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Reduction of Heat Stress Effect on Hematological and Antioxidant Indicators by Acetaminophen and Vitamin C Supplement for Dairy Buffalo of Al-Chybaish Area

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ABSTRACT

This study was conducted in a field in Al-Chibayish Swamp, Thi-Qar Province, Iraq, from July 23, 2022 to August 21, 2022. The objective was to investigate the impact combination of acetaminophen and vitamin C adding in a water drink for several days a week to minimize the effects of summer heat stress (HS) and to examine its effects on hematologic biochemical and hormonal biomarkers relate to oxidative status of the body. Sixteen water buffalo, aged 4 to 7 years and weighing between 450 and 500 kg, were fed according to their dietary requirements and randomly assigned to four treatments (T1, T2, T3 and T4). , each treatment consisted of four animals. The first treatment (T1) drank water regularly (control group), while treatment T2, T3 and T4 drank water mixed with acetaminophen and vitamin C (AVC) at a dose of 500g/1000 liters for treatment 3, 5 and 7 consecutive days per week, respectively, over a 30-day period. The environmental climatic conditions (temperature, humidity and temperature-humidity index) were recorded daily at 15:00. Complete blood counts and several biochemical parameters (ALT, AST, ALP, urea and creatinine) were monitored, as well as cortisol and glutathione measurements as antioxidant markers. The results of the present study revealed that the administration of AVC significantly impacted the hematological indices and the total and differential leukocyte values. The 7-day AVC supplementation led to increased values of RBC, Hb, and HCT, while WBC, neutrophils, and monocytes values decreased, in comparison to the control group. A significant decrease ($P \leq 0.05$) in liver enzymes was appear in the fourth group showed lower levels of ALT, AST, and ALP compared to the other three groups after 10, 20, and 30 days of AVC administration, while the control group showed the highest values of these HS markers. Whereas, the results indicated there were no significant differences in renal function markers (creatinine and urea) along the study periods. While, treatments 2, 3, and 4 showed significant differences compared to the control group in cortisol hormone and glutathione enzyme levels. Animals treated with 7 days of AVC had the highest values, while the control group had the lowest values. According to above results can conclude that use of acetaminophen and vitamin c combinations is safe for buffaloes and reduce the deleterious effect of heat stress and improve water dairy buffalo physiology and oxidative status.

Introduction

Heat stress is one of the most important types of stress that faced animal and human. In a study dealing with the causes of climate change in Iraq were revealed unprecedented rise in temperatures for the last two decades, and the general trend of rainfall is decreasing in all regions of Iraq (Namdar *et al.,* 2021). It is clear that the phenomena of desertification and drought have worsened in recent years through the climate budget (Al hussaini , 2012). This is what makes Iraq one of the most countries in the world facing heat stress. The combined effects of environmental temperature and relative humidity on livestock are measured by knowing the temperature and humidity index (THI). Therefore, the temperature and humidity index is considered a measure for evaluating the risks of heat stress and preventing its effects on producing animals (Habeeb et al., 2018). Recent studies show that cows is exposed to heat stress when the THI value at rate of 68 and sever effect of heat stress appeared with the increase in of THI values when it exceeds 72 (Dash *et al.,* 2016). Naturally, the buffalo's body absorbs a higher amount of sunlight than cows due to several reasons including its dark color and lack of sweat glands. The regulatory system of the animal's body begins with activities through the rate of breathing and the number of heartbeats, and increase in evaporation through the surface of the skin so that the animal may be can acclimate to the new environment (Al-Zarkan and Al-Anbari, 2020). The response to heat stress is divided into two phases: acute and chronic. The acute response is driven by the autonomic nervous system that stimulates the release of catecholamine and glucocorticoids that alter metabolism and activate factors involved in the acute response (Thorp & Schlaich, 2015). As for the response to chronic stress, it stimulates the endocrine system, which is linked to a receptor, to change the sensitivity of tissues to signals, which leads to a new physiological state, These responses are called acclimatization or adaptation (Fregly & Zajac, 1996).

