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Impact of Heat Stress on Lactating Egyptian Buffaloes (*Bubalis bubalis*): Physiological, Hormonal and Oxidative Responses

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ABSTRACT

Heat stress typically causes substantial financial losses due to the reduction in animal performance and the increase in morbidity or mortality. It is essential to comprehend the physiological reactions and biochemical alterations through different body homeostasis during various environmental seasons to prevent harmful impacts of heat stress. Objective of this experiment was to evaluate the different seasonal dynamics of the Egyptian buffaloes' oxidative, hormonal, metabolic and physiological responses to the country's summer season climate. Throughout the year, the physiological reactions of twenty lactating water buffaloes were measured. These responses included rectal temperature (RT), respiration rate (RR) and body surface temperature (BST), in addition to serum hormonal, oxidative/antioxidant, and metabolic indices. Significant increases in RR and BST were detected in summer season in relation to winter season when the temperature-humidity index (THI) was higher (>80). The heat stress during the summer season was associated with lower serum antioxidant enzyme levels and higher oxidative stress. Serum cortisol and hepato-renal functional bio-indicators were also significantly elevated in the summer and autumn. In comparison to former seasons, the levels of growth hormone (GH), triiodothyronine (T3), thyroxine (T4), and adrenocorticotrophic hormone (ACTH) were also much reduced in the autumn and summer. THI was found to have a negative relation with physiological and antioxidant indices, but a positive relation with serum cortisol and malondialdehyde (MDA) levels. According to our findings, lactating Egyptian buffaloes have considerable heat stress during the summer season, which necessitates greater managerial measures to prevent animal welfare concerns and economic losses.

1. INTRODUCTION

Environmental status is an arrangement of numerous aspects as temperature, air movement, humidity, rainfall, radiation, ionization, and barometric pressure. Heat stress brought on by extreme weather conditions at higher temperatures can result in significant reductions in livestock productivity (Napolitano et al., 2023). Animals' reproduction and productivity are significantly impacted by global warming. By 2100, an increase in temperature of 1.8 to 4 Â°C is predicted (IPCC, 2013). Egypt has experienced heat waves over the past ten years, stressing the animals and reducing their output; in which upper Egypt is classified as a tropical region and lower Egypt as a subtropical region (Omran and Fooda, 2023). Heat stress is typically linked to problems with animal welfare and large financial losses from livestock's decreased productivity, illness, and mortality (Li et al., 2021). Given the current climate change scenario, which will likely raise the probable concerns of humid and hot circumstances in upcoming days and increase the frequency of heat stress episodes, the problem of heat stress is anticipated to get inferior (Gonzalez-Rivas et al., 2020). A few reviews of the detrimental impacts of heat stress on the physiology of animals have been conducted before on different ruminant species (Omran et al., 2020; Hussain et al., 2023; Napolitano et al., 2023). Egyptian buffalo (*Bubalus bubalis*) is an essential tropical species adjusted to environmental and managerial circumstances in Egypt and further developed countries. It is a primary source of 45–50% of milk production and roughly 35–40% of meat output (MALR, 2017). The FAO (2019) estimates that there are roughly 3.7 million buffaloes in Egypt. A cow emits 376 mg of methane per day, compared to the buffalo's 157 gm, or 58% less. This means that buffaloes are more environmentally friendly than cows (Omran et al., 2020). According to Atwa et al. (2019), buffaloes are valuable livestock for Egyptian farmers and breeders because of their ability to withstand disease and adapt to harsh environments. They are also thought to be an effective way to transform low-value roughages into extremely nourishing foodstuffs, such as meat and milk.

Buffaloes have thick epidermis on their skin that shields them from UV radiation. Their shiny covering, which reflects heat radiation to prevent overheating, is caused by secretions from many sebaceous glands (Li et al., 2021). Nevertheless, buffalo are less tolerant to heat than cattle because they have fewer sweat glands and a darker body color (Bombade et al., 2018). Furthermore, buffaloes are more vulnerable to heat stress because of enhanced milk production in addition to experiencing to humid, hot settings, particularly when there is a lack of adequate protection, wallowing space, or swimming facilities (Das et al., 2014). Dairy animals are extra vulnerable to heat stress due to their greater metabolic rates and the more heat produced throughout rumen fermentation (Shenhe et al., 2018). This is because higher milk yields are linked to higher levels of metabolic heat production in the body (Lakhani et al. 2018).

