

## PRODUCTIVITY AND GENERAL RESISTANCE OF LACTATING COWS UNDER CHRONIC HEAT STRESS AND THEIR CORRECTION WITH MAGNESIUM NANOCOMPOUNDS

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### ABSTRACT

The aim of this study was to scientifically substantiate the prevention of chronic heat stress in high-yielding lactating cows through the use of a magnesium nanocompound. The research was conducted under production farm conditions with natural fluctuations of the microclimate and temperature-humidity index (THI 65–79). In a controlled experiment, Ukrainian Black-and-White cows (n=10, peak lactation) were divided into control and experimental groups (additionally fed with a nanoform of magnesium citrate 0.5 mg/kg of body weight per day for 10 days). Productivity (daily milk yield), clinical-physiological, hematological, and biochemical parameters were measured, and statistical analysis along with correlation-regression modeling was performed, accounting for the lag effect of THI.

A strong association was identified between milk yield and THI with a two-day lag ( $r \approx -0.45$ ), confirming the cumulative effect of heat stress. Under chronic heat stress, cows in the control group experienced a 17.7% decrease in productivity, while those supplemented with Mg exhibited only an 11.7% decline, with a significant intergroup difference (+1.8 L/day,  $p < 0.05$ ). Mg supplementation for 10 days prior to heat stress had a stabilizing effect on blood oxygen transport function and mitigated immune and platelet changes in high-yielding cows under chronic heat stress. Mg supplementation may have contributed to better metabolic adaptation to the stressor, which was manifested by a decrease in the negative effect of heat stress on blood metabolic parameters. These changes suggest a possible hepatoprotective and anti-stress effect of prophylactic magnesium nanocompound supplementation under chronic heat stress conditions. In particular, an improvement in the A/G ratio was established under stress conditions, and a decrease in the intensity of TBIL, ALT/AST, BA, CK under conditions of heat stress on the body of lactating cows, and an improvement in lipid metabolism (TG, CHOL), respiration (tCO<sub>2</sub>), and Ca and Mg content in the blood.

The novelty of the work lies in the combination of real production monitoring of THI with the assessment of lag effects and the demonstration of the corrective action of the nanoform of magnesium according to CHS.

**Key words:** Heat stress, THI, dairy cow, magnesium nanocompounds, productivity, hematology, blood biochemistry, prevention.

## Introduction

Progressive climate change including rising temperatures, more frequent droughts, floods, and soil degradation exerts systemic negative impacts on agriculture, especially the livestock sector. FAO (2013) estimated that livestock supply chains produce approximately 7.1 Gt CO<sub>2</sub>-eq per year, representing 14.5% of global anthropogenic greenhouse gas emissions. Cattle production accounts for a major share of these emissions, mainly due to enteric fermentation and feed-related processes. More recently, FAO (2023) emphasized the need to develop pathways for reducing greenhouse gas emissions from livestock agrifood systems while maintaining productivity and food security. This places unprecedented pressure on agricultural systems, necessitating adaptation through innovative technologies, feeding strategies, improved housing, and efficient water management.

During heat waves and climatic stress, animal productivity—particularly in dairy herds—declines significantly, threatening food security. Against this backdrop, mineral metabolism, especially the role of magnesium (Mg) as a key macroelement, gains increasing importance. Mg is the second most abundant intracellular cation after potassium and plays a critical role in nervous, cardiovascular, endocrine, and immune functions (Pinotti, Cazzola, & Givens, 2021). In cattle, Mg regulates energy metabolism, protein and enzyme synthesis, neuromuscular conduction, myocardial contraction, and the activity of enzymes in glycolysis and the citric acid cycle (Seely *et al.*, 2023).

Magnesium deficiency in ruminants is a well-documented issue, most commonly manifesting as hypomagnesemic tetany on pastures with high potassium content (Mayland, 1988). However, recent studies highlight a broader spectrum of Mg functions: it is a cofactor for over 300 enzymes, including those responsible for energy metabolism (hexokinase, pyruvate kinase, ATPases), protein and nucleic acid synthesis (RNA polymerase, ribosomal enzymes), and antioxidant defense (superoxide dismutase, glutathione peroxidase). Under modern climatic extremes—high temperatures and drought—Mg may serve a strategic role in maintaining physiological stability, resilience, metabolic efficiency, hepatic protection, immune response, and sustained milk production in dairy cows.

The novelty of this study lies in the integrated assessment of chronic heat stress in lactating cows under real production conditions. In particular, the study combines field monitoring of the temperature-humidity index, analysis of the delayed 1-2-day lag effect of THI on milk yield, and evaluation of the corrective potential of a magnesium nanocompound in high-yielding dairy cows. This approach makes it possible to assess not only the direct impact of heat stress, but also its cumulative effect on productivity and physiological resistance.

## Materials and Methods

Experimental research was carried out on a commercial farm located in the forest-steppe zone of Ukraine, housing Ukrainian Black-and-White cattle in loose housing with deep bedding. Feed and water were freely available. The average milk yield per lactation on the farm was 7,000–8,000 L.

