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Effects of Acetaminophen and Vitamin C Combination on Clinical Vital Signs and Behavioral Traits of Heat-Stressed Dairy Buffalo

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Acetaminophen; Vitamin C; Physiological; Behavioral; Heat stress; Buffalo

Abstract

Background: Heat stress (HS) presents significant challenges to water buffalo breeding, particularly in harsh environments like southern Iraq. HS adversely impacts physiological and behavioral aspects in dairy buffaloes, leading to economic losses in productivity and welfare. Interventions such as acetaminophen and vitamin C supplementation have been suggested to alleviate HS impacts due to their anti-stress and anti-inflammatory characteristics.

Methods: This study was conducted in Al-Chibayish Marshes southern Iraq and aimed to evaluate the effects of acetaminophen and vitamin C combination (AVC) on heat-stressed dairy buffalo. Sixteen lactating buffaloes were divided into four treatments. One treatment received regular water (control), while other treatments were given water mixed with AVC at a dose of 500g/1000 liters for 3, 5, and 7 consecutive days per week, respectively, over 30 days. Environmental (temperature, humidity, and THI) and physiological data (body temperature, respiration rate, and pulse rate) were recorded daily, while feed and water consumption were recorded weekly.

Results: Buffaloes experienced significant HS, with temperatures reaching up to 53.2°C and corresponding THI values ranging from 88.05 to 99.31. AVC administration for 5 consecutive days led to increased feed intake by 1.15 kg daily per treatment and decreased water consumption by 16.43 liters daily per treatment compared to the control. Additionally, rectal temperature, respiration rate, and heart rate showed progressive improvement during the second and third weeks, with the most notable improvement observed in the fourth week of the study. These improvements in vital signs and behavioral traits indicate the ability of AVC to improve thermoregulation and reduce heat stress impact.

Conclusion: The investigation highlights the potential of AVC supplementation showing a promise in alleviating HS effects on dairy buffaloes by positively influencing physiological and behavioral parameters. Further research is needed to elucidate the underlying mechanisms and optimize supplementation strategies for enhancing buffalo welfare and productivity under heat stress.



Introduction

The rearing of water buffalo (*Bubalus bubalis*) stands out as the most important farm animal and is considered an economic and national wealth, by providing valuable milk and meat, particularly in the marshlands within southern Iraq. This region, identified as one of the harshest environments globally [1], underscores the critical contemporary issue of the Earth's surface temperature rise due to climate change and global warming [2]. In livestock, a phenomenon called heat stress (HS) occurs when the sum of metabolic heat production and environmental heat exceeds the heat dissipated by the animal [3]. Climatic factors such as ambient temperature, relative humidity, solar radiation, precipitation, and wind speed are reasons associated with HS [4]. Such factors tend to affect the equilibrium values of an animal's behavioral, physiological and metabolic parameters [5]. Therefore, breeders regard the challenge of increasing ambient temperature during harsh environmental periods which caused economic losses in milk yield and components [6], reduced rumination and increased risk of metabolic diseases [7]. The dark color of buffalo and the absence of sweat glands contribute to its higher absorption of sunlight compared to cows. Alterations that occur by HS involve behavioral changes in buffalo flocks, reduced feed intake, increased water consumption, increased respiratory rate, pulse rate, and high body temperatures which lead to deterioration in its economic, productive, and reproductive characteristics [3].

Throughout ruminants, regulatory mechanisms within the body respond to environmental changes, commencing with adjustments in breathing rate, heartbeats, and enhanced evaporation from the skin's surface. These adaptations allow animals to acclimate to novel environments [8]. Consequently, numerous investigations have explored dietary and management interventions aimed at mitigating the impacts of HS on buffalo performance [6]. Contemporary studies have proposed the use of a combination of acetaminophen (paracetamol) and vitamin C in drinking water to alleviate heat stress. This product, known for its antipyretic, analgesic, anti-stress, and anti-inflammatory properties, serves as a supportive treatment for conditions such as rheumatism, arthritis, and viral infections induced by various pathogens, environmental factors, and climatic stressors. Meanwhile, [9] investigated the potential effects of coadministration of vitamin C to protect against paracetamol-induced oxidative stress, examining alterations in hematological, biochemical, and oxidative stress parameters. Some studies have suggested that this combination could effectively manage stress and inflammation triggered by

