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RESEARCH TITLE

Relationship between glutathione levels on the milk composition and production in heat-stressed Holstein cows

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Abstract

This study was conducted at the Taj Al-Nahrain dairy farm station / Al-Qadisiyah Governorate, and aimed to determine the relationship between glutathione levels in the blood and changes in milk production and its components and the behavior of Holstein cows under heat stress. The results showed a high significant relationship ($P \leq 0.01$) between high (56.41-41.41 µmol. L-1) and medium (41.41-26.41 µmol.L-1)of glutathione levels in the blood with the percentage of protein reaching 4.71% and 4.31% and lactose 6.62 % and 5.77%, and non-fat solids 12.83% and 11.94%, respectively, and significantly ($P \leq 0.05$) with the percentage of fat in milk, which recorded 4.32% and 4.01%, respectively. While the results did not record any significant relationship between the glutathione levels in the blood with the percentage of specific density and the degree of freezing of milk. The results showed a significant relationship ($P \leq 0.05$) between low and medium of glutathione levels in the blood of cows and total milk production (720 and 700 kg, respectively). The results also showed a relationship between a low glutathione levels in the blood and an increase in rumination time. These results indicate the strong relationship between glutathione levels in the blood and the performance and behavior of Holstein cows under exposed to heat stress.

Key Words: Heat stressed-Holstein cows, Glutathione.

عنوان البحث

العلاقة بين مستويات الجلوتاثيون على تركيب الحليب وإنتاجه في أبقار الهولشتاين المعرضة للإجهاد الحراري

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المستخلص

أجريت هذه الدراسة في محطة مزرعة ألبان تاج النهرين / محافظة القادسية، وهدفت إلى تحديد العلاقة بين مستويات الجلوتاثيون في الدم والتغيرات في إنتاج الحليب ومكوناته وسلوك أبقار الهولشتاين تحت الإجهاد الحراري. أظهرت النتائج وجود علاقة عالية المعنوية (P≤0.01) بين المستويات المرتفعة (41.41−56.41 ميكرومول لتر−1) والمتوسطة (26.41−41.41 ميكرومول.لتر−1) في الدم مع وصول نسبة البروتين إلى 4.71%. و4.31% واللاكتوز 6.62% و5.77%، والمواد الصلبة غير الدهنية 12.83% و11.94% على التوالي، وبشكل معنوي (P≤0.05) مع نسبة الدهن في الحليب التي سجلت 4.32% و4.01% على التوالي. بينما لم تسجل النتائج أي علاقة معنوية بين مستويات الجلوتاثيون في الدم ونسبة الكثافة النوعية ودرجة تجميد الحليب. أظهرت النتائج وجود علاقة معنوية (P≤0.05) بين انخفاض ومتوسط مستوى الجلوتاثيون في دم الأبقار وإنتاج الحليب الكلي (720 و700 كغم على التوالي). وأظهرت النتائج أيضًا وجود علاقة بين انخفاض مستويات الجلوتاثيون في الدم وزيادة وقت الاجترار. تشير هذه النتائج إلى العلاقة القوية بين مستويات الجلوتاثيون في الدم وأداء وسلوك أبقار الهولشتاين تحت التعرض للإجهاد الحراري.

الكلمات المفتاحية: الإجهاد الحراري ، أبقار الهولشتاين، الجلوتاثيون.