To assess the effect of chronic heat stress (CHS) on productivity, daily milk yield of the herd (n=167) was recorded throughout July 2025, alongside weather data from the nearest meteorological station (<https://www.visualcrossing.com/>). July was characterized by pronounced contrasts: from cool days (July 19–20, minimum 16 °C, average 16–17 °C) to heat waves (July 7–8, maximum 35.7 °C, average 27.1–27.5 °C). Humidity ranged from very low (40–50%) on hot days to critically

high (>90% on July 18 and 29). Thus, the month was characterized by marked contrasts: a combination of periods of heat and dryness with cool and humid periods. These data are important for assessing the impact of heat stress on farm animals, as it is the combination of high temperature and humidity that creates critical conditions for the welfare of cows. In the room where lactating cows were kept during July 2025, microclimate parameters were measured daily at 2:00 p.m., which corresponds to the peak heat load.

Air temperature (°C) and relative humidity (%) were determined using a Testo 608-H1 digital thermohygrometer (Testo SE & Co. KGaA, Germany), pre-calibrated according to the manufacturer's instructions. The thermal stress index (THI) was calculated based on the air temperature and relative humidity values. The indoor temperature ranged from 18.5 °C (July 18) to 30 °C (July 7), and the humidity ranged from 48.1% (July 7) to 99.3% (July 18). THI values ranged from 65 to 79, indicating both moderate and chronic heat stress during certain periods (above 72). The most critical conditions were observed on July 7–8 and July 22–26, when the THI reached 78–79.

The THI was calculated using the standard formula:  $THI = 1.8 \times T - (1 - RH/100) \times (T - 14.3) + 32$ , where THI is the temperature and humidity index, T is the air temperature in °C, and RH is the relative humidity in % (Bohmanova, 2006). The obtained THI values were classified according to international criteria: THI < 68 – no heat stress, 68–72 – mild stress, 73–77 – moderate stress, > 78 – severe/chronic heat stress.

The average milk productivity of the herd of cows under heat stress was determined daily.

To study the effect of Mg nanocomposites on the productivity and stress resistance of lactating high-yielding cows under conditions of chronic heat stress, 10 cows of the Ukrainian Black-and-White dairy breed were selected in the peak lactation phase (6–9 weeks after calving), with an average body weight of 530–560 kg and an average productivity of 30–35 liters/day. The animals were divided into two groups according to the principle of analogues: control (n=5) and experimental (n=5). DIM (Days in Milk) averaged 88–92 days, and the body condition score (BCS) was 3.0–3.1 points. Housing conditions were tethered, with free access to water and a balanced diet in accordance with NRC (2001) standards. During July 2025, high thermal stress index (THI 72–78) values were recorded in the livestock building, confirming the presence of CHS.

The animals in the experimental group were given a nanoform of magnesium citrate in a dose of 0.5 mg/kg of body weight per day (TU U 15.8-35291116-014:2011) with water for 10 days (July 10–19, 2025), provided by Nanomaterials and Nanotechnologies LLC. The selected dose of the magnesium nanocompound, 0.5 mg/kg body weight per day, was used as a short-term prophylactic dose aimed at supporting magnesium homeostasis during the period of increasing heat load. This dosage was chosen to provide additional magnesium supply without excessive mineral intake or risk of metabolic overload. The 10-day supplementation period was considered sufficient for preventive support of adaptive mechanisms before the development of chronic heat stress, while remaining safe for lactating cows under production conditions. Daily milk yields (based on the results of morning and evening milking: starting at 6:00 a.m. and 6:00 p.m.), respiratory rate, and rectal temperature at 2:00 p.m. were recorded daily.

Blood samples were collected twice: under normal conditions, before exposure to heat stress in lactating cows (July 19, 2025); during chronic heat stress (CHS) (July 28, 2025). Blood was taken from the jugular vein before morning feeding at 06:00 into tubes with anticoagulant. Stabilized blood in a refrigerated container was immediately delivered to a specialized biochemical laboratory

for testing. In blood plasma, 24 parameters were determined using the MNCHIP Pointcare V3 biochemical analyzer (China) with Comprehensive Profile (CP) reagent discs: TP, Alb, Glob, A/G ratio, ALT, AST, AST/ALT, GGT, ALP, TBIL, BA, CREA, UREA, UCR, CK, AMY, GLU, TG, CHOL, Ca, P, Ca×P, Mg, tCO<sub>2</sub>. In whole blood, morphological parameters were determined using the Mindray BC-20 Vet (China) automatic hematology analyzer: RBC, HGB, HCT, MCV, MCH, MCHC, WBC, LYM, GRN, MON, PLT, MPV, PCT.

Data processing was performed using MS Excel 2019 and Statistica 10.0. The mean value ( $M \pm SD$ ) was determined, and normality was tested using the Shapiro-Wilk criterion. The reliability of differences was assessed using Student's t-test ( $p \leq 0.05$ ). Additionally, correlation analysis ( $r$ ) and regression modeling were performed to identify the relationship between THI, productivity, and blood parameters.

The study was conducted in accordance with Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruel Treatment" and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986).