vaccination in cattle calves, potentially leading to a decrease in circulating stress hormones and inflammatory markers [10]. In a recent study, [11] investigated the effects of administering this combination in drinking water over several days per week to alleviate the impacts of heat stress. They propose that this approach is safe for dairy buffaloes and can reduce the harmful effects of heat stress by improving hematologic, biochemical, and hormonal biomarkers associated with the body's oxidative status. Although studies investigating the use of paracetamol and vitamin C as stress-relieving agents in cattle remain limited, further research is warranted to ascertain the efficacy of this therapy. Despite the proliferation of manufacturers offering this product, information regarding its physiological and productive effects on animals in this context remains weak. The present study was designed to determine whether the physiological signs as well as drinking and eating behavioral characteristics of heat-stressed dairy buffaloes could be enhanced by the administration of a combination of acetaminophen and vitamin C.

Methods

This investigation was carried out during the summer season to evaluate the use of a combination of acetaminophen and vitamin C to alleviate the negative effects of heat stress on physiological and behavioral parameters in Iraqi dairy buffaloes. A preparation period of two weeks was allocated for animal acclimatization and measurement training. The experimental part of this study was conducted for a period of thirty days during the harshest climate (23/7/2022 to 21/8/2022) in a buffalo herd in Al-Chibayish Marshes, Thi-Qar Province, Southern Iraq.

Farm and management

The buffalo farm is located in Chibayish 30°58'32.3"N 47°00'59.0"E closer to Al-Wista Marshes serving as a natural environmental habitat for Iraqi water buffaloes. The farm comprises a semi-open barn measuring 60 L × 30 W × 3 H meters, with a fenced area for exercises. The barn is divided into four sections, each part (4 animals) containing a water-drinking trough (2 L × 1 W × 0.7 H meters) and a feed basin (2 L × 1 W × 0.4 H meters) under the shed.

Animals of the study

Sixteen lactating buffaloes were used in this study. The age of animals ranged from 4-7 years and the weight ranged from 450-500 kg with an average parity of 2-3 times. All animals were clinically examined to ensure they were healthy with no signs of any disease. The studied animals were given free access to dietary formalizations and water. The components of the feed

(concentrate and green fodder) were introduced in the morning and evening in concrete basins for all studied groups. The vital signs of animals (rectal body temperature, pulse rate and respiratory rate as well as behavioral parameters (feed intake, and water consumption) were recorded daily.

Animal feeding

The animals in the present study consumed a mixed ration consisting of brown flour (45%), wheat bran (24%), corn cob (13%), hay (6%), molasses (6%), soybean (5%), and mineral mixture (1%). The chemical composition of this concentrated feed was measured to obtain a crude protein content of 12.8% and metabolic energy of 2157 Mcal/kg. In addition, green fodder such as reeds, papyrus, and green weeds was used, available in the area, and introduced in the same manner as concentrated feed. The quantities of concentrated feed estimated depended on 2% of body weight, while green fodder was introduced freely. Each group used a concrete water pool with dimensions 2 L × 1 W × 0.7 H meters, which was exchanged and filled three times daily with tap water and added acetaminophen and vitamin C according to the study protocol. Water consumption was estimated using a meter-long ruler, numbered in centimeters.

