Comprehensive Analysis of the effects of heat stress on some blood parameters in dairy cows (Literature review)

A hőstressz hatásának átfogó elemzése néhány vérparaméter változására tejtermelő tehenekben (Irodalmi összefoglalás)

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SUMMARY

Heat stress in dairy cows represents one of the most significant challenges within the agricultural sector, particularly in dairy farming. Numerous studies have investigated its impact on blood parameters and overall body metabolism, yet the findings remain highly contradictory. Some research indicates that heat stress leads to elevated levels of blood markers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), β-hydroxybutyrate (BHB), and urea, while other studies report conflicting results, highlighting a lack of consistent patterns. The primary objective of this study is to synthesise and critically evaluate the effects of heat stress on selected blood parameters in dairy cows, with a focus on the robustness of existing studies. The inconsistencies in the literature emphasise the need for further research applying larger sample sizes and more rigorous experimental designs. It is imperative that future studies consider key influencing factors such as photoperiod, feeding regimens, and the timing of sample collection, as these variables can significantly affect outcomes. By addressing these limitations, researchers can contribute to a deeper understanding of how heat stress impacts blood parameters in dairy cows. Such insights are crucial for developing evidence-based strategies to mitigate the adverse effects of heat stress, ultimately improving the welfare and productivity of dairy herds.

Keywords: BHB, blood parameters, dairy cows, heat stress, urea

ÖSSZEFOGLALÁS

A tejtermelő teheneket érintő hőstressz az egyik legjelentősebb kihívás a mezőgazdaságban, különösen a tejtermelésben. Számos tanulmány vizsgálta a vérparaméterekre és a szervezet általános anyagcseréjére gyakorolt hatását, de az eredmények továbbra is erősen ellentmondásosak. Egyes kutatások azt mutatják, hogy a hőstressz a vérparaméterek, például az alanin-aminotranszferáz (ALT), aszpartát-aminotranszferáz (AST), β-hidroxi-butirát (BHB) és a karbamid megemelkedett szintjéhez vezet, míg más tanulmányok ellentmondó eredményekről számolnak be, rámutatva az egységes minták hiányára. Ennek a tanulmánynak az elsődleges célja, hogy összefoglalja és kritikusan értékelje a hőstressz hatását a tejtermelő tehenek kiválasztott vérparamétereire, a meglévő vizsgálatok megbízhatóságára összpontosítva. A szakirodalomban tapasztalható ellentmondások hangsúlyozzák a további kutatások szükségességét, nagyobb mintaméreteket és szigorúbb kísérleti terveket alkalmazva. Elengedhetetlen, hogy a jövőbeni tanulmányok figyelembe vegyék az olyan kulcsfontosságú befolyásoló tényezőket, mint a fotoperiódus, a takarmányozási rend és a mintagyűjtés időpontja, mivel ezek a változók jelentősen befolyásolhatják az eredményeket. E korlátok kezelésével a kutatók hozzájárulhatnak annak mélyebb megértéséhez, hogy a hőstressz hogyan befolyásolja a tejtermelő tehenek vérparamétereit. Ezek a felismerések kulcsfontosságúak a hőstressz káros hatásainak enyhítésére irányuló, bizonyítékokon irányuló stratégiák kidolgozásához, ami végső soron a tejtermelő állományok jólétének és termelékenységének javításához vezet.

