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Association of Sleeping Time with Determination of Estrus in Riverine Buffaloes (*Bubalus bubalis*)

S.K. Joshi[1], T.K. Mohanty[1*], M. Bhakat[1] and S. Sathapathy[2]

[1]Division of Livestock Production Management, National Dairy Research Institute, Karnal, Haryana, INDIA
[2]Department of Veterinary Anatomy, CVSc. and A.H., OUAT, Bhubaneswar, Odisha, INDIA

**Corresponding author: TK Mohanty; Email: drtusarmohanty@gmail.com*

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ABSTRACT

The present study was conducted on the Murrah buffaloes maintained at Livestock Research Centre of ICAR-National Dairy Research Institute (NDRI), Karnal. The animals were classified into heifer, primipara and pluripara. For this experiment a total of 50 Murrah buffaloes were followed, out of which 8 were heifers, 12 were primipara and 30 were pluripara. Daytime was considered to be from 6.00 am to 6.00 pm and night from 6.00 pm to 6.00 am. It was confirmed that the mean daily sleeping time of buffaloes during the reference day in heifer, primipara, pluripara and overall of buffaloes was 76.63±1.80, 72.93±2.04, 74.64 ±1.44 and 74.40±1.06 min for heifer, primipara, pluripara and overall buffaloes, respectively. The total sleeping time decreased significantly ($p<0.05$) one day before estrus by 52.37%, 39.86%, 38.41% and 40.53% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. The difference between -1 d and day of estrus was significant ($p<0.05$) for all group except for heifers. The decrease in total sleeping time on estrus day was by 62.59%, 57.78%, 46.13% and 51.37% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. From the above study, it can be concluded that sleeping behaviour around estrus indicated their potential for useful markers in early detection of estrus. The changes in sleeping behavior of individual buffalo can be used for early detection of change of reproductive status as it is significantly affected by estrus and as the change in sleeping behavior.

Keywords: Association, estrus, Murrah buffaloes, progesterone, sleeping

Efficient estrus detection forms the core determinant of a successful reproduction program. Among all, heat detection has historically been an important component of a successful reproduction program. Inefficient heat detection causes significant economic losses (Karir *et al.,* 2006). The efficiency of reproductive biotechnologies, such as artificial insemination (AI), for buffalo herds based only on visual estrus detection is seriously compromised due to the discrete estrous behavior of the buffalo species, especially among buffalo heifers (Campanile *et al*. 2010). Detection of estrus is a key determinant of profitability of dairy herds, but estrus is increasingly difficult to observe in the modern dairy cow with shorter duration and less-intense estrus. Concurrent with the unfavorable correlation between milk yield and fertility, estrus-detection rates have declined to less than 50% larger herd sizes and less labor per cow reduce opportunities for visual observation, and detection is often below 50% (Van Eerdenburg *et al.*, 2002; Roelofs *et al.*, 2006). As, incorrect and poor estrus detection is related to financial losses due to extended calving intervals, milk loss, veterinary costs and an increase in the number of cows culled due to infertility (Walker *et al*., 1996). A 5% increase in conception rates alone was predicted to increase net annual income by 1226 US dollar whereas, decreasing days to first service from 80 to 60 days, increasing efficiency of heat detection from 50 to 60%, and increasing conception rates from 35 to 50%, combined to yield an increased net income of 18,485 US dollar in a farm of 300 breedable dairy cows (Hady *et al*., 1994). Covert or silent estrus constitutes the single largest factor responsible for poor reproductive efficiency in buffalo. Aside from seasonal factors affecting the display of estrus, the most important contributing factor is an inability to detect estrus. Silent heat is common among buffaloes, which means that ovulation occurs without any visual heat signs. The proportion of buffaloes coming in to heat in morning, the mid-day period and evening or night was 48.08, 18.17 and 33.65% respectively (Agarwal and Purbey, 1983). Among Murrah buffaloes diurnal pattern of estrus behaviour have been observed with 59% of estrus recorded between 10 PM and 6 AM (Prakash, 2002). Homar, (2013) reported 44% of estrous behavior to occur during unsociable hours between 1800 and 0600 h when the herdsmen may not be present which makes the detection more difficult and inefficient. The changes in general behavior of buffaloes are good indicators of the reproductive status of animal. This technique necessitates continous survillence of animal. Videography provides more accurate detection of estrus as there is 24 hr surveillance of the animal; however it requires more time and labour compared to other methods. A continuous observation of 24 h per day is important, because the beginning of oestrus is mostly in the early morning (Sambraus, 1978) and for this continuous observation videography is required.