Heat stress is commonly detected when environmental temperature surpasses the thermoneutral zone of the body of the animal and causes a difference amongst heat produced and/or gains in the body and its dissipation. In addition to causing changes in hormonal and metabolic secretions and a reduction in the intake of food, heat stress in buffaloes also raises their rectal temperature (RT), body surface temperature (BST), respiration rate (RR), and oxidative stress (Omran and Fooda, 2023; Soliman et al., 2024). Temperature-humidity index (THI) is a frequently applied metric to assess thermal comfort. It has been used with several livestock species to comprehend the physiological phases of heat-stress and thermo-neutral zones. To illustrate degree of thermal stress, THI standards have been divided into three phases: Severe stress is >89, moderate stress is 79–89 and mild stress is 72–79 (Bombade et al., 2018). It is advised to take management measures (such as cooling) when the THI value is higher than 70 in order to prevent dairy cows from producing less milk (Alhussien and Dang, 2018).

Buffaloes exhibit heat stress symptoms even though they have an efficient thermoregulatory system when they are subjected to high ambient temperatures (36 °C or beyond), intense direct sun radiation, and high relative humidity (Das et al., 2014). Although buffaloes are thought to have more thermotolerance than dairy cattle, a THI score of ≤74 is ideal for raising buffaloes (Hussain et al., 2023). Research on how resilient animals may be to these new conditions under gradual global warming scenarios and climate variation is needed, especially in subtropical weather zones (such as lower Egypt), where the mean environmental temperature is predicted to upsurge the most throughout the coming decades (IPCC, 2013).

2. MATERIAL AND METHODS

2.1. Ethical approval

The committee of animal care (AU 13-2024-307) at Alexandria University provided guidelines and rules for the care and management of experimental animals.

2.2. Animals, ration, and experimental design

Twenty mature, healthy, lactating Egyptian buffaloes in total were chosen at random for this experiment. Each buffalo weighed 580 ± 20 kg on average, and they were all in their mid-lactation period (3–4 months). Every buffalo had four lactations on average (4 ± 1) and was milked two times a day in the morning and afternoon. The experiment was conducted between April 2023 and March 2024, the animals were bred at a private farm in the El-Beheira governorate, which is in lower Egypt's northernmost region and consists of the Nile Delta between Upper Egypt and the Mediterranean Sea. The animals were kept in loose, shaded quarters with adjoining outdoor yards. The total mixed ration that was sold commercially was fed to the experimental animals. There was always clean water available for the animals. Table 1 provides an overview of the ration's components and analysis. Throughout the trial period, buffaloes produced an average of 8.13 kg of milk/day. Adherence to a plan for deworming and immunization served as a preventative measure against parasitic infestation and illnesses.

2.3. Measurements and sampling

2.3.1. Meteorological data:

The meteorological data incorporated air temperature (AT, Â°C) and relative humidity (RH, %), which was weekly documented on average to estimate the temperature-humidity index (THI) (Li et al., 2021). The buffalo shed's AT and RH were measured at 06:00, 12:00, and 18:00 hours using a digital hygrometer-thermometer instrument at a predetermined time. Throughout the year, four seasons were identified as follows: winter (December to February), autumn (September to November), summer (June to August), and spring (March to May).

2.3.2. Physiological parameters recording

Using an animal infrared thermometer, the average BST/week was measured every Wednesday at 8:00 AM and 2:30 PM from three distinct locations (the forehead, left thoracic region, and abdomen). In parallel, RR was calculated as times/minute by watching thoracic movements with a counter and stopwatch (for two minutes), while RT was measured by a veterinary rectal thermometer (by putting it in rectum for 15 sec.) (Omran and Fooda, 2023).