Kulcsszavak: BHB, vérparaméterek, tejtermelő tehenek, hőstressz, karbamid

1. Introduction

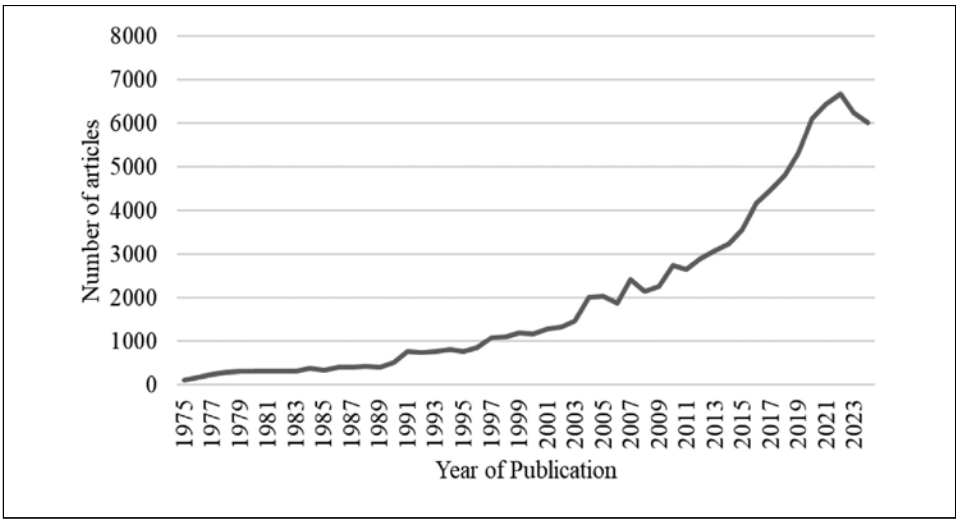
Global temperatures have experienced a 1.0°C increase since the 19th century. Furthermore, projections indicate that temperatures will continue to rise, with a predicted increase of 1.5°C between the years 2030 and 2052 (*Pradhan et al,* 2022). Heat stress, primarily caused by elevated ambient temperature and relative humidity can have an immediate impact on the productivity of farm animals (*Bernabucci et al,* 2010). As a result of the physiological and metabolic abnormalities caused by heat stress, dairy cows are prone to decreased reproductive and productive abilities, resulting in substantial financial losses for the dairy industry (*West,* 2003). When an animal experiences heat stress, it undergoes hyperthermia, which results in a reduction of its capacity for reproduction and growth to maintain thermal balance. In order for physiological processes and biochemical reactions to function optimally, it is crucial for animals to regulate their internal body temperature within the normal range (*Shearer and Beede,* 1990). For the body to remain in a state of physiological balance, blood is an essential indicator of any changes in metabolism (*Geneser,* 1985). The fluctuation of the components in blood is impacted by various factors that either impede or enhance the circulatory system (*Radkowska and Herbut,* 2014). Blood serves as the crucial component in preserving the physiological stability of the body (*homeostasis*), while hematological indicators are regarded as the key factor in showing an animal's ability to adapt to its environment and, as a result, its welfare (*Anderson et al,* 1999*; Sattar and Mirza,* 2009). Biochemical tests, on the other hand, provide insight into the internal state of the body, the functioning of various organs, including the kidneys and liver, and the progression of metabolic changes that occur within the body (*Scamell,* 2006). However, certain physiological and pathological situations have the potential to upset this homeostasis. As changes in biochemical and hematopoietic components are crucial indications of an animal's healthy or pathological status (*Ahmad et al,* 2003). The haematological values can be used to diagnose a variety of pathological and metabolic conditions that can negatively impact a cow's ability to reproduce and produce milk, costing dairy farmers significant amounts of money (*Dutta et al,* 1988*; Pyne and Maitra,* 1981). Confirmed by *Ahmad et al* (2003) as a change in biochemical and hematopoietic components are crucial indications of an animal's healthy or pathological status. However, numerous studies have investigated the impact of heat stress on blood parameters, yet the findings have often been inconsistent and contradictory. For example, studies on ALT levels have reported varying results, with some showing an increase *Srikandakumar and Johnson* (2004) and others indicating no changes *Hooda and Singh* (2010) under heat stress conditions. Similarly, research on BHB levels has revealed contrasting outcomes, with *Ronchi et al* (1999) and *Cartwright et al* (2023) documenting divergent responses to heat stress. These discrepancies highlight the complexity of physiological responses to heat stress and suggest that multiple factors, including species, breed, individual variability, and experimental conditions, may influence these outcomes. Given the wealth of research in this area, it is essential to synthesise existing findings to better understand the relationship between heat stress and blood parameters. Therefore, the primary objective of this study is to summarise and critically analyse the effects of heat stress on selected blood parameters published different authors in the literature. This approach aims to identify patterns, address inconsistencies, and provide a comprehensive overview of how heat stress influences metabolic and physiological processes as reflected in blood markers.

