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SK Joshi
Division of Livestock Production Management, National Dairy Research Institute, Karnal, Haryana, India

TK Mohanty
Ph.D. Principal Scientist, Animal Reproduction, Gynaecology and Obstetrics Officer In-charge, Artificial Breeding Research Center ICAR-NDRI, Karnal, Haryana, India

A Kumaresan
Division of Livestock Production Management, National Dairy Research Institute, Karnal, Haryana, India

M Bhakat
Division of Livestock Production Management, National Dairy Research Institute, Karnal, Haryana, India

Correspondence
TK Mohanty
Ph.D. Principal Scientist, Animal Reproduction, Gynaecology and Obstetrics Officer In-charge, Artificial Breeding Research Center ICAR-NDRI, Karnal, Haryana, India

Association of feeding and drinking time in the determination of estrus in Murrah buffaloes (*Bubalus bubalis*)

SK Joshi, TK Mohanty, A Kumaresan and M Bhakat

Abstract
The present study was conducted on the Murrah buffaloes maintained at Livestock Research Centre of National Dairy Research Institute (NDRI), Karnal. The animals were classified into two groups viz. group I and group II basing on the lactation number as there were some morphological changes in the udder and teats of buffaloes with increasing lactations. In group I, animals up to 3rd lactation were there, whereas the animals from 4th to 5th lactation were included in group II. There were 20 buffaloes under group I and 30 buffaloes under group II. It was confirmed that the mean daily feeding time of buffaloes during the reference day in heifer, primipara, pluripara and overall of buffaloes was 191.97±3.43, 262.55±3.61, 212.88±4.11 and 222.47±4.43 min, respectively. The mean night feeding time of buffaloes during the reference day was 64.72±1.85, 100.95±3.29, 77.68±6.18 and 82.64±4.18 min for heifer, primipara, pluripara and overall buffaloes, respectively. The total feeding time (min) in Murrah buffaloes decreased significantly (P<0.05) on the day of estrus (160.66±3.95) compared to that during the reference period (222.47±4.43). The total drinking time was decreased significantly (P<0.05) one day before estrus by 37.86%, 31.32% and 31.70% for primipara, pluripara and overall buffaloes, respectively compared to the day of reference. Further, the feeding time (r=0.252, p=0.779) was found to be positively correlated with estrogen concentration.

Keywords: Association, feeding, drinking, estrus, Murrah buffaloes

Introduction

Almost the entire world population of buffaloes (*Bubalus bubalis*) is found in the developing countries of Asia where they are the back bