolerance decreased by 5.48%, 22.1% and 18.63%, and the level of ruminating decreased relative to the Normal by 4.9%, 2.1% and 17.6%, respectively. This aligns with the study of Hut et al. (2022) who observed similar behavioral patterns and even reduced rumination at THI 56 and above. Although this study was carried out in a temperate region, it leads to the hypothesis that Holstein cows which are identified a highly sensitive dairy cows are victims of environmental change as a result of import. To counteract this, crossbreeding with local breeds may produce offspring that retain the dairy quality of Holstein cows and the endurance of local breeds. Future research should also focus on exploring wider THI ranges to properly ascertain the lowest limit of heat stress and make calculated decisions. At the same time, statistically significant increase in rectal temperature was characteristic only for animals with medium and low stress tolerance, which was 0.6% in both groups (Table 1).

The decrease of water in feces detected in animals of all groups indicates stagnation in the digestive tract (Table 2). This is also indicated by an increase in the amount of undigested neutral-detergent fiber in the feces. This is due to the fact that heat stress reduces not only the intensity of mastication, but also the activity of the rumen and the mobility of reticulo-rubic tissue, collectively affecting the speed of fractional passage of digested product in the gastrointestinal tract (Shebeko et al. 2025). This aligns with Antanaitis et al. (2022) who concluded that heat stressed cows in addition to decreased chewing also experienced lower rumen pH and acetate concentration, increased respiration, greater ruminal temperature and reduced digestion.

Against this background, in animals with a high degree of stress resistance, a decrease in the level of starch in fecal masses was observed compared to the pre-stress condition. The content of some biogenic macronutrients was also heterogeneous. The cows in all groups showed a decrease in the concentration of phosphorus in feces, while, on the other hand, the amount of magnesium in feces increased in animals with medium and low resistance to heat stress, as well as potassium in cows with high stress tolerance (Table 2). Indirectly, these results may indicate an increase in phosphorus assimilation and excretion of magnesium and potassium.

**Table 1:** Feed intake, cud level, motor activity and rectal body temperature in Holstein cows under heat stress

Indicators	Unit meas.	Degree of stress resistance (M+m)					
		high		average		low	
		Normal n=36	Stress n=36	Normal n=27	Stress n=27	Normal n=27	Stress n=27
Feed consumption	kg/day	48.7±0.76	46.5±0.93**	45.8±0.91	43.7±0.89**	45.4±0.88	42.0±0.76**
Chewing gum level	%	63.20	58.30	66.10	58.70	64.30	46.70
Lying cows	%	38.15	32.67	48.10	26.00	38.30	19.67
Body temperature	$^{\circ}$ C	38.9±0.08	39.1±0.11	39.0±0.07	39.6±0.08***	39.1±0.08	39.7±0.11***

Values are expressed as mean±SE. Asterisks indicate statistically significant differences between Normal and heat stress conditions within each group: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001

**Table 2:** Chemical composition of feces in Holstein cows under heat stress (%)

Fecal indicators	Degree of stress resistance (mean±SE)					
	High		average		low	
	Normal n=36	Stress n=36	Normal n=27	Stress n=27	Normal n=27	Stress n=27
Moisture	84.30±0.75	83.63±0.33	84.23±0.87	84.30±1.17	86.53±0.15	83.13±0.89***
Crude protein	18.17±0.44	17.43±0.54	18.03±0.50	17.77±0.08	18.60±0.63	17.80±0.10
Starch	0.27±0.02	0.19±0.01***	0.29±0.01	0.27±0.04	0.44±0.09	0.34±0.03
ADF	33.50±0.75	34.83±0.55	33.60±0.68	33.93±0.40	32.10±0.19	32.63±1.99
aNDF	50.93±1.57	53.40±1.21	51.37±1.23	52.30±0.24	49.50±0.37	51.43±0.43**
Raw ash	9.38±0.36	9.74±0.83	9.86±0.76	10.16±0.65	9.74±0.44	9.85±0.72
Ca	1.79±0.14	1.88±0.10	1.74±0.13	1.85±0.13	1.77±0.17	1.81±0.04
P	0.93±0.03	0.81±0.04*	0.90±0.03	0.78±0.02**	0.82±0.02	0.76±0.02*
Mg	0.89±0.03	0.97±0.08	0.88±0.02	1.05±0.08*	0.85±0.03	0.97±0.03**
K	0.65±0.01	0.73±0.03*	0.69±0.02	0.72±0.01	0.69±0.02	0.73±0.01

