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Effects of Betaine Supplementation on Heat Stress-associated Blood Metabolites, Antioxidant Profiles and Milk Production in Dairy Cows

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Abstract

THIS study aimed to assess the impact of betaine supplementation on blood biomarkers and metabolism in dairy cows exposed to humid, hot conditions. Thirty lactating cows from an Egyptian industrial dairy farm were divided into two groups: a control group receiving no betaine (n=15) and a supplemented group (80g betaine/day for four weeks, n=15). Blood samples were collected at three-time points: baseline zero week (0w), two weeks (2w), and four weeks (4w). The levels of cortisol, superoxide dismutase (SOD), malondialdehyde (MDA), albumin, total protein, insulin, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, non-esterified fatty acids (NEFA), and beta-hydroxybutyric acid (BHBA) were measured and compared between the groups using linear t-test and across time points using repeated measure ANOVA. Additionally, milk production was recorded throughout the study period. The results indicated that cortisol levels were significantly lower in the betaine group at 2w and 4w compared to the control group. Urea and ALT levels were consistently more down in the betaine group at all time points. BHBA and NEFA levels showed significant decreases in the betaine group at 4w. SOD level was notably higher in the betaine group at 2w and 4w. Finally, milk yield was improved in the betaine-supplemented cows than in the control group. In conclusion, supplementing dairy cows with betaine positively influences blood metabolites and antioxidant profiles, improving homeostatic response to heat stress and increasing milk production.

Keywords: Heat stress, Blood parameters, Dairy cattle, Betaine.

Introduction

Climate change presents a significant challenge for humanity, leading to more frequent heat stress episodes in various regions [1]. As global warming escalates, heat stress episodes are becoming increasingly systematic and widespread across multiple areas [2]. Dairy cows are particularly susceptible to heat stress, which occurs when a combination of external conditions raises the effective ecological temperatures above the animal's "thermoneutral" zone [3]. Recent studies have emphasized maintaining an optimal environmental range for cattle, typically between 5 and 25 °C. However, when the temperature humidity index (THI) surpasses 72 units, the intake of dry matter and milk yield in lactating dairy cows tend to decline significantly [1, 4-9].

Heat stress can significantly affect animal homeostasis, and researchers have utilized physiological variables, such as hormone levels, body temperature, and respiratory rate, to analyze these effects [10]. Studies have demonstrated several negative consequences of heat stress on food-producing animals [1, 4, 11, 12]. Heat stress has been shown to cause a decrease in milk production and have detrimental economic implications for the dairy industry. Additionally, heat stress increases the risks of metabolic-related health issues, such as rumen acidosis mortality. Furthermore, sluggish heifer growth is another consequence of heat stress. Lastly, heat stress can lead to diminished reproductive effectiveness, irregular estrous cycles, reduced conception rates, and reduced milk production in dairy cows [13] [14].In response to heat stress, dairy

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cows employ self-adaptation mechanisms to mitigate the effects [15]. These mechanisms include reducing daily dry matter intake, suppressing rumen fermentation activities, minimizing physical exertion, decreasing milk yield, and altering the composition of milk components such as fats, proteins, and lactose [15].

Therefore, understanding the physiological impacts of heat stress on animals is crucial. By studying body temperature, respiratory rate, and hormone levels, researchers can gain valuable insights into the effect of heat stress on animal health, productivity, and general well-being. Additionally, implementing effective interventions to mitigate the adverse effects of heat stress is crucial for maintaining the well-being and productivity of dairy cattle [1, 16].

Betaine, known as trimethyl glycine or betaine anhydrous, plays an essential role in homocysteine synthesis by providing methyl groups necessary for methionine production [17]. With its multiple actions, betaine offers potential benefits in mitigating heat stress and enhancing milk production in lactating cows [18-20]. The transport of betaine across cell membranes is facilitated by a sodium-coupled transporter, which is involved in hyper-osmotic stress responses [21]. Due to its polar regions and neutral charge, betaine can retain intracellular water molecules despite concentration gradients [22]. As observed in pigs, this osmolyte function of betaine reduces the energy requirements for ion pumping, thus minimizing basal heat production in animals [23, 24].