## Results

The effect of magnesium nanocomposites on cow productivity during chronic heat stress. Chronic heat stress is a major cause of reduced milk yield in summer, associated with impaired welfare, reduced feed intake, diminished anabolic processes, and lower lactation. Under such conditions, Mg can be quite effective in correcting the stress resistance of lactating cows to heat stress on their bodies. Its action is quite multifaceted and ranges from neurohumoral regulatory mechanisms to individual links in energy, protein, and lipid metabolism. Therefore, the use of nanofoms of trace elements opens up new opportunities for increasing their bioavailability and correcting the negative effects of heat stress.

Milk production in cows is one of the most sensitive indicators of heat stress. Figure 1 shows the relationship between average milk yield per herd (blue line, liters) and the heat stress index (THI, orange line) during July 2025. At the beginning of the month (July 1–5), milk yield peaks at  $\approx 27.8$  liters (day 3), after which it gradually declines. At this time, the THI rose from 65 to 75, accompanied by slight fluctuations in productivity. In the middle of the month (July 10–18), there is a noticeable drop in milk yield (to 26 liters and below), which coincides with an increase in THI above 70–75, indicating the negative impact of heat stress. The most pronounced decline in productivity occurred on July 25–29, when the THI consistently exceeded 75–78: milk yield fell to a minimum of  $\approx 25.3$ – $25.5$  liters. After the THI decreased at the end of the month (July 30–31), a partial recovery in milk yield to  $\approx 26.4$ – $26.5$  liters was observed, indicating the reversible nature of the impact of climatic stress.

The graph confirms the close relationship between an increase in THI and a decrease in milk yield: at  $THI > 72$ , there is a noticeable decline in productivity, and when THI exceeds 75 for a long time, chronic heat stress develops.

Therefore, lactating cows are very sensitive to sudden changes in temperature and humidity, but the physiological response to milk yield is delayed—the drop in milk yield becomes apparent 1–2 days after peak THI values. The calculation showed that the Pearson correlation coefficient between milk yield and THI for July 2025 is  $\approx 0.016$ , which is a very low value (almost no linear correlation). Therefore, milk yield did not change in direct proportion to THI (i.e., it cannot be said that each increase in THI immediately reduces milk yield in the same proportion). However, the

graph shows that there is a delayed (lag) effect: the decline in milk yield occurs 1–2 days after peak THI values. That is why the simple correlation for the same day is almost “smoothed out”.

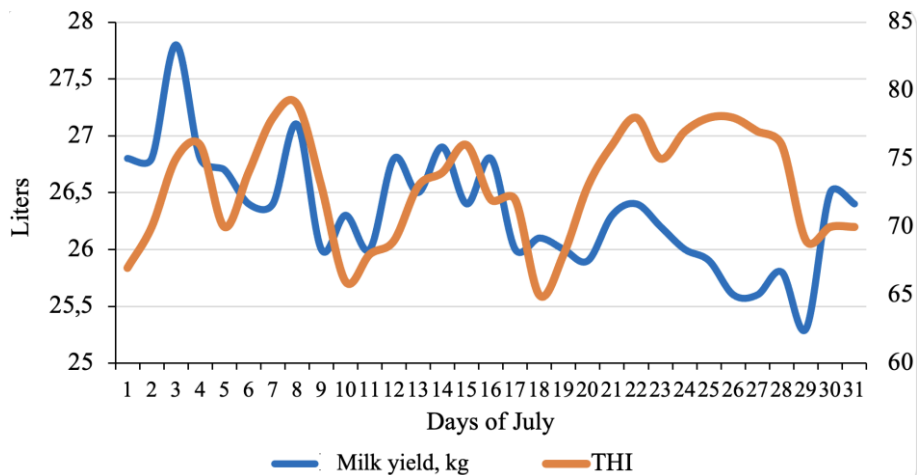


Figure 1: Dependence of milk yield on the heat stress index during July (n=167).

After conducting a correlation analysis with lags (shift of 1 and 2 days), it was found that THI with a shift of 1 day is inversely correlated with milk yield –  $r = -0.18$  (weak negative correlation;  $p=0.34$ ). THI with a lag of 2 days –  $r = -0.45$  (moderate negative correlation;  $p=0.01$ ). This confirms that the cumulative effect of heat stress must be taken into account – the intensity of milk production in the mammary gland reacts with a delay. On the day of the THI increase or the following day, changes in productivity are not yet statistically significant.

The results of the regression analysis (THI with a lag of 2 days) showed that the regression coefficient is  $-0.07$ , which means that when the THI increases by 1 point (after 2 days), milk yield decreases by approximately 0.07 liters of milk per cow on average for the herd.  $R^2 = 0.20$  (20% of the variation in milk yield is explained by changes in THI with a lag of 2 days). Thus, THI after 2 days has a noticeable but not absolute effect on milk yield. In addition to climatic factors, productivity is also determined by diet, physiological condition, productivity level, management, etc.

It is known that under conditions of heat stress, high-yielding cows lose more milk than low-yielding cows due to their more intense metabolism, which is associated with high levels of endogenous heat production and negative energy balance. In the initial state, before the stressor, no significant differences were observed between the control and experimental animals, and productivity corresponded to their genetic potential (about 30–35 liters). The increase in the heat stress index during the experiment to critical values (72–78) was accompanied by a gradual decrease in milk productivity in cows of the control group (from 32.5 to 27.0 liters per day). At the same time, in animals that were given Mg nanocomposites, the decline in productivity was less intense: in dairy cows, the average daily milk yield remained at 28.5–29.0 liters and was 1.8 liters/day higher ( $P<0.05$ ) than in the control group of cows (Fig. 2).

