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High yielding cows metabolism peculiarities under climate change conditions with the use of feed additive with protein protected from rumen degradation

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Article's History: Received: 25.04.2023 Revised: 25.08.2023 Accepted: 27.09.2023 **Abstract.** The research relevance is determined by the problem of thermal load on the body of dairy cows by increasing the content of protein protected from breakdown in the rumen. The research aims to determine the metabolism and level of delivery of various forms of protein and energy to the body of highly productive cows in the diets of cows in the second half of lactation under conditions of climate change. Research methods include zootechnical (assessment of feed intake, rationing, selection of

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groups, organisation of the experiment, determination of milk production), biochemical (determination of the content of basic nutrients in feed, milk quality indicators), statistical, and mathematical. The compensation of protein in the diet of high-yielding cows under climate change conditions was carried out using the protein feed additive TEP-mix with protein protected from rumen breakdown. It has been proven that its inclusion in the cow's diet can increase average daily milk production by 9.1% in the cold season (0-10°C) and by 28.0% in the summer (21-36.4°C). In addition, the fat and protein content in milk increased in cows receiving the high-protein feed additive TEP mix. It was found that the increase in protein nutrition of cows from 23.18% to 33.53% with the use of the feed additive TEP-mix with protein protected from scar breakdown ensures a reduction in the impact of climate change on the metabolism of dairy cows. Thus, it has been proved that the use of specific protein feed sources that regulate the level of non-cutting protein in the diet can be considered a factor in combating heat stress and increasing milk production of dairy cows throughout the physiological cycle of lactation, including in extreme conditions of high temperatures

Keywords: metabolism; dairy cows; heat stress; protected protein

INTRODUCTION

Studies of the physiology of cow nutrition show that animals exposed to a significant excess of thermal stress have a negative nitrogen balance mainly due to a decrease in feed intake, which makes this study relevant. O. Borshch (2021) noted that nitrogen deficiency can be compensated for by increasing the protein content in the dry matter of the diet. At the same time, such an increase is significantly limited by the inability of rumen microorganisms to quickly process soluble crude protein that has been supplied with dietary components to the cow's rumen into microbial protein, and the resulting excess nitrogen leads to its absorption into the bloodstream, which negatively affects metabolism, dry matter intake and milk production. This means that the solution to increasing the intake of protein in the body of a dairy cow lies not in the plane of physical quantity, but rather in the plane of dietary protein quality and, in particular, its rumen solubility. M. Gauly & S. Ammer (2020), A. Iqbal et al. (2021) and P. Skliarov et al. (2022) pointed out that most modern protein nutrition systems when determining the protein requirement of animals, do not proceed from the content of crude and digestible protein in the diet, but from the amount of protein that is broken down in the small intestine and is defined as the sum of the breakdown and non-breakdown protein fractions in the rumen. This approach to protein nutrition for high-yielding cows is based on providing the animal body with readily soluble nitrogen compounds in feed protein and non-protein nitrogen sources based on the proteolysis of microbial protein and feed protein.

J. Garner *et al.* (2022) noted that rumen degradable protein is a source of nitrogen for microorganisms that use it to synthesise their protein amino acids, which provide 50% to 90% of cows' amino acid requirements after absorption in the small intestine. With high milk production, the synthesis of milk protein from microbial amino acids accounts for only 40-50% of milk protein, the rest must be provided by non-hydrolysed rumen protein. J. Meneses *et al.* (2021) considered that this was largely impossible to achieve by feed selection.

Therefore, to protect protein from rumen degradation, feeds, especially high-protein feeds, are treated with various physical and chemical methods.

O. Borshch (2021) points out that at high and even average levels of cow milk production, protein synthesis by rumen microorganisms does not meet the animals' need for amino acids, while a simple increase in the protein content of feed (not considering protein breakdown) causes to excessive breakdown in the rumen and ultimately to its irrational use, while a high proportion of dietary proteins that are not broken down in the rumen but digested in the intestine improved the productivity of dairy cows even in adverse climatic conditions.