2. Blood biochemical parameters, blood tests

Heat stress has consistently posed a significant challenge for dairy cows, affecting their productivity, health, and overall well-being. In our research, an extensive analysis of studies was conducted examining the impact of heat stress on blood parameters. This investigation utilised the Web of Science database and applied Boolean operators, specifically the "AND" operator, to refine our search and focus on studies linking heat stress and blood parameters. As a result, a total of 99,951 publications were identified, including both research articles and review papers, with the earliest studies dating back to 1975. This comprehensive exploration highlights the growing body of literature on the subject, reflecting the increasing awareness of the detrimental effects of heat stress on dairy cattle. The number of studies has steadily increased over the years until 2024, reflecting the growing interest in climate change and the increase in global average temperatures over the years (Figure 1).

The focus on blood parameters is crucial because numerous physiological processes are regulated by blood biochemical components. Deficiencies or imbalances in these parameters can lead to impaired physiological functions and reduced performance, ultimately resulting in structural and systemic health issues (*McDowell,* 2003). The blood indicators that may indicate the nutrient state of the cow, include glucose, fructosamine, insulin, non-esterified fatty acid (NEFA), β-hydroxybutyric acid (BHBA), and cholesterol, as well as enzymes and

Figure 1. **The publication trend on the topic "heat stress and blood parameters" in dairy cows**



1. ábra: Tudományos cikkek tendenciája a „hőstressz és vérparaméterek" témában tejelő teheneknél proteins that disclose liver function (*Stengärde et al,* 2008). The levels of certain substances in the blood of cattle can provide insight into the animal's energy and protein metabolism as well as the functioning of certain organs especially when the cows are in negative energy balance caused by stressful factors such the heat stress (*El-Nouty et al,* 1990). Beta-hydroxybutyric acid, free fatty acids and glucose are markers of energy metabolism. Meanwhile, the presence of urea, total protein, and albumin reflects protein metabolism. To assess liver health, indicators such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase, total lipids (TL), triglycerides (TG), and glycogen and total bilirubin concentration are used (*Douglas et al,* 2007; *Petit et al,* 2007; *Stojević et al,* 2005). The kidney function is evaluated by measuring creatinine levels (*Stojević et al,* 2005). According to several studies, BHBA and the enzymes AST and ALT are among the most accurate markers of metabolic stress in blood serum (*Mordak and Nicpoń,* 2006; *Van Den Top et al,* 1995). However, research findings on the effects of heat stress on these markers are highly contradictory.