Protocol of the study

The veterinarian drug, MS-VCPARA, MOOSUN, China (Paracetamol 30% and Vitamin C 30% soluble powder) was used as a source of acetaminophen and vitamin C combination (AVC). The sixteen dairy buffaloes used in this study were divided into four groups as the following:

- **Control:** four dairy buffaloes were fed on a regular ration and drank clear tap water for 30 days.
- **3 days AVC:** dairy buffaloes were fed on a regular ration and drank tap water supplemented with acetaminophen and vitamin C (500 mg/1000L water for 3 days weekly) for a long 30 days of the experiment.
- **5 days AVC:** dairy buffaloes were fed on a regular ration and drank tap water supplemented with acetaminophen and vitamin C (500 mg/1000L water 5 days weekly) for a long 30 days of the experiment.
- **7 days AVC:** dairy buffaloes were fed on a regular ration and drinking tap water supplemented with acetaminophen and vitamin C (500 mg/1000L water along the week) during the period of 30 days of the experiment.

Parameters of the study

Meteorological data

The ambient temperature (°C) and relative humidity (%) were recorded by using HTC-2 (China) digital LCD electronic thermo-hygrometer. This device was suspended inside the barn at a height of 2 meters to measure the temperature and humidity that the animal experienced. The meteorological data were recorded on a daily basis at two to three o'clock in the evening (at the same time that the physiological characteristics of the animal were measured). The temperature-humidity index (THI) was determined using the formula provided by the National Research Council, which takes into account the dry bulb temperature (T °C) and relative humidity (RH %) as follows [12]:

$$THI = (1.8 * T ^\circ C + 32) - [(0.55 - 0.0055 * RH \%) * (1.8 * T ^\circ C - 26)]$$

This equation was also used by [4] and [13] for evaluating the genetic and environmental effects of HS on German cattle. [14] compared seven different THI models and found that these models varied in their ability to measure heat load and its impact on dairy buffalo performance. They identified the THI model developed by the National Research Council in 1971 as the most effective for studying the effects of HS on lactating buffaloes.

Vital signs measurements

All studied animals were clinically monitored by recorded pulse rate, respiratory rate and body temperature during the hottest period of the day (14:00 to 15:00) once daily. The vital signs of buffaloes were examined simultaneously with the meteorological data recording, that's due to their response rapidly to changing weather conditions. The pulse rate, denoting the frequency of heart beats per minute, was assessed manually by placing fingertips on the caudal artery. Respiratory rate, on the other hand, was determined by observing the number of flank movements of the buffalo within a one-minute period while the animal was at rest [15]. A high-quality electronic digital veterinarian thermometer (ME13002, Asia Connection Co., Ltd., China) was used for recording the rectal temperature.

Feed and water consumption

Feed for the studied buffalo was introduced in two intervals by the percentage of 2% of the body weight for the concentrated feed. While the green fodder was given unlimited quantity. Feed intake was estimated by the following equation through 24 hours:

$$\text{Feed consumption} = \text{Total feed weight} - \text{weight of feed residue}$$

Water was put to each treatment in a concrete pool with dimensions (2 L × 1 W × 0.7 H meters) that have a

capacity of about 200 L. The drinking water pool was connected to a water tank, with a 1000 L capacity. All animals drink clear tap water directly from the pool *ad libitum*. This pool was filled 3 times daily after adding acetaminophen and vitamin C for the studied treatments and water consumption was estimated one time during 24 hours by the following equation:

$$\text{Water consumption} = \text{Total water add to pool (L)} - \text{water residue in the pool (L)}$$

Statistical analysis

The dataset was subjected to statistical analysis employing a two-way analysis of variance (ANOVA) within a completely randomized design (CRD), utilizing SPSS software version 27 (SPSS, 2020). Post-hoc comparisons were conducted using the least significant difference method (LSD) to ascertain differences between means. Significance levels were set at $P \leq 0.01$ and $P \leq 0.05$.

Results

In this study, weather data collected within the animal's barn indicated that buffaloes experienced heat stress, with THI values ranging from 88.05 to 99.31. The highest ambient temperature of 53.2 °C occurred on August 4th, 2022, while the lowest temperature of 47.7 °C and corresponding THI were recorded on the final day of experimentation (August 21st, 2022). Between July 29th and August 3rd, 2022, prevailing seasonal southern winds originating from the Arabian Gulf carried heightened humidity levels, exacerbating heat stress among the animals, as reflected by the highest recorded THI value.