Therefore, this study aimed to evaluate the impact of betaine supplementation on blood variables and metabolism in lactating dairy cows exposed to hot and humid conditions.

Material and Methods

Ethics approval

This study was conducted according to the research ethics approved by the research committee of the College of Veterinary Medicine, Kafr-Elsheikh University, Kafr El-Shaikh, Egypt (KFS-IACUC/95/2019).

Animals, Diets, and betaine supplement

Healthy Holstein lactating cows (n=30) were selectively chosen from a commercial dairy farm in Egypt during the hot, humid period (between August and September 2019). The cow selection criteria included matched parity (1st and 2nd lactations) and thorough health assessment through detailed physical examinations. The cows were housed in tie stalls following the farm standard protocol for milk and feed. They were randomly allocated into two groups: the control group (receiving no betaine, n=15) and the betaine group (supplied with 80 g betaine/day for 4w, n=15). In this study, we selected this high dosage of betaine to explore its potential impact on the observed parameters, considering previous research findings [25, 26]. In a powdered form, the betaine supplement was well mixed with 50 ml of cane molasses to ensure thorough integration of all betaine particles. The blend was applied as a top dressing on the feed to enhance their intake. Also, to augment the bioavailability of the given betaine, it was divided into two halves (40 g each) and administered at two separate times (with morning and evening diets).

On the other hand, the control group was given the same amount of cane molasses using the same technique used in the betaine group but without any betaine. The cows were fed a total mixed ration (TMR) thrice daily at 7:30 am, 2:30 pm, and 9:30 pm. The amount of the TMR was calculated for each cow based on their body weight, and it consisted primarily of alfalfa hay, corn silage, and corn grain, formulated to meet or exceed the nutritional requirements based on the National Research Council criteria (Table S1, NRC, 2001). In addition, the cows had unrestricted access to fresh and clean water. The experiment's on-farm personnel and researchers regularly monitored the cows' health. Furthermore, the milk yield was obtained and recorded three times throughout the day: in the morning, afternoon, and evening.

The cows were subjected to blood sampling at three time points: baseline zero weeks (0w) at the comfort zone (temperature: 23±1 °C and relative humidity: 40±5%), two weeks (2w), and four weeks (4w) (temperature: 41±1 °C and relative humidity: 45±5%). Throughout the study, it was observed that the barn's average temperature humidity index exceeded 72, with the highest recorded index reaching 88.7. These findings indicate that the cows experienced heat stress during the experiment (Figure S1).

Ambient conditions

To evaluate the environmental conditions inside the cowshed, the ambient temperatures and relative humidity were measured 1.5 m above the floor. The Elitech US BT3 Digital Thermometer - Hygrometer, USA, was used to record the air temperature and relative humidity at various locations throughout the experiment. This device has an accuracy of ±0.35 °C. Daily records were maintained to track the highest and lowest temperatures. The Temperature Humidity Index (THI) was calculated using the formula employed by Landi, Maggiolino [27]. The maximum THI value was determined based on the highest temperature recorded within 24 hours. Simultaneously, the minimum relative humidity and minimum THI were calculated using the lowest temperature and maximum relative humidity [12].

Blood sampling and analysis

Blood samples were collected from the tail vein in a sterile condition using 10 ml vacutainers. Plasma was collected on K3-EDTA vacutainers, while serum was collected using plain vacutainers and left to clot at a refrigerated temperature for 10 minutes before centrifugation. Plasma tubes were immediately centrifuged at 1,500 × g for 10 minutes, while serum tubes underwent the same centrifugation process after clotting [28]. All plasma and serum aliquots were stored at -20 °C until further analysis.

