

Original Research Paper

# Prevention of Heat Stress in Lactating Cows

<sup>1</sup>Vladimir Zaitsev, <sup>2</sup>Vasily Korotkiy, <sup>3</sup>Nadezhda Bogolyubova, <sup>1</sup>Lilia Zaitseva and <sup>2</sup>Viktor Ryzhov

<sup>1</sup>Department of Bioecology and Physiology of Farm Animals, Faculty of Biotechnology and Veterinary Medicine, Samara State Agrarian University, Ust-Kinelsky, Russia

<sup>2</sup>Khinvest Science and Technology Center LLC, Nizhny Novgorod, Russia

<sup>3</sup>Department of Physiology and Biochemistry of Agricultural Animals, Federal Research Center of Animal Husbandry Academician L.K. Ernst, Podolsk, Russia

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Corresponding Author:

Viktor Ryzhov

Khinvest Science and

Technology Center LLC,

Nizhny Novgorod, Russia

Email: vikt.ryzhov@gmail.com

**Abstract:** In summer, when the air temperature rises, dairy cattle are very susceptible to heat stress, which results in a significant decline in productivity. The goal of the study is to assess how a Coniferous Energy Supplement (CES) made from raw materials derived from forestry waste affects lactating cows' ruminal digestion, milk production, and quality in extreme temperatures. Two sets of black-and-white cows were studied for a preliminary period after calving. The cows of the control group received the main diet, which consisted of hay, green mass, and grain mixtures. In addition to the main food, the coniferous energy supplement was introduced into the diets of dairy animals of the experimental group at a dose of 150 g/head per day. During the study period, the given feed's chemical composition, ruminal digestion parameters, productivity, and quality of cows' milk were determined. By incorporating the coniferous energy supplement into the cows' diet during the hot season, the rumen's enzymatic processes were strengthened, the blood serum's bactericidal activity and lysis percentage increased and the average daily milk yields of natural fat content increased by 11.9-12.2%.

**Keywords:** Heat Stress, Nutritional Supplement, Ruminal Digestion, Milk Productivity, Milk Quality

## Introduction

Stress factors are any stimuli that cause a reaction to stress or deviation from homeostasis. The stress factors are diverse in nature, time, and constancy (Eyck *et al.*, 2019). During life, animals are affected by various internal and/or external stress factors that can disrupt the normal physiological balance, thereby causing a threatening homeostatic state (McManus *et al.*, 2022). Stress leads to the activation of physiological mechanisms necessary to maintain homeostasis and can harm functions such as reproduction, immunity, and growth (Bhimte *et al.*, 2018). The most acute stress factor is temperature and elevated temperatures negatively affect animals (Rashamol and Sejian, 2018). During heat stress, animal productivity and reproductive ability decrease dramatically.

The negative impact of high temperatures in the summer can be reduced by introducing immunomodulating energetic supplements into the diet of cows, which will optimize the energy balance and allow

additional energy to be extracted from feed due to the effective splitting of fat and fiber (Min *et al.*, 2019).

One of the options may be the use as a raw source of such products, namely coniferous needles (branches with coniferous needles) of the plants that have undergone the extraction process with a composition of polyatomic alcohols. Such raw materials are a kind of forestry waste (produced about 10 million tons annually) that is usually burned in logging areas, causing fires and poisoning the atmosphere with combustion products. The basis of the technology is the concentrated extraction of active substances of therapeutic and immune profile (coniferous needle biogenic extract) specific to woody greens (needles) from coniferous raw materials by extraction with a composition of polyatomic alcohols (a glucoplastic component), which simultaneously act as the energy component of the supplement and contain additional auxiliary ingredients (Korotkii *et al.*, 2018).

Dairy cattle are very sensitive to heat stress, which entails significant economic losses for agricultural production, not

only directly in the form of a decrease in milk productivity and quality (somatic cell content) but also in a hidden way (health-related losses) (Toledo *et al.*, 2022).

The study aimed to evaluate the response to a Coniferous Energy Supplement (CES) based on raw materials obtained from forestry waste on ruminal digestion, and the amount and quality of milk produced by cows while they are giving milk. under extreme temperature conditions.