2.3.3. Serum samples collection:

Every season—spring, April 18th, summer, July 23rd, autumn, October 19th, and winter, January 29th—blood samples were drawn from the jugular vein prior to morning feeding (Li et al., 2021). After collection, the samples of blood were placed on ice and sent straight to lab so that the serum could be separated. The serum was kept cold at –20 Â°C, awaiting additional examination.

2.3.4. Hormonal profile estimation:

Utilizing commercial ELISA Kits (CUSABIO BIOTECH CO., Wuhan, China) by using ELISA assay (ELISA microplate reader) in accordance with manufacturer's instructions, the serum hormones, including adrenocorticotropic hormone (ACTH) (Weina et al., 2018) (cat. No.: CB13–01), insulin (cat. No.: CB03–14), cortisol (cat. No.: 057-24), triiodothyronine (T3) (cat. No.: CB62-03), thyroxine (T4) (Koulouri and Gurnell, 2013) (cat. No.: CB62-02), and growth hormone (GH) (Lee et al., 2020) (cat. No.: 112-01) were examined.

2.3.5. Oxidative/antioxidants status:

Following the manufacturer's instructions, the concentration of oxidative stress marker malondialdehyde (MDA) (Buege and Aust, 1978) (cat. No.: NB003-81) and the levels of serum antioxidant enzymes, including glutathione peroxidase (GSH-Px) (Beutler et al., 1063) (cat. No.: NB005-25), total superoxide dismutase (SOD) (cat. No.: NB001-22), and catalase (CAT) (Aebi, 1984) (cat. No.: NB007-36), were measured using a spectrophotometer and the Nanjing Built-in Kits (www.njjcbio.com).

2.3.6. Metabolic parameters measurement:

Standard diagnostic kits (Biodiagnostics Company, Egypt) were used to determine the serum total protein, albumin, liver function tests (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)) (Young et al., 2008), kidney function test (blood urea nitrogen (BUN)) (de Castro et al., 2014), and glucose (Trinder, 1969) values spectrophotometrically (Perkin Elmer Model No. 186, Germany).

2.4. Statistical analysis

The mean ± SE is the average values for all buffaloes for each season. Via SAS software type 9.2 (SAS Institute Inc., Cary, NC, USA), values of physiological parameters were evaluated using recurrent measure analysis of variance and then Tukey's post hoc test to relate the levels between various seasons. As previously mentioned, the data on blood hormones, metabolic parameters, and oxidative stress markers were compared between seasons using the student's t test (SAS, 1994).

3. RESULTS

3.1. Meteorological data and Physiological parameters

Table 2 demonstrates the average shed THI levels and the physiological characteristics of lactating buffaloes through various seasons. With the greatest THI value (81) in the summer, THI in spring, autumn, and summer was substantially greater than in winter ($P < 0.05$). Summer season RR and BST values were substantially greater than winter BST values ($P < 0.05$). Nevertheless, there was no noticeable variation ($P > 0.05$) in the buffaloes' RT between seasons.

3.3. Serum hormonal profile

Table 3 shows that the concentration of ACTH was substantially lower in the summer and significantly greater ($P < 0.05$) in the winter relative to further seasons. Summer and autumn months had considerably greater ($P < 0.05$) cortisol contents than the spring and winter months. But there was no discernible variation in insulin concentrations between the seasons ($P > 0.05$). T3 value was lowest ($P > 0.05$) in autumn and summer, whereas it was highest in spring and winter. Autumn and winter had a significantly ($P < 0.05$) greater T4 content than summer and spring. Winter had the highest level of GH, while summer had the lowest ($P < 0.05$).

3.4. Serum oxidative marker and antioxidant enzymes

Table 4 displays the average values of antioxidant enzymes in the serum during the various seasons. The summer months had considerably

higher ($P < 0.05$) MDA levels than the other seasons. In comparison to winter, the higher MDA levels suggested a significant level of oxidative stress. Additionally, autumn's MDA contents were considerably greater than those of spring and winter ($P < 0.05$). The MDA levels of the winter and spring did not alter significantly (P > 0.05). When compared to other seasons, winter had the highest contents (P < 0.05) of GSH-Px, CAT, and SOD, while summer had the lowest average concentrations of all three enzymes (P < 0.05).