Introduction

Animal products are an important source of human food, and as a result of population growth and rising income, especially in developing countries, this has led to an increase in demand for animal protein (Saeed et al., 2023a) but this industry faces major challenges, most notably heat stress (HS), which is known as a result of the imbalance between the heat produced or gained from the environment and the amount of heat lost to the environment (Brown-Brandl, 2018).Global warming has caused temperatures to rise in all regions of the world (Segnalini et al., 2011), it is well known that Holstein cows are characterized by their high milk production, so they are sensitive to changes in temperature and will suffer from heat stress (HS) as a result of an increase in body temperature (Kaufman et al., 2018 ; Saeed et al., 2023c), and a decrease in milk production (Nascimento et al., 2019). Also, heat stress causes disruption in the body's normal organ functions, metabolism, antioxidant efficiency (Ranjitkar et al., 2020), and may lead to death (Dikmen et al., 2015). Heat stress alter in the natural regulation of oxidants/antioxidants, causing severe damage to cells. High temperature also leads to an enhancement of the occurrence of oxidative stress, which is attributed to the high production of oxygen radicals by the organism and the decrease in antioxidant defenses. Antioxidant enzymes oxidative stress protects the organism from oxidative stress (Pragna et al., 2017). Glutathione (GSH) is a short peptide consisting of three amino acids: glutamic, cysteine, and glycine. It is found in animal tissues and plays an important role as an antioxidant within the body. It protects the cell from oxidative damage by reducing peroxides (Allen et al., 2015). The increase in antioxidant enzymes in the body of cows under heat stress reduces cell damage and reduces the production of oxygen radicals (Yu et al., 2020). Due to high metabolic processes, high-producing dairy cows are highly vulnerable to the effects of heat stress, which causes disturbances in their ability to thermoregulate through behavioral, productive and physiological changes. The expression of thermoregulation behavior may be reduced activity, feed intake, and rumination. Furthermore, maintaining a standing or lying position may be a sign of the negative effect of heat stress, as cows prefer to stand rather than lie down even after being deprived of lying down (Allen et al., 2015). Researchers noticed that rumination time decreases with increasing ambient temperatures, causing a significant decrease in cows' milk production (Wadhwani et al., 2023). Therefore, monitoring and analyzing behavioral changes achieves a greater understanding of heat stress and may be a key factor for developing effective strategies to reduce the effects of heat stress on dairy cow (Herbut et al., 2021). The response of dairy cow may vary depending on the genetic makeup of individual breeds, stage of lactation, milk production, housing system, climate zone, diet and cooling management strategies, with the severity of environmental conditions playing a crucial role in determining the extent of this response (Broucek et al., 2020; Saizi and Idowu 2020 ; Theusme et al., 2021). As a result of the importance of the issue of heat stress and its significant economic losses, the current study aimed to determine the relationship between glutathione levels in the blood and the production and components of milk and some behavioral parameters of Holstein cows under conditions of heat stress.

Material and Methods

All experimental procedures and protocols were approved by the Animal Care Committee of the University of Kufa, Iraq with code number 20579/2022.

Location:

The study was conducted at Taj Al-Nahrain Dairy Farm Station (latitude: 32°02'23.4''N and longitude: 44°56'28.5''E), which is located in the Al-Qadisiyah Governorate, Iraq, during the summer from 1 July to 1 September 2022.

Experimental Animal

Forty German Holstein dairy cows from the herds of Taj Al-Nahrain Dairy Farm with healthy status in third lactation were selected for the current experiment. All cows were maintained and raised under the same nutrition and management as on a dairy farm station.

Samples and Data Collection

Blood samples were collected from the udder vein of all cows through 10 ml tubes without EDTA and centrifuged at 1400 rpm for 10 minutes, and were immediately transferred to the laboratory using the cold box. Glutathione enzyme activity was estimated using the ELISA technique (BioTek, USA) according to the method (Christopher et al., 1999). Glutathione levels were classified based on its level in the blood of cows into low, moderately high, or high above the normal level. Milk samples were obtained from the same cows 3 times daily, preserved by refrigeration, and transported directly to the laboratory. Then the milk components were analyzed for protein, fat, lactose, non-fat solid contents, freezing point, and specific density using a Lacto Flash device (Funk Geber Company, Germany).

Milk production:

The daily milk production was calculated from the total milk production in cows exposed to heat stress during two months, divided by the days of production, and the peak production was estimated by calculating the first increase in production during the period, provided that it does not exceed 60 days, while the stability in milk production was calculated from the average production of the last three weeks of the period divided by the average production of the first three weeks of the period, and the level of heat stress was determined by measuring the values of the temperature and humidity index (THI) (3 reading. Season-1). According to the method (Segnalini et al., 2011) using the equation: THI=(1.8× Ambient Temperature +32)- (0.55-0.55× Relative Humidity)-[(1.8× Ambient Temperature +32)–58] the meteorological data were obtained from the station records. According to (Wierama , 1990), the levels of thermal stress imposed on cows are determined according to the values of THI. If it is less than 72, the cows do not suffer from heat stress, but they suffer from heat stress if the value of THI ranges from 73-79, and you suffer from moderate stress if the value reaches 80-89, and you suffer from severe stress if the THI value reaches greater than or equal to 90.