Consequently, many studies have been conducted on feeding and management treatments to reduce the impact of HS on buffalo performance (sparke *et.al.* 2001). Recently, some studies refer to using a combination of acetaminophen (paracetamol) and vitamin C in drinking water for alleviating HS in animals. This product is used as an antipyretic, analgesic, anti-stress and anti-inflammatory, and is used as an adjunct to the treatment of rheumatism, arthritis, and anti-viral caused by viruses, bacteria, parasites, climatic dust, and increasing animal immunity and improving adaptability to heat stress (Matic ,2016). Park *et al.,* (2020) suggested that a combination of acetaminophen and vitamin C may be beneficial for controlling stress and inflammation caused by FMD vaccination. Studies on the use of paracetamol and vitamin C as an anti-stress drug in cattle are still limited. Scientists need more effort to prove the pontifical of this therapy, despite the increase in manufacturers of this product, the information about the physiological effects on animals is still weak and unclear in this area.

Aim of the study: this study aimed to reduce the effect of heat stress on dairy buffalo by supplement of acetaminophen and vitamin C and evaluating the hematological, antioxidant, and organs function status of adult dairy buffaloes.

Material and methods:

This study was carried out during the summer season during July and August months (23/7/2022 to 21/8/2022) for a period of thirty days herd in Al-chyebaish Marshes, Thi-Qar Province - Southern Iraq. Sixteen lactating buffaloes were used, Age range from (4-7) years and weight range (450-500) kg. All animals were clinically examined by a specialized veterinarian to ensure healthy animals. The studied animals were given free access to the dietary formalizations and water Table (1). Dairy buffaloes were divided into four groups randomly (4 buffalo for each group) as following:-

Treatment 1 (T1; Control): dairy animals were fed on regular ration and drinking clear tape water for 30 day.

Treatment 2 (T2): dairy buffalos were fed on regular ration and drinking tape water supplemented with acetaminophen and vitamin C (500 mg/1000L water for 3 days at a week) a long 30 day of the experiment .

Treatment 3 (T3): dairy buffalo were fed on regular ration and drinking tape water supplemented with acetaminophen and vitamin C (500 mg/1000L water along 5 days) along 30 days of the experiment .

Treatment 4 (T4): dairy buffalo were fed on regular ration and drinking tape water supplemented with acetaminophen and vitamin C (500 mg/1000L water along week) along period of 30 days of the experiment.

Table (1) the ingredients and chemical composition of the concentrate ration used in the study

	Inclusion level (%)
Wheat bran	24
Hay	6
Corn cob	13
Brown flour	45
Molasses	6
Soy bean	5
Mineral mixture	1
Crude Protein %	12.8
Metabolic Energy (Mcal/ kg)	2157

Sample collection:

Blood samples were collected at three intervals along the study through the jugular vein after control procedures for animal and sterilize the area with alcohol. Blood drawn by using disposable syringe 5 ml, and divided into two parts: 2 ml of blood was placed in a tube containing the anticoagulant EDTA for hematological examination. The other 3 ml of blood was put into Gel/clot Activator (without anti-coagulant) which immediately centrifuged to separate serum for subsequent biochemical analysis.

The weather temperature, relative humidity and temperature- humidity index (THI) were recorded by use HTC-2 (China) digital LCD electronic thermo-hygrometer. The THI was calculated by applying the formula of National Research Council (1971), which considers dry bulb temperature (T °C) and relative humidity (RH %) as follows:THI = (1.8 * T °C + 32) – [(0.55 - 0.0055 * RH %) * (1.8 * T °C - 26)]

Hematological Parameters Study:

Complete blood picture analysis were done in Alahwar laboratory to estimate erythrocyte count (RBC), hemoglobin (Hgb), Packed cell volume (PCV) , platelet (PLT), total white blood cell (WBC), neutrophils, lymphocytes, monocytes, basophils). The above hematological parameters were measured each ten days by Sesmex autoanalzer device, Japan.

Biochemical Parameters Study:

The biochemical tests that required for the present study were represented by total protein, urea, glucose, ALP, AST, ALT, and creatinine. All these parameters were measured by using spectrophotometer (Apel,Japan) and specific kit for each parameters used belong to Biolab company, Thailand . After thawing the gel activator tube of serum, samples were mix with reagents. Cortisol and glutathione were measured by using ElISA kit (BT, Bioassay technology Laboratory, China) and read the results by using BioTeck ELISA reader, USA.