3.5. **Blood metabolites**

Table 5 displays seasonal differences in the lactating buffaloes blood metabolites. Seasonal variations in total protein, albumin, and globulin levels were not significant. Conversely, summer season ALT and AST concentrations were noticeably greater than those of further seasons (P < 0.05). Summer has the maximum concentration of urea nitrogen, with autumn, spring, and winter following. Between the seasons, there was no discernible variation in the glucose contents.

Table 1: Constituents, nutrient's composition, and calculated analysis of experimental ration.

Experimental ration	**% DM**
Ingredient	DM
Corn silage	17.5
Beet sugar pulp	9.5
Yellow corn	18.4
SBM (44%)	10.2
Berseem hay	40.7
Salt	0.54
Sodium bicarbonate	1.00
Limestone	0.68
Calcium phosphate (monobasic)	0.50
Magnesium oxide	0.35
Premix*	0.13
Calcium bentonite	0.50
Total	100
Calculated analysis	
NE_l (Mcal/kg)	1.28
CP (% DM)	12.8
Forage NDF (% DM)	32.61
Ether‑extract (% DM)	2.10
RUP (% CP)	33.45
Ca (% DM)	0.90
P (% DM)	0.37

*Contained 20.0% Cl, 13.0% Na, 10.0% Ca, 8.0% Mg, 8.0% S, 1.0% K, 0.62% Zn, 0.54% Mn, 0.20% Fe, 0.08% Cu, 0.07% I and 0.2% Co, 551 IU/g Vitamin A, 132 IU/g Vitamin D, 3 IU/g Vitamin E. NEl=Net energy for lactation, CP=Crude protein, NDF=Neutral detergent fiber, RUP=Rumen un‑degradable protein, DM=Dry matter

Table 2. Average Temperature–humidity index (THI) and physiological measurements of lactating buffaloes during different seasons.

Parameters	**Spring**	**Summer**	**Autumn**	**Winter**
THI	72.38 ± 3.25^{a}	81.00 ± 0.84^{a}	73.25 ± 3.38^{a}	59.67 ± 2.94^{b}
Respiratory rate (times/min)	12.67 ± 1.49^{ab}	16.48 ± 1.11^{a}	14.85 ± 2.27^{ab}	9.97 ± 2.94^{b}
Body surface temperature (Â°C)	28.62 ± 1.38^{b}	32.33 ± 0.75^{a}	30.78 ± 2.42^{ab}	24.42 ± 0.52^{c}
Rectal temperature (Â°C)	37.57 ± 0.13	37.27 ± 0.09	37.38 ± 0.05	37.57 ± 0.21

Means ± S.E. Means with different superscripts in the same row vary significantly (P < 0.05) (N = 20).

Table 3. Serum hormonal profile in lactating buffaloes throughout different environmental seasons

Hormones	**Spring**	**Summer**	**Autumn**	**Winter**
Corticotrophin (ACTH) (pg/ml)	49.04 ± 6.48^{b}	26.43 ± 3.54^{c}	44.64 ± 5.27^{b}	73.24 ± 6.34^{a}
Cortisol (ng/ml)	38.13 ± 3.55^{b}	52.24 ± 4.51^{a}	51.36 ± 4.18^{a}	35.02 ± 2.51^{b}
Insulin (nIU/ml)	13.06 ± 1.48	13.34 ± 2.87	15.49 ± 2.84	13.27 ± 1.65
Triiodothyronine (T3) (ng/ml)	2.42 ± 0.33^{a}	1.21 ± 0.12^{c}	1.33 ± 0.31^{c}	1.79 ± 0.22^{b}
Thyroxin (T4) (ng/ml)	67.28 ± 4.16^{ab}	50.38 ± 3.31^{b}	77.18 ± 5.23^{a}	73.74 ± 3.22^{a}
Growth hormone (GH) (ng/ml)	12.48 ± 1.61^{ab}	7.89 ± 1.83^{c}	10.28 ± 2.11^{bc}	14.45 ± 1.03^{a}

Means ± S.E. Means with different superscripts in the same row differ significantly (P < 0.05) (N = 20).