3. AST and ALT

The blood test, which evaluates liver function or liver damage, is one of the most popular blood tests. A straightforward blood test indicating liver functions measures the quantity of specific enzymes, which control key chemical reactions in the body. The aminotransferases are among the more used and most sensitive liver enzymes. Mainly are alanine aminotransferase (ALT or SGPT) and aspartate aminotransferase (AST or SGOT) (*Gaina et al,* 2020). These enzymes catalyse the transition of an amino radical from an amino acid to a keto acid. They are broadly distributed in animal tissues and are present in trace activities in all animals' serum as a result of regular tissue breakdown and subsequent enzyme release. Since these enzymes largely act within cells, a rise in their level in the serum frequently signifies cellular damage (*Coles,* 1967). In case of subclinical liver damage, increased AST activity in the serum is a sensitive indicator of liver injury (*Meyer and Harvey,* 1998). The normal range of AST and ALT levels in the blood of various species has been the subject of several studies According to (*Gaina et al*, 2020) standard level of AST in cows is 60-125 U/l and ALT is 6.9-35 U/l. Another study by *Whitaker et al* (2005) mentioned that for cows the reference blood activities of AST range from 58–100 U/dm^3, meanwhile for ALT range from 25 to 74 U/dm^3. Despite AST is a prevalent enzyme found in several tissues and organs, exhibiting notably elevated activity in the liver, and heightened blood AST activity serves as a marker for liver impairment (*Cincović et al,* 2011; *Zimmerman et al,* 1968). Several factors may affect the activities of AST and ALT in the blood of dairy cows. The study by *El-Ghoul et al* (2000) revealed that the AST activity significantly increases during the final week of pregnancy compared to the first week following birth. Conversely, during the seventh and eighth months of pregnancy and at the start of lactation, the activity of the ALT enzyme dramatically decreases, while that of the AST may fluctuate irregularly (*Tainturier et al,* 1984). The findings of *Stojević et al* (2005) indicate that milk production and the dry period significantly impact the levels of AST and ALT activities in the blood. Their study showed that the level of AST activity was higher at early lactation, decreased until reaching its lowest level during the dry period, while the level of ALT activity was higher in the middle of lactation and was also at its lowest during the dry period. This changing pattern of AST and ALT activities during lactation was explained by *Sakowski et al* (2012) as being linked to liver injury, which is typically caused by a poor energy balance during early lactation stages and intensifying protein-focused metabolic alterations, respectively. It is widely recognized that a variety of internal and external factors, including the animal's age, nutrition, season, management, and sex, can alter the normal range of these liver enzymes (*Doornenbal et al,* 1988; *Hawley and Peden,* 1982; *Quintela et al,* 2011; *Tibbo et al,* 2008). According to *Radkowska and Herbut* (2014), the system of cattle management may have an impact on the levels of AST and ALT in the blood. The study found that cows kept in pasture exhibited elevated levels of AST and ALT in comparison to those housed in barns. The authors attributed this difference to the higher content of nitrogen compounds in pasture forage, which may affect the liver's ability to process nitrogen, leading to increased serum levels of the AST and ALT enzymes in these cows. Heat-stressed cows may have a higher susceptibility to fatty liver disease due to compromised ATP synthesis, alterations in gluconeogenesis precursor availability, elevated oxidative stress, and an accumulation of hepatic lipids in the liver (*Skibiel et al,* 2018). *Berian et al* (2019) reported that heat stress significantly increases AST and cholesterol levels in the blood of cows, while ALT levels remain unaffected. These findings align with previous research. For example, *Hooda and Singh* (2010) observed an increase in AST levels with no changes in ALT when cows were exposed to a chamber temperature of 40°C. Similarly, *Mohapatra et al* (2021) and *Garcia et al* (2015) found elevated AST activities in heat-stressed cows, with no effect on ALT. In the case of ruminants, the liver cells do not exhibit high levels of ALT activity in contrast to AST, and the high level of ALT following liver injury, such as necrosis, is negligible as noted by *Forenbacher* (1993). However, contrasting results have been reported in some studies, where significant increases in both AST and ALT activities were observed under heat stress conditions. This was noted in the work of *Singh et al* (2012), *Kamal et al* (1989) and *Yadav et al* (2016). Furthermore *Kim et al* (2022), mention that the calves under heat stress show a high level of cortisol in their blood with increasing AST, and ALT levels of activities, where they are used as markers for liver-damaging (*Panteghini,* 1990). It is also important to note that many of these studies relied on relatively small sample sizes, which could influence the reliability of their findings. For example, *Mohapatra et al* (2021) included 24 animals per group in their study, *Singh et al* (2012) analysed only 6 animals per group, and *Kamal et al* (1989) examined just 8 animals. These limited sample sizes may reduce the statistical power of the results, highlighting the need for larger-scale studies to validate these findings and establish more robust conclusions. In other studies where they focused on seasonal effect and taking summer as heat stress period, *Marai et al* (1995) showed a seasonal variation in the function of the liver as the activities of AST and ALT were higher in summer. *Garcia et al* (2015) studied the relationship between heat stress and blood parameters, they found as a result the heat-stressed cows had higher levels of cholesterol, glucose, albumin, and total protein and higher AST activity compared to thermoneutral cows. Contrary, *Alameen and Abdelatif* (2012) found a decrease in the activities of AST in the blood of crossbreed dairy cows in summer compared to winter and the activity of ALT was higher in winter. In the study by *Srikandakumar and Johnson* (2004), the authors conducted a systematic investigation into the impact of heat stress on the blood parameters of three distinct bovine breeds, Holstein, Jersey and Australian Milking Zebu. The authors measured the subjects' blood parameters during both winter and summer months to account for the possible influence of seasonal variations on their findings. The results show a decrease in the level of AST observed in all three breeds under heat stress conditions. In summary, the majority of research confirms an increase in AST activity under heat stress conditions, while results for ALT remain highly contradictory. However, previous studies have not considered the potential effect of photoperiod, which may significantly influence enzyme and hormone activities in the body. This oversight raises concerns about the accuracy of studies conducted across different seasons. Although photoperiod and heat stress are closely related, accounting for photoperiod in research could help distinguish the specific effects of heat stress. Given the high number of conflicting results, a study with a larger sample size that accounts for the influence of photoperiod is essential to obtain more reliable and conclusive findings.