The levels of cortisol (g/dl), glucose (mg/dl), total protein (g/dl), albumin (g/dl), insulin (uU/ml), urea (mg/dl), globulin (g/dl), Alanine Aminotransferase (ALT) (U/L), and Aspartate Aminotransferase (AST) (U/L), SOD (U/ml), MDA (nmol/ml) were measured using a multi-parameter analyzer (Seracal, Gesan Production Kit, Campobello di Mazara, Trapani, Italy). The analyzer was calibrated with standard assay kits before each analytical session. Internal accuracy was verified using two multi-parameter control sera (Seracontrol N and Seracontrol P, Gesan Production Kit, Campobello di Mazara, Trapani, Italy). Each sample was analyzed three times, and the raw dataset recorded the arithmetic mean of the three measurements for each parameter [29]. The NEFA level was determined using diagnostic kits on photometric systems (DIA Lab, Austria), while the BHBA level was analyzed using diagnostic kits from POINTE Scientific INC, USA."

Statistical Analysis

The reported data were presented as mean ± standard error. The normality of the data was assessed using the Shapiro-Wilk test, while the homogeneity of variances was tested using the Brown-Forsythe test. All variables exhibited normal distribution and nearly equal variances. A linear t-test was employed to compare the tested parameters between the two groups, while a repeated-measure ANOVA was utilized to identify differences across various time points. A significance level of $P<0.05$ was set to determine statistical significance. The statistical software SPSS version 22 was used to conduct these analyses.

Results

Betaine supplementation significantly reduced rectal temperature and respiratory rate compared to the control group, as depicted in Figs. 1A & 1B. No significant differences were observed in the total protein levels (Fig. 2A) and albumin (Fig. 2B) between the two groups at 0w, 2w, and 4w. However, in the betaine group, there were significantly lower levels of urea at 0w, 2w, and 4w compared to the control group (Fig. 2C). Similarly, ALT levels were significantly lower in the betaine group at 0w, 2w, and 4w compared to the control (Fig. 2D). The AST levels did not exhibit significant variances between the groups at 0w and 2w. However, at 4w, the betaine group demonstrated significantly lower AST levels than the control group (Fig. 2E).

The cortisol level between both groups was not significantly different at 0w. Nevertheless, at 2w and 4w, the betaine group showed a significantly lower cortisol level compared to the control group (Fig. 3A). There were no notable differences in glucose levels between the groups at 0w and 2w (Figure 3B). However, at 4w, the betaine group exhibited a significant increase in glucose levels compared to the control group (Fig. 3B). Insulin levels showed no significant differences between the groups at 0w, 2w, and 4w (Fig. 3C). At 0w, there were no significant differences in SOD levels between the groups (Figure 3D). However, at 2w and 4w, the betaine group demonstrated significantly higher SOD levels compared to the control group (Fig. 3D). MDA levels did not differ substantially between the groups at 0w (Fig. 3E). However, at 2w and 4w, the betaine group displayed considerably lower MDA levels than the control group (Fig. 3E).

At 0w and 2w, there were no notable variations in the BHBA levels between the two groups (Fig. 4A). However, at 4w, the betaine group exhibited a significant reduction in BHBA levels compared to the control group (Fig. 4A). Similarly, there were no significant differences in NEFA levels between the two groups at 0w and 2w (Fig. 4B). However, at 4w, the betaine group displayed significantly lower NEFA levels than the control group (Fig. 4B). The milk yield for the dairy facility increased to 28.87 kg/day and 29.16 kg/day in the betaine group, whereas the control group had yields of 27.70 kg/day and 28.1 kg/day at week two and week 4, respectively (Table 1).

Discussion

Cortisol is an important hormone released by the adrenal glands that helps the body respond to heat stress[30]. This hormone serves multiple functions in mitigating the effects of heat stress. Under heat stress conditions, cortisol stimulates the liver to produce glucose, improving blood glucose levels. In addition to promoting gluconeogenesis, cortisol supports lipolysis in adipose tissue[31]. This mechanism aids in fulfilling the heightened energy needs of the body and sustaining energy equilibrium during demanding thermal circumstances [32].

