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AMELIORATION OF PHYSIOLOGICAL RESPONSES, CORTISOL AND MILK YIELD IN BUFFALOES BY REGULATING THEIR DRINKING WATER TEMPERATURE DURING SUMMER SEASON

Ganesh Hanumant Gore, Anagha Sandeep Nagvekar, Shailesh Dayaram Ingole*, Simin Vispi Bharucha, Prakash Madhukar Kekan and Shambhudeo Dadarao Kharde

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ABSTRACT

The one-month trial incorporated during the summer season included twelve healthy lactating Murrah buffaloes equally divided into two groups. Drinking water previously cooled in earthen pots was offered to the experimental group, and the control group was given water available on the farm. The milk yield, serum cortisol, serum cholesterol, pulse and respiratory rates, rectal temperature, and water consumption in both groups were regularly quantified. Significant differences were found in the milk yield and serum cholesterol concentrations, and highly ($P<0.01$) significant variations were discerned in the serum cortisol concentrations, water consumption, pulse and respiratory rates, and rectal temperature. This study demonstrated the beneficial effects of offering precooled drinking water on lactating buffaloes studied physiological parameters during the summer. It further demonstrated that this heat stress management technique which is easy to emulate and is decidedly economical, also facilitated an increase in the milk yield.

Keywords: *Bubalus bubalis*, buffaloes, water, milk yield, cortisol, physiological responses

INTRODUCTION

Morphological adaptations help animals in thermoregulation, such as skin colour, hair/wool, the intensity of sweat glands, and subcutaneous adipose tissue. Regarding thermoregulatory mechanisms, buffaloes are at a disadvantage due to their skin colour and negligible sweat glands and, thus must cope with compounded stress. Under natural conditions, this is overcome by wallowing in water and mud. But in semi-organized or poorly managed herds, there is hardly any scope for such activities, and buffaloes must adjust themselves accordingly, which tells upon their growth, reproductive and lactational performances.

Low productivity, poor growth, and reproductive efficiency of dairy animals from tropical climates, as compared to animals from subtropical and temperate climates, are well established (Fuquay, 1981). Primarily high ambient temperature of the tropics, along with the poor nutritional and managemental practices, contributes significantly towards the low productivity of tropical animals (Ingraham *et al*., 1979; De Dios and Johnson, 1984). Information on systematic studies on various aspects of physiological and biochemical adaptations of buffalo to thermal

Department of Veterinary Physiology, Mumbai Veterinary College, Mumbai, Maharashtra Animal and Fishery Sciences University, India, *E-mail: sdingole@mvc.ac.in; ingoleshailesh@gmail.com

stress from different agro-climatic conditions of Maharashtra in general and from the hot humid climate of the konkan region is not available. These studies, if carried out systematically, will be useful to develop appropriate nutritional and managemental practices for the buffalo in the field.

The morphological, structural, and functional buffalo physiognomies make these animals compatible with hot and humid environmental conditions, except that heat stress has damaging consequences on buffalo reproduction (Kaur and Arora, 1982; Tailor and Nagda, 2005). In addition to the direct climate change effects on the production and reproduction, far-reaching ancillary concerns of the climatic influences on pastures, forages, grain, and crop by-residues affect the feed and fodder quantity and quality as well as affect the severity and distribution of parasitic infestations and livestock afflictions.