## Materials and Methods

### General Study Design

The protocol of the study was discussed and approved at the meeting of the ethical committee of the Federal Research Center of animal husbandry named after academician L.K. Ernst, Russia (protocol No. 5 dated 22.03.2021).

Two groups of black and white cows (10 heads each) were the subject of a year-long study at the farm of AST group LLC in Verkhniye Belozerki village, Stavropol district, Samara region, following calving with a preliminary (equalization) period (10 days). Determination of the quality of the milk of experimental animals was carried out in the testing research laboratory of the Samara State Agrarian University.

The cows of the control group received the Main Diet (MD), which included legume hay, green mass, and feed mixture (barley, oats, and sunflower meal). Cows of the experimental group, in addition to the MD, received a CES at a dose of 150 g/head per day.

### Composition of the Nutritional Supplement

The CES was developed at the Khiminvest Science and Technology Center LLC (Nizhny Novgorod) (Bogolyubova *et al.*, 2018; Zaitsev *et al.*, 2022) and is a feed agent of the following composition, by weight %: Coniferous energy extract, 78%; propylene glycol, 10%; crushed active charcoal, 5%; linseed oil, 2%; sugar, 5%.

### Methods

Following the experiment, five animals from each experimental group had their whole and stabilized blood samples taken. The Department of Physiology and Biochemistry of Farm Animals collected these samples in order to analyze markers that describe the metabolic status. The level of nonspecific immunity of the blood of experimental animals ( $n = 5$ ) was determined in the laboratory of the microbiology of Blood Serum Bactericidal Activity (BSBA).

Milk yield (gross, average daily yield) was calculated based on control milking from all experimental animals ( $n = 10$ ).

To determine the milk quality of experimental animals ( $n = 10$ ), we took average milk samples and determined

the Molecular Dynamics (MD) of fat, MD of protein, and somatic cell content.

The design of the experiments module of statistica 6 software (StatSoft, Inc., USA) was used to mathematically process the experimental data and the Student's t-test was used to assess the reliability of the results.

## Results

To study the effect of the tested supplement on the fermentation processes in the rumen, at the end of the experiment, using an esophageal probe, we took the rumen contents, in which the pH, the total content of VFA, the molar ratio of individual fermentation acids, the concentration of ammonium nitrogen, the content of the total number of microorganisms and their species were determined (Figs. 1-3).

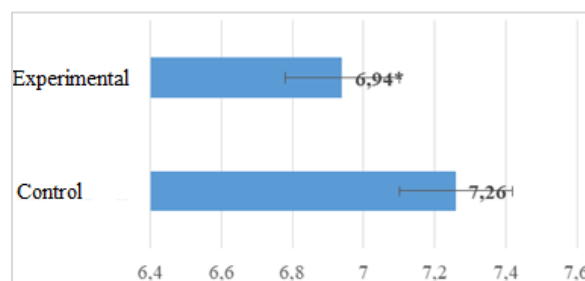


Fig. 1: The pH of the cow rumen contents 3 h after feeding

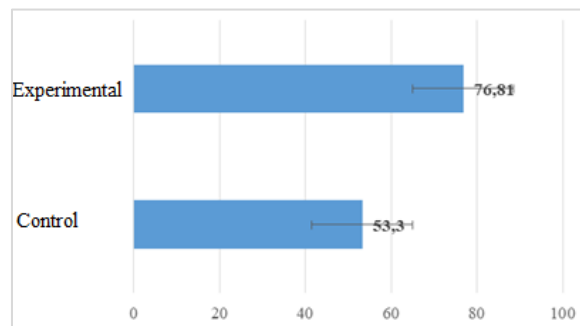


Fig. 2: Total VFA content in the rumen fluid 3 h after feeding, mmol/g

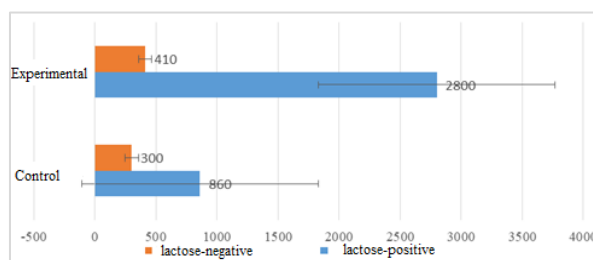


Fig. 3: The content of lactose-positive and lactose-negative microorganisms in the contents of the rumen of cows 3 h after feeding, CFU/g