Table 4. Seasonal variations in serum antioxidant enzymes in lactating buffaloes

Parameters	Spring	Summer	Autumn	Winter
MDA (nmol/ml)	2.90 ± 0.21^{c}	11.29 ± 0.43^{a}	8.32 ± 0.60^{b}	2.88 ± 0.25^{c}
GSH-Px (U/ml)	206.22 ± 9.84^{b}	110.16 ± 8.35^{d}	149.67 ± 12.07^{c}	243.53 ± 13.18^{a}
SOD (U/ml)	31.11 ± 2.14^{b}	12.14 ± 1.13^{d}	25.48 ± 3.22^{c}	37.37 ± 2.04^{a}
CAT (U/ml)	10.48 ± 2.33^{b}	2.88 ± 1.24^{c}	3.16 ± 0.54^{c}	19.33 ± 1.01^{a}

Means ± S.E. Means with different superscripts in the same row differ significantly (P < 0.05). (N = 20). CAT, catalase; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase.

Table 5. Values of various blood metabolites in lactating buffaloes throughout different seasons

Parameters	Spring	Summer	Autumn	Winter
Total protein (g/L)	70.12 ± 4.32	70.15 ± 5.87	71.77 ± 4.11	68.53 ± 7.00
Albumin (g/L)	30.96 ± 1.84	33.73 ± 3.02	30.23 ± 2.08	33.02 ± 2.80
Globulin (g/L)	39.15 ± 4.91	36.62 ± 5.85	41.54 ± 4.07	35.51 ± 6.09
Alanine aminotransferase (ALT) (U/L)	51.54 ± 8.89^{b}	59.82 ± 10.68^{a}	52.11 ± 9.61^{b}	37.05 ± 6.37^{c}
Aspartate aminotransferase (AST) (U/L)	146.23 ± 11.64^{b}	177.29 ± 28.62^{a}	154.90 ± 13.26^{b}	113.88 ± 25.02^{c}
Blood urea nitrogen (BUN) (mmol/L)	6.56 ± 0.81^{c}	14.50 ± 1.31^{a}	9.81 ± 1.09^{b}	5.25 ± 1.19^{c}
Glucose (mmol/L)	3.34 ± 0.24	3.14 ± 0.12	3.47 ± 0.46	3.55 ± 0.38

Means ± S.E. Means with different superscripts in the same row differ significantly (P < 0.05). (N = 20)

4. DISCUSSION

Among the many measures of an animal's ability to adapt to its surroundings, physiological state is one of the most significant. The reproductive and production efficiency of farm animals is adversely influenced by hot environment conditions (Hussain et al., 2023). In the current study, significant differences were found in the lactating buffaloes' physiological responses in various environmental seasons of the year. increased THI values (>80) were seen in meteorological data for four months (June to September), and these values were well correlated with increased RR and BST in lactating buffaloes. Increased BST in the summer and fall, compared to other seasons, suggested that increased THI impacted lactating buffaloes. The temperature-humidity index (THI), according to Omran et al. (2020), is frequently applied as a useful indicator of the grade of climatic stress on animal physiology. Environmental sections of Egypt are classified by this index into three sectors, with Lower Egypt having a more stressful climate than Upper and Middle Egypt (because of increased relative humidity). Furthermore, the ability to consume these parameters as predictive markers for animal environmental adaptation in each sector was demonstrated by the strong correlation found amongst THI and tested parameters, especially the animal hematocrit value and RR (Soliman et al., 2024).