The animals under investigation in this study did not display any significant differences ($P \leq 0.05$) in their water intake throughout the three-week experimental period when compared among groups (Table 1). However, during the fourth week, a notable decrease in water consumption was observed among all animals treated with acetaminophen and vitamin C compared to those in the control group. Further analysis of water consumption patterns within each group supplemented with AVC revealed a significant decline ($P \leq 0.05$) from the second to the fourth week compared to the baseline established in the first week of the study. The administration of AVC for 5 consecutive days led to a significant decrease in water consumption, estimated at 16.43 liters (2.87%) daily per treatment compared to the control group. Conversely, animals in the control group exhibited fluctuating water consumption levels over the course of the experiment.

Table (2) illustrates the impact of HS on the feed consumption of dairy buffaloes during the hot

season, a subject investigated through the follow-up feeding of the control group. The results indicate that the supplementation of AVC led to an enhancement in feed intake among the treated groups, resulting in a significant increase ($P \leq 0.05$) compared to the control group. Moreover, the groups supplemented with AVC demonstrated a consistent and gradual increase in feed intake over the four-week duration of the experiment. The most interesting finding was that administration of AVC for five consecutive days led to a significant increment in feed intake by 1.15 kg (2.13%) daily per treatment. In contrast, the control group exhibited fluctuations in feed intake quantity throughout the same period.

Groups/ weeks	1 st week	2 nd week	3 rd week	4 th week	L.S. D
Control	583.57 ^a ± 1.79	560.00 ^b ± 5.93	570.00 ^{ab} ± 3.61	573.57 ^{Aa} ± 1.42	18.57
3 days AVC	586.42 ^a ± 1.42	567.85 ^{bc} ± 4.34	575.00 ^b ± 1.54	557.85 ^{Bc} ± 3.24	11.42
5 days AVC	590.00 ^a ± 1.88	569.28 ^b ± 2.54	571.42 ^b ± 2.36	557.14 ^{Bc} ± 2.64	14.28
7 days AVC	589.28 ^a ± 2.54	566.42 ^b ± 3.89	565.00 ^b ± 2.18	561.42 ^{Bb} ± 2.36	22.85
L.S.D.	N.S	11.42	10.00	12.14	-

Capital letters represent significant differences vertically at ($P \leq 0.05$) level. Small letters represent significant differences horizontally at ($P \leq 0.05$) level.

Table 1: Impact of Acetaminophen and Vitamin C (AVC) Administration (500 mg/1000L) for 0, 3, 5, and 7 Days a Week on Daily Water Consumption (L/day/group) in Heat-Stressed Dairy Buffaloes (Mean ± S.E.).

The body temperature of dairy buffaloes was diligently recorded daily throughout the entire duration of the study and is graphically depicted in Figure (1). This figure serves to investigate the efficacy of AVC supplementation in mitigating body temperature elevation despite prevailing environmental heat stress, as indicated by the temperature-humidity index (THI). Notably, the figure visually represents a statistically significant decrease ($P \leq 0.05$) in body temperature across all treatment groups when compared with the control group. Furthermore, a notable and statistically significant progressive decline in body temperature was observed among the animals over the course of the four-week experimental period for the 3-day AVC, 5-day AVC, and 7-day AVC groups in comparison to their respective earlier weeks.

Of particular interest, the 7-day AVC group exhibited the most favorable body temperature outcomes ($P \leq 0.05$) by the conclusion of week 4, showcasing a return to normalcy in comparison to both the other experimental groups and its own body temperatures in the preceding weeks of the experiment. It is noteworthy that animals within the control group displayed the highest and statistically significant ($P \leq 0.05$) elevation

in body temperature throughout the duration of the study when compared against all other experimental groups, highlighting the pronounced impact of climatic heat stress on this group.