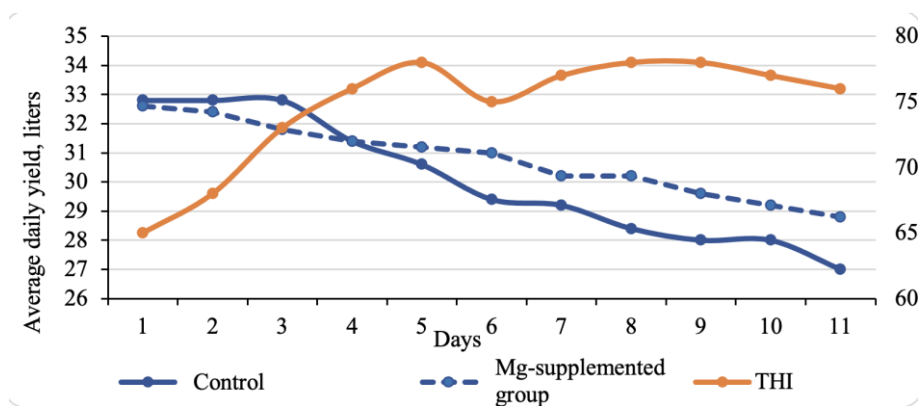


Figure 2: Productivity of cows fed magnesium nanocomposites depending on the heat stress index.

Thus, the addition of Mg nanocomposites partially mitigated the negative effects of heat stress in high-yielding cows, thereby reducing productivity losses by almost 6% compared to the control animals.

The effect of Mg nanocomposite administration on the overall resistance of high-yielding cows under chronic heat stress. The dose of Mg nanocomposites used did not significantly affect the blood picture of the experimental group animals compared to the control group. According to the studies, 10 days of Mg supplementation did not have a negative effect on the number of blood cells, their ratio, cell volume, thrombocrit, and is completely safe (Table 1). The number of red blood cells in the experimental group increased slightly (by 7.7%), but this difference was not statistically significant. Hemoglobin and hematocrit levels remained virtually unchanged, indicating the stability of the oxygen-carrying function of the blood. There was a tendency toward a decrease in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), which may indicate a more compact erythrocyte morphology under the influence of Mg, but without significant changes. The number of leukocytes and their populations under normal conditions also did not differ between groups, confirming the absence of a pronounced effect.

Heat stress on lactating cows can negatively affect the oxygen-transport function of blood. Thus, under heat stress conditions, lactating cows in the control group showed a significant decrease in red blood cell count by 11.5% ( $P < 0.05$ ) and hemoglobin by 7.2% ( $P < 0.05$ ), indicating the development of anemia. Obviously, the removal of excess heat from the body of a lactating cow due to the expansion of peripheral blood vessels and increased sweating may be accompanied by accelerated aging of red blood cells, but this issue requires further study.

It should be noted that prophylactic feeding of Mg nanocomposites for 10 days before the onset of heat stress had a stabilizing effect on the oxygen-transport function of the blood of lactating cows under CHS conditions. In animals that received Mg nanocomposites, the number of red blood cells during heat stress did not change significantly compared to their pre-stress level, but compared to animals in the control group with signs of heat stress, it was 13.8% higher ( $P < 0.05$ ). The hematocrit in the experimental group was also slightly higher (by 8%) than in the blood of control animals. MCV, MCH, and MCHC values decreased under stress conditions in both control and experimental animals, but the changes were only a trend. This may indicate greater stability of erythropoiesis under the influence of Mg, although complete compensation for the negative changes could not be achieved.

The redistribution of blood flow in the bloodstream of lactating cows in the control group under intense heat stress was accompanied by a corresponding reaction of white blood cells to the increased stress on the digestive system. In the blood of control cows, under the action of a heat stressor, the number of leukocytes increased by 20.6% ( $P < 0.001$ ) due to granulocytes (+49.3%,  $P < 0.001$ ), while in the experimental group this increase was significantly less pronounced: the number of leukocytes increased by only 7.9% ( $P < 0.05$ ), and granulocytes by 31.2% ( $P < 0.05$ ), indicating the hepatoprotective effect of Mg. The platelet count in the blood of control animals under heat stress decreased significantly by 11.9% ( $P < 0.001$ ), while in the blood of the experimental group, there was a less significant decrease in their number (+5.9%,  $P < 0.05$ ), which may indicate a positive effect of Mg supplementation on the blood coagulation system.

MPV and PCT values did not change significantly. Thus, feeding Mg nanocomposites for 10 days before exposure to a heat stressor ensured the preservation of erythrocyte parameters and mitigated immune and platelet changes in high-yielding cows under chronic heat stress, indicating a potential protective physiological effect.