Statistical analysis:

Statistical analysis was performed using analysis of variance (T-test) via the SPSS computer package version 21. The difference was considered significant at ($p < 0.05$) and the difference between means was assessed by LSD (SPSS 2016).

Results and discussion:

Table (2) represents the climatic environment recorded inside the buffalo barn during the study period. The lowest ambient temperature (47.7℃) recorded in this study was on the last day of the experiment, resulting in the lowest recorded temperature-humidity index (THI) value of 88.05. In contrast, the highest temperature of 53.2℃ was observed on August 4, 2022. During the period from July 29 to August 3, 2022, there was prevalence of seasonal southerly winds. from the Persian Gulf. These winds often carry high humidity, thus exacerbating HS for animals. The highest recorded THI value of 99.31 was observed on July 31, 2022.

The results of some hematological parameters revealed significant increase ($P \leq 0.05$) in RBC, Hb, and HCT values for the buffalos supplemented with AVC in certain days of week compared with buffalos of control group (table 3). In addition, 7 days AVC group had the best values ($P \leq 0.05$) for the above parameters along the four weeks of the study than the other studied groups. Whereas, the results referred to non- significant difference ($P \leq 0.05$) among periods (10,20 and 30 day) of the same group.

Total leukocyte account that represented by table 4&5 showed significantly reduce ($P \leq 0.05$) in their values during the 10 days of experiment for the 7 days AVC group when compared with other studied groups that appeared non-significant difference ($P \leq 0.05$) among each other. After 30 days of the experiment, the 3 days AVC and 7 days AVC groups had less significant values in leukocyte count than the control and 5 days AVC groups. In contrast, neutrophils of buffalos supplemented with AVC showed significant decrease (P≤0.05) in their values when compared with control group along the periods of the study. The Elevation in monocyte of control group reduced significantly (P≤0.05) after 30 days of the experiment and showed significant (P≤0.05) with groups supplemented by AVC.

The table 6 represents the effect of HS and AVC supplement on liver enzymes activities of dairy buffalos. The activity of ALT showed significant elevation (P≤0.05) for the control, 3 days AVC and 5 days AVC groups, respectively when compared with values of ALT in 7 days AVC group in different three periods of the study. While AST activity improved in 3 days AVC group after 30 days of the experiment to show significant decrease (P≤0.05) with control group. In contrast, the 5 days AVC and 7 days AVC supplemented groups improved their values significantly (P≤0.05) after 10 days of experiment periods compared with control group. From the other side, ALP enzyme activity was recorded significant elevation (P≤0.05) for control, 3 days AVC and 5 days AVC groups after 10 days of experiment when compared with 7 days AVC group which appear significantly reduce (P≤0.05) in ALP enzyme activity among all the studied groups along the periods of experiment.

The markers for kidney function of heat stressed dairy buffalos supplemented with acetaminophen and vitamin C represented by table (7). The results revealed non-significant differences in urea and creatinine levels between control group and AVC supplemented groups, also, there was no significant difference in creatinine and urea levels between periods of study when compared each with other.

The levels of cortisol hormones and glutathione enzyme are illustrated in table (4-10). This table showed significant reduce (P≤0.05) in cortisol hormone level and glutathione enzyme level for the buffalos supplemented with AVC in different periods of the study compared with buffaloes of control group that appeared high significant (P≤0.05) levels of cortisol and glutathione. While the results revealed there was no significant differences (P≤0.05) among the periods of the study for each group alone.

Discussion:

It is clear that temperature, humidity and THI remained elevated throughout the extended study period (THI from 88.05 to 99.31). These conditions far exceed the recommended comfort levels for buffalo, with a THI of less than 77 considered optimal for their well-being (Matera et al., 2022; Stefani et al., 2022; Yadav et al., 2022; Behera et al., 2023; Omran and Food., 2023; Shu et al., 2023). The Iraqi buffalo's ability to survive and endure in such extreme temperatures, where temperatures reach above 52℃, highlights the buffalo's ability to adapt to extreme environmental conditions. Many other buffalo breeds would struggle to withstand such extreme temperatures and could face extinction under similar conditions.