**Table 1:** Blood parameters of test subjects: Biochemical ( $M \pm m$ ,  $n = 5$ )

| Indicator                    | Group       |              | Standard*     |
|------------------------------|-------------|--------------|---------------|
|                              | Control     | Experimental |               |
| Total protein, g/L           | 90.10±02.70 | 92.50±01.9   | 70-92 g/L     |
| Albumins, g/L                | 33.90±01.10 | 35.10±01.6   | 25-36 g/L     |
| Globulins, g/L               | 56.20±03.70 | 57.40±02.1   | 40-63 g/L     |
| Albumin/Globulin (A/G) ratio | 0.60        | 0.61         | 0.4-0.8       |
| Urea, mm/L                   | 7.62±00.50  | 6.86±00.3    | 2.4-7.5 mm/L  |
| Creatinine, µm/L             | 91.40±06.30 | 77.00±11.1   | 62-163 µm/L   |
| ALT, IU/L                    | 36.30±02.40 | 36.50±02.8   | 10-36 IU/L    |
| AST, IU/L                    | 92.50±08.10 | 91.20±12.2   | 41-107 IU/L   |
| Alkaline phosphatase, IU/L   | 60.90±16.10 | 52.30±08.0   | 31-163 IU/L   |
| Total cholesterol, mm/L      | 6.90±00.50  | 05.10±00.9   | 2.1-8.2 mm/L  |
| Glucose, mm/L                | 2.16±00.09  | 02.50±00.1   | 2.0-4.8 mm/L* |

\*Normative values of biochemical parameters were taken from the L.K. Ernst federal science center for animal husbandry

Figure 1, which shows the acidity of the contents of the rumen, shows that this indicator was 6.94 units in the experimental group's cows and 7.26 units in the control group's. The shift of the indicator to the acidic side when feeding them with the supplement is probably associated with a more advanced stage of development of VFAs (by 44%), which is reflected in Fig. 2. Using CES led to an increase in the proportion of acetic acid in the rumen fluid and a decrease in the proportion of propionic and butyric acids.

The introduction of the CES in the amount of 150 g had a beneficial effect on the microflora of the rumen (Fig. 3). All animals at the time of the experiment were healthy and their biochemical blood parameters were within acceptable physiological norms (Table 1). Analyzing the state of nitrogen metabolism in the body of cows, it should be noted that the animals receiving CES were distinguished by an increased concentration of total protein (2.7% difference from the control group) and albumin (3.5% difference).

Given the antitoxic effects of CES components on the body, a drop in urea levels in the experimental group's cows indicates improved liver function.

The concentrations of glucose (which increased by 15.7% compared to the control group) and cholesterol (which decreased by 26% compared to the control group) were impacted by modifications in the body's carbohydrate-lipid metabolism in cows caused by CES, the investigated feed complex called. These facts indicate the positive effect of the fed complex on providing the body with energy and the intensity of metabolic processes. The animals under study had blood glucose levels that were both close to and within the lower reference values for cows. This fact is explained by the high consumption of glucose for the formation of milk fat at the beginning of lactation, which causes low values of the metabolite in the blood. In the observed group of test subjects, the concentration of glucose is higher than in the control, which is justified by the use of energy components in the diet. Table 2 displays the mineral composition of cow blood.

The data displayed indicates that the blood levels of phosphate and magnesium in the experimental animals rose. The biologically active substances of the coniferous extract, as well as glycerin, which is part of the supplement, can affect the metabolism intensity, including the deposition of calcium and phosphorus in the body. This ultimately results in shifts in the content of inorganic phosphorus in the blood serum during the study relative to the control group receiving a standard diet. With the exchange of calcium and phosphorus in the body of animals, the exchange of magnesium is associated with the metabolism of vitamin D. Magnesium is an important factor in the activation of oxidative phosphorylation enzymes, including ATPase and affects the processes of protein biosynthesis and beta-oxidation of fatty acids. It is associated with muscle function and is necessary to maintain the vital activity of cicatricial microflora and other physiological functions, which, when added to the cows' diet, has a positive effect on their metabolic processes.