**Table 1: Blood morphology parameters in high-yielding cows after magnesium nanocompound supplementation (M±SD; n=5)**

Indicators	Animal groups			
	Control	Experiment	Control	Experiment
	Under normal conditions		Under CHS	
RBC, T/L	6,37±0,53	6,86±0,57	5,64±0,43*	6,42±0,62 <sup>^</sup>
HGB, g/L	102,6±4,0	102,2±7,1	95,2±3,8*	97,5±4,2
HCT, %	31,19±1,22	31,9±2,36	29,35±1,6	31,7±1,9
MCV, fL	49,11±2,65	46,8±6,0	52,24±3,96	49,6±3,2
MCH, pg	16,16±1,05	14,9±1,05	16,96±1,53	15,3±1,3
MCHC, g/L	3,29±0,1	3,21±0,24	3,25±0,18	3,08±0,21
WBC, 10 <sup>9</sup> /L	7,88±0,35	7,67±1,01	9,5±0,33***	8,28±0,54* <sup>^^</sup>
GRN, 10 <sup>9</sup> /L	4,12±0,31	3,91±0,68	6,15±0,6***	5,13±0,56 <sup>^</sup>
LYM, 10 <sup>9</sup> /L	3,46±0,46	3,45±0,56	3,02±0,48	2,81±0,24
MON, 10 <sup>9</sup> /L	0,29±0,02	0,31±0,03	0,33±0,05	0,34±0,06
PLT, 10 <sup>9</sup> /L	386,8±16,3	390,6±20,5	340,6±14,8***	360,6±19,5*
MPV, fL	5,93±0,22	6,01±0,37	6,55±0,65	6,06±0,55
PCT, ml/L	2,29±0,07	2,35±0,15	2,22±0,16	2,18±0,16

*Note: RBC – red blood cell count; HGB – hemoglobin; HCT – hematocrit; MCV – mean corpuscular volume; MCH – mean corpuscular hemoglobin; MCHC – mean corpuscular hemoglobin concentration; WBC – white blood cells; GRN – granulocytes; LYM – lymphocytes; MON – monocytes; PLT – platelets; MPV – mean platelet volume; PCT – thrombocrit. 2. Data are presented as M±SD, n=5. \*, \*\*, \*\*\* indicate statistically significant differences within the same group between the values before and during chronic heat stress: \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . <sup>^</sup>, <sup>^^</sup> indicate statistically significant differences between the control and experimental groups under the same physiological condition: <sup>^</sup>  $P < 0.05$ , <sup>^^</sup>  $P < 0.01$ .*

Under comfortable conditions for lactating cows, the biochemical blood parameters in the control and experimental groups did not differ significantly, indicating that 10 days of feeding Mg nanoparticles at a dose of 0.5 mg/kg body weight per day had no effect on basic metabolic processes (Table 2). Total protein, albumin, globulins, and the albumin/globulin ratio in the blood of cows in the control and experimental groups remained at the same level, indicating no negative effect of

Mg supplementation on protein metabolism. A similar situation was observed with the catalytic activity of the studied enzymes (ALT, AST, GGT, ALP), the content of bile acids, triglycerides, cholesterol, and glucose.

Under CHS conditions, pronounced changes were observed in the blood of animals in the control group, indicating the development of stress-induced liver dysfunction and metabolic disorders. Apparently, the redistribution of blood flow in favor of peripheral organs under CHS causes a number of undesirable phenomena in the digestive tract, which negatively affects the functional state of the liver.

Thus, the level of albumin in the blood of cows in the control group under stress conditions decreased by 11.7% ( $P<0.05$ ), which led to a decrease in the albumin/globulin ratio by 22% ( $P<0.05$ ), while in the experimental animals these indicators were significantly higher: albumin by 3.8% and A/G by 14% ( $P<0.05-0.01$ ). Total bilirubin in the control group increased by 72% ( $P<0.05$ ), while in the experimental group it was 10.5% lower, indicating the hepatoprotective effect of Mg.

Regardless of the research conditions, heat stress causes an increase in the activity of certain enzymes in the blood, in particular, the catalytic activity of ALT in the control group increased by 36.5% ( $P<0.001$ ), in the experimental group – by 28.2% ( $P<0.001$ ), and AST activity increased by 32.1% and 24.9%, respectively ( $P<0.05$ ). Creatine kinase activity also increased by 39.6% in the control group ( $P<0.05$ ) and by 25% in the experimental group, confirming a lower degree of muscle tissue damage. Similarly, the level of bile acids in the blood increased by 26% in the control group ( $P<0.05$ ) and by 11% in the experimental animals. Thus, Mg supplementation may have partially mitigated stress-related metabolic disturbances.

A decrease in the intensity of digestion under conditions of CHS negatively affects the energy metabolism of cows in general and the condition of the animal in particular. Under the conditions of the study, CHS in dairy cows of the control group was accompanied by a decrease in glucose levels by 15.9% ( $P<0.05$ ), triglycerides by 27.9% ( $P<0.01$ ), and cholesterol by 33.8% ( $P<0.05$ ). In the experimental group of animals, these indicators were 1.8%, 9.9%, and 10.8% higher than in the control group, respectively.

The intensity of lung ventilation significantly affects the content of gases in the blood of animals, therefore, during CHS, these indicators can change significantly. Thus, total  $\text{CO}_2$  in the blood of cows in the control group decreased by 20.7% ( $P<0.001$ ), while in the experimental animals it was 10.4% higher ( $P<0.05$ ).