Behavioral measurements:

Behavioral measurements were carried out through visual observation 3 times. d-1 and repeated for 6 days throughout lactation period, where the duration of jaw movements was monitored and measured to calculate the time of rumination (1 min. d-1), and the time of lying down and standing (min. d-1).

Statistical Analysis:

The data were statistically analyzed using the Statistical Analysis System program SAS software V9.4 (SAS Institute Inc., Cary, NC, USA). To study the relationship between glutathione levels and changes in milk production and components of heat-stressed Holstein cows using a completely randomized design (CRD) and comparing the significant effect using the Duncan test using the same program.

Results and Discussion

It was noted that during the period of heat stress in the summer, the THI value was 87.65, meaning that they were suffering from moderate stress. It is clear from Table. 1 that there is a significant relationship between the level of glutathione in the blood and the percentage Protein, lactose, fat and non-fat solids in the milk of Holstein cows exposed to heat stress, The results showed a highly significant relationship (P≤0.01) between high and medium levels of glutathione in the blood (56.41-41.41 µmol. L-1) (41.41-26.41 µmol. L-1) with the protein percentage reaching 4.71% and 4.31%, lactose 6.62% and 5.77%, and non-fat solids 11.94% and 12.83%, respectively, and significantly (P ≤ 0.05) with the percentage of fat in milk, which recorded 4.32% and 4.01%, respectively. While the results did not record any significant relationship between the level of glutathione in the blood, the specific density ratio and the degree of freezing of milk. These results indicate a relationship between the enzyme glutathione and heat stress, as glutathione acts as a low-molecular weight antioxidant and serves as a defense mechanism for tissues against free radical damage (Altan et al., 2003). Therefore, it is observed in cows with a high glutathione level that the percentages of the main milk components such as the percentage of protein, lactose, fat, and the percentage of non-fat solids are higher compared to cows with a low glutathione level. This was explained by the apparent decrease in milk production in cows that had a high percentage of glutathione, as the percentage of water in the milk is lower. Milk, which leads to an increase in the proportion of components, and this is consistent with what some have argued, and that the increase in the specific density of milk when heat stress increases is the result of an increase in the percentage of fat in the milk as a result of the decrease in the amount of milk and its containment of a low percentage of water (Ahmed , 2020).

Table 1: Effect of glutathione levels on the milk characteristics and components of heat-stressed Holstein cows (Mean ±SE)

Glutathione levels	Protein (%)	Fat (%)	Lactose (%)	Specific Density of Milk (%)	Milk Freezing (C°)	Solids Non-Fat (%)
High	4.71 ±0.832[a]	4.32 ±0.142[a]	6.62 ±0.236[a]	0.997 ±0.0465	-0.779 ±0.154	12.83 ±0.927[a]
Intermediate	4.31 ±0.737[a]	4.01 ±0.254[a]	5.77 ±0.189 [a]	1.022 ±0.0462	-0.29 ±0.132	11.94 ±0.651[a]
Low	2.62 ±0.605[b]	3.42 ±0.127[b]	3.69 ±0.117[b]	1.159 ±0.0459	-0.413 ±0.191	7.48 ±0.393[b]
Significant	**	*	**	NS	NS	**

a,b with different superscripts in the same column are significantly different (P≤0.05). NS: Not significant *: P≤0.05; **: P≤0.01; High: 56.41-41.41 µmol. L^{-1}; intermediate :41.41-26.41 µmol. L^{-1}; low: 26.41-11.4 µmol. L^{-1}

The results of Table 2 showed that there is a significant relationship between the glutathione levels and total milk production, and the period of lactation. The results indicated that there is a significant relationship (P≤0.05) between low (26.41-11.41 µmol. L-1) and medium (41.41-26.41 µmol. L-1), levels of glutathione, total milk production (720 and 700 kg) and lactation period. While the lactation period was observed to be higher for cows with low and medium glutathione levels, 303.53 and 303.18 days, respectively, compared to cows with high glutathione levels, which amounted to 294.48 days, with no significant difference in production, daily milk, consistency, and peak production in heat-stressed Holstein cows. These results indicate the relationship between the glutathione levels and heat stress on the animal and its reflection on the properties of the milk. It is noted that the total milk production increased in cows with a low and medium glutathione levels, reaching 720 and 700 kg, respectively, compared to cows with a high glutathione levels, which reached 661 kg, which indicates that the cows were suffering from high heat stress, which was reflected in a decrease in their milk production, This is consistent with the findings of (Muschner-Siemens et al., 2020) who indicated a decrease in milk production in cows exposed to heat stress, and explained that part of the harmful effects of heat stress on milk production can be attributed to a decrease in feed intake and nutrients, and their depletion from the cow's intestines. It spreads in the blood that flows to the tissues, which works to cool the body away from the digestive system, which ultimately leads to a decrease in milk production. While (Dikmen et al., 2015) stated that the decrease is due to changes in the metabolic process and physiological changes as well as the amount of feed, this is due to a decrease in the activity of metabolic hormones in cases of heat stress and an imbalance in metabolic processes, which causes a decrease in milk production (Pragna et al., 2017).