The effects of drinking water temperature on the health and performance of dairy animals are sparingly studied. Huuskonen *et al.* (2011) studied the effect of drinking water temperature on water intake and performance of dairy calves. A few authors have examined the effect of offering heated (Andersson, 1985; Osborne *et al*., 2002) or chilled (Baker *et al*., 1988; Wilks *et al*., 1990) drinking water on the performance of dairy cows. Andersson (1985) conducted a study on Swedish Red and White cows and found that the coldest water temperature (3^{o}C) led to a decrease in milk yield compared to other temperature treatments. In contrast, Osborne *et al.* (2002) reported that cows consumed more heated water (30 to 33^{o}C) than water at ambient temperatures (7 to 15^{o}C), but this increased water intake did not affect milk yield. In the case of sheep, Shiga (1986) discovered that in cold environments, they preferred warmer water when given a choice between ambient and heated drinking water. Similarly, Olsson and Hydbring (1996) found that goats preferred warmer water when given the option between ambient and heated drinking water. However, there is limited information available regarding the effects of drinking water temperature, specifically on dairy buffaloes. Overall, these studies indicate that different species of dairy animals may have varying preferences and responses to drinking water temperature. While cold water temperature decreased milk yield in cows, heated water did not affect milk yield but increased water intake. Further research is needed to understand the specific effects of drinking water temperature on dairy buffaloes and its implications for their production.

There are many therapeutic benefits of consuming water from an earthen pot, and it is also an excellent way to store and help cool the water naturally. These earthen pots not only cool the liquid filled in it but contribute health benefits by providing earth elements. The pot transfers chill to water based on the climate. This quality of earthenware pot is unique, and no other container has the same quality. Earthenware pot is porous because clay is porous. When water is stored in earthenware pots, evaporation takes place, which leads to a cooling effect. The process of evaporation involves the absorption of heat energy from the surroundings, causing the water particles to gain energy and change into a gaseous state. As the water evaporates and mixes with the air, it results in a decrease in temperature. Earthenware pots have small, microscopic holes or pores, allowing water to seep. This seepage provides a constant water supply to the pot's outer surface. The water on the surface evaporates, further aiding in the cooling process. In addition to the cooling effect, earthenware pots have another benefit. The clay used in these pots is alkaline in nature. When water comes into contact with the alkaline clay, it helps to neutralize the acidity of water and provides a more balanced pH. This alkaline water is believed to have potential health benefits, including relieving gastronomic pains associated with acidity. Drinking water from an earthenware pot can help improve metabolism without dangerous chemicals like BPA (Bisphenol A in plastic that cling to the water). It helps as water tastes natural, pleasantly chilled and helps to improve metabolism and virility. After work, exercise, or at the time of drinking water, when an animal drinks ice-cooled water, it causes sunstroke and makes the animal sick. Cooling drinking water with ice is more expensive. So chilled and cooled water in an earthenware pot on summer days is less expensive to farmers.

In view of the immense importance of studies of the climatic physiology of dairy animals to improve the health, reproductive performance, and milk production and the nonavailability of adequate information on the effect of climatic parameters on lactating buffaloes and the effect of feeding cooled water, the current trial was conceptualized to examine the milk yield, water intake, serum cortisol, and cholesterol concentrations and gauge the physiological responses in Bottom of Form

buffaloes offered pre-cooled drinking water (earthenware pot), and those offered normal drinking water.

MATERIALS AND METHODS

Animals for experiment

Twelve healthy Murrah buffaloes in early lactation ranging from dairy farms, Aarey colony, Goregaon, Mumbai was selected. The selected animals were lactating but nonpregnant. The average milk yield of these animals ranged between 3.0 to 12.0 kg per day. The experiment was conducted in the hot months of April and May. The buffaloes were divided into two groups of six buffaloes each. The first group (experimental) was provided with cooled drinking water from an earthenware pot, and the second group (control) was provided with normal (naturally warm) water. The buffaloes on the farm resided in asbestos roof sheds underneath natural daylight and temperature, were fed a maintenance ration of paragrass (15 kg) and concentrate mixture (2.5) twice daily (morning and evening) as well and had access to ad-lib paddy straws. An additional 0.5 kg of concentrate mixture per kg of milk production was supplied to all the lactating animals. Clean drinking water was provided.

Sampling

Blood samples (10 to 12 mL) were collected in vacutainers aseptically from experimental buffaloes by jugular vein puncture. Serum was separated after centrifugation at 2000 rpm and was stored at -20^{o}C until used for analysis.