The concentration of certain chemical elements in the blood of cows was also quite sensitive to the level of heat stress on the animal's body. In particular, the Ca content in the blood of cows in the control group decreased by 12% ( $P<0.001$ ). A similar situation was observed with the Mg content in the blood of cows in the control group under heat stress, which decreased significantly by 17.2% ( $P<0.01$ ) compared to the previous study period. As for P, the content of this chemical element in the blood of cows under TSS also decreased slightly in both groups of experimental animals.

It should be noted that preventive feeding of Mg nanocomposites had a positive effect not only on the content of this element in the blood of cows under heat stress, but also on the Ca content. Thus, additional feeding of the element somewhat smoothed out the situation with regard to the drop in its level (only by 10.1%,  $P<0.05$ ), and the Ca concentration was also at a slightly higher level (by 6.9%,  $P<0.05$ ), which indicates the effectiveness of feeding nanocomposites to maintain

homeostasis. Thus, the addition of Mg nanocomposites to cows for 10 days to prevent heat stress helped to maintain protein, lipid, energy, and mineral metabolism, and also suggested a possible hepatoprotective effect.

**Table 2: Biochemical blood parameters of high-yielding cows under chronic heat stress (M±SD; n=5)**

Indicators	Animal groups			
	Control	Experiment	Control	Experiment
	Under normal conditions		Under CHS	
TP, g/dL	7,96±0,34	7,87±0,27	8,0±0,62	7,82±0,48
Alb, g/dL	3,60±0,21	3,62±0,12	3,18±0,27*	3,3±0,27*
Glob, g/dL	4,36±0,15	4,26±0,16	4,82±0,59	4,51±0,29
A/G ratio,	0,82±0,04	0,85±0,02	0,64±0,11*	0,73±0,05**
TBIL, mg/dL	0,22±0,04	0,23±0,05	0,38±0,11*	0,34±0,09*
ALT, U/L	43,74±3,11	44,0±2,2	59,7±4,9***	56,4±4,5***
AST, U/L	129,6±27,2	130,8±23,8	171,2±29,9*	163,5±18,4*
AST/ALT	3,02±0,86	3,00±0,70	2,92±0,8	2,93±0,56
GGT, U/L	37,92±7,83	38,2±8,4	49,34±10,66	49,6±9,8
ALP, U/L	41,24±10,66	40,1±11,6	52,46±13,12	48,3±7,1
BA, umol/L	33,82±5,43	33,7±5,8	42,6±2,96*	41,5±2,5*
CK, umol/L	213,5±29,4	211,9±27,0	298±52,97*	265,5±26,6*
AMY, U/L	389,4±69,1	384,7±68,5	421,3±87,3	428,8±97,7
TG, mg/dL	38±5,36	37,7±5,7	27,4±3,2**	30,1±3,7*
CHOL, mg/dL	175,8±41,3	171,6±38,4	116,4±16,1*	129,0±9,5*
GLU mg/dL	66,56±5,84	66,7±7,8	56,0±5,5*	56,98±4,9*
CREA, mg/dL	1,02±0,13	1,02±0,15	0,92±0,11	0,90±0,12
UREA, mg/dL	14,44±2,06	14,6±2	12,22±2,06	12,0±1,9
UCR	14,06±2,64	14,6±3,1	13,24±2,41	13,4±2,6
tCO <sub>2</sub> , mmol/L	27,74±1,7	27,6±2,0	22,0±1,7***	24,3±1,5*^
Ca, mg/dL	10±0,22	9,94±0,26	8,8±0,42***	9,41±0,42*^
P, mg/dL	6,56±1,3	6,65±1,43	6,1±1,24	6,08±1,18
Mg, mg/dL	2,56±0,11	2,57±0,17	2,12±0,22**	2,31±0,17*

Note: 1. TP – Total protein (g/dL); Alb – Albumin (g/dL); Glob – Globulin (g/dL); A/G ratio – Albumin/globulin ratio; TBIL – Total bilirubin (mg/dL); ALT – Alanine aminotransferase (U/L); AST – Aspartate aminotransferase (U/L); AST/ALT – AST/ALT; GGT – GGT (U/L); ALP – Alkaline phosphatase (U/L); BA – Bile acids (μmol/L); CK – Creatine kinase (μmol/L); AMY – Alpha-amylase (U/L); TG – Triglycerides (mg/dL); CHOL – Cholesterol (mg/dL); GLU – Glucose (mg/dL); CREA – Creatinine (mg/dL); UREA (BUN) – Urea (mg/dL); UCR – Urea/Creatinine; tCO<sub>2</sub> – CO<sub>2</sub> Titre (mmol/L); Ca – Calcium (mg/dL); P – Phosphorus (mg/dL); Ca/P – Ca/P; Mg – Magnesium (mg/dL). 2. Data are presented as M±SD, n=5. \*, \*\*, \*\*\* indicate statistically significant differences within the same group between the values before and during chronic heat stress: \* P<0.05, \*\* P<0.01, \*\*\* P<0.001. ^, ^^ indicate statistically significant differences between the control and experimental groups under the same physiological condition: ^ P<0.05, ^^ P<0.01.