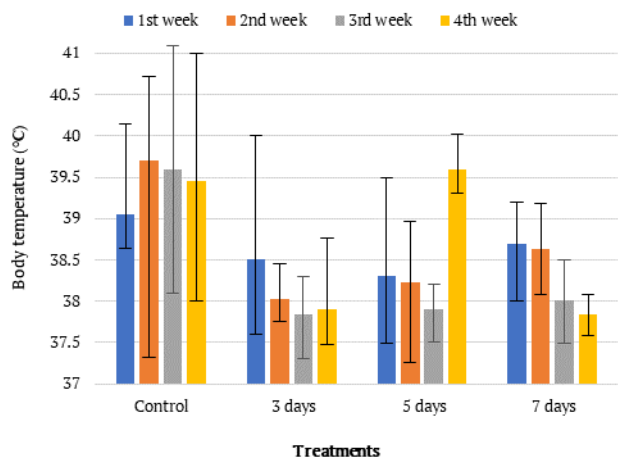


Figure 1: Body temperature (°C) of heat-stressed dairy buffaloes supplemented with Acetaminophen and Vitamin C (AVC) (Stripes in the bars indicate the daily minimum and maximum values for body temperature; °C).

Groups/weeks	1 st week	2 nd week	3 rd week	4 th week	L.S.D
Control	44.14 ^b ± 0.26	51.00 ^{Aa} ± 0.31	52.14 ^a ± 0.63	52.85 ^{Ba} ± 0.74	1.28
3 days AVC	44.42 ^c ± 0.48	50.57 ^{Ab} ± 0.81	53.71 ^a ± 0.47	54.85 ^{Abba} ± 0.40	2.85
5 days AVC	44.85 ^b ± 0.54	53.85 ^{Aa} ± 1.53	53.42 ^a ± 0.20	54.00 ^{Abba} ± 0.51	1.28
7 days AVC	44.42 ^c ± 0.30	48.85 ^{Bb} ± 0.96	52.42 ^a ± 0.20	54.28 ^{Abba} ± 0.42	2.00
L.S.D.	N.S.	3.14	N.S.	1.85	

Capital letters represent significant differences vertically at (P≤0.05) level.

Small letters represent significant differences horizontally at (P≤0.05) level.

Table 2: Impact of Acetaminophen and Vitamin C (AVC) Administration (500 mg/1000L) for 0, 3, 5, and 7 Days a Week on Concentrated Feed Consumption (Kg/day/group) in Heat-Stressed Dairy Buffaloes (Mean ± S.E.).

The impact of AVC administration on pulse rate in heat-stressed dairy buffaloes over a span of four weeks, administered at varying doses, was explored as depicted in Figure 2. The findings unveiled a significant decrease (P ≤ 0.05) in heart rate among animals supplemented with AVC at different doses throughout the study weeks when contrasted with those in the control group. Additionally, the treatment groups exhibited significantly declining pulse rate values over the course of the study weeks compared to their respective values in the initial week. Conversely, the control group consistently demonstrated significantly elevated (P ≤ 0.05) pulse rate values throughout the study period, both internally and in comparison, to other treatment

groups supplemented with AVC as reported in prior studies.

Figure 3 elucidated the impact of heat stress, manifesting as a significant increase (P ≤ 0.05) in respiratory rate among dairy buffaloes in the control group over the four-week study period, in contrast to buffaloes supplemented with AVC on varying days of the week throughout the study duration. Furthermore, during the fourth week of the experimental period, a noteworthy decrease (P ≤ 0.05) in respiratory rate was observed in buffaloes supplemented with AVC compared to their respiratory rates in the preceding weeks of the study. Specifically, the 3-day AVC and 5-day AVC groups exhibited a significant reduction in respiratory rate earlier than the 7-day AVC group, with discernible effects appearing as early as the second week of treatment for the former groups, while the latter group experienced reduced respiratory rates after the third week of the study.