Furthermore, cortisol inhibits glucose uptake in skeletal muscles and adipose tissues. This suppression of glucose uptake preserves glucose for vital organs, such as the brain, during heat stress. By reducing glucose uptake in skeletal muscles and fatty tissues, cortisol ensures that glucose is prioritized for essential functions and prevents excessive glucose utilization in non-essential tissues.

Based on our observations, using betaine affects cows' blood cortisol levels. Incorporating betaine into the diet was observed to decrease cortisol levels in cows experiencing heat stress, which is in line with the findings of previous studies [33, 34]. Furthermore, research conducted by Lakhani, Kumar [35] examined the impact of betaine supplementation on cortisol levels during heat stress. The results revealed that the treatment group displayed lower cortisol levels (9.82 ± 0.25 ng/ml) than the control group (10.36 ± 0.16 ng/ml). These findings are consistent with a study conducted by Deshpande et al. (2020), which reported a significantly lower mean value (4.92 ± 0.48 ng/ml) in the treated group compared to the control group (7.18 ± 0.455 ng/ml).

Moreover, even after ceasing the betaine supplementation, the group treated with betaine displayed lower cortisol levels than the control group ($p < 0.05$). However, according to the study by Zhang, Ying [36], incorporating betaine into the feed at doses of 0, 10, 15, or 20 g/day did not yield noticeable effects on the plasma cortisol levels of dairy cows experiencing heat stress. Nevertheless, they found that supplementing heat-stressed cows with a daily dosage of 15 mg of betaine helped stabilize their endocrine hormone levels [36]. The primary mechanism through which corticosterone exerts its biological effects is activating the growth hormone. The growth hormone acts as a transcription factor, interacting with the gene regulatory element in the promoter region of its target genes [37].

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In the present study, glucose concentrations tend to decrease significantly in high ambient temperatures, consistent with other studies [38, 39]. This decrease can be attributed to higher blood insulin activity or lower feed intake [40]. Non-esterified fatty acids (NEFA) in the serum and plasma are crucial metabolic regulators [41]. Dairy cows experiencing heat stress often exhibit low NEFA concentrations to promote glucose utilization and reduce metabolic heat generation [42, 43]. However, Shehab-El-Deen, Leroy [44] observed an increase in NEFA levels in dairy cows during the summer compared to winter, suggesting the animals' efforts to maintain energy balance. The concentration of NEFA in the blood can provide insights into energy balance and mobilization [25, 45, 46]. Our study found that using betaine resulted in decreased NEFA levels compared to the control group, indicating enhanced energy availability. This finding aligns with the investigation conducted by Shah, Ma [47], which demonstrated that betaine supplementation reduces NEFA levels in cows experiencing heat stress. However, a study by Davidson, Hopkins [17] examined the plasma NEFA and BHBA levels in lactating Holstein cows that were provided with a total mixed ration supplement containing 45 g of rumen-protected betaine per day despite the diet being low in methionine. They found that dairy cows experience increased release of endogenous free radicals during thermal stress, reducing their capacity for antioxidants. This is reflected in the serum's antioxidant enzymatic activities, showing a decline in superoxide dismutase (SOD) and an increase in malondialdehyde (MDA) levels in heat-stressed cows [47-49].

In our current study, we observed that betaine supplementation improved the activity of superoxide dismutase (SOD) and reduced malondialdehyde (MDA) levels compared to the control group. Heat stress can lead to an increased production of endogenous free radicals and reduced antioxidant capacity [36, 50, 51]. These changes are reflected in the activity of antioxidant enzymes in the serum, including a significant increase in SOD activity and a decrease in MDA activity.