The hematological finding of the study is consistent with the research by Sunil Kumar et al. in 2011, which showed that vitamin C increased feed consumption and subsequently improved the number of red blood cells and certain blood parameters. Abdullah et al., (2009) suggested that the decrease in the number of red blood cells in ruminants during the summer period may be attributed to the reduction in thyroid hormone secretion, leading to decreased factors for the formation and production of blood cells. Studies related to HS in Mora buffaloes in India conducted by (Vijayakumar et al., 2011) and(Imran et al., 2011) also showed a decrease in the percentage of hemoglobin. Hemoglobin plays a crucial role in gas exchange within living organisms by transporting oxygen from the lungs to the cells of the body. High environmental temperatures were found to negatively affect hemoglobin levels in the blood.

The increase in the number of WBC during the summer periods compared to the winter season during exposure to HS may be attributed to the effect of the hormone cortisol, which increases the production of white cells (Al-busaidi et al., 2008). While Omar et al., (2021) demonstrated that the high rate of white blood cells is influenced by HS on thyroid hormone levels and the secretion of thyroxin. While Ashour et al, (2004) found there was drop in neutrophils % concomitant with the rise in lymphocytes. The white blood cells count was significantly elevated (around 50%); in extent there was increasing in neutrophils and monocytes while lymphocytes and eosinophils have decreased with marked elevation in neutrophils/lymphocytes ratio (Omran et al., 2011). These studies agreed with the results of present study in drop of RBC,HB and HCT due to HS, and the ameliorative effect of AVC supplement appear clear by repair the hematological indices values .

Verma et al. (2022b) reported significant alterations in the serum levels of urea, phosphorus, and alkaline phosphatase activity in six lactating Murray buffaloes in response to elevating temperature humidity index (THI). These findings suggest that HS can affect the metabolism and biochemistry of buffaloes, leading to changes in various biochemical parameters, it is important to note that these alterations in biochemical parameters can have significant health consequences for the buffaloes, and can potentially affect their productivity and reproductive performance. Therefore, it is crucial for animal producers and caretakers to monitor and manage environmental conditions to minimize the risk of HS and associated health issues in their livestock. Umar et al. (2021) studied the impact of thermal stress on 96 Murrah and graded Murray buffaloes over a period of one year in Jammu, India. The author reported that the plasma levels of AST and ALT were relatively stable in the THI range of 65-72, but the AST levels decreased while the ALT levels increased significantly in response to higher THI levels (>73). This suggests that HS may lead to liver damage in buffaloes, as ALT is an indicator of liver injury, Additionally, Umar and his team observed a reduction in the levels of both glutathione peroxidase and superoxide dismutase in response to thermal stress. These enzymes are involved in the antioxidant defense system of the body, and their reduction suggests that buffaloes are under oxidative stress during heat stress, which may result in cellular damage and dysfunction, Overall, the findings of this study suggest that thermal stress has significant negative effects on the metabolic, liver function, and antioxidant defense systems of buffaloes, which may impact their health and productivity. Also, Li et al. (2020) conducted a study on the effect of high temperature and relative humidity on 20 non-lactating Nile-Ravi buffaloes during the months of June to August 2017 in Nanning, China, the results of the study showed that the high temperature and humidity levels had lower levels of serum glutathione peroxidase. These studies agreed with the results of the present study when showed high levels of cortisol and glutathione as marker for oxidative status of the buffaloes that suffered from thermal stress. The hypothalamic-pituitary axis controls the secretion of cortisol and adrenal hormones by secreting the adrenal stimulating hormone known as ACTH (Adrenocorticotropic hormone). Cortisol plays a crucial role in numerous physiological processes, particularly energy production and thermoregulation. (Abdel-Samee et al., 1992). Researcher was discovered that the concentration of the hormone cortisol increased in cattle when they were exposed to severe environmental conditions, such as high temperatures. (Alvarez, 1997). Haque et al. (2012) found that the concentration of cortisol in blood plasma increases with temperature, as the cortisol concentration at 22 degrees Celsius was 7.77 ng/ml and at 45 degrees Celsius it was 16.12 ng /ml. In other study done by Kumar and Singh (2021) that examined the effect of a high temperature humidity index (THI) on the development of

Murrah buffaloes in India. The results revealed an increase in cortisol levels and a decrease in growth hormone and IGF-I levels, indicating that thermal stress may negatively impact the health and growth of buffaloes.