The animal's physiological response to harsh climatic conditions, particularly in a hot climate, and its thermotolerance are directly correlated with the respiratory rate (RR). In our study, RR was significantly greater in the summer than it was in the winter. This demonstrated that elevated body temperature, or THI, altered respiratory and cardiovascular functioning, and that animals' adaptive response to maintain body temperature by evaporative cooling was to increase respiration rate (RR) (Hussain et al., 2023). Buffaloes surrounded by higher temperatures in climatic chamber had reduced pulse rate (PR) but greater RR and RT, according to earlier research (Napolitano et al., 2023). In a similar vein, cattle have been shown to react to heat stress by dramatically increasing their RR, RT, and PR (Gonzalez-Rivas et al., 2020). In our study, there was no seasonal variation in either RT or RR (as RR was constant over three seasons). The results are consistent with previous research (Das et al., 2014; Omran and Fooda, 2023) showing that swamp buffaloes can control their body heat balance, as the impacts of THI on RR, RT, and PR are not significant at 30 Â°C. When outside temperatures surpass 30 Â°C, buffaloes' respiratory rates rise noticeably. This is because the activation of peripheral receptors (the thermo-regulatory system) causes panting to begin without causing an increase in respiratory rate (Hall, 2015).

Buffaloes displayed more variations in BST and RT than cattle when they were subjected to higher environmental temperatures due to their decreased sweating capability and dark skin (Gonzalez-Rivas et al., 2020). For this reason, at greater ambient temperatures, buffaloes have evolved an adaptation mechanism that causes them to search for water to immerse in (Dash et al., 2016). Barros et al. (2015) stated that there were no signs of thermal discomfort in Murrah buffalo bulls that were monitored throughout summer months of April through August, with an average THI level of 79.7 (range from 78.2 to 80.6). However, THI values greater than 75 in female buffaloes have been linked to increases in RT and RR (Albenzio et al., 2024) as well as negative impacts of heat stress on a drop in fertility in subtropical climates (Dash et al., 2016).

The activity of the thyroid gland has also been demonstrated to be impacted by climate stress, and this is linked to changes in the serum T3 and T4 values in buffaloes. Thyroid function is suppressed by exposure to high ambient temperatures (Hall, 2015). The control of body temperature and the increase in metabolic heat is brought about by thyroid activity. Climate stress causes physiological strain, which raises the metabolic burden and influences animals' feed consumption (Soliman et al., 2024). Adjustments in levels of metabolic hormones including T3, T4, and insulin show these metabolic alterations. The current study demonstrated the clear impacts of heat stress and THI on lactating Egyptian buffaloes by observing a considerable drop in T3 and T4 levels in the summer compared to the winter. A previous study showed similar findings about the reduction of T3 and T4 concentrations in buffaloes throughout the summer heat stress season (Lakhani et al., 2018). To lower the basal metabolic rate and hence the net body heat generation during heat stress, the body naturally reduces its contents of T3 and T4. (Napolitano et al., 2023). However, no change was recorded between the serum T3 and T4 concentrations in Nili-Ravi buffaloes through humid hot, and hot, dry seasons (Das et al., 2014). Heat stress causes T3 and T4 values to fluctuate, but these changes take longer to manifest, and it can take several days for the levels to return to normal (Omran et al., 2020).

Heat stress causes the hypothalamus-pituitary-adrenal axis of the central nervous system to release corticotrophin releasing factor, which in turn causes the anterior pituitary glands corticotrope to emit ACTH. The adrenal gland cortex produces and secretes glucocorticoids like cortisol in response to further stimulation from ACTH release (Albenzio et al., 2024). Lactating buffaloes were shown to have a much lower amount of ACTH during the summer than during other seasons. This could be explained by the greater levels of cortisol that are seen during the summer, which can negatively feedback the hypothalamus-pituitary-adrenal axis (Hussain et al., 2023). It was further demonstrated by the similar inverse association between ACTH and cortisol levels that our investigation reported.