In this study, we observed that betaine supplementation improved the activity of ALT and AST compared to the control group. Previous studies have reported a significant increase in ALT and AST levels during the hot and dry seasons [52-54]. In the present study, the rise in ALT and AST levels in the control group may be attributed to increased gluconeogenesis or some negative impact of heat stress on liver function [52, 54, 55]. The activity of these enzymes is believed to be associated with the animals' greater capacity to adapt to heat stress [56].

During hot, humid, and dry seasons, the body's increased metabolic needs can lead to protein catabolism, resulting in elevated urea levels [55, 57]. In the present study, increased urea production in the control group can indicate dehydration [58]. Similarly, studies have shown urea levels tend to rise during the summer or other hot months [54, 59]. Serum total protein and albumin concentrations are often used as indicators of nutritional health [60]. In our study, no significant differences were observed in the total protein and albumin levels between the groups at 0w, 2w, and 4w, which is consistent with the findings of Attia [60] and Sejian, Srivastava and Varshney [61].

Thermal stress can impact milk composition and decrease milk production, particularly in high-production dairy cows, as highlighted by Ravagnolo and Misztal [62] [63, 64].

Several factors contribute to these effects. Firstly, heat stress causes a reduction in feed intake among dairy cows, resulting in decreased digestion and absorption of nutrients and a lower supply of nutrients to the udder. Approximately 50% of the decline in milk production is estimated to be attributed to reduced feed consumption by dairy cows

[65]. Heat stress also contributes to metabolic issues in dairy cows. The hypothalamus, which regulates the release of endocrine hormones, plays a role in mitigating heat stress in cows. It reduces the release of T3 and T4 hormones, reducing the cows' appetite and energy usage. At the same time, the hypothalamus inhibits the production of progesterone and prolactin, leading to a decrease in milk secretion and overall milk production. In this study, betaine supplementation increased milk yield to 28.87 and 29.16 kg/day compared to 27.70 kg/day and 28.1 in the control group at week two and week 4, respectively. In line with our results, previous research has shown that betaine supplementation can increase animal milk production. For instance, studies have demonstrated that supplementing Holstein dairy cows with 100 or 150 g of anhydrous betaine during early lactation significantly increased milk yield [63, 64]. Another study by Zhang, Ying [36] found that adding 15 and 20 g/day of betaine resulted in higher milk output than the control group.

Conclusions

In conclusion, the impact of thermal stress on dairy cows, particularly concerning milk production and composition, is primarily demonstrated. Heat stress can lead to decreased milk yield, attributed to reduce feed consumption and impaired nutrient absorption. However, previous research has indicated that the supplementation of betaine can help counteract the adverse effects of heat stress on milk production. Previous studies have also shown that adding betaine to the dairy cow's diet increases milk yield. This suggests that incorporating betaine supplementation could be a valuable approach to enhance milk production in heat-stressed dairy cows. Further research and practical implementation of such interventions can enhance the resilience and productivity of dairy cows facing thermal stress.

Acknowledgement

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Conflict of Interest

The authors declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical approve

This study was conducted according to the research ethics approved by the committee on research of the College of Veterinary Medicine, Kafr-Elsheikh University, Kafr El-Shaikh, Egypt (KFS-IACUC/95/2019).

Funding statement

Not applicable.

Authors contribution

Conceptualization, W.E., M.N., M.A, M.Z., and R.S.; validation, N.G., A.S and R.S.; investigation, M.D., M.Z., W.E. and M.A.; resources, M.A. and W.E.; writing—original draft preparation, W.E., M.Z, M.D., N.G., and M.A.; writing—review and editing, R.S., M.Z., and M.A.; supervision, M.N., N.G., M.A., and M.Z.; project administration, M.N, M.A. and R.S ; All authors have read and agreed to the published version of the manuscript.

