International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2022; 7(6): 53-58

www.veterinarypaper.com
Received: 13-10-2022
Accepted: 18-11-2022

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Potential of water spray in improving welfare of dairy subtropical buffaloes: Behavioral and biochemical evidence

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DOI: https://doi.org/10.22271/veterinary.2022.v7.i6a.457

Abstract
Heat stress is the main challenge for buffaloes as animals. The buffaloes wallow in water to abate the heat load. The use of sprayed water from sprinklers/soakers or misters has been the most common method of cooling in dairy animals during summer. This study aimed to evaluate the effects of a cooling strategy on milk yield, physiological, behavioral and biochemical activities of buffaloes. Thirty dairy buffaloes aged (4-7) years old were used. The animals were divided into two groups; a control group (15 buffaloes) was reared on a dusty floor. The buffaloes were kept in an open yard throughout the day all over the year. The yard was provided with a holding pen for veterinary inspection and another cooled group (15 buffaloes) was managed as the control group in addition to a system of water tubes at 1.5 meters over the animal's head ended with nozzles to allow automatic spraying of water over the animal body surface for 15 minutes (twice daily) to decrease body heat stress by evaporation. We concluded that the eating, rumination, and resting time (min/3hrs) were higher in cooled group buffaloes than in control group buffaloes. While the aggression was decreased with the cooling management system. The total milk yield was improved with the water spray cooling system on the buffalo farm. The onset of post-partum oestrus was faster in the cooled group.

Keywords: Water spray, buffaloes, milk yield, behavioral evidence, biochemical evidence

Introduction

The thermal comfort region for the greatest animals is between 4 °C and 25 °C and when environmental temperature surpasses 25 °C, animals suffer from heat stress conditions and so the heat stress has become a major concern for dairy producers because of the associated decreases in milk production and large economic losses (Habeeb, 2020) [19].

Under the hot climate conditions in tropical and subtropical areas, exposure to elevated ambient temperature is the major constraint on animal productivity and depression in feed consumption is the most important reaction (Marai *et al*., 2002) [23]. Particularly, exposure to elevated temperature evokes a series of changes in the animals' biological functions that include depression in feed intake, efficiency, and utilization, disturbances in the metabolism of water, protein, energy, and mineral balances, hormonal secretions, enzymatic reactions, and blood metabolites. Such changes result in the impairment of production and reproduction performances (Marai *et al*., 2002; Marai and Haeeb, 2010) [23, 22]. Heat stress can cause changes in hormone profiles like prolactin, thyroid hormones, glucocorticoids, growth hormone, adrenocorticotropic hormone, oxytocin, estrogen, and progesterone (Farooq *et al*., 2010; Habeeb, 2020) [16, 19].

In severe heat stress, growth, milk yield, milk composition, and reproductive traits are reduced because of the extreme changes in biological functions affected by heat stress (Habeeb, 2020) [19]. The body temperature of buffaloes in the hot sun could only be kept normal in the shade or by wallowing or by quasi-continuous application of water. In the shade or in the wallow, buffaloes cool off quickly, perhaps because their black skin, which is rich in blood vessels, conducts and radiates heat efficiently (Marai and Haeeb, 2010) [22].

Water buffaloes are adjusted to hot and humid environmental conditions by wallowing in water to decrease heat stress (Napolitano *et al*., 2007) [25].