The data presented in Table 3 demonstrates that the experimental group's cows had an 8.0% rise in lysis percentage when compared to the control group. The blood serum of calves receiving CES exhibited a 2.2% increase in bactericidal activity compared to the control group. Coniferous extract, a potent phytobiotic included in the CES, improved the cows' nonspecific immunity indicators.

The outcomes of the control milking demonstrated that the CES in the diet had a beneficial impact on the cows' dairy productivity. In contrast to the cows receiving the investigated complex, whose average daily milk yield was reduced by 1 kg, the average daily milk yield of the cows receiving a diet without the addition of CES declined by 2.2 kg one month after the experiment began (average daily ambient temperature: 33°C, relative humidity: 63%).

**Table 2:** Mineral content in the blood ( $M \pm m$ ,  $n = 5$ )

| Indicator        | Group      |              | Standard*       |
|------------------|------------|--------------|-----------------|
|                  | Control    | Experimental |                 |
| Calcium, mm/L    | 02.62±0.12 | 02.62±0.09   | 2.06-3.16 mm/L  |
| Phosphorus, mm/L | 01.73±0.16 | 02.10±0.30   | 1.13-2.91 mm/L  |
| Ca/P ratio       | 1.51       | 1.25         | 0.82-2.39       |
| Magnesium, mm/L  | 01.18±0.07 | 01.29±0.16   | 0.75-1.34 mm/L  |
| Iron, µm/L       | 31.10±2.40 | 28.40±4.10   | 12.9-37.1 µm/L* |

\*Normative values of biochemical parameters were taken from an L.K. Ernst federal science center for animal husbandry

**Table 3:** Nonspecific blood resistance indicators in the test subjects ( $M \pm m$ ,  $n = 5$ )

| Indicator  | Group        |              |
|------------|--------------|--------------|
|            | Experimental | Experimental |
| BSBA, %    | 81.4±4.5     | 83.2±2.5     |
| % of lysis | 19.4±3.7     | 21.1±1.3     |

**Table 4:** Economic efficiency of the use of CES in the diets of cows (per head)

| Indicator   | Group     |              |
|---|-----------|--------------|
|   | Control   | Experimental |
| Duration of the experiment, days  | 85        | 85           |
| On average during the experimental period                                   |           |              |
| Average daily milk yield, kg  | 14.66±0.2 | 16.26±0.4*   |
| % compared to the control group   | 100.0     | 110.9        |
| % fat   | 3.82±0.2  | 3.83±0.04    |
| % protein   | 2.99±0.02 | 3.01±0.02    |
| Number of somatic cells, thousand/cm <sup>3</sup>                           | 277±38    | 261±5.2      |
| Gross milk yield for the period, kg   | 1,246.1   | 1,382.1      |
| Milk fat yield, kg  | 47.60     | 52.93        |
| Gross milk yield of 3.4% milk, kg   | 1,400.0   | 1,557.0      |
| Selling price of 1 kg of milk of basic fat content and protein content, rub | 25.5      | 25.5         |
| Revenue from the sale of milk, rub  | 35,700-00 | 39,703-50    |
| Cost of CES   | -         | 1,785-00     |
| Conditional net income, rub   | -         | 2,218-50     |

On the 45<sup>th</sup> day of the experiment, milk yield decreased in cows of the control group by 1.2 kg and in the experimental group by 0.9 kg. By the time the experiment reached its 60<sup>th</sup> day, milk productivity had dropped by 0.9 and 0.3 kg, respectively.

The average daily milk yield in general for the experiment with natural fat content and after conversion to 3.4% fat content in cows of the experimental group was higher by 10.9 and 11.2%, respectively, compared with animals of the control group. The use of CES also had a positive effect on the composition of cows' milk, slightly increasing the fat and protein content. A positive fact of the influence of CES is a decrease in the number of somatic cells due to the bacteriostatic effect of the coniferous extract included in its composition.