MATERIALS AND METHODS

The present study was conducted on Murrah buffaloes maintained at Livestock Research Centre of National Dairy Research Institute (NDRI), Karnal. The animals were classified into heifer, primipara and pluripara. For this experiment a total of 50 Murrah buffaloes were followed, out of which 8 were heifers, 12 were primipara and 30 were pluripara. Daytime was considered to be from 6.00 am to 6.00 pm and night from 6.00 pm to 6.00 am. The experimental animals were maintained in loose housing system under group management practice. The feeding mangers and resting area were covered with asbestos sheets at moderate height with low slope inclination. At one corner of paddock, there was provision of drinking water trough with running fresh tap water. There were trees shades within the paddock for shelter of the animals as per their preferences. The flooring of the paddock was "brick on edges" and the manger area is concrete with grooves. Buffaloes were fed as per requirements the available green fodders (Maize, Jowar, berseem, Lucerne and Oats) with concentrates based on their body weight and milk yield. Milking buffaloes were given additional concentrate at the rate of 1.0 kg for every 2.5 kg milk production, above 5.0 kg milk yield and maintenance ration. The concentrate to the milking animals was fed in divided allowances during times of milking. For this study buffaloes were numbered individually on the flanks and back with white water paint twice a week so that they could be recognized. Sleeping behavior patterns were recorded by digital video recording done by 4 CCTV outdoor cameras (HIKVISION). The cameras had digital zoom for close viewing. The cameras were enabled with array infrared technology for night vision. Two extra infra red lights were installed for better night vision. Cameras were installed at different places and different angles in the experimental shed so that whole shed can be covered in viewing angle. The cameras were used to shoot images at a capturing speed of 4 frames per second (FPS). All videos were stored in digital video recorder (DVR) having hard disk of 1 TB space. All parameters were recorded in minutes: seconds format which were later converted to minutes as per the need of the parameters. Sleeping behaviours of buffaloes like day sleeping time, night sleeping time were monitored from 7 days before estrus to 7 days after estrus. The day of estrus was confirmed by serum progesterone assay. Daily mean values from d −7 to −3 before day of estrus and from d +3 to +7 after day of estrus were averaged, resulting in one individual day of reference per buffalo for sleeping time. The individual reference values were then compared with each of the 5 d around the day of estrus (d −2, −1, 0, +1, +2).

The definite time of estrus was confirmed by progesterone concentration. All video recordings were analyzed by continuous observation. The sleeping patterns of each individual buffalo were read by 1X to 4X speed depending on the activities of the buffalo. The start and end point of sleeping was observed by both forward and back ward replay. The duration of each activity was the difference between start and end point of that activity. Animals were considered sleeping when they were lying on its belly or on their side, with head leaning on the interior, on the floor or along the body with eyes closed. Again it was divided into day sleeping time and night sleeping time. For the estimation of P4, blood samples were collected on the day of heat. After the collection of blood, samples were left to stand for some time at 4 °C, up to the oozing out of serum, and then it was centrifuged at 4 °C at the rate of 3000 rpm for 20 minutes to separate the serum. The separated serum samples were stored in cryo-vials at -20 °C till the assay for Progesterone hormone (P4). Estrus stage was confirmed by serum progesterone concerntration, i,e. if the serum progesterone concerntration was < 0.1 ng/ml, then the animal was said to be in heat (Vukovic *et al*., 2016). The data were analysed statistically by using the standard statistical methods of Snedecor and Cochran (1994**)** and by doing two way ANOVA and post hoc test with Systat Software Inc, USA and SPSS 16.0 version software.

RESULTS AND DISCUSSION

Sleeping Time

The data on the mean value of total sleeping time during the reference period, on the day of estrus and during the peri-estrus period has been presented in Table 1 and Fig. 1.