I. Suprun & Y. Kurylenko (2022) determined that determining the amino acid requirements for high-yielding cows in both the productive and dry periods requires indepth knowledge of the protein composition of feed and its role in rumen fermentation. Q. Shan et al. (2020) found that during lactation, the mammary glands require a significant number of amino acids, the metabolism of which is a rather complex process, amino acids can be converted into other amino acids or used for energy through oxidation, but most of the amino acids absorbed from the blood are used by the mammary glands for milk protein synthesis. Scientists (Van den Bossche et al., 2023) found that methionine intake improves milk production and antioxidant capacity, reduces lymphocyte apoptosis, promotes Bcl-2 gene expression in lymphocytes and suppresses the Bax gene. Moreover, methionine perfusion has been shown to increase milk protein synthesis.

It is practically impossible to regulate the supply of methionine and lysine from the soluble part of crude protein, as it is impossible to control the quantity and quality of rumen microorganisms to increase the synthesis of individual amino acids. That is why the issue of amino acid nutrition of dairy cows has recently been addressed by adding special feed additives to the diet, which use protected methionine. However, as noted above, an increase in the level of protein in the diet of cows should be accompanied by an increase in the intake of not only methionine but also other essential amino acids, because the use of protected methionine alone is not sufficient to improve the quality of protein nutrition. Given the research relevance, the research aims to determine the peculiarities of metabolism and delivery of various forms of protein and energy to the body in the diets of highly productive cows in the second half of lactation under different climatic conditions.

MATERIALS AND METHODS

The possibility of reducing the negative impact of high temperature on the metabolism and productivity of animals with the help of the protein feed additive TEPmix (Arnika Feed LLC, Ukraine) with a protein protected from rumen cleavage was investigated in the conditions of Pechenizke LLC, Chuhuiv district, Kharkiv region. The scientific and economic experiment was conducted during 2022 (January-September) on high-yielding cows of the Ukrainian Red and White dairy breed, considering productivity, with an average live weight of 550-600 kg in the second, third and fourth lactations. Control and experimental groups of 70 cows were formed. During the experiment (243 days), the control group was fed a basic diet with the addition of sunflower meal, and the experimental group - a basic diet with the addition of TEP-mix. The diets of both groups were balanced so that the concentration of the main nutrients was as similar as possible, with a difference of 1% to 3%. The difference was in the use of a traditional protein supplement in the control group - sunflower meal with a low degree of protein protection (15.2%) against rumen degradation, and in the experimental group – a high-energy protein supplement TEP mix with a high degree of protein protection (65.3%).

Before the experiment, feed samples were taken to determine their chemical composition and nutritional value in the laboratory for assessing the quality of feed and animal products of the Institute of Animal Husbandry of the National Academy of Agrarian Sciences of Ukraine. Considering the chemical composition and nutritional value of the feed, the main diet of the experimental animals of both groups was equally complete and balanced in terms of nutrition, the amount of organic and mineral substances and had no significant differences, except for the quality of protein nutrition, in particular, the level and ratio of protected protein, which was 10.35% higher than in the control group.

The diet of the control group of cows in the second half of lactation included 8.0 kg of corn silage, 12.0 kg of triticale + oats haylage, 1.0 kg of pea straw, 5 kg of beer pellet, and 7.6 kg of mixed fodder. This diet contained 17.66 kg of DM, 186.7 MJ of ME, and 2841 g of CP, including 2182 g of DM and 658 g of NDF, or 23.18% of the total crude protein. The diet of the experimental group of cows included 8.0 kg of corn silage, 12.0 kg of triticale + oats haylage, 1.0 kg of pea straw, 5 kg of beer pellets, 1.7 kg of TEP-mix, and 5.8 kg of mixed fodder (1.6 kg of sunflower meal was replaced by a high-energy protein supplement TEP-mix in the amount of 1.7 kg). The diet of the experimental group contained 17.50 kg of DM, 185.5 MJ of ME, 2836 g of CP, including 1885 g of DM and 951 g of NDF, or 33.53% of the total crude protein.

The daily feed intake of the experimental animals during the experimental feeding was almost complete – the residues were only 1 to 3% of the target. Housing conditions, feeding, and watering regimes, and microclimate parameters were the same between the groups of experimental animals. The cows were kept in a typical four-row barn in a tethered manner. Milking was carried out twice a day using a milk pipeline, and control milking was carried out using portable buckets and a milk meter. Feed distribution was carried out by mobile feeders, and manure removal was carried out by scraper conveyors. Watering was carried out by automatic drinkers. Cows were fed with complete feed mixtures twice a day. The research plan is shown in Table 1.

| Table 1. Research plan | | | | | | | | |
|------------------------|--------------------------|---------------------|---------|-----------------|--|--|--|--|
| Research stage | Research period, days | Air temperature, °C | Group | Amount heads | Feeding conditions | | | |
| | 83 | Up to +10 - | Control | 70 | Basic ration (BR) + sunflower meal (NRP-23.18%) | | | |
| I | | | Studied | 70 | Basic ration (BR) + TEP-mix supplement (NRP-33.53%) | | | |
| II | 60 | From +20 to +30 _ | Control | 70 | OP + sunflower meal (NRP-23.18%) | | | |
| | | | Studied | 70 | OR + TEP-mix additive (NRP-33.53%) | | | |
| 111 | 100 | More than +30 _ | Control | 15 | OP + sunflower meal (NRP-23.18%) | | | |
| | | | Studied | 15 | OR + TEP-mix additive (NRP-33.53%) | | | |

Source: compiled by the authors

During the experiment, the following parameters were determined: actual chemical composition and

nutritional value of feed; actual feed consumption – daily, by conducting control feeding during two adjacent

days, to determine the difference between the specified amount of feed and its residues in the context of each group; level of milk production of cows – monthly, by conducting control milking with subsequent selection of average milk samples to determine its quality. Milk was analysed for chemical composition, nutritional and energy value, and physical and technological properties using a Bentley-150 device, and the content of somatic cells in milk was determined using a Somacaunt 150 device using the flow cytometry method.

The economic efficiency of milk production with the use of various feed additives with protected protein in cattle diets was calculated by the difference between the cost of feed and sold products. The statistical processing of the research results was carried out using biometric methods to determine the level of probability of the difference (Baranovsky *et al.*, 2017).

All experimental studies were carried out by modern methodological approaches and in compliance with relevant requirements and standards, in particular, they meet the requirements of DSTU ISO/IEC 17025:2005 (2006). The animals were kept, and all manipulations were carried out by the Order of the Ministry of Health of Ukraine No. 416/20729 "On Approval of the Procedure for Conducting Animal Experiments in Research Institutions" (Law of Ukraine No. 249, 2012), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1986).

RESULTS AND DISCUSSION

It was found that an increase in the non-degradable protein fraction in cow diets by 10.35% in January-March at an average air temperature of +7°C increased the average milk yield of cows by 9.1% (Table 2). Thus, at the beginning of the experiment, cows in the control group had an average milk yield of 19.6 kg of milk (with an average fat content of 3.93% and protein content of 2.98%), and in the experimental group - 19.6 kg of milk (with an average fat content of 3.93% and protein content of 3.01%). This means that there was no difference between the average milk yield and fat in the experimental and control groups and minimal and unreliable difference in protein. During the 83 days of the first stage of the experiment, the daily productivity of cows in the control group increased by only 0.2 kg. In the experimental group, the daily productivity of cows increased by 1.80 kg (9.1%). At the same time, there was a tendency to increase both protein and fat in milk.

Table 2. Dynamics of milk production of experimental cows at the first stage of the experiment

| Indicator | Actual air temperature 0°C – +10°C | | |
|--|---------------------------------------|------------|-------------------------|
| | control | studied | +/- relative to control |
| Cows in the group, heads | 70 | 70 | - |
| Research length, days | 83 | 83 | - |
| Average daily natural milk yield at the beginning of the period, kg | 19.6±0.56 | 19.8±0.47 | +0.2 |
| Average daily natural milk yield at the end of the period, kg | 19.8±0.51 | 21.6±0.51 | +1.8 |
| Average mass fraction of fat in milk at the beginning of the period, $\%$ | 3.93±0.009 | 3.93±0.008 | 0 |
| Average mass fraction of fat in milk at the end of the period, % | 4.07±0.006 | 4.14±0.008 | +0.07 |
| Average mass fraction of protein in milk at the beginning of the period, % | 2.98±0.005 | 3.01±0.007 | +0.03 |
| Average mass fraction of protein in milk at the end of the period, % | 3.22±0.003 | 3.27±0.006 | +0.05 |

Source: compiled by the authors

In the second stage of the study, from 26 March to 26 May (60 days), with a gradual increase in the average air temperature to +21°C, the effect of experimental feeding became even more significant. Feeding a diet with a 10.35% higher degree of protection against protein breakdown in the rumen was found

to increase the milk yield of each cow in the experimental group by another 1.5 kg of milk, while in the control group, this figure decreased by 1.1 kg. On average, during the second stage of the study, the cows of the experimental group produced 4.4 kg more milk, or 23.5% more (Table 3).

Table 3. Dynamics of milk production of experimental cows at the second stage of the experiment

| Indicators | Actual air temperature +11°C – +20°C | | |
|--------------------------|---|---------|-------------------------|
| | control | studied | +/- relative to control |
| Cows in the group, heads | 70 | 70 | - |
| Research length, days | 60 | 60 | - |

| | | | Table 3, Continued |
|--|---|------------|-------------------------|
| Indicators | Actual air temperature +11°C - +20°C | | |
| | control | studied | +/- relative to control |
| Average daily natural milk yield at the beginning of the period, kg | 19.8±0.51 | 21.6±0.51 | +1.4 |
| Average daily natural milk yield at the end of the period, kg | 18.7±0.40 | 23.1±0.52 | +4.4 |
| Average mass fraction of fat in milk at the beginning of the period, $\%$ | 4.07±0.006 | 4.14±0.008 | +0.07 |
| Average mass fraction of fat in milk at the end of the period, $\%$ | 4.12±0.003 | 4.19±0.008 | +0.07 |
| Average mass fraction of protein in milk t the beginning of the period, % | 3.22±0.003 | 3.27±0.006 | +0.05 |
| Average mass fraction of protein in milk at the end of the period, % | 3.24±0.003 | 3.29±0.006 | +0.05 |
| Source, compiled by the authors | | | |

Source: compiled by the authors

The productive effect of the experimental feeding was even more pronounced at the third stage of the research when the air temperature rose to +24.5 °C - +36.4 °C. Thus, within 60 days of the experimental stage, the average milk yield of each cow increased by 1.3 kg of natural milk, while in the control group, this figure decreased by 2.3 kg (Fig. 1). The amount of fat and protein in

the milk of cows in the experimental group also increased by 0.14% and 0.06%, respectively, while in the control group, the fat content in milk increased by only 0.08% and the protein content decreased by 0.03% (Figs. 2-3). Thus, the productivity of cows in terms of basic fat content in the experimental group increased by 2.64 kg, and in the control group – decreased by 2.2 kg (Fig. 4).

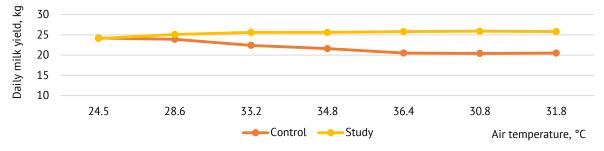
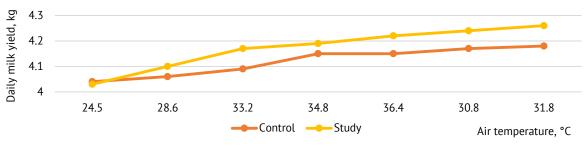


Figure 1. Average daily milk yield of natural milk



Source: compiled by the author

Figure 2. Mass fraction of fat in milk, %

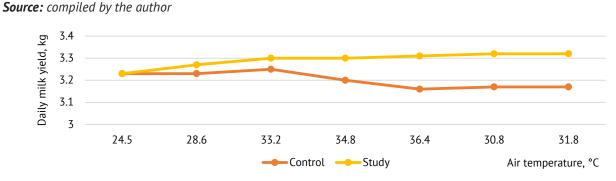


Figure 3. Mass fraction of protein in milk, %



Source: compiled by the author

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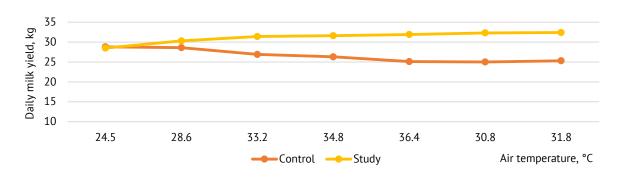


Figure 4. Average daily milk yield of basic fat content

Source: compiled by the author

The analysis of the results of the study by groups of cows shows that the amplitude of milk yield fluctuations in the control group animals was 9.2 kg (max-min), while in the experimental group animals it decreased to 6.0 kg. The amplitude of fat and protein fluctuations in the groups was almost the same. The obtained results prove that cows respond positively and significantly to changes in protein nutrition by increasing the level of non-degradable protein in the diet by adding TEP-mix to the basic diet.

The high difference in productivity between the control and experimental groups at the third stage of the study proved that the degree of resistance to heat stress also increases with increasing temperature. This fact can be explained by the stabilisation of metabolism in the body in general and a decrease in the intensity of protein fermentation in the rumen. During the stressful period of high ambient temperature, the organism of the experimental animals was better supplied not only with protein but also with energy. This is caused by the use of less energy for the digestion of non-digestible protein and, in addition, an increase in the flow of amino acids absorbed into the intestine contributes to the use of their share for energy purposes. That is why the energy supply of cows during heat stress with the use of TEP-mix in the diet was more significant, aimed at the synthesis of additional milk. At the same time, the increase in the amount of milk was not accompanied by losses in its quality (in terms of fat and protein mass fraction). Thus, it can be argued that the use of specific protein feed sources that regulate the level of non-degradable protein in the diet can be considered a factor in counteracting heat stress and increasing milk production of dairy cows throughout the physiological cycle of lactation, including in extreme conditions of high temperatures.

Stress caused in the body of ruminants by changes in climatic conditions hurts the general condition of the organism and, accordingly, the level of milk production and reproductive function. The solution to the problem of reducing the impact of heat stress on the body of animals requires the search for effective feed factors that can slow down the production of heat energy in the body. Thus, S. Gao *et al.* (2017) proved the effectiveness of using protein supplements of animal origin with a higher level of rumen-degradable protein fraction in the rearing of dairy calves. S. Salo (2018) confirms the importance of optimising both rumen-degradable and non-degradable protein content and synchronising protein with energy, which affects cow reproduction rates and environmental emissions. G. Conte *et al.* (2018) highlighted that feeding protected proteins reduces metabolic heat production and provides the right nutrient profile for high-yielding cows in early lactation.

Using experiments on dairy cows in climate-controlled chambers, S. Williams *et al.* (2021) showed that adding ingredients to the diet of lactating cows that increase energy density and reduce internal heat production can reduce the negative effects of hot weather on milk production. In recent years, an increasing number of studies have been devoted to the testing of feeds and feed additives with rumen-protected substances (Bypass products) in ruminant feeding, and they confirm our hypothesis that such products reduce the negative effects of heat stress on the animal body and contribute to increased milk production in the face of climate change.

For example, M. Lata & B. Mondal (2021) show that the use of feed additives with protected protein in feeding medium and high-performance dairy cattle increases dry matter intake and milk production by 10-15%, reduces milk cost and contributes to better live weight gain in young animals. J. Meneses *et al.* (2021) point out that the use of a rather expensive substance, protein, in the diet of dairy cows requires the calculation of the optimal ratio of degradable and non-degradable protein in the rumen. Based on the results of their research, they proposed the following ratio: 60% degradable protein and 40% non-degradable protein. They also suggest the use of supplements in the diet to improve rumen fermentation and digestibility.

Based on experiments on rationing cow diets with feeds with different rates of rumen decomposition, A. Shah *et al.* (2020) and J. Garner *et al.* (2022) argue that feeds with slowly decomposing starch, which reduces the amount of heat generated during digestion, reduce

the impact of heat stress. The most recent data on the use of Byrass products in dairy cow diets under heat stress was published by P. Roskopf *et al.* (2023). In their experiments on cows in mid-lactation, the replacement of fermentable energy (corn grain) in the rumen with non-fermentable energy (protected fat) improved milk yield and milk fat content, while the respiratory rate and body temperature of cows did not change. The authors emphasise the need to clarify the mechanism of increasing milk production in cows by using protected fat in feeding without improving heat balance under heat stress.