Table 2: Effect of glutathione levels on milk production characteristics of heat-stressed Holstein cows (Mean ±SE)

Glutathione levels	Daily milk yield (kg)	Total milk yield (kg)	Persistency (%)	Peak yield (kg)	Lactation period (d)
High	11.00±0.662	661±39.7^{b}	14.09±0.695	14.18±0.311	294.48±2.012^{b}
Intermediate	11.66±0.657	700±39.4^{a}	14.56±0.687	14.75±0.592	303.18±1.997^{s}
Low	12.00±0.652	720±39.1^{a}	15.27±0.684	14.35±0.462	303.53±1.982^{a}
Significant	NS	*	NS	NS	*

^{a,b} with different superscripts in the same column are significantly different (P≤0.05). NS: Not significant *: P≤0.05; High: 56.41-41.41 µmol. L^{-1}; intermediate :41.41-26.41 µmol. L^{-1}; low: 26.41-11.4 µmol. L^{-1}

Table 3 shows the relationship between the glutathione levels and the behavior of Holstein cows, as the results showed that significant relationship (P≤0.01) between low levels (26.41-11.41 µmol. L-1) and time of rumination, which reached 466.80 min.day-1 compared to the medium levels (41.41-26.41 µmol. L-1) and high levels of glutathione (56.41-41.41 µmol. L-1), reaching 456.71 and 447.0 min.d-1, respectively. In addition, the superiority of cows with medium glutathione levels on cows with a high glutathione level at the time of rumination, as it reached 456.71 min.d-1. These results indicate that increasing the levels of antioxidant enzymes in conditions of heat stress reduces the occurrence of oxidative stress resulting from heat stress, which causes a decrease in rumination time as a result of decreased feed consumption due to heat stress, and this is consistent with the results reached by (Ayemele et al., 2021; Saeed et al., 2023) who reported that ruminant animals under stress conditions reduce their level of physical activity, feed consumption, and rumination, while at the same time increasing their water consumption. The results indicated that there is no significant relationship between the time of lying down and standing and the level of glutathione in the blood of cows under conditions of heat stress. This is due to increased lying time during the night and standing time during the day in conditions of heat stress.

Table 3: Effect of glutathione levels on some behavioral parameters of Holstein cows exposed to heat stress (mean±SE)

Glutathione levels	Stand up (min. d^{-1})	Lying down (min. d^{-1})	Rumination time (min.d^{-1})
High	778.45 ± 28.41	661.50 ±40.21	447.0 ±7.84 [c]
Intermediate	756.91 ±30.89	682.99 ±38.80	456.71 ±4.578[b]
Low	747.32 ±32.26	692.67 ±36.37	466.80 ±5.54[a]
Significant	NS	NS	**

a,b with different superscripts in the same column are significantly different (P≤0.05). NS: Not significant **: P≤0.01; High: 56.41-41.41 µmol. L^{-1}; intermediate :41.41-26.41 µmol. L^{-1}; low: 26.41-11.41 µmol.

Conclusion

In conclusion, our study elucidates significant associations between blood glutathione levels and productive performance amidst heat stress conditions, particularly pertaining to milk production and key milk constituents. Moreover, our findings underscore a notable correlation between glutathione level and behavioral parameters, notably rumination duration. Importantly, we observed a direct link between rumination time and overall milk yield, which was notably impacted under heat stress circumstances. These insights highlight the pivotal role of glutathione in modulating dairy cow performance under thermal stress, emphasizing the multifaceted interplay between biochemical factors, behavior, and milk production in challenging environmental conditions.

Conflict of Interest

The authors have no conflict of interest.

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