Physiological parameters like rectal temperature, respiratory rate, and pulse rate were measured and recorded daily, from 1.00 P.M to 3.00 P.M.

Blood analysis

The serum cortisol was estimated by the electrochemiluminescence immunoassay “ECLIA” by using Elecsys and Cobas E Immunoassay analyzer. The serum cholesterol was estimated by CHOD- PAP methodology using the biochemical analyzer.

Milk yield

Individual morning and afternoon milk yields were recorded, and the total yield was calculated by weighing the quantity (kg) at each milking.

Water consumption

The first group (experimental) was provided with cooled drinking water from an earthenware pot, and the control group was provided usual (naturally warm) water. The consumption of drinking water was recorded with the help of a graduated bucket. The temperature of the pre-cooled drinking water (earthenware pot water) and normal (naturally available on the farm warm) drinking water was recorded by thermometer twice a day and on all days.

Temperature-humidity index

During the experiment, the data of atmospheric temperature, relative humidity, vapour pressure, dry bulb, and wet bulb temperatures were collected from the Meteorological Observatory, Santacruz, Mumbai 400 099. The temperature-humidity index (THI) was calculated by using the formula of McDowell as reported by Amakiri and Funsho (1979) as given below:

$$THI = 0.72\ (C_{db} + C_{wb}) + 40.6$$

Where THI - Temperature-humidity index
C_{db} - Dry bulb temperature
C_{wb} - Wet bulb temperature

Statistical analysis

Analysis of the variance of the data was done using a completely randomized design. Differences in means were tested using the student T-test to study the effect of pre-cooled water on serum cortisol, serum cholesterol, rectal temperature, respiratory rate, pulse rate, water consumption, and milk yield in buffaloes.

RESULTS AND DISCUSSIONS

Rectal temperature

The Mean ± S.E. rectal temperature was non-significant in experimental buffaloes (100.3±0.01°F) compared to control buffaloes (100.7±0.017°F). No research work could be traced in the available literature on the effect of precooled drinking water using earthenware pots on rectal temperature in buffaloes. Rectal temperature increases due to a rise in ambient temperature have been published by Radadia *et al.* (1980); Wankar *et al.* (2014) in buffaloes, Salam (1980) in dairy animals, Bayer *et al.* (1980) in lactating German Friesian cows, by providing showering, provision of cooled drinking water, Radadia *et al.* (1980) in Murrah buffaloes, Singh *et al.* (1980) in Haryana x Jersey, Haryana x Brown Swiss and Haryana x Holstein heifers, Hassan *et al.* (1981) in lactating buffaloes, Otani (1983) in Holstein-Friesian cows, Balakrishnan and Nagarcenkar (1988) by water splashing and showering and shade recorded decrease in rectal temperature. These works agree with the present study observations wherein the experimental group had constant rectal temperature from the beginning to the end of the experiment. In contrast, in the Control group, the rectal temperature (100.7°F), which was constant at the beginning of the experiment, subsequently showed a slight increase (100.8°F) at the end of the experiment.

Respiratory rate

The Mean ± S.E. respiratory rate in experimental buffaloes was 20.33±0.25 and in control buffaloes was 28.91±0.39 per minute. The respiratory rates recorded were highly ($P<0.01$) significant in experimental buffaloes compared to control buffaloes. The respiratory rate indicated that the animals were under heat stress and that the body was taking measures to elevate heat stress. No research work could be traced in the available literature on the use of cool drinking water (precooled in earthenware pot) on the respiratory rate in buffaloes. Increased respiratory rate due to rise in ambient temperature has been reported by Mostageer *et al.* (1974); Radadia *et al.* (1980); Badreldin and Ghany (1982); Gudev *et al.* (2007) in buffaloes. These opinions agree with those of our study. The relationship between behavioural and physiological markers can be employed to gauge the adaptive capacity and subsequently, the buffaloes' well-being when exposed to diverse environments (Broom and Johnson, 1993). Some system stressors are quantifiable as amendments of cardiac or respirational velocities which can be acceptable indexes of social stress (Guyton, 1995).