4. β-hydroxybutyrate (BHB)

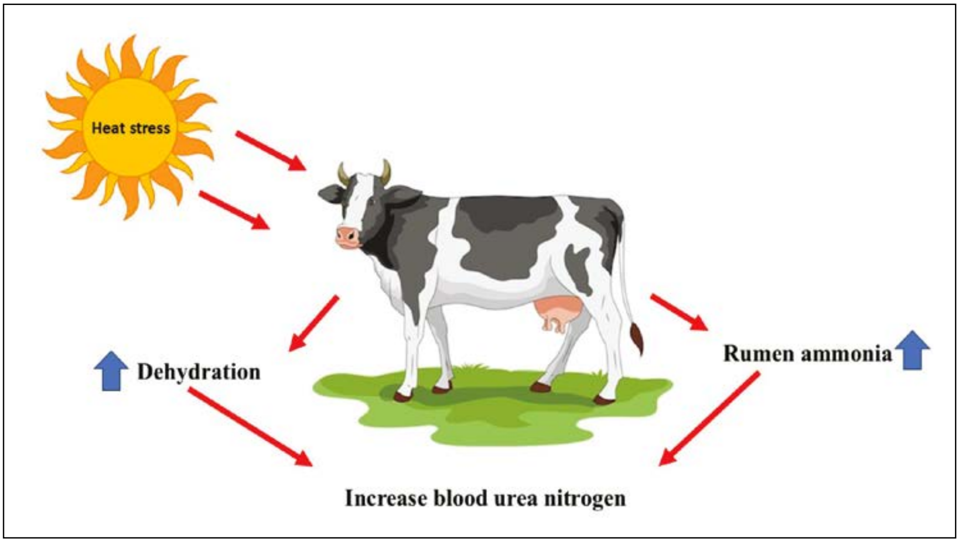
The β-hydroxybutyrate (BHB) is a crucial component of the primary ketone bodies found in dairy cows, accounting for approximately 70% of the total volume of ketone bodies present in this compound (*Guliński,* 2021). The BHB levels serve as an indicator of metabolic disturbances such as ketosis and fatty liver (*Song et al,* 2016). The increase in BHB in the blood is caused by the cow's inability to adapt to negative energy balance (NEB) and declining the metabolic sources of glycogenotic substances, which results in excessive mobilization of adipose reserves and the release of abnormal amounts of non-esterified fatty acids (NEFA) and ketone bodies, including BHB, into the bloodstream (*Benedet et al,* 2019). The primary source of BHB is the ketogenesis process, as well as the action of butyrate-producing bacteria in the rumen (*Puppel et al,* 2019). However, BHB is only partially metabolized in the liver of dairy cows, leading to an accumulation of BHB in the blood and liver, resulting in hyperketonemia (*Song et al,* 2016). The BHB and NEFA are two key diagnostic markers of ketosis, with normal BHB levels in healthy cows being below 1.2 mmol/L, elevated levels, exceeding 1.2 or even 1.5 mmol/L, may indicate the presence of clinical or subclinical disease (*Iwersen et al,* 2013). The increased concentration of BHB in the blood has a detrimental effect on the cow's immune system, overall health, and milk supply (*McArt et al,* 2013). *Mečionytė et al* (2022) investigated the impact of elevated BHB levels on cow performance and found that an increase in BHB concentration was associated with a delay in the first insemination day and an increase in the insemination rate. The results of recent studies have demonstrated that dairy cows subjected to a prolonged BHB infusion experienced modifications to both systemic and local mammary metabolism as well as an impact on the immunological response of the mammary gland (*Zarrin et al,* 2013). Furthermore, this BHB infusion resulted in a decrease in the concentrations of plasma glucose and glucagon (*Zarrin,* 2014). According to *Compton et al* (2015), the cows whose BHB levels exceeded 1.2 mmol/L within the first five days post-partum were at a 2.5 times greater risk of developing a uterine infection by the first month after calving. Additionally, those cows that had BHB levels exceeding 1.2 mmol/L at any point during the first five weeks post-partum had a 7% lower pregnancy rate within the first six weeks of breeding. Several factors affect the level of BHB in the blood of cows as according to *Rodriguez et al* (2021) the highest concentrations of blood BHB post-partum have been found at 7 days after calving in cows with increased body condition score (BCS) loss during the late dry season and greater parity. The level of BHB in the blood can also be affected by a cow's lactation stage. Cows that are in early lactation had higher levels of BHB compared to those in late lactation. Also, it increases over parities (*Ranaraja et al,* 2016). According to the research conducted by *Dhiman et al* (1991), it was established that there was a significant increase in the concentration