Values are expressed as mean±SE. Asterisks indicate statistically significant differences between Normal and heat stress conditions within each group: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001

**Table 3:** Hematological parameters in Holstein cows under heat stress

Indicators	Unit meas.	Degree of stress resistance (M+m)					
		High		average		low	
		Normal n=36	Stress n=36	Normal n=27	Stress n=27	Normal n=27	Stress n=27
WBC	10 <sup>9</sup> /L	9.95±0.545	9.35±0.442	9.85±1.044	9.69±0.414	9.51±0.614	9.97±0.628
LYM	10 <sup>9</sup> /L	3.96±0.488	3.32±0.448	3.56±0.829	3.08±0.831	3.52±0.495	3.40±0.572
MID	10 <sup>9</sup> /L	0.44±0.022	0.50±0.028	0.43±0.047	0.49±0.057	0.46±0.027	0.49±0.043
GRA	10 <sup>9</sup> /L	5.54±0.200	5.61±0.201	5.86±0.290	6.12±0.382	5.55±0.257	6.08±0.274
LYM	%	37.09±2.261	32.37±2.595	34.96±3.439	28.05±2.359	35.25±2.131	30.98±2.88
MID	%	4.52±0.126	5.34±0.145***	4.38±0.137	5.03±0.168**	4.64±0.153	4.85±0.192
GRA	%	58.55±2.283	62.57±2.505	63.07±2.245	65.77±2.757	60.11±2.115	64.17±2.956
RBC	10 <sup>12</sup> /L	5.96±0.079	6.02±0.091	6.00±0.107	6.07±0.170	5.88±0.109	6.04±0.138
HGB	h/g	88.31±1.017	85.94±1.098	87.73±1.364	84.04±2.202	87.96±1.662	85.12±1.857
MCHC	h/g	338.03±2.545	334.54±2.971*	337.92±3.00	332.26±5.670*	338.26±4.269	333.73±4.508
MCH	pg	14.73±0.143	14.27±0.149*	14.59±0.184	13.81±0.270*	14.71±0.233	14.09±0.231
MCV	fL	47.17±0.530	46.83±0.505	46.55±0.673	45.67±0.687	47.67±0.811	46.55±0.832
RDW CV	%	14.66±0.114	14.69±0.103	14.50±0.089	14.66±0.081	14.50±0.124	14.71±0.113
RDW SD	fL	31.78±0.309	31.66±0.317	31.13±0.421	30.87±0.411	31.71±0.410	31.39±0.475
HCT	%	28.04±0.333	28.10±0.342	27.83±0.426	27.59±0.706	27.90±0.495	27.98±0.612
PLT	10 <sup>9</sup> /L	503.31±14.303	516.26±16.882	491.27±25.261	537±43.394	534.04±26.216	494.92±26.496
MPV	fL	6.77±0.060	6.94±0.043*	6.81±0.067	6.94±0.050	6.90±0.061	7.08±0.072
PDW	fL	12.05±0.298	13.06±0.269*	12.98±0.470	13.97±0.461	12.03±0.266	14.05±0.528***
PCT	%	0.34±0.010	0.360±0.011	0.33±0.016	0.37±0.029	0.37±0.019	0.349±0.017
P-LCR	%	5.80±0.424	5.40±0.263	5.56±0.303	5.81±0.312	6.15±0.537	6.50±0.510

Values are expressed as mean±SE. Asterisks indicate statistically significant differences between Normal and heat stress conditions within each group: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001