As a medicine commonly used for antipyretic, it is believed that the acetaminophen mechanism of action in lowering body temperature lies in its effect on the anteroposterior hemisphere, the part of the brain responsible for temperature regulation (Bührer et al., 2021). This is done by affecting the central nervous system and the thermoregulatory center in the brain, in addition to suppressing the production of chemicals responsible for temperature regulation in the body (Vallejo et al., 2010).

Acetaminophen as a medicine commonly used for antipyretic, it is believed that the acetaminophen mechanism of action in lowering body temperature lies in its effect on the anteroposterior hemisphere, the part of the brain responsible for temperature regulation (Bührer et al., 2021). This is done by affecting the central nervous system and the thermoregulatory center in the brain, in addition to suppressing the production of chemicals responsible for temperature regulation in the body (Vallejo et al., 2010). It's suggested that the commercial combination of vitamin C and paracetamol can potentially remain stable while preserving the properties of both components at room temperature for extended periods of up to 11 days in its liquid formulation (Golonka et al., 2015). Whereas the powder forms of vitamin C and paracetamol combination, normally, could show stability for a longer time. Under stressful conditions (such as high thermal load, restricted water intake, physiological changes, and diseases) the demand for vitamin C may exceed its natural production capacity. Consequently, this imbalance can result in reduced performance, heightened vulnerability to infections, and increased mortality rate (Akinmoladun, 2022). Emerging evidence suggests that the levels of ascorbic acid in blood and other tissues decline during periods of stress (Sinha et al., 2017). Vitamin C plays a crucial role in numerous physiological processes in animals. One of the biochemical role of vitamin C is to act as an antioxidant (a reducing agent) by donating electrons to various enzymatic and non-enzymatic reactions, which will help protect cells and tissues from oxidative damage caused by the generation of reactive oxygen species responsible for destroying the body cells (Guo et al., 2018). Many researchers have highlighted the potential of supplementing with vitamin C to reduce the severity of HS in ruminants during hot weather. The mechanisms by which vitamin C alleviates HS involve enhancing heat dissipation through improved blood flow to the skin, increased sweating, and better regulation of body temperature (Akinmoladun, 2022). Vitamin C has also been suggested to modulate hormonal responses, including the reduction of stress hormones like cortisol and the improvement of thyroid function, which may help mitigate the negative effects of heat stress (Martinez et al., 2022). Despite its potential benefits, studies investigating the impact of acetaminophen supplementation on alleviating HS in dairy animals is very limited and almost non-existent. Except Park et al. (2020) examined the impact of acetaminophen and vitamin C combination on stress and inflammation in Korean native cattle vaccinated against foot-and-mouth disease (FMD) .The results indicated that the acetaminophen and vitamin C combination at the level of 1.0 kg/ton feed for 5 days after FMD vaccination could effectively manage HS and inflammation in calves.

Conclusion: Through the study, the effect of heat stress on the blood, biochemical and oxidative properties of dairy water buffalo was shown to be affected, as well as the effective positive role of adding paracetamol and vitamin C in improving the performance of animals in terms of physiology and reducing oxidative stress.

Table (2) Heat stress during the period of experiment represented by environmental temperature, relative humidity and Temperature and humidity index

Date of measurement	Ambient Temperature °C	Relative Humidity %	THI
23/07/2022	48.2	10	88.68
24/07/2022	47.5	12	88.70
25/07/2022	46.4	10	87.05
26/07/2022	46.9	10	87.50
27/07/2022	47.8	10	88.32
28/07/2022	47.8	10	88.32
29/07/2022	47.9	21	92.05
30/07/2022	47.8	22	92.28
31/07/2022	52.1	29	99.31
01/08/2022	52.5	15	94.48
02/08/2022	52.5	15	94.48
03/08/2022	52.8	14	94.38
04/08/2022	53.2	10	93.23
05/08/2022	51.9	10	92.05
06/08/2022	52.3	10	92.41
07/08/2022	52.1	10	92.23
08/08/2022	52.2	10	92.32
09/08/2022	51.3	10	91.50
10/08/2022	51.2	10	91.41
11/08/2022	48.2	10	88.68
12/08/2022	47.6	10	88.14
13/08/2022	49.5	10	89.87
14/08/2022	48.4	10	88.87
15/08/2022	50.2	10	90.50
16/08/2022	48.6	10	89.05
17/08/2022	49.4	10	89.77
18/08/2022	48.4	10	88.87
19/08/2022	49.2	10	89.59
20/08/2022	48.1	10	88.59
21/08/2022	47.5	10	88.05