Water buffaloes wallow in water to conflict heat stress during summer, the intensification of buffalo farming systems (De Rosa *et al*., 2009) [14] and the reduction in potholes and ponds in villages have led to limited or no access to water for wallowing (Bah *et al*., 2021; Bah, *et al*. 2022) [4, 5]. So, to decrease pools used for wallowing, the farmers use sprinklers to cool the buffaloes. These sprinklers use a large amount of groundwater, which is becoming rare (Bah *et al*., 2021) [4].
Recently, there has been a focus on reducing water use on cooling dairy animals with minimal impact on productivity and welfare (Tresoldi *et al*., 2019; Bah *et al*., 2021) [35, 4]. The use of sprayed water from sprinklers/soakers or misters has been the most common method of cooling in dairy cows during summer (Anderson *et al*., 2013; Tresoldi *et al*., 2018; Bah *et al*. 2022) [3, 34, 5]. Also, Heat stress has become a major concern for dairy producers because of the associated decreases in milk production and large economic losses (Habeeb, 2020) [19].
Using sprinklers as a managerial factor for Egyptian buffaloes with water to cool their bodies decrease the adverse effect of heat load during the peak of milk production and composition was affected by water spray during the hottest period of the day. Managerial factors modification through water spray is associated with both hematological and biochemical responses in cooled buffaloes (Imbabi *et al*., 2019) [20]. Reproductive power is affected in female buffaloes by inherent late maturity, poor oestrous signs in the hot season, distinct seasonal reproductive patterns, and long inter calving intervals (Singh *et al*., 2000) [32].
Reproductive efficiency is the main agent affecting the productivity of the livestock, this is affected by delayed puberty, silent oestrous, changing of oestrous length, genital prolapse, difficult birth, retention of fetal membranes, seasonality of calving, long calving heat and subsequent calving interval. Despite the development of buffaloes' managerial techniques, the improvements in milk or meat production were poor and mainly due to an improvement in rearing techniques rather than genetic selection (Barile, 2005) [6]. Wallowing pools help the dairy buffaloes to cool themselves by cutaneous evaporation. The application of a water spraying system was considered an effective method of attenuating heat load, and the cortisol levels as stress indicating hormones were always higher in the serum of buffaloes that were managed in the open yard without wallowing than those managed in the shed and open space which provided with a wallow (5x2x1 m^3). Buffaloes managed at hot climatic conditions resulted in a reduction in reproduction and production performances. Buffaloes were reared successfully when adequate shade is available (Imbabi *et al*., 2019) [20]. The physiological and behavioral responses were taken as the proxy indicators of animal welfare (Bah *et al*., 2022) [5].

Material and Methods

The research was designed to investigate the effect of water spray as a cooler application on some behavioral and biochemical activities and the productive performance of dairy buffaloes in Egypt. This experiment was carried out on 30 female dairy buffaloes at Samy Asaed dairy farm which locate at Abo-Hamad city at Sharkeya governorate, Egypt. The experimental procedure was achieved following the general guidelines of the National Institutes of Health for the care and use of animals in scientific investigations and was approved by Zagazig University - Institutional animal care and Use committee (Approval number: ZU-IACUC/2/F/292/2022). All the human and ethical respects were taken into consideration and performed. The farm includes two dairy buffaloes' stables, one milking room, two isolation rooms, and two calf pens.

Buffaloes used and identification

Thirty female dairy buffaloes aged (4-7) years old were used. The animals were marked by painted-colored materials applied on the back of each buffalo.

Animal management and feeding system

Dairy buffaloes were kept loose in two shaded yards. The shelters represented 70% of the yards. The concrete manager was present outside the steel fence, allowing 2 m for each buffalo (Grasso *et al*., 2001) [18]. The animals were divided into two groups; a control group (15 buffaloes) was reared on a dusty floor. The buffaloes were kept in an open yard throughout the day all over the year. The yard was provided with a holding pen for veterinary inspection and another cooled group (15 buffaloes) was managed as the control group in addition to a system of water tubes at 1.5 meters over the head of the animals ended with nozzles to allow automatic praying of water over the animal body surface for 15 minutes (twice daily) to decrease body heat stress by evaporation (cooled group) (Imbabi *et al*., 2019) [20].
The animals were provided with 15-20 kg concentrated ration per head divided into two times daily every 12 hours (was given half an hour prior to the milking). The formulation of the ration was done as the recommendation of the NRC (2003) [26]. The buffaloes were milked by an automatic milking machine. The buffaloes were milked twice daily (the first one at 6 am and the second at 6 pm. The watering system of the farm through a water trough located in the yard provided with clean water ad-libitum.
Post-partum oestrus detection in this farm was done by the direct observer who recorded the oestrus signs (decreased milk yield, bellowing, vulvar swelling, the raising of the tail, frequent urination, mucus discharge, and mounting another female) (Borghese *et al.*,1997) [9].