As for hematological indicators, it is known that the total number of erythrocytes and hemoglobin level in blood increases under stress (Bekseitov et al. 2025). (Table 3). However, in our case, the change in these parameters was random in nature. Nevertheless, a statistically significant decrease in the mean hemoglobin content in erythrocyte (MCH) and mean hemoglobin concentration in erythrocyte mass (MCHC) in animals with high and medium stress tolerance was observed under heat stress, which can be explained by a probable decrease in iron content in the body of cows exposed to heat stress. It is noteworthy that in the same two groups an increase in the relative total level of monocytes, eosinophils and basophils was observed, on average by 0.82% and 0.65%. This reaction is usually associated with an immune response to an inflammatory state (Table 3). Zeng et al. (2023) concluded that heat stress effect in dairy cows hematological parameters are not randomized but poorly understood and requires more research. From their study, they highlighted that heat stress caused a reduction in oxygen concentration by reducing hemoglobin concentration and red blood cell count, thus

instigating oxidative stress and hypoxia.

In the group of highly stress-resistant animals, an increase in the mean platelet volume (MPV) was observed. This usually occurs with their enhanced production and increased size, which increases their aggregation activity (Bodrova et al. 2023). The increase in platelet cell width by PDW volume (%) observed in cows with high and low stress tolerance indicates the variability in their size and may be indicative of thrombopoiesis disorders (Table 3).

Any stress, representing a set of complex physiological reactions, affects endocrine processes in the animal organism. One of the biomarkers of stress is an increase in the level of cortisol (Caroprese et al. 2010; Iqbal et al. 2023; Grelet et al. 2025), the increased concentrations of which are associated with the emergence of anxiety (Mösl and Palme 2002), however, no significant intragroup differences in this hormone were found (Table 4). The decrease in cortisol concentration is consistent across various studies but is also specific to dairy cow species. This reinforces the role of cortisol in maintaining stability and adaptive responses. Future research is required to

understand the genetic mechanism associated with different reactions to heat stress on cortisol levels in different dairy species as highlighted in the study by Mylostyvyi et al. (2024).

Under conditions of metabolic disorders caused by stress, one of the fast reacting links of biochemical homeostasis is enzyme activity (Yarovan and Novikova 2012), which reflects the slightest changes in animal metabolism and helps to detect pathological processes before the deviation of other biochemical indicators. In particular, the enzymes AST and ALT play an important role in amino acid metabolism. It is indicative that the significant decrease in ALT activity observed in the experiment in cows with different degrees of stress resistance is also characterized by a significant decrease in the amount of total protein in their blood (Table 4). The results align with the study of Kim et al. (2022), who concluded that heat stress directly affected protein metabolism and growth. They also observed a decrease in blood glucose, non-esterified fatty acids, and blood amino acids profiles and increased blood urea nitrogen and HSP70 gene expression during heat stress. In addition, Kim et al. (2022) suggested dietary protein supplementation as a way to counteract this decrease in protein level.

The enzyme creatinine is involved in energy metabolism of muscle and other tissues of the organism and is a fairly constant value. However, under the action of heat stress, a significant increase in its activity was observed in all experimental groups of cows; this can be observed under significant physical load or due to disturbance of energy balance in the animals' organism (Table 4). Another enzyme under consideration, alkaline phenylphosphatase, is a phosphatase with a physiological role of dephosphorylating compounds, which detaches phosphate from many types of molecules and is an indicator of phosphorus-calcium metabolism of bone tissue. However, in our case, changes in the activity of this enzyme in all groups of animals considered were not significant (Table 4).

It is known that during stress, mineral substances are actively consumed. Nevertheless, blood urea nitrogen (BUN), which is an important marker used to assess the general metabolic state, did not change compared to the measurement parameters before the experiment. A very different picture is observed for magnesium, a significant decrease in blood magnesium levels was found in animals of all groups. Lactating cows are very sensitive to changes in the balance of this element, as their organism does not have easily accessible magnesium reserves, which is

further aggravated by the impact of stress factor. Nevertheless, in the group of highly stress-resistant cows, a statistically significant decrease in the concentration of calcium and phosphorus in blood was a marker of heat stress (Table 4).