Table (3) some hematological indices (RBC, Hb and HCT) of heat stressed dairy buffalos supplemented with Acetaminophen and Vitamin C (AVC) (Mean ± S.E)

Treatments/ weeks	RBC (10^{12}/L)			HB (g/dl)			HCT %		
	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
Control	2.35 B ±0.24	3.18 B ±0.37	2.73 B ±0.26	8.75B ±0.35	9.10 ±0.33	9.12 B ±0.34	25.65 ±0.71	26.55 B ±0.92	27.47 B ±0.51
3 days AVC (500 mg/1000L)	4.33 A ±0.09	5.13 A ±0.33	4.77 A ±0.17	10.95A B ±0.99	10.25 ±0.45	10.25A B ±0.38	28.75 ±2.45	28.72A B ±0.55	27.90 B ±0.58
5 days AVC (500 mg/1000L)	4.10 A ±0.30	5.21 A ±0.44	4.43 A ±0.25	9.72 AB ±0.88	7.47 ±2.55	10.27A B ±0.449	28.15 ±2.11	29.85A ±0.64	31.27 A ±0.87
7 days AVC (500 mg/1000L)	4.7 A ±0.28	4.79 A ±0.25	5.03 A ±0.49	12.08 A ±0.44	11.85 ±0.22	11.31 A ±0.47	27.85 ±0.59	30.27 A ±0.86	31.69 A ±0.67

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

Table (4) Total and differential leukocytes (WBC, Neutrophils and Monocyte) values of heat stressed dairy buffalos supplemented with Acetaminophen and Vitamin C (AVC) **(Mean ± S.E)**

Treatments/ Weeks	WBC (10^9/L)			Neutrophils (10^9/L)			Monocyte (10^9/L)		
	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
Control	13.13 A ± 0.59	10.47 ±0.87	10.65 A ±0.47	2.84 A ±0.50	2.89 A ± 0.58	3.17 A ± 0.69	1.34 A ±1.49	1.99 A ±3.41	1.20 ±0.135
3 days AVC (500 mg/1000L)	8.72 A ±1.93	7.40 ±1.42	7.20 B ±1.54	1.89 AB ±1.24	1.07 B ±0.39	1.76 B ±0.27	1.05 Ba ±0.28	1.02 Ba ±0.11	0.26 b ±0.177
5 days AVC (500 mg/1000L)	12.45 A ±1.38	9.05 ±0.54	9.57 A ±0.56	2.22 AB ±0.25	1.67 B ±0.22	1.95 B ±0.25	1.27 Ba ±0.76	1.55 Ba ±0.41	0.85 b ±0.499
7 days AVC (500 mg/1000L)	8.30 B ±1.09	7.73 ±3.89	7.39 B ±3.82	1.00 B ±0.17	1.27 B ±0.31	1.81 B ±0.34	0.42 B ±0.07	0.65 B ±0.15	0.22 ±1.155

Capital letters represent significant difference vertically at ($P \leq 0.05$) level

Table (5) Total and differential leukocytes (Lymphocyte **and** Basophil) **values of heat stressed dairy buffalos supplemented with Acetaminophen and Vitamin C** (AVC) **(Mean ± S.E)**

Treatments	Lymphocyte (10^9/L)			Basophil (10^9/L)		
	10 days	20 days	30 days	10 days	20 days	30 days
Control	8.67 ±1.09	6.92 ±0.97	6.45 ±1.52	0.21 ±0.03	0.35 ±0.07	0.70 ±0.05
3 days AVC (500 mg/1000L)	7.43 ±2.35	6.60 ±1.38	5.81 ±0.28	0.27 ±0.01	0.02 ±0.11	0.50 ±0.12
5 days AVC (500 mg/1000L)	7.28 ±0.48	6.10 ± 0.16	6.04 1.17	0.20 ±0.08	0.12 ±0.04	0.75 ±0.26
7 days AVC (500 mg/1000L)	6.80 ±2.34	6.82 ±1.99	6.04 ±1.95	0.12 ±0.02	0.57 ±0.09	0.17 ±0.04