Mean daily total sleeping time of buffaloes during the reference day was 76.63±1.80, 72.93±2.04, 74.64 ±1.44 and 74.40±1.06 min for heifer, primipara, pluripara and overall buffaloes, respectively. The total sleeping time decreased significantly ($p<0.05$) one day before estrus by 52.37%, 39.86%, 38.41% and 40.53% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. The difference between -1 d and day of estrus was significant ($p<0.05$) for all group except for heifers. The decrease in total sleeping time on estrus day was by 62.59%, 57.78%, 46.13% and 51.37% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. Heifers spent more time in sleeping compared to other groups of animals on the day of reference, but on the day of heat pluripara spent significantly ($p<0.05$) more time in sleeping compared to other groups of animals. The decrease in total sleeping time can be explained by the reports of Thind and Gill (1988), who compared 4 pre- and post oestral days and reported a lower resting time. The decrease in total sleeping time quantifies behavioral changes and completes the picture of increased activity and restlessness for which this parameter is compromised. This area of research has remained mostly untouched, so comparison of result with other studies is limited.

Day sleeping time

The data on the mean value of day sleeping time during the reference period, on the day of estrus and during the peri-estrus period has been presented in Table 2.

Mean day sleeping time of buffaloes during the reference day was 29.38±0.48, 30.49±0.79, 28.61±0.50 and 29.23±0.39 min for heifer, primipara, pluripara and overall buffaloes, respectively. On reference day, heifers slept for significantly ($p<0.05$) higher duration than primipara and pluripara. The day sleeping time decreased significantly ($p<0.05$) one day before estrus by 54.62%, 41.65%, 36.97% and 40.47% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of

Table 1: Change in total sleeping time in min (Mean ± SE) in periestrus period in comparison to reference period through 24 hr videography in Murrah buffaloes

Days	Heifer	Primipara	Pluripara	Overall
Reference	76.63±1.80^{aA}	72.93±2.04^{aA}	74.64±1.44^{aA}	74.40±1.06^{a}
-2	65.50±1.93	60.71±2.02	64.67±1.47	63.66±1.09
-1	36.50±2.62^{bB} (52.37%)	43.86±1.33^{bAB} (39.86%)	45.97±1.80^{bA} (38.41%)	44.24±1.24^{b} (40.53%)
0	28.67±1.80^{bB} (62.59%)	30.79±1.21^{cB} (57.78%)	40.20±1.61^{cA} (46.13%)	36.18±1.25^{c} (51.37%)
1	66.33±2.36	66.79±1.74	67.20±1.23	66.98±0.91
2	75.17±2.46	73.29±1.53	77.60±1.11	76.10±0.87

Values bearing different superscript (a,b,c) differ significantly in column P< 0.05; Values bearing different superscript (A,B,C) differ significantly in rows P< 0.05

Table 2: Change in day sleeping time in min (Mean ± SE) in periestrus period in comparison to reference period through 24 hr videography in Murrah buffaloes

Days	Heifer	Primipara	Pluripara	Overall
Reference	29.38±0.48^{aB}	30.49±0.79^{aA}	28.61±0.50^{aC}	29.23±0.39^{a}
-2	26.17±1.45	23.43±0.90	25.87±0.66	25.22±0.52
-1	13.33±0.71^{bB} (54.62%)	17.79±0.68^{bA} (41.65%)	18.03±0.81^{bA} (36.97%)	17.40±0.57^{b} (40.47%)
0	10.50±0.62^{bB} (64.26%)	11.86±0.31^{cB} (61.10%)	15.30±0.73^{cA} (46.52%)	13.76±0.52^{c} (52.92%)
1	26.50±1.77	26.71±1.07	26.20±0.60	26.38±0.50
2	30.33±1.82	28.50±0.64	30.53±0.56	29.94±0.44

Values bearing different superscript (a,b,c) differ significantly in column P< 0.05; Values bearing different superscript (A,B,C) differ significantly in rows P< 0.05

Table 3: Change in night sleeping time in min (Mean ± SE) in periestrus period in comparison to reference period through 24 hr videography in Murrah buffaloes