In buffaloes (Atwa et al., 2019; Soliman et al., 2024) and dairy cattle (Alhussien and Dang, 2018), serum cortisol is a well-documented indicator of status of physiological stress. In this study, summer and autumn showed significantly greater serum cortisol levels than winter and spring did. Since buffaloes were similarly stressed by higher humidity in the fall, similar amounts of serum cortisol in the autumn and summer demonstrated the impact of climatic stress in both seasons. An increase in serum cortisol signals that the hypothalamic-pituitary-adrenal axis is being stimulated, which in turn helps animals sustain homeostasis and modify their body physiology in response to stress. Previous research on Murrah buffaloes revealed comparable results on elevated cortisol values through harsh summer circumstances (relative to other seasons) (Lakhani et al., 2018; Bombade et al., 2018) and Sahiwal cows (Alhussien and Dang, 2018). Furthermore, it has been noted that animals thermo-tolerant exhibit higher levels of cortisol than their thermo-sensitive counterparts. For example, crossbred buffaloes (Nili-Ravi × Murrah) were shown to have higher plasma cortisol than Mediterranean buffaloes (Shenhe et al., 2018).

Animals must acclimate to prolonged heat stress conditions to achieve physiological equilibrium, which is partially accomplished by lowering GH and catecholamine levels (Omran et al., 2020). Given that GH is a key hormone involved in the metabolism of energy and that its function is limited to lower body heat output, it would seem essential (Hall, 2015). As per previous research, our experiment found a significant reduction in serum GH levels in lactating buffaloes throughout the summer as opposed to winter and spring (Lakhani et al., 2018; Li et al., 2021). Along with calorigenesis, GH is also in charge of producing heat via promoting thyroid gland activity (Atwa et al., 2019). To support the homeotherms physiological homeostasis like lactating buffaloes in increased environmental temperatures, a reduction in GH is therefore unavoidable.

Consistent with previous findings, we observed non-noticeable differences in the serum insulin and glucose values in the serum in lactating buffaloes throughout seasons (Omran and Fooda, 2023). Nevertheless, there have been prior reports of contradictory results, such as a reduction (Atwa et al., 2019) or an enhancement (Ghavi et al., 2013) in heat stressed cows serum insulin concentration. The primary causes of these inconsistencies include the animals' varying nutritional level, metabolic state, physiological stage, and capacity for thermotolerance.

Concentrations of serum SOD and GSH-Px were found to be lowest in summer and greatest in winter in the current investigation. According to earlier research, heat stress causes a drop in GSH-Px levels, which causes H_2O_2 to build up (Waiz et al., 2016). The antioxidant defense system is disrupted by exposure to high ambient temperatures, which results in a reduction in the antioxidant enzymes activity (CAT, GSH-Px, and SOD). Redox imbalance and ultimately oxidative stress are eventually caused by this, which leads to the gathering of significant quantities of reactive oxygen species (ROS) that cannot be eliminated in time (Lakhani et al., 2018). Consequently, ruminant levels of antioxidant and oxidative enzymes are typically utilized as biomarkers to indicate the severity of oxidative stress (Son et al., 2023). One of the most significant byproducts of membrane lipid peroxidation is MDA, and elevated amounts of this compound can exacerbate damage to cell membranes. Accordingly, the level of MDA in an animal is utilized as a biomarker for oxidative stress severity (Li et al., 2021).

In the current experiment, markedly enhanced MDA values in lactating buffaloes were recorded in summer than in further seasons, which indicates an exceeding level of oxidative stress as previously recorded in Murrah buffaloes (Lakhani et al., 2018). The values of heat shock proteins and antioxidant enzymes are the most essential stress biomarkers that expose climate adaptability and animal comfort (Waiz et al., 2016; Son et al., 2023). MDA increased concentrations accompanied by reduced values of antioxidant enzymes (CAT, GHS-Px, and SOD) in summer than in further seasons show a state of oxidative stress in buffaloes caused by the enhanced THI.