In cattle conveyed a positive correlation between temperature, relative humidity and rainfall with pulse and respiration rates and body temperature. He further explained that this augmented respiratory rate can be used as an index of discomfort in large animals. Singh and Upadhyay (1997) noticed that during heat stress Karan Fries had a higher rectal temperature and respiration rate as compared to Sahiwal cattle. McDowell (1972) reported that when exposed to hot weather, lower respiratory rates indicate that the animals suffer lesser distress. Pandey *et al.* (2017) also found higher values of physiological responses of Tharpakar and Karan Fries heifer's exposed to the temperature of 42^{o}C in a climatic chamber.

The Experimental group and Control group of buffaloes had higher respiratory rates at the beginning of the experiment. Subsequently, the buffaloes which were provided cooled drinking water (pre-cooled in an earthenware pot) showed a decline in the respiratory rate at the end of the experimental period (19.04 per minute). The Control group, provided with drinking water that was naturally warm, showed a persistently high respiratory rate till the end of the experiment. This indicates that buffaloes consuming cooled drinking water (pre-cooled in an earthenware pot) experienced less stress due to heat.

A major channel of thermoregulation in these animals is the respiratory process which aids the buffaloes in diminishing their heat load by amplifying pulmonary evaporative loss and this appeared to higher in the Control group. On the other hand, the experimental buffaloes sustained their respiratory rate at a lower level which was considerably near normal for cool weather. Thus, there measures taken were, beneficial in providing thermal comfort to animals not necessitating their respiration activity to be subjected to severe test (Radadia *et al.*, 1980). Thus, this drinking water precooled in earthen ware pot provided to the experimental animals is one of the measures to reduce heat stress in buffaloes. The difference in the respiratory rate in experimental and Control group of animals was almost equal to 8 per minute as shown which was highly ($P<0.01$) significant.

Pulse rate

The Mean ± S.E. pulse rate in experimental buffaloes was 53.10±0.10, and in control buffaloes was 60.67±0.34 (beats per minute). The difference in the value of pulse rate recorded between the experimental and control group of buffaloes was highly ($P<0.01$) significant. No research work could be traced in the available literature on the effect of cooled drinking water (precooled in an earthenware pot) on pulse rate in buffaloes. An increase in the pulse rate due to heat stress has been reported by Harris *et al.* (1960) in lactating Holstein and Jersey cows, Raghavan and Mullick (1961) in Murrah buffaloes, Aziz *et al.* (1979) in Friesian cattle and buffaloes, Bayer *et al.* (1980) in lactating German Friesian cows, Verma and Hussain (1988) in buffaloes.

The experimental animals and control animals showed nearly constant pulse rates throughout the experimental period. However, the pulse rate reported in the control animals was higher than in the experimental group of animals. This indicates that the animals which were given cooled drinking water had lower pulse rates than the animals which were provided with drinking water that was naturally warm. This is due to the reduced level of stress in buffaloes that consumed cooled drinking water. The physiological responses are essential to cope with the animals to the environments. The respiration rate (RR), heart rate (HR), pulse rate (PR), rectal temperature (RT), and skin temperature are recorded higher in crossbred cattle as compared to Zebu cattle. Broom and Johnson, 1993 proposed that the correlation between the behavioural and physiological markers could be a valuable gauge to evaluate the adaptive capacity and, accordingly, the animals' welfare in relation to varied atmospheric conditions. Thakur *et al.* (1989) reported that air temperature rise results in pulse rate acceleration this increasing trend persisted even when the ambient temperature declined, suggesting that this physiological response was slow to return to its normal range even when animals were late placed in favourable comfort zones.

Cortisol

The Mean ± S.E serum cortisol in the experimental group was 3.60±0.32 (ng/ml) and in Control group was 6.38±0.70 (ng/ml). The serum cortisol was highly ($P<0.01$) significant in experimental buffaloes as compared to control buffaloes. No research work could be traced in the available literature on the effect of cooled drinking water (precooled in earthenware pot) on serum cortisol in buffaloes. An increase in serum cortisol levels due to heat stress has been reported by Silva *et al.* (2014) in lactating buffaloes and Vijaykumar *et al.* (2011) in buffalo heifers. In the present study, the experimental and control group buffaloes showed high cortisol levels due to heat stress at the beginning of the experiment. Subsequently, the buffaloes which were provided cooled drinking water showed a decline in serum cortisol levels, and by the end of the experiment, serum cortisol levels had come to normal levels. Whereas in the control group provided with drinking water that was naturally warm, showed persistently high serum cortisol levels till the end of the experiment. This indicates that buffaloes with cooled drinking water experienced reduced stress due to heat. Cortisol is synthesized in the adrenal cortex and is regarded as an indicator of stress in different species of animals (Okeudo and Moss, 2005; Mohamed, 2006). Blood cortisol level is a classical index of the reaction of animals to any environmental stressors and higher cortisol concentration may be required to meet the energy crisis during physical stress to the animals. It is the magnitude of stress challenge and the magnitude of change in metabolism (Saeb *et al.*, 2010). Serum cortisol decreased due to heat ameliorative measures in buffalo heifers (Vijaykumar *et al.*, 2011). Similarly, Caldron *et al.* (2004) reported reduced serum cortisol values in Holstein and Brown Swiss heat-stressed dairy cows after using two different cooling systems, and these pronouncements conform to the results obtained in our study, concluding that the provision of cool drinking water is an easy and convenient method to ameliorate heat stress.

Cholesterol

The Mean ± S.E serum cholesterol in the experimental group was 107.66±6.26 (mg/dl), and the Control group was 132.79±8.76 (mg/dl). The concentration of serum cholesterol was significant ($P<0.05$) between the two groups. No research work could be traced in the available literature on the effect of cooled drinking water (precooled in an earthenware pot) on serum cholesterol in buffaloes. Serum cholesterol is the intermediate in the biosynthetic pathway of steroid hormones precursors. Serum cortisol is a stress hormone produced by adrenal cortex, and therefore it is likely that the level of serum cholesterol may rise and fall due to stress (McDonald, 1980). The variation in the values of serum cholesterol in both groups of animals may be due to stress.

Milk yield

The Mean ± S.E milk yield (kg/day) in experimental buffaloes was 10.38±0.69 (kg/day), and control buffaloes were 8.06±0.48 (kg/day) and showed a statistical difference. No research work could be traced in the available literature on the effect of cooled drinking water (precooled in an earthenware pot) on milk yield in buffaloes. Optimum milk production usually is obtained within the comfort zone. Even a moderate increase in temperature above the comfort zone results in a measurable decline in milk production. Temperature below the comfort zone also has adverse effects on milk production. Reduction in feed intake is mainly responsible for the decreased milk production in heat-exposed lactating animals. The milk production of buffaloes provided with cooled drinking water (pre-cooled in an earthen pot) increased, while it decreased in the control group. The level of stress caused due to heat / hot environment was reduced in animals given cooled water to drink and this reduced stress may have restored and increased milk production. Cooling can elevate heat stress and can improve thermal balance and productive performance in dairy cows. High-yielding cows are more vulnerable to heat stress (Silanikove *et al*., 1997), and this effect is more pronounced during early lactation (McDowell *et al*., 1975; Sharma *et al*., 1988; Barash *et al*., 2001). This could be because cows during the first trimester of lactation are either in negative or near negative or near negative energy balance and decrease in energy intake due to low feed intake would reduce milk production.