The costs of concentrated feed for the production of 1 kg of milk in the rations of cows of the experimental group were lower by 10.1%, compared with analogs from the control group.

Table 4 shows data on the economic efficiency of the use of CES in the diets of cows.

Table 4 displays the data indicating that after 85 days of feeding cows with CES, we additionally received 157 kg of milk of 3.4% fat content from one animal, the cost of which will be 4,003.5 rubles. At the same time, over the entire period of the experiment, we spent 12.75 kg of CES (150 g/day) in the amount of 1,785 rubles (the cost of the supplement is 140 rubles per kg). It follows that for 85 days of the experiment, one cow received a conditional net income of 2,218.5 rubles.

## Discussion

Stress affects the energy balance (i.e. the difference between energy consumption and expenditure). Depending on stress, the energy balance may be increased (pregnancy, lactation, cold) or decreased (heat, social, immune, calving) (Collier *et al.*, 2017). To reduce heat production and increase heat loss during heat stress, the cow's feed intake decreases, and the respiratory rate increases. A respiratory rate of >60 breaths per minute is an indicator of heat stress in

lactating dairy cows (Collier *et al.*, 2017). Since lactating cows exposed to heat stress have reduced consumption of dry matter in feed and, consequently, they do not get enough energy, minerals, and vitamins (Min *et al.*, 2019). CES is a source of energy (propylene glycol, sugar) and vitamins A, C, B2, K, E and P (coniferous extract) (Korotkii *et al.*, 2018).

Other authors also observed a positive effect of feeding feed supplements containing glycerin, propylene glycol, and others on milk productivity without a negative impact on the metabolic status of animals (Girard *et al.*, 2016; Klebaniuk *et al.*, 2016). The immunomodulating feed ingredient Omnigen-AF being introduced into the diet of lactating cows led to a decrease in vaginal temperature and a lower number of somatic cells in milk. Feed consumption increased (by 7%) (Leiva *et al.*, 2017).

The rise in the overall protein content in cows that are given CES (Table 1) was associated with intensive metabolic processes and sufficient or excessive intake of protein with feed. The protein-metabolizing enzyme aspartate aminotransferase (AST) was found to exhibit a small increase in activity in experimental animals, which is acceptable with a high protein metabolism characteristic of new calves (Morgunova and Chusova, 2018). A high level of aminotransferase activity may also be associated with the effect of heat stress on the body (Kuzminova *et al.*, 2022). The end products of protein metabolism (creatinine, urea) did not exceed the norm, which indicates intense protein metabolism.

To increase the dairy productivity of animals, preserve productive health, and reduce the effects of heat stress in specialized livestock enterprises, we recommend using the CES in a dose of 150 g/head per day in feeding lactating cows.

## Conclusion

The study indicates that incorporating a CES composed of natural ingredients led to an 8.7% boost in Volatile Fatty Acid (VFA) formation and an increase in the overall count of mesophilic aerobic and facultative anaerobic microorganisms in the rumen.

The addition of the CES to the meals improved the lactating cows' metabolism of protein, fat, and carbohydrates, as evidenced by a 2.7% rise in the concentration of total protein, albumin by 3.5% and glucose by 15.7% with a decrease in urea content by 10.1%, creatinine by 15.7% and cholesterol by 26.0%. The blood serum's bactericidal activity increased by 2.2% and its lysis percentage increased by 8.8% as a result of the CES.

Future research should explore the long-term effects of coniferous energy supplements on dairy cow health, reproductive performance, and environmental sustainability. Additionally, investigating optimal dosage and formulation for various cattle breeds and climates would be valuable.

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## Author's Contributions

**Vladimir Zaitsev:** Proposed the main idea and drafted the manuscript.

**Vasily Korotkiy:** Contributed to the conceptualization and the theoretical development.

**Nadezhda Bogolyubova:** Secured funding, validated the analytical methods, performed the model derivation and data analysis.

**Lilia Zaitseva:** Managed data curation, participated in the theory development, conducted the model derivation and data analysis.

**Viktor Ryzhov:** Verified the analytical methods, wrote and revised the manuscript, carried out the model derivation and data analysis.

## Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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