In summer lactating buffaloes, enhanced MDA values but reduced antioxidant enzyme levels show antagonistic impacts of heat stress. The importance of buffaloes MDA levels as a biomarker for heat stress is confirmed by their higher value when combined with enhanced THI and reduced antioxidant enzymes. Previous research has documented the impact of heat stress on buffalo's fertility, with acyclic buffaloes exhibiting lower antioxidant capacity but greater MDA levels (Son et al., 2023). Additionally, it has been shown that buffaloes are more vulnerable to lower fertility when their THI is higher than 75 (Dash et al., 2016). Additionally, previous studies have demonstrated a correlation between reproductive acyclicity in buffaloes and ROS and the overall antioxidant capacity of follicular fluids, as this oxidative-antioxidative imbalance severely damages follicular cells (Hafez, 2019; Soliman et al., 2024).

Since the plasma protein retains enough water in intravascular fluids and keeps blood's viscosity, during heat stress, it offers an effective means of removing heat from the inside body to skin's external surface for heat dissipation by non-evaporative processes (Omran et al., 2020). Our research revealed a non-significant difference in total protein, albumin, and globulin between the seasons, which may help to understand how buffalo's physiology adapts to heat stress in the summer. In contrast, the summer season enhanced hepato-renal functional biomarkers (ALT, AST, and BUN). This is consistent with earlier studies by Napolitano et al. (2023) that found a marked increase in liver and kidney function markers because of slight consumption of heparo-renal damage in the summer to combat elevated oxidative/nitrosative stress when an animal was attempting to lessen the negative impacts of heat stress. In our research, spring, summer, and autumn reported an average THI above 75, which was likewise linked to the oxidative and physiological reactions of lactating buffaloes. These results, which show that the same animals were examined in every season, may point to the likelihood of cumulative stress. This supports previous findings of Dash et al. (2016) indicating buffalo inhabit a heat-stressed zone that runs from April to September. Comparable results were also reported in Murrah buffaloes raised in the Amazon tropical Eastern region of Brazil; these buffaloes displayed heat stress symptoms during both the rainy and non-rainy climates (Silva et al., 2014). Therefore, it is advised that buffaloes should be well maintained from spring to autumn to mitigate the negative impacts of heat stress on their ability to reproduce and be productive, particularly in the summer. Additionally, it is consistent with past research that recommended using efficient stress-reduction techniques from April to September to lessen the negative effects of heat stress on dairy animals' productivity (Gonzalez-Rivas et al., 2020). According to our research, lactating buffaloes could tolerate a THI level up to 75 (the zone of thermal comfort). However, environmental factors that result in a THI level higher than 75 might produce heat stress, which calls for the use of efficient cooling techniques to lessen its harmful effects. To the best of our knowledge, our research offers useful insights into the adaptive physiology of Egyptian buffaloes, helping to improve the productivity and reproduction of these animals in summer season heat-stressed environments. Therefore, a livestock owner can choose the right breed to rise to achieve optimal productivity based on compatibility with his farm's location.

5. CONCLUSIONS

Summer season had a higher THI level in lactating buffaloes along with notable rises in RR, BST, serum cortisol, and MDA concentrations. Furthermore, throughout the summer, greater THI caused a decrease in serum total antioxidant capacity. Heat stress also decreased serum T3, T4, and GH levels.

According to our research, the four months of June through September are crucial for managing buffalo since their THI values are higher than 80. As a result, proper cooling measures must be used to reduce heat stress and prevent animal welfare problems and performance losses. To investigate the THI thresholds in relation to lactating buffaloes' reproductive and productive performance in summer climate circumstances, more research is necessary.

Publication consent

Each author has demonstrated their consent for the publication of the current manuscript.

Data and material availability:

All data of this study is provided.

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All data of this study is provided.

CONFLICT OF INTERESTS

All authors have stated the absence of any conflicts of interest.

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AUTHORS' CONTRIBUTIONS

K.A.E: Conceptualization, Formal Analysis, Investigation, Resources, Writing – original draft. **H.A.E**: Data collection, Formal Analysis, Resources, Writing – original draft. **M.H.H**: Conceptualization, Data curation, Formal Analysis, Project administration, Resources, Supervision, Writing – final draft, review and editing.

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