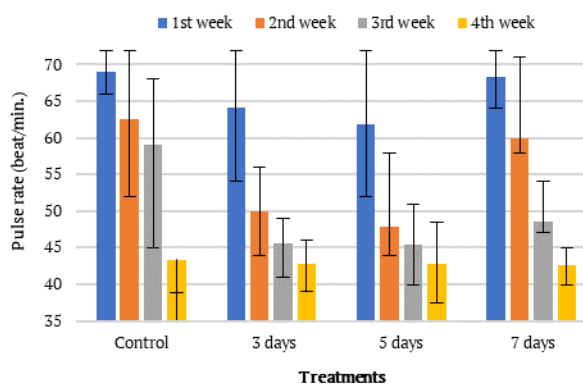


Figure 2: Pulse rate (beat/min.) of heat-stressed dairy buffaloes supplemented with Acetaminophen and Vitamin C (AVC) (Stripes in the bars indicate the daily minimum and maximum values for pulse rate; beat/min.).

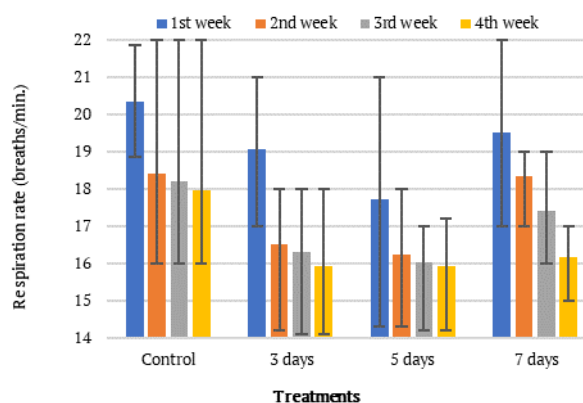


Figure 3: Respiration rate (breaths/min) of heat-stressed dairy buffaloes supplemented with Acetaminophen and Vitamin C (AVC) (Stripes in the bars indicate the daily minimum and maximum values for respiration rate; breaths/min.)

Discussion

A substantial portion of research within the domain of HS impact on dairy buffalo has typically relied upon meteorological data obtained from the nearest meteorological station, often located several kilometers distant from the animal husbandry facility. Our investigation into weather patterns, however, illuminated the pervasive presence of HS throughout the entire 30-day study duration within the barn and at levels perceptible to the animals. Notably, temperatures, humidity, and THI levels consistently persisted at elevated thresholds during the prolonged study period with THI values ranging from 88.05 to 99.31. These conditions markedly exceeded the recommended comfort thresholds for buffaloes, with a THI below 77 deemed optimal for their physiological well-being [16-21].

The resilience of Iraqi buffaloes in enduring and thriving under such extreme thermal exigencies, where temperatures soared above 52 °C, underscores their remarkable adaptive ability in navigating harsh environmental adversities. Conversely, many other buffalo breeds would likely struggle to survive in the face of comparable temperature extremes and could potentially face the risk of extinction under similar circumstances. Prolonged exposure to heightened temperatures and humidity levels may precipitate the degradation of several economic traits within the Iraqi buffalo populace. Consequently, proactive measures aimed at attenuating the deleterious impacts of HS and fortifying the welfare and productivity of buffaloes warrant earnest consideration.

During periods of elevated temperatures, ensuring sufficient water intake becomes crucial to prevent dehydration and support vital physiological processes in animals [22]. Water consumption plays a pivotal role in maintaining physiological balance and regulating body temperature, particularly in hot conditions [22, 23]. In this study, the groups of dairy buffaloes supplemented with AVC did not exhibit significant differences in water consumption compared to the control group during the initial three weeks of the experiment. This indicates that the initial administration of acetaminophen and vitamin C did not notably impact water intake in heat-stressed buffaloes. However, by the fourth week, all treated animal groups showed a notable decrease in water consumption compared to the control group. The findings revealed a reduction in water consumption, estimated at 16.43 liters (2.87%) per treatment per day when AVC was administered for five consecutive days compared to the control group. This decline in water consumption among the treated groups in the later weeks of the experiment suggests that the combination of acetaminophen and vitamin C might have influenced

the animals' thermoregulation, resulting in reduced reliance on water intake for cooling their bodies. Additionally, when comparing water consumption within each supplemented group over the study period, a significant decrease in water intake was observed from the second to fourth weeks, in contrast to the first week. This decline could be attributed to the buffaloes' physiological adaptation to heat stress and the efficacy of AVC supplementation in alleviating the heat stress response. During heat stress, lactating buffaloes increase sweat evaporation to dissipate heat, thus elevating water consumption to compensate for increased moisture loss through sweating and evaporation [24]. Despite having fewer sweat glands than cattle, buffaloes primarily utilize water loss through sweating and respiration as their main thermoregulatory mechanism [25, 26].