Table (6) liver function enzymes activities of heat stressed dairy buffalos supplemented with Acetaminophen and Vitamin C (AVC) (Mean ± S.E)

Treatments/ weeks	ALT (u/L)			AST (u/L)			ALP (u/L)		
	10 days	20 days	30 days	10 days	20 days	30 days	10 days	20 days	30 days
Control	45.00 A ± 3.53	42.00 A ±3.24	48.15 A ±2.01	37.75 A ±6.57	48.25 A ±3.77	50.50 A ±4.11	48.75 A ±6.22	46.55 A ±2.39	52.50 A ±2.62
3 days AVC (500 mg/1000L)	36.50 B ±3.12	41.25 A ±4.15	51.25 A ±3.27	35.72 A ±6.55	46.37 A ±3.45	43.50 B ±3.22	37.75 B ±6.34	42.40 A ±2.45	45.75 A ±2.62
5 days AVC (500 mg/1000L)	49.50 A ±4.09	41.50 A ±0.95	41.50 A ±6.58	42.45 A ±3.02	33.85 B ±2.15	42.50 B ±1.75	42.50A B ±4.48	48.42 A ±1.55	51.50 A ±2.84
7 days AVC (500 mg/1000L)	19.25 B ±4.13	28.15 B ±4.62	24.50 B ±3.59	24.72 B ±2.96	32.82 B ±2.66	24.50 C ±2.72	20.25 C ±1.10	22.87 B ±1.80	23.25 B ±2.65

Capital letters represent significant difference vertically at ($P \leq 0.05$) level.

Table (7) Renal function markers of heat stressed dairy buffalos supplemented with Acetaminophen and Vitamin C (AVC) (Mean ± S.E)

Treatments/ weeks	**Creatinine (mg/ml)**			**Urea (mg/ml)**		
	10 days	**20 days**	**30 days**	**10 days**	**20 days**	**30 days**
Control	1.00 ±0.20	1.62 ±0.22	1.06 ±0.41	27.25 ±4.19	39.70 ±0.78	39.00 ±2.48
3 days AVC (500 mg/1000L)	1.32 ±0.36	1.97 ±0.07	1.47 ±0.21	30.00 ±6.57	36.50 ±2.95	33.25 ±2.83
5 days AVC (500 mg/1000L)	1.60 ±0.10	1.99 ±0.164	1.82 ±0.28	34.75 ±3.83	33.00 ±2.48	38.50 ±1.55
7 days AVC (500 mg/1000L)	1.16 ±0.79	1.42 ±0.34	1.55 ±0.62	30.50 ±2.68	38.00 ±2.11	36.00 ±3.13

Table (8) Cortisol and glutathione levels of heat stressed dairy buffalos supplemented with Acetaminophen and Vitamin C (AVC) (Mean ± S.E)

Treatments/ Weeks	Cortisol (g/L)			Glutathione (g/L)		
	10 days	20 days	30 days	10 days	20 days	30 days
Control	8.14 ^{A} ±0.68	7.77 ^{A} ±0.1	6.88 ^{A} ±0.57	12.30 ^{A} ±1.02	13.77 ^{A} ±1.30	12.83 ^{A} ±0.71
3 days AVC (500 mg/1000L)	7.00 ^{AB} ±0.56	5.57 ^{B} ±0.05	5.10 ^{B} ±0.45	9.62^{B} ±0.81	8.62 ^{B} ±0.72	10.23 ^{A} ±0.82
5 days AVC (500 mg/1000L)	5.59 ^{B} ±0.72	6.15 ^{B} ±0.72	6.25 ^{AB} ±0.25	9.65 ^{B} ±1.11	10.80 ^{B} ±1.03	4.95 ^{B} ±0.07
7 days AVC (500 mg/1000L)	4.84 ^{B} ±0.26	4.73^{B} ±1.48	4.32 ^{B} ±1.63	9.31 ^{B} ±1.66	4.85 ^{C} ±0.11	4.32 ^{B} ±0.06

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