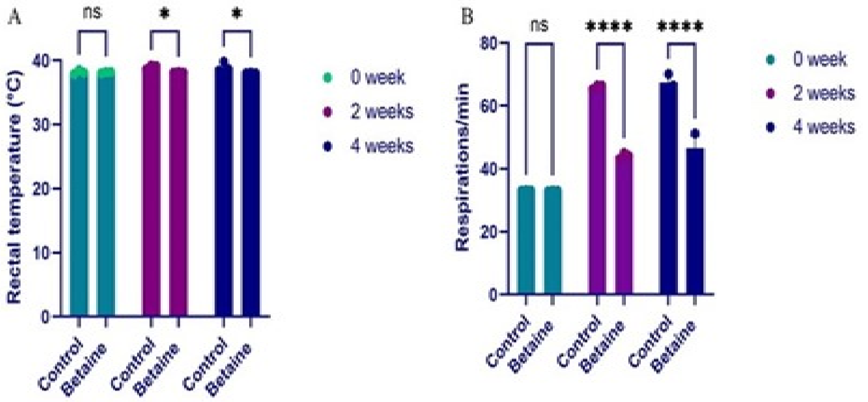

Fig. 1. The values of rectal temperature (A) and the respiratory rate (B) in control and betaine groups at 0w, 2w, and 4w. Results are reported as means ± standard error. ns: non-significant, *p < 0.05, ****p < 0.0001.

TABLE 1. The impact of betaine supplementation on both body weight and milk yield.

Parameters	**2week**		**4week**	
	Control	**Betaine**	**Control**	**Betaine**
Milk yield (kg/day)	27.70±2.1^{b}	28.87±3.2^{b}	28.1±1.5^{b}	29.16±3.1^{a}
Body weight, kg	735±12.1^{b}	743±10.2^{ab}	712±15.1^{c}	750±14.4^{a}

Results are reported as means ± standard error. Mean with different letters are significantly different at $p < 0.05$.

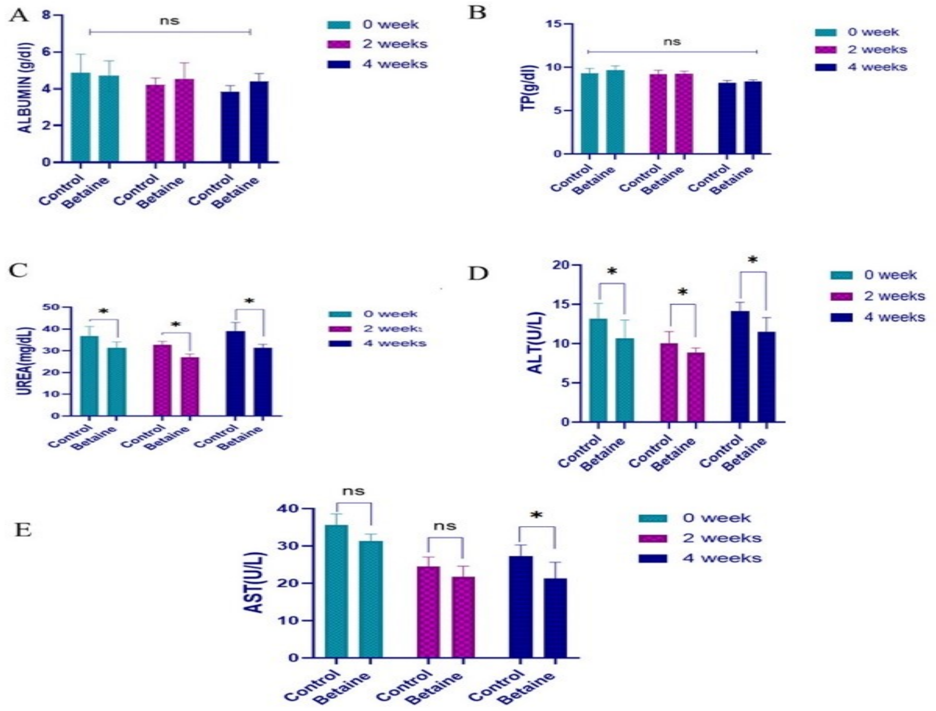

Fig. 2. The levels of albumin (A), total protein (TP) (B), urea (C), ALT (D), and AST (E) levels in in control and betaine groups at 0w, 2w, and 4w. Results are reported as means ± standard error. ns: non-significant, *p < 0.05, **p < 0.01.