One major drawback is that the study was limited to a single region with a distinctly continental climate, which might limit the results' applicability to dairy systems in regions with milder or more humid climates. The study's concentration on a single hormonal indicator (cortisol) may also have overlooked the complexity of the stress response, even though cortisol was found to be a useful biomarker for stratification. To develop a more complete phenotyping model, future research could include a wider panel of biomarkers, such as those linked to inflammation (such as acute phase proteins) and oxidative stress. Understanding the long-term physiological and productive effects, such as effects on fertility, longevity, and intergenerational effects, as suggested by other literature, is further limited by the relatively brief duration of the heat stress exposure period.

Yet, despite the large number of available physiological biomarkers of stress, the simplest and most effective way to identify the stress state in cattle is still considered to be a decrease in milk yield (Grelet et al. 2022), which was also confirmed in our experiment. Along with the decrease in productivity, in all groups of animals with different stress tolerance, a decrease in such physical and chemical parameters as fat, protein, SOMO and milk density was observed. And the greatest decrease in fat was observed in the group with medium stress resistance, in protein and SOMO in the group of low stress-resistant cows and in milk density in the highly resistant group.

Undoubtedly, the reduction of protein and fat in milk affects the cost of production, but to a much greater extent, the economic losses revealed under heat stress are caused by the reduction of milk productivity of dairy cows (Table 5). At similar decrease of average milk yields under heat stress: 2.18L - highly resistant cows, 2.24L - medium resistant cows and 2.26L - low resistant cows, the highest milk yields are characterized, first of all, by highly stress-resistant animals (Table 5). Hence, identification and selection of resistant cows is an important strategy to minimize the effect of heat stress on dairy cattle performance (Rong et al. 2019; Sigdel et al. 2019). Accordingly, the numerical predominance of highly stress-tolerant animals in the herd structure creates prerequisites for reducing economic losses of the dairy herd in the summer period.

**Table 4:** Biochemical parameters of blood of Holstein cows under heat stress

Indicators	Unit meas.	Degree of stress resistance (mean±SE)					
		High		average		low	
		Normal n=36	Stress n=36	Normal n=27	Stress n=27	Normal n=27	Stress n=27
Cortisol	nmol/L	36.45±1.683	36.66±1.713	65.51±1.132	66.90±1.177	106.37±4.504	110.75±4.504
Total Protein	h/g	91.00±2.654	73.00±1.149***	94.11±1.438	73.68±1.316***	92.83±1.266	70.33±0.894***
ALP	ME/L	68.46±5.480	59.36±2.983	64.93±9.060	50.85±3.190	48.35±8.922	60.93±4.297
ALT	ME/L	160.09±7.376	37.95±1.414***	151.42±18.207	34.67±1.339***	131.74±8.128	33.08±0.929***
AST	ME/L	167.76±12.177	148.06±9.804	153.73±12.254	142.40±14.247	114.37±4.000	129.97±7.805
Creatinine	µmol/L	79.58±1.659	92.69±4.097***	79.67±0.940	94.76±5.379***	78.52±1.334	103.54±6.314***
BUN	mmol/L	4.98±0.140	4.96±0.256	4.99±0.161	4.84±0.294	4.30±0.128	3.78±0.299
Calcium	mmol/L	2.16±0.025	2.10±0.015**	2.06±0.020	2.10±0.030	2.11±0.034	2.08±0.023
Magnesium	mmol/L	1.16±0.068	0.77±0.073***	1.25±0.086	0.67±0.044***	1.48±0.053	0.65±0.110***
Phosphorus	mmol/L	2.34±0.080	2.04±0.046**	2.16±0.092	2.02±0.033	2.22±0.114	2.04±0.048

Values are expressed as mean±SE. Asterisks indicate statistically significant differences between Normal and heat stress conditions within each group: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001