of BHB in the blood during the initial four weeks of lactation. The increase was observed when the proportion of forage in the diet was raised from 38.2% to 98.2%. Further, the study conducted by *Hutjens and Schultz* (1971) also confirms that an increase in BHB concentration in the blood can be achieved by incorporating silages with high moisture content in the diet. These findings suggest that dietary modifications play a crucial role in determining the concentration of BHB in the blood. Focusing on the effect of the heat stress on the levels of BHB in dairy cows, *Turk et al* (2020) conducted a comparative study between two groups of cows; one group calved in the summer while the other group calved in autumn. The results of the study indicated that the autumn group had significantly higher levels of beta-hydroxybutyrate (BHB) in their blood. Additionally, *Garcia et al* (2015) conducted research to examine the levels of BHB in cows under both thermoneutral conditions and heat stress conditions. The findings of their study showed that there was no significant difference in the levels of BHB between the two conditions. *Moreira et al* (2015) concluded that heat stress does not significantly affect BHB levels in dairy cows, suggesting that the metabolic processes regulating BHB production and utilisation remain stable under environmental challenges. These findings are consistent with studies by *Cartwright et al* (2023), *Stefanska et al* (2024) and *Ellett et al* (2024), which highlighted the resilience of BHB levels under heat stress, reinforcing the idea that dairy cows may employ physiological mechanisms to maintain energy balance during periods of elevated temperatures. Collectively, this growing body of research indicates that BHB levels may not reliably reflect the impact of heat stress in dairy cows. In contrast, *Ronchi et al* (1999) observed elevated BHB levels in cattle subjected to heat stress, a finding corroborated by *Belić et al* (2011), who reported similar increases in cows exposed to high temperatures. They attributed these results to the fact that heat stress exacerbates NEB by reducing dry matter intake (DMI) while simultaneously increasing energy demands for milk production (*West,* 2003; *Xu et al,* 2015). When NEB becomes severe, fat mobilization intensifies, leading to elevated blood concentrations of (NEFA) and (BHB) (*Liu et al,* 2014). This metabolic imbalance increases the risk of ketosis and fatty liver disease, which can further compromise cow health and productivity. However, these contradictory results could stem from methodological limitations. For example, *Ronchi et al* (1999) studied only 16-month-old heifers, with five animals per group, while *Belić et al*

(2011) included just 12 cows in their experiment. In comparison, studies reporting no effect of heat stress on BHB levels generally employed larger sample sizes, lending greater reliability to their conclusions. Consequently, the evidence strongly suggests that heat stress does not have a substantial impact on BHB levels, with findings from well-designed studies supporting this conclusion, indicating that BHB levels may not serve as a reliable indicator of heat stress in dairy cows.