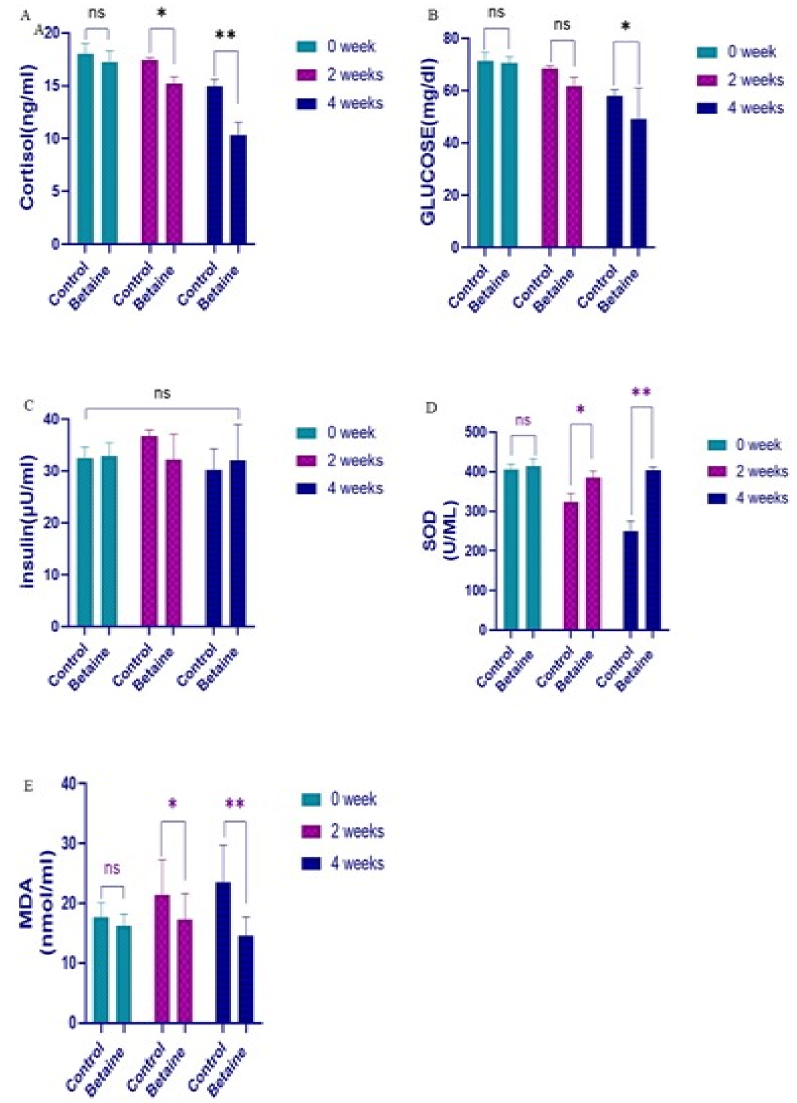

Fig. 3. The levels of cortisol (A), glucose (B), insulin (C), SOD (D), and MDA (E) in in control and betaine groups at 0w, 2w, and 4w. Results are reported as means ± standard error. ns: non-significant, *p < 0.05, **p < 0.01.