Observation techniques

Behavioral activities of female buffaloes from each group were observed for 3 hours weekly, through a focal sample observation. The observations were carried out far enough from the herd to avoid any disturbance (Overton *et al*., 2002) [27]. The time spent and frequencies in different maintenance activities were recorded by using a stopwatch, multipurpose counter, and a field notice. As well as the productive and reproductive traits were collected for the detection of biochemical parameters.

The maintenance activities were recorded under the following classification

Ingestive behavior: as described by (Fukasawa and Tsukada, 2010) [17].

Eating

Number of occurrence (frequency) during the observation time.
Eating time: Time (minute) spent in eating during the observation time.

Drinking

Frequency of drinking / observation time.
Drinking time: Time (minute) spent in drinking by buffaloes/ visit of trough.

Rumination

Was defined as the period in which the animal regurgitate the bolus of feed, chewed and reswallowed it, furthermore it occur in standing or in sternal recumbancy.

Number of occurrence during the observation time.

Rumination time: Time (minute) spent in rumination during the observation time.

Recumbancy (lying)

Was classified according to (Overton *et al*. 2002) [27].

Sternal recumbancy: When the buffaloes lying on sternum with fore limbs and curled under the body and one hind leg is tucked forward under the body, the other hind leg is stretched out to the side of the body with partial flexion of stifle and hock joint.

Lateral recumbancy: When the buffaloes lie fully on their side while holding their head forward.

Number of occurrence (frequency/ observation time).

Time (minute) spent in recumbancy during the observation time.

Body care behaviour (grooming) was classified according to (Laister *et al*. 2011) [21].

Self - grooming: Was defined as the duration when the buffaloes licks herself or rubs its body against object.

Mutual grooming: Was defined as the duration when the buffaloes licks other buffaloes body.

Number of occurrence (frequency/ observation time).

Time (minute) spent in grooming during the observation time.

Aggressive behaviour: was classified according to (Val-Laillet *et al*. 2009) [37].

Head butting: The event in which the buffaloes butts other buffaloes (with body contact).

Head throwing: The event in which the buffaloes throws head to turn other buffaloes away (without body contact).

Number of occurrence (frequency/ observation time).

Productive traits

Milk yield per buffaloes: The whole amount of milk yield in kg/day.

Milking time (minute) spent in milking per buffaloes: by computing the time (min) taken during the process of milking through the morning and evening milking per buffaloes.

Milking rate (g/min): The amount of milk let down (g) divided by the time (min) taken during the process of milking.

Collection of blood samples

The blood samples were obtained from 15 female buffaloes of each group (control and cooled groups) as random samples to beat the circadian variation in hormone levels collected in the morning (Bertoni *et al.*, 2002) [8].

The samples were obtained from jugular vein puncture at the end of the neck base of female buffaloes and were allowed to coagulate at the temperature of the pen for 30 minutes. The blood samples were centrifuged at 3000 r.p.m for 10 minutes. The sera were aspirated and transferred into a dry and sterile labeled tube then were preserved in a deep freezer at -20 °C till the analysis for cortisol, prolactin and estrogen hormones by using commercial kits with the help of a UV spectrophotometer (Shimadzu UV spectrophotometer 1600 Double beam, Shimadzu Company, Japan).

Statistical analysis

Results were statistically analyzed by using one-way ANOVA) Snedcor and Cochran, 1989) [33] and by using SPSS program (2001) to make statistical analysis for comparison between groups by using a t-test.

Results

Table 1: Effect of water spray on the maintenance behavior of buffaloes

Behavioral pattern	Control Group	Cooled Group	Sig.
Feeding time (min)/3 hrs	60.14±0.22	84.12±0.07	**
Feeding frequency/3 hrs	5.77±0.94	7.32±0.75	**
Drinking time (min)/3 hrs	3.20±0.85	2.55± 0.33	*
Drinking frequency/3 hrs	5.14±0.10	1.80±0.50	**
Ruminating time (min)/3 hrs	90.43±0.22	144.22±0.54	**
Ruminating frequency/3 hrs	6.64±0.33	9.11±0.56	N.S
Resting time/3 hrs	116.34±0.23	119.18±0.57	N.S
Resting frequency/3 hrs	4.32±0.48	6.24±0.77	*
Grooming frequency	13.13±0.57	9.44±0.90	*
Aggression frequency	2.12±0.25	1.11±0.56	*

N.S = non-significant.