**Table 5:** Dairy productivity and milk quality in Holstein cows under heat stress

Indicators	Unit meas.	Degree of stress resistance (mean±SE)					
		high		average		low	
		Normal n=36	Stress n=27	Normal n=27	Stress n=36	Normal n=27	Stress n=27
Yield	L	29.31±0.519	27.13±0.570***	27.11±0.691	24.87±0.470**	25.39±0.576	23.13±0.595***
Fat	%	3.42±0.080	3.22±0.055*	3.42±0.138	3.18±0.035*	3.33±0.140	3.25±0.062
Protein	%	3.26±0.018	2.85±0.035***	3.27±0.022	2.96±0.111**	3.25±0.017	2.80±0.025***
Lactose	%	4.79±0.044	4.79±0.033	4.79±0.100	4.78±0.045	4.79±0.030	4.73±0.044
SOMO	%	8.15±0.074	7.13±0.042***	8.18±0.100	7.40±0.045***	8.13±0.071	7.00±0.050***
Density	A°	35.66±0.226	30.19±0.197***	35.42±0.233	30.29±0.225***	35.00±0.280	30.15±0.238***
SCC	thousand/cm <sup>3</sup>	281.31±8.845	292.72±20.011	264.25±9.690	280.26±11.264	265.30±8.008	263.63±12.623

SCC: somatic cell count. Values are expressed as mean±SE. Asterisks indicate statistically significant differences between Normal and heat stress conditions within each group: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001

It is important to note that currently, to address heat stress on dairy farms, measures are usually taken to stabilize the physiological state of the animals and reduce the stress effect. First of all, these are various ways to reduce metabolic heat production by cows, including changes in their diet and feeding and drinking regimes, as well as various measures to cool animals (canopies, installation of fans, water showers for animals, etc.) (Buryakov et al. 2016). However, due to the fact that the herd of dairy cows is genetically heterogeneous in terms of stress resistance, in addition to the above-mentioned measures, systematic monitoring of individual stress resistance of cows with possible selection of resistant animals is necessary to improve the state of the dairy farm. In this case, cortisol level could be the main biomarker for selection of animals for stress tolerance. In addition, this parameter correlates with milk productivity (Tallo-Parra et al. 2018), which significantly increases the importance of its determination. At the same time, various behavioral, hematological, biochemical and physiological disorders in cows caused by stress are additional parameters characterizing animals with different degrees of stress resistance, and the decrease in milk productivity and milk quality parameters are the most characteristic symptoms of its impact.

According to the results of our work it can be seen that under heat stress in Holstein cows, depending on the degree of their stress resistance, both general group diagnostic signs of stress and specific intragroup ones are detected. The group-wide indicators of heat stress, typical for all Holstein cows, regardless of their stress resistance, first of all, include various behavioral reactions aimed at reducing heat dissipation. These are reduced feed intake, reduced resting time in the lying position and reduced cud level. Also, for animals of all groups, a decrease in phosphorus content in fecal masses, creatinine and magnesium in blood, milk productivity and some milk quality indicators (protein, fat, SOMO and density) were noted.

As for specific within-group changes induced by stress, the most of them were found in Holstein cows with high stress tolerance: decrease in starch and potassium concentration in fecal matter, increase in MPV and decrease in calcium and phosphorus in blood. For cows with low stress tolerance, the increase in neutral-detergent fiber in fecal masses was specific and no characteristic changes in diagnostic parameters were found in animals with medium tolerance to heat stress.

## Conclusion

Based on the results obtained, it can be concluded that blood cortisol level serves as an effective biomarker for determining the degree of heat stress resistance (high, medium, low) in Holstein cows. The study identified both general (intergroup) and specific (intragroup) diagnostic indicators of heat stress that vary depending on this resistance level. The most consistent markers observed across all animals, regardless of their stress resistance, were behavioral reactions and a reduction in milk yield and quality. Notably, cows classified with a high degree of stress tolerance were found to be the most sensitive to heat stress based on a comprehensive set of diagnostic indicators, yet they simultaneously maintained the highest milk productivity under such conditions. Consequently, selectively breeding animals with a high stress resistance and increasing their proportion within the herd emerges as a promising strategy to mitigate the detrimental effects of heat stress.

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