Days	Heifer	Primipara	Pluripara	Overall
Reference	47.25±1.41^{aA}	42.14±2.07^{aA}	47.66±0.97^{aA}	45.69±0.88^{a}
-2	39.33±1.58	37.29±1.34	38.30±0.95	38.06±0.70
-1	23.17±1.94^{bA} (50.97%)	26.07±1.26^{bA} (38.13%)	27.60±1.08^{bA} (42.08%)	26.62±0.79^{b} (41.73%)
0	18.17±1.19^{Bb} (61.55%)	18.93±1.03^{Cb} (55.08%)	24.67±0.91^{bA} (48.24%)	22.22±0.75^{c} (51.36%)
1	39.83±1.62	40.07±1.31	40.50±0.86	40.24±0.65
2	44.83±1.72	44.79±1.10	46.50±0.99	45.82±0.70

Values bearing different superscript (a,b,c) differ significantly in column P< 0.05; Values bearing different superscript (A,B,C) differ significantly in rows P< 0.05

reference. The difference between -1d and day of estrus was significant (p<0.05) for all group except for heifers. The decrease in the day sleeping time on estrus day was by 64.26%, 61.10%, 46.52% and 52.92% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. Primipara spent significantly (p<0.05) more time in sleeping compared to other groups of animals on the day of reference, but on the day of heat pluripara spent significantly (p<0.05) more time in sleeping compared to other groups of animals. The decrease in day sleeping time is a reflection of the decrease in total sleeping time on -1 d and 0 d.

Night sleeping time

The data on the mean value of night sleeping time during the reference period, on the day of estrus and during the peri-estrus period has been presented in Table 3.

Mean night sleeping time of buffaloes during the reference day was 47.25±1.41, 42.14±2.07, 47.66±0.97 and 45.69±0.88 min for heifer, primipara, pluripara and overall buffaloes, respectively. On reference day, heifers and pluripara slept for higher duration in night than primipara and pluripara. The night sleeping time decreased significantly (p<0.05) one day before estrus by 50.97%, 38.13%, 42.08% and 41.73% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. The difference between -1d and day of estrus was significant ($p<0.05$) for primipara and overall animals. The decrease in night sleeping time on estrus day was by 61.55%, 55.08%, 48.24% and 51.36% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. During estrus, pluripara spent significantly ($p<0.05$) more time in sleeping compared to other groups of animals. The decrease in night sleeping time is also a reflection of the decrease in total sleeping time on -1 d and 0 d.

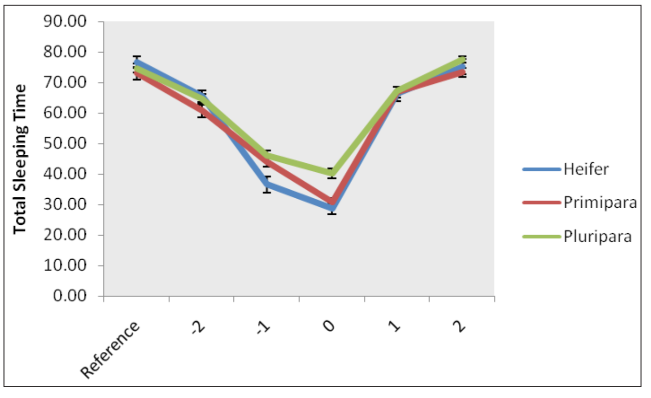

Fig. 1: Total sleeping time (min) in periestrus and reference period in Murrah buffaloes

CONCLUSION

From the above study, it can be concluded that sleeping behaviour around estrus indicated their potential for useful markers in early detection of estrus. The changes in sleeping behavior of individual buffalo can be used for early detection of change of reproductive status as it is significantly affected by estrus and as the change in sleeping behavior. The total sleeping time for overall buffalos was decreased significantly ($p<0.05$) one day before estrus by 40.53% and on the day of estrus it was decreased to a level of 51.37%. As there was observed decrease in total, day and night sleeping time of Murrah buffaloes one day prior to the day of estrus, the change in this parameter can be used for early detection of estrus in Murrah buffaloes.

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