5. Blood urea nitrogen content

In the field of livestock production research, the measurement of blood urea nitrogen content in ruminants has become a conventional approach for evaluating the protein status of cattle (*Hammond,* 1983). According to *Kaneko* (2008) the reference values for urea concentration in the blood of cows is typically within the range of 1.11 to 1.67 mmol/L. According to *Wheelock et al* (2010), the production of plasma urea nitrogen can occur through two primary mechanisms: hepatic deamination of amino acids released from skeletal muscle, or an inadequate absorption of rumen ammonia into microbial protein. The increase in urea concentration in the blood can be a result of various physiological and pathological conditions such as acidosis, pregnancy toxaemia, urinary tract infections, diarrhoea, thirst, and dehydration (*Igbokwe,* 1993; *Nauriyal and Baxi,* 1978; *Radostits et al,* 2007; *Singh et al,* 1992). As documented by *Park et al* (2010), the rise in blood urea nitrogen levels has the potential to augment the danger of lipidmobilization. Furthermore, elevated plasma urea concentrations signify increased liver ammonia detoxification (*Law et al,* 2009). There are several determinants that can influence the concentration of urea in the blood, including the degradation of rumen, the quantity and solubility of dietary carbohydrates, the degradation of muscle tissue, the renal and hepatic function, the quantity of amino acids in the diet, and the protein intake (*Van Saun,* 1997). The correlation between dietary intake and urea concentration has been established, and as observed in a study by *Folman et al* (1981), an increase in nitrogen in the diet of lactating dairy cows leads to a proportionate increase in blood urea nitrogen levels. However, consuming a diet that is excessively rich in protein (>16%) is not recommended as it may result in heightened excretion of urea and negatively impact energy balance, as noted in a study by (*Van Saun,* 1997). Multiple studies have indicated that heat stress significantly impacts the levels of urea in the blood of cows. For instance, *Gao et al* (2017) observed heightened blood urea nitrogen levels in multiparous cows exposed to heat stress. Similarly, *Wheelock et al* (2010) reported an increase in urea levels in a group of cows subjected to heat stress. *Garner et al* (2017) noted that short-term heat stress led to an increase in plasma non-esterified fatty acids (NEFA) and urea, findings that were also supported by *Kim et al* (2022) and *Roths et al* (2023). In contrast, *Alameen and Abdelatif (2012)* found decreased blood urea levels in crossbred dairy cows during summer compared to winter. Similarly, *Srikandakumar and Johnson* (2004) and *Valencia et al* (2024) reported a decrease in blood urea levels in cows under heat stress. However, these studies had small sample sizes and lacked information on the feed provided to the cows. In conclusion, studies that found heat stress increases blood urea nitrogen levels tend to have clearer methodologies compared to others. The increase

Figure 2. **Factors affecting blood urea nitrogen in cows**



2. ábra: A tehenek vér karbamid-nitrogéntartalmát befolyásoló tényezők

in blood urea nitrogen levels during heat stress can be explained, on the one hand, by dehydration caused by elevated temperature, which concentrates urea in the blood (*Tshuma et al,* 2023). On the other hand, heat stress often reduces rumen motility and microbial efficiency, impairing microbial protein synthesis and increasing ammonia absorption from the rumen (Fig. 2). This ammonia is then converted into urea in the liver (*Baumgard and Rhoads,* 2013; *Cowley et al,* 2015; *Zhao et al,* 2019).

6. Conclusion

The relationship between heat stress and its impact on various blood parameters in dairy cows remains a subject of debate. The existing literature is inconsistent, with some studies suggesting that hot weather conditions can lead to increased levels of AST, ALT, BHB, and urea, while others report the opposite. Based on current findings, AST and urea appear to be negatively affected by heat stress and could potentially serve as indicators of heat stress in dairy cows. Conversely, evidence strongly suggests that heat stress does not have a significant impact on BHB levels. Findings from well-designed studies indicate that BHB levels may not be reliable indicators of heat stress in dairy cows. For ALT, however, further research is needed to draw clear conclusions. This disparity underscores the necessity for additional studies with larger sample sizes and robust study designs that account for critical factors such as photoperiod, feed, and sampling time, as these variables can influence results. Such efforts are essential to develop a more comprehensive understanding of the effects of heat stress fluctuations on these blood parameters.

7. References

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