The study's observations reveal that the supplementation regimen exhibited a cumulative effect on the animals' feed intake behavior. The ability of the supplemented treatment to consistently promote increased feed intake bears great significance for sustaining the energy demands of heat-stressed dairy buffaloes and supporting their overall productivity. In contrast, the control group displayed fluctuating feed intake quantities throughout the four weeks of the experiment. This variability in feed consumption could be attributed to the animals' physiological responses to heat stress, potentially resulting in reduced appetite and feed intake. Several studies have reported a significant decline in feed intake among dairy buffaloes during hot environments [27]. Researchers have indicated that reduced feed intake in buffaloes during heat stress stems from nerve impulses sent to the appetite center in the hypothalamus, leading to decreased feed intake [28-30]. Additionally, [16] have suggested a decrease in feeding behavior activities during the heat stress period as one of the reasons for the deterioration in feed consumption.

Numerous studies and research have demonstrated that measuring average body temperature, pulse, and respiration rates is a common method utilized to assess the health status of animals, including dairy buffaloes [31, 32]. According to the researcher, vitamin C and acetaminophen contributed to reducing heat stress by lowering rectal temperature. Similarly, researchers have also noted that high body temperatures can impact the heart rate by increasing blood flow and requiring more pressure on blood vessels to carry blood through the heart, thus resulting in a significant increase in heart rate [33, 34]. Buffaloes are particularly sensitive to heat stress due to their lack of sweat glands and the thickness of their skin [35]. The study indicated that the addition of vitamin C through drinking water aided in decreasing high body temperatures by

dissipating heat through surrounding blood vessels and maintaining body temperature. Vitamin C's role in regulating and improving body hormones and enhancing immunity also contributes to reducing protein breakdown. Moreover, vitamin C can increase the percentage of oxygen in the body, resulting in an increase in metabolic processes. These findings are consistent with research conducted on HS in Egyptian buffaloes, which observed elevated body temperatures and increased pulse and respiration rates during the summer period [36]. A study on cattle and buffaloes exposed to high temperatures in Egypt likewise reported elevated body temperature, respiration rate, and pulse rate correlating with rising air temperature, noting buffaloes as more susceptible than cows [37].

Our previous study [11] indicates that administering 500g/1000 liters of AVC for 30 days mitigating the negative impacts of heat stress and enhancing the physiological and oxidative status and does not pose any adverse effects for dairy buffaloes. Notably, the treated groups with AVC exhibited the highest values of cortisol hormone and glutathione enzyme levels, as well as the lowest liver enzymes (ALT, AST, and ALP) levels compared to control groups. However, there were no notable differences in renal function markers (creatinine and urea) throughout the study duration.

In conclusion, this study represents a pioneering investigation into the impact of acetaminophen and vitamin C supplementation on mitigating the effects of HS on dairy buffaloes. It underscores the significance of monitoring physiological parameters such as body temperature, pulse, and respiration rates as indicators of HS, along with providing animals with ad libitum access to water and feed. The study elucidated the valuable role of combining acetaminophen and vitamin C in enhancing both physiological and behavioral traits during HS resulting from hot conditions.

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Author Contributions

Wisam K. Salih Sangor: Contributed to the research and field work.

Adnan Jabbar J. Al-Kanaan: The corresponding author, analysis of the data and writing the manuscript draft.

Nameer A. Khudhair: Design the study and contributed to writing of the manuscript.

All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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