Thus, in highly productive cows, chronic heat stress causes disturbances in protein, lipid, energy, and mineral metabolism, manifested by a decrease in albumin, glucose, Ca, and Mg levels, an increase in liver enzyme activity, bile acid content, bilirubin, and creatine kinase activity. Feeding Mg nanocomposites significantly mitigated these changes, helped maintain protein composition stability, supported lipid and energy metabolism, and had a hepatoprotective and anti-stress effect.

## Discussion

Summer periods of keeping dairy cows with an elevated temperature-humidity index lead to a significant decrease in milk productivity in highly productive animals. Heat stress (THI >72) consistently reduces feed intake and milk yield (Collier *et al.*, 2019; West, 2020). Kim *et al.* (2023) noted that under CHS conditions, bull calves show reduced dry matter intake and body weight gain, reflecting the overall negative energy effect of heat. Stefańska *et al.* (2024) demonstrated that elevated THI in the pre-calving period reduces milk yield and reproductive performance in lactating cows. Habimana *et al.* (2023) recorded a drop in milk yield and a change in the profile of milk metabolites in Holsteins during heat stress. The general conclusion is that cow productivity decreases by 10–25% depending on the duration and intensity of heat. Our results confirm these data: in the control group of high-yielding cows, milk yield under CHS conditions decreased by 17.7% ( $p < 0.001$ ). A lag effect was observed: the sharpest drop in productivity occurred 1–2 days after peak THI values. This is consistent with the findings of Collier *et al.* (2019), who pointed to the delayed nature of the mammary gland's response.

As mentioned above, Mg is a cofactor in more than 300 enzymatic reactions in various metabolic pathways (Pinotti *et al.*, 2021). It influences neuroendocrine regulation, energy metabolism, glucose and lipid metabolism, and ion balance. Kovács *et al.* (2023) showed that adding Mg butyrate before calving reduces inflammatory markers and promotes a better start to lactation. Seely *et al.* (2021) demonstrated that maintaining physiological concentrations of ionized Mg in cows during the transition period is critical for maintaining feed intake and milk production. Experiments with different sources of Mg (MgO, CaMg(OH)<sub>2</sub>) have shown that the balance of minerals in the diet directly affects milk yield and lactation stability (Schonewille *et al.*, 2013). In our experiment, the use of Mg nanoform reduced productivity losses to 11.7% compared to 17.7% in the control group. After heat stress, milk yield in the experimental group was significantly higher (+1.8 l/day;  $p < 0.05$ ), confirming its anti-stress and anabolic role. Thus, Mg contributed to the preservation of milk productivity in cows even under conditions of high heat stress.

The possible protective effect of magnesium under chronic heat stress may be associated with several physiological mechanisms. Magnesium is involved in energy metabolism as a cofactor of ATP-dependent enzymatic reactions and may support cellular energy balance during increased thermal load. It also contributes to membrane stability, regulation of calcium-magnesium homeostasis, and maintenance of neuromuscular and cardiovascular functions. In addition, Mg may indirectly support antioxidant defense and reduce stress-related cellular damage. The more stable erythrocyte parameters observed in the experimental group may be related to the role of Mg in maintaining membrane integrity and supporting erythropoiesis. The less pronounced changes in leukocyte and granulocyte counts, as well as liver-related biochemical indicators, suggest that Mg supplementation may also moderate the inflammatory response and support liver function under chronic heat stress conditions.

Chronic heat stress reduces cow productivity by decreasing feed intake, disrupting energy balance, and altering metabolism. During severe heat stress, there is a decrease in intestinal blood flow, disruption of the intestinal barrier, and endotoxemia (Lambert, 2009). The level of detoxification in the liver of cows under CHS is of paramount importance. It is known that detoxification mechanisms are quite energy-intensive (Yuchen *et al.*, 2024). Therefore, the use of Mg supplements, especially in forms with high bioavailability, plays a key role in maintaining milk yield by

supporting liver function, energy, protein, and mineral metabolism, as well as reducing inflammatory responses. Our results confirm these findings and demonstrate the effectiveness of Mg nanocomposites as a means of maintaining productivity during CHS.

An analysis of recent studies (2018–2025) shows that heat stress is one of the key factors that reduces productivity and disrupts homeostasis in high-yielding cows. Under its influence, pronounced changes occur in the blood system, affecting morphological, biochemical, immune, and mineral indicators. Most authors (Jo *et al.*, 2024; Zeng *et al.*, 2023) note a decrease in the number of red blood cells, hemoglobin, and hematocrit, which indicates the development of anemic manifestations. In our experiment, a similar trend was observed in control animals: RBC decreased by 11.5% ( $p<0.05$ ), HGB by 7.2% ( $p<0.05$ ). In contrast, in the group that was given Mg nanocomposites, these indicators remained stable, which is consistent with the conclusions about the role of antioxidant support in maintaining erythropoiesis (Zeng *et al.*, 2023).

Under conditions of heat stress, there is an increase in the number of leukocytes and granulocytes along with an increase in pro-inflammatory cytokines and HSP70 (Chen *et al.*, 2023; Blond *et al.*, 2024). In our opinion, this is primarily due to the deployment of a protective response to digestive disorders and an increase in the flow of toxins into the bloodstream. In our conditions, the WBC index in control animals increased by 20.6% ( $p<0.001$ ), and GRN by 49.3% ( $p<0.001$ ). In experimental cows with Mg administration, these changes were less pronounced (WBC +7.9%, GRN +31.2%), confirming the anti-stress effect of Mg (Chen *et al.*, 2023).