In the course of scientific and production testing, the high efficiency of using the high-protein additive TEP-Mix in dairy cattle feeding under conditions of climate change, in particular, critical air temperatures, has been proven, which has resulted in an increase in average daily milk production, fat and protein content in the milk of experimental cows in both cold and warm seasons.

CONCLUSIONS

The use of specific protein feed sources that regulate the level of non-degradable protein in the diet can be considered as a factor in counteracting heat stress and increasing milk production of dairy cows throughout the physiological cycle of lactation, including in extreme conditions of high temperatures. It has been proven that the inclusion of high-protein feed additives with a high degree of protection against rumen breakdown in the diet of cows can increase average daily milk production by 9.1% in the cold season (0-10°C) and by 28.0% in the summer (21-36.4°C). In addition, cows treated with the high-protein feed additive TEPmix increased the fat and protein content in milk. It has been determined that an increase of protein protected from rumen decomposition by 10.35% in the feed ration of dairy cattle helps to reduce the effect of heat stress on the animal body, which is reflected in an increase in milk production and improvement of milk quality. The high difference in productivity between the control and experimental groups at the third stage of the study proved that the degree of resistance to heat stress also increases with increasing temperature. This fact can be explained by the stabilisation of metabolism in the body in general and a decrease in the intensity of protein fermentation in the rumen.

During periods of high ambient temperature, when the body is exposed to heat stress, experimental animals fed a high-protein feed additive with a high degree of protection against rumen breakdown were more supplied with protein and energy. This is caused by lower energy consumption for digestion of non-digestible protein, and an increase in the flow of amino acids absorbed into the intestine helps to use a portion of them for energy purposes. That is why the energy supply of cows during heat stress with the use of TEPmix in the diet was more significant and aimed at the synthesis of additional milk. The prospect of further research is to test feed additives with protected protein against a wide range of climatic indicators in different climatic zones of Ukraine, under different conditions of keeping dairy cattle of different breeds, and different levels of milk production throughout lactation.

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CONFLICT OF INTEREST

None.

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Особливості метаболізму високопродуктивних корів в умовах кліматичних змін за використання кормової добавки із захищеним від розщеплення в рубці протеїном

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Анотація. Актуальність досліджень полягає у вирішенні проблеми термічного навантаження на організм молочних корів за рахунок збільшення вмісту захищеного від розщеплення в рубці протеїну. Мета роботи – визначити метаболізм та рівень доставки в організм високопродуктивних корів різних форм протеїну та енергії в раціонах корів другої половини лактації в умовах кліматичних змін. Методи дослідження – зоотехнічні (оцінка споживання кормів, складання раціонів, підбір груп, організація досліду, визначення молочної продуктивності), біохімічні (визначення вмісту основних поживних речовин у кормах, показників якості молока), статистичні, математичні. Компенсацію протеїну у раціоні високопродуктивних корів в умовах кліматичних змін здійснювали за допомогою білкової кормової добавки ТЕП-мікс із захищеним від розщеплення в рубці протеїном. Доведено, що включення її до раціону корів дає змогу збільшити середньодобову молочну продуктивність на 9,1 % у холодний період року (0-10 °C) та на 28,0 % влітку (21-36,4 °C). Також у корів, що отримували високобілкову кормову добавку ТЕП-мікс, підвищувався вміст жиру і білка у молоці. Встановлено, що підвищення протеїнового живлення корів з 23,18 % до 33,53 % за використання кормової добавки ТЕПмікс із захищеним від рубцевого розщеплення протеїном, забезпечує зменшення впливу кліматичних змін на метаболізм організму молочних корів. Таким чином, доведено, що використання специфічних білкових кормових джерел, що регулюють рівень нерозщеплюваного протеїну в раціоні, можна розглядати як фактор протидії тепловому стресу і підвищення молочної продуктивності дійних корів впродовж усього фізіологічного циклу лактації, у тому числі і в екстремальних умовах підвищених температур

Ключові слова: метаболізм; молочні корови; тепловий стрес; захищений протеїн