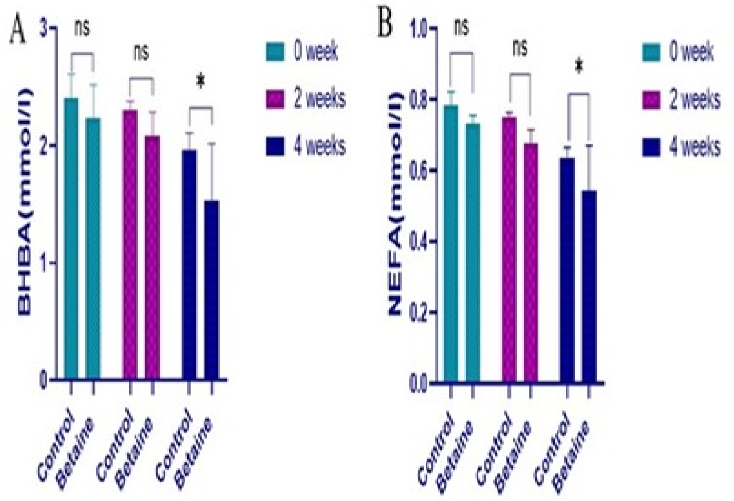

Fig. 4. The levels of BHBA (beta-hydroxybutyric acid) (A) and NEFA (non-esterified fatty acid) (B) levels in control and betaine groups are at 0w, 2w, and 4w. Results are reported as means ± standard error. ns: non-significant, *p < 0.05, **p < 0.01.

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تأثيرات إضافة البيتين على المستقبلات الدموية المرتبطة بالإجهاد الحراري، ومضادات الأكسدة وإنتاج الحليب في الأبقار الحلوب

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هدفت هذه الدراسة إلى تقييم تأثير إضافة البيتين على مؤشرات الدم والأيض في الأبقار الحلوب المعرضة لظروف حارة ورطبة. تم تقسيم ثلاثين بقرة مدرة للحليب من مزرعة ألبان صناعية مصرية إلى مجموعتين: مجموعة تحكم لم تتلقى البيتين (عدد 15) ومجموعة مكملة (80 غرام بيتين/اليوم لمدة أربعة أسابيع، عدد 15). تم جمع عينات الدم في ثلاث نقاط زمنية: الأساس صفر أسبوع (0أ)، أسبوعين (2أ)، وأربعة أسابيع (4أ). تم قياس مستويات الكورتيزول، سوبر أوكسيد ديسموتاز (SOD)، مالونداyalدهايد(MDA) ، الألبومين، البروتين الكلي، الإنسولين، اليوريا، أسبارتات أمينوترانسفيراز(AST) ، ألانين أمينوترانسفيراز(ALT) ، الجلوكوز، الأحماض الدهنية غير المسترة(NEFA) ، وحمض بيتا هيدروكسي بوتيريك (BHBA)وتمت مقارنتها بين المجموعتين باستخدام اختبار تي الخطي وعبر نقاط الزمن باستخدام تحليل التباين المتكرر ANOVA.بالإضافة إلى ذلك، تم تسجيل إنتاج الحليب طوال فترة الدراسة. أشارت النتائج إلى أن مستويات الكورتيزول كانت أقل بشكل ملحوظ في مجموعة البيتين في 2أ و4أ مقارنة بمجموعة التحكم. كانت مستويات اليوريا و ALTأقل بشكل متسق في مجموعة البيتين في جميع نقاط الزمن. أظهرت مستويات BHBA و NEFAانخفاضات ملحوظة في مجموعة البيتين في 4أ. كان مستوى SOD أعلى بشكل ملحوظ في مجموعة البيتين في 2أ و4أ. وأخيراً، تحسن إنتاج الحليب في الأبقار المكملة بالبيتين مقارنة بمجموعة التحكم. في الختام، تعزز إضافة البيتين للأبقار الحلوب تأثيرات إيجابية على المستقلبات الدموية وملفات مضادات الأكسدة، محسنة الاستجابة المتوازنة للإجهاد الحراري وزيادة إنتاج الحليب.

الكلمات الدالة: الإجهاد الحراري، معايير الدم، الأبقار الحلوب، البيتين