It is known that heat stress is accompanied by a decrease in glucose and cholesterol levels, an increase in urea content, and activation of liver enzymes in the blood of animals (Stefańska *et al.*, 2024; Habimana *et al.*, 2023; Penev & Stojnov, 2024). In our study, the control group of animals also showed a decrease in blood glucose (−15.9%;  $p<0.05$ ) and cholesterol (−33.8%;  $p<0.05$ ) levels and an increase in ALT (+36.5%;  $p<0.001$ ) and AST (+32.1%;  $p<0.05$ ), and bile acid content (+26%;  $p<0.05$ ). The regulatory effect of Mg on the physiological state of the liver was manifested in the blood of cows in the experimental group by less pronounced deviations of the studied indicators compared to their pre-stress level, which may indicate a possible hepatoprotective effect of the supplement.

Studies (Antanaitis *et al.*, 2024; Blond *et al.*, 2024) demonstrate a decrease in the concentrations of Ca, Mg, and  $\text{CO}_2$  in the blood during heat stress. Our data confirm this pattern: in the blood of control cows, the level of  $\text{CO}_2$  decreased by 20.7% ( $p<0.001$ ), Ca by 12% ( $p<0.001$ ), and Mg by 17.2% ( $p<0.01$ ). In the experimental animals, the deviations were significantly smaller (Mg −10.1%, Ca −6.9%), which is consistent with the results of studies on the key role of Mg in maintaining calcium homeostasis and preventing subclinical hypomagnesemia (Pinotti *et al.*, 2021; Seely *et al.*, 2021).

Literature data show that the addition of Mg, particularly in the form of Mg butyrate or organic salts, reduces the level of inflammation markers, particularly haptoglobin, and has a positive effect on protein and lipid metabolism (Kovács *et al.*, 2023). A link between low levels of ionized Mg and metritis and postpartum disorders has also been demonstrated (Rodríguez *et al.*, 2017). Our study confirmed that Mg stabilizes protein and lipid metabolism, reduces the risk of increased liver enzyme activity in the blood, and protects against a sharp drop in Ca and Mg levels in the blood during heat stress. This suggests that Mg may have multifaceted anti-stress and hepatoprotective effects under heat stress conditions.

A limitation of this study is the relatively small number of animals included in the experimental trial, with five cows per group. Therefore, the obtained results should be interpreted as field experimental evidence demonstrating the potential corrective effect of the magnesium nanocompound under production conditions. Further studies involving a larger number of animals, different productivity levels, and longer observation periods are needed to confirm these findings and to more precisely determine the practical effectiveness of this approach.

The literature data and our results are consistent: heat stress in cows causes anemic changes, leukocytosis, and disturbances in protein, lipid, and mineral metabolism. The use of Mg (especially in highly bioavailable forms) appeared to be safe under the conditions of this study and may reduce the negative effects of these changes, supporting physiological liver activity, blood gas transport, homeostasis indicators, and productivity. Our study expands this knowledge by demonstrating the effectiveness of Mg nanocomposites in maintaining physiological blood parameters and milk productivity under conditions of chronic heat stress.

### **Conclusion**

The results of the study indicate that chronic heat stress is a powerful destabilizing factor for highly productive lactating cows, causing a significant decrease in milk productivity, the development of anemia, the activation of inflammatory reactions, and disturbances in protein, lipid, and mineral metabolism. It has been proven that even relatively short-term exposure to a high temperature-humidity index (THI) leads to complex changes in the blood system, including a decrease in the number of red blood cells and hemoglobin, an increase in the leukocyte response due to granulocytes, a decrease in albumin, Ca and Mg, and activation of liver and muscle enzymes. This indicates the systemic nature of stress-induced disorders that directly threaten the sustainability of milk production in the context of climate change.

At the same time, the use of Mg nanocomposites in a prophylactic regimen (0.5 mg/kg body weight per day for 10 days) showed a pronounced prophylactic effect. In experimental animals, productivity losses were significantly lower, and morphological and biochemical blood parameters remained at levels much closer to the physiological norm. Mg supplementation showed potential anti-anemic, immunomodulatory, hepatoprotective, and regulatory effects, reducing the intensity of negative changes caused by heat stress. This supports the assumption that Mg plays an important role in maintaining cellular homeostasis, antioxidant protection, and energy metabolism, and highlights the need to review traditional mineral support regimens for high-yielding herds during the summer period.

The novelty of the study is determined by the combination of field THI monitoring, analysis of the 1-2-day delayed effect of heat stress on milk yield, and evaluation of a magnesium nanocompound as a corrective strategy under real production conditions.

Thus, the results of the study have a dual significance: on the one hand, they deepen the understanding of the pathogenesis of heat stress in high-yielding cows, and on the other hand, they suggest the practical potential of using Mg nanocomposites as a supportive tool for adaptive support. This opens up prospects for the development of scientifically based protocols for the prevention and correction of heat stress, which will contribute to maintaining the productivity, health, and resistance of animals in the face of global climate challenges.

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## Conflict of Interest

The authors have not declared any conflict of interests.

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