* = significant difference at level $p \leq 0.05$.

** = highly significant difference at level $p \leq 0.01$

The results in Table (1) revealed that the eating time (min/3hrs) was higher at the buffaloes sprayed with water in the cooled group (84.12±0.07 min) than the buffaloes in the control group (60.14±0.22min). where the differences were highly significant at $P \leq 0.01$. There was a highly significant difference at $p \leq 0.01$ in the rumination time between the control and the water-sprayed group as the rumination time was higher in water sprayed group which was 144.22±0.54 mins versus 90.43±0.22 mins in the control buffaloes.

There was a non-significant difference between the two groups in resting time and frequency. There was a significant difference between the control and water-sprayed groups in aggression and grooming frequency. As the aggression decreased with water sprinkling on the body surface of the buffaloes.

Table 2: Daily morning and evening milk yield (kg/day) in relation to managerial factor

Milk yield periods	Control Group	Cooled Group	Sig.
Morning milk	7.12± 0.46	8.66±0.22	*
Evening milk	4.19± 0.77	4.99±0.14	N.S
Total daily milk yield	10.91±0.22	12.30±0.09	*
Cortisol hormone level (ug/dl)	1.31±0.42	0.95±0. 010	*
Prolactin hormone level (ng/ ml)	0.67±0.32	0.99±0.33	*
Estrogen hormone level (ng/ml)	3.87±0.23	4.36±0.46	*

N.S = non-significant.

* = significant difference at level $P \leq 0.05$.

The results in Table (2) showed that the total milk yield was improved with water spray cooling system as it was 12.30±0.09 in cooled group versus 10.91±0.22 in control group. The stress indicating hormone (cortisol level) was superseded with water spraying and the difference between the 2 studied groups was significant. The level of serum prolactin (ng/ml) and serum estrogen (ng/ml) were significantly higher at cooled buffaloes than at control ones.

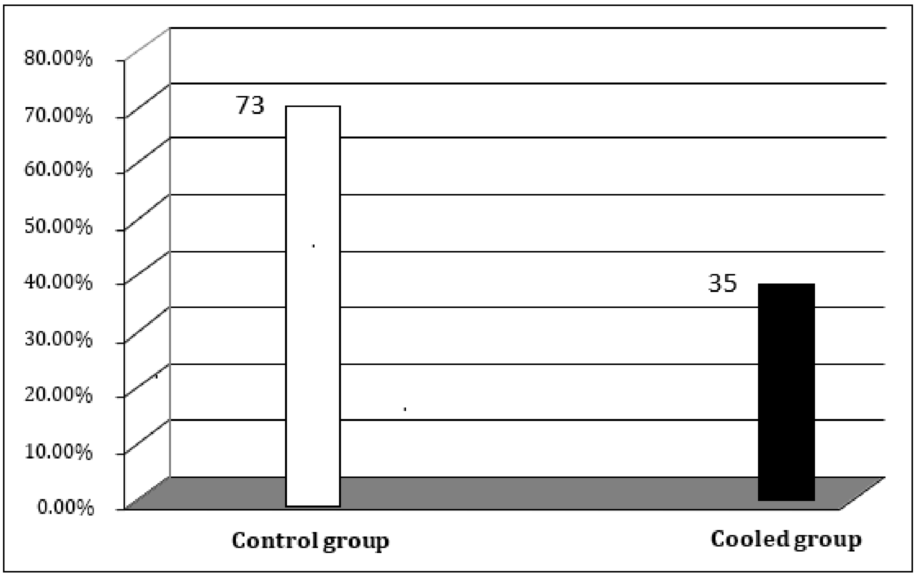

Fig 1: influence of water spray on the onset of post-partum oestrus (days)

Figure (1) shows the onset of oestrus post-partum was earlier at cooled buffaloes as it was 35 days versus 73 days in control buffaloes.