Water consumption

The Mean ± S.E water consumption in experimental buffaloes was 30.6±0.23 (litres/day), and in control, buffaloes were 41.7±0.44 (litres/day) and were highly ($P<0.01$) significant. No research work could be traced in the available literature on the effect of cooled drinking water (precooled in an earthenware pot) on water consumption in buffaloes. Water is directly involved in reticulo-rumen cooling and serves as the primary vehicle for heat transfer. Increased environmental temperature activates the hypothalamic thirst center leading to higher water consumption in the acclimating buffaloes to heat stress.In the present study, the temperature of the cooled drinking water (precooled in an earthenware pot) provided to the experimental group of buffaloes was between 26 to 27^{o}C in 1st week, 25 to 27^{o}C in 2nd week, between 27 to 28^{o}C in 3rd and 4th week respectively. The temperature of drinking water (naturally warm) provided to the Control group of buffaloes was between 32 to 33^{o}C in 1st week, 31 to 33^{o}C in 2nd week, and between 32 to 33^{o}C in 3rd

Table 1. Mean physiological response, cortisol, and milk yield of buffaloes during summer having cooled and normal drinking water.

Parameters	Control (Normal water)	Experimental (Water in earthen pot)
Rectal temperature	100.7±0.017	100.3±0.014
Respiratory rate/per min	28.91±0.39**	20.33±0.25**
Pulse rate (beats/min)	60.67±0.34**	53.10±0.10**
Serum cortisol (ng/ml)	6.38±0.70**	3.60±0.32 **
Serum cholesterol (mg/dl)	132.79±8.76 *	107.66±6.26 *
Water consumption (litres/ day)	41.7±0.44**	30.6±0.23**
Milk yield (kg/day)	8.06±0.48*	10.38±0.69*
THI	82.97	82.97
Water temperature	31 - 33°C	25 - 27°C

and 4th week, respectively. It was evident that the experimental buffaloes drank less water compared to the Control group of buffaloes. This was due to the fact that the temperature of the cooled water was pleasant enough to satisfy or quench their thirst due to the high environmental temperature. The Control group of animals which were provided with naturally warm water drank more water, which signifies the important role of water in heat dissipation (Winker *et al*., 2014). Minnet (1947); Mullick and Kehar (1952); Raghavan and Mullick (1961); Ramachandra *et al*. (1966); Mehta and Ganwar (1978) reported physiological reactions and blood metabolites values to explain the effects of different methods of cooling the buffaloes and their thermal balance. All these studies specify that measures such as wallowing, showering etc. are effective in lessening the thermal stress to some extent in buffaloes during summer. Providing cool drinking water (precooled in an earthenware pot) serves as a better measure to ameliorate the thermal stress in buffaloes during summer.

Temperature humidity index (THI)

The mean THI during the experimental period recorded was 82.97. The temperature-humidity index (THI) expresses the effect of temperature and humidity together on animal production performance. The primary tool used in the dairy industry to predict the degree of thermal stress on animals is the temperature humidity index (THI). Normal THI in buffaloes is below 64. When THI is 64 to 71 causes a minimum stress level at that time, buffaloes are likely to begin experiencing heat stress, and buffaloes show little discomfort. When THI is 72 to 79 causes mild stress levels at that time, buffaloes become uncomfortable and increase respiration rate and dilation of blood vessels. Milk yield and feed intake are decreased.

Thus, it can be concluded that the buffaloes were under stress during summer and tried to deviate from heat stress by increasing respiratory rate resulting in evaporative cooling, increasing body temperature so as to reduce uptake of heat from the environment, and increasing heart/ pulse rate, thereby, increasing flow of blood to periphery. Further, the cooled drinking water from earthenware pot is suitable for buffaloes, particularly during summer, as the temperature of water in the earthen ware pots are lower, which helps to minimize the heat stress caused by exposure of animal to high environmental temperature and hot wind so as to obtain maximum milk production from lactating buffaloes.

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