Discussion

The average eating behavior of buffaloes was improved with body cooling by water spray (Ahmad *et al*., 2017) [1]. It has been noticed that the buffaloes spent the most time in feeding and rumination bouts when water spray system and fans under shade, the buffaloes were fed once daily in the morning and for short periods during the diurnal hours, which might have explained the similarity in the total eating time despite the difference of daily variable periods. Slightly higher feeding time in cows could be attributed to different managerial factors and milking process. (Drwencke *et al*., 2020) [15]. Cooling with sprinklers and fans in the shaded area had a best effect on the maintenance behavior during cooler hours compared to the hotter hours of the daytime (Tucker *et al*., 2008; Butt *et al*., 2020) [36, 10]. Although (Anderson *et al*.,2013) [3]. found that the cooling system didn't affect the feeding neither than buffaloes performance. Dairy cows showed a decrease in their feeding time with a higher ambient heat load (Chen *et al*., 2016) [11].
The buffaloes resting behavior was increased in hot sand bedding due to the heat load. However (Chen *et al*., 2016) [11]. Water spray systems showed to be more functional in cooling the skin of buffaloes, as they produce higher performance, physiological, and good behavioral activities than that using less water or not. The buffaloe farmers can invent their showering technique during the subtropical summer using a water spray system (Bah *et al*., 2021) [4].
Enrichment the Egyptian buffaloes with sprinklers to cool its skin decrease the adverse effect of heat load during milk production and especially during the hottest period of the day. Environmental modification through sprinkles is associated with both hematological and biochemical blood parameters in buffaloes (Imbabi *et al*., 2019) [20]. The blood buffaloes' cortisol level increased with buffaloes' management in the hot building due to physiological heat stress. (Choudhary and Sirohi, 2019) [12]. as well as concluded that wallowing pool enables buffaloes to cool themselves by skin evaporation. The use of a wallowing pool and water spray were effective strategies for suppressing heat stress, as well as the cortisol levels were always higher in serum buffaloes that were managed in the open yard without wallowing than those managed in a shed and open space provided with a wallow (5 x 2 x 1 m3). The tendency of a higher cortisol level in the buffaloes could be attributed to more heat stress (Silva *et al*. 2014; Bah *et al*. 2022) [30, 5].
The increased cortisol concentration in heat-stressed dairy animals was found to be associated with reduced milk production and this could be attributed to the fact of deviation of available energy for coping up mechanisms to heat stress challenges (Silanikove, 2000; Habeeb, 2020) [29, 19]. The concentration of cortisol is altered by acute and chronic heat exposure (Christison and Johnson, 1972; Marai and Haeeb, 2010) [13, 22].
Data of the current work demonstrated that the level of serum prolactin and serum estrogen were significantly higher at cooled buffaloes than at control ones. This result is in harmony with the results of previous studies which reported that estrogen hormone levels reached a high level during the heat period to reach the maximum concentration (5.5 ng/ml) on day 17th of oestrous cycle (Beg and Totey, 1999) [7]. The concentration of serum prolactin hormone (ng/ml) was the highest level in buffaloes sprayed with water which improve the total milk yield (Singh and Madan 1993) [31]. It has been also documented that prolactin is directly affecting the reproductive performance of buffaloes as hyper prolactinaemia causes infertility in buffaloes during summer months due to severe heat stress as prolactin-induced suppression of estrogen secretion through poor luteal development (Mondal *et al*. 2006; Roy and Prakash, 2007) [24, 28]. Changes in the prolactin secretion during heat stress were correlated with body temperature changes with increased rectal temperature reducing the prolactin concentration (Alamer, 2011; Habeeb, 2020) [2, 19]. Prolonged heat exposure suppresses the production of hormone-releasing factors from the hypothalamic centers causing reductions in pituitary prolactin (Marai and Haeeb, 2010) [22].
The shortage of hormonal levels in heat-stressed lactating animals may be responsible for the depression in milk yield and composition. Besides, a high level of cortisol in animals exposed to high ambient temperature may be associated with

the depression in quantity and quality of milk (Habeeb, 2020) [19].

Conclusions

In conclusion, the feeding, rumination, and resting time (min/3hrs) were higher in cooled group buffaloes than in control group buffaloes. While the aggression was decreased with the cooling management system. The total milk yield was improved with the water spray cooling system on the buffalo farm. The onset of oestrus post-partum was faster, the serum cortisol levels was lower, and the level of serum prolactin and serum estrogen were higher at cooled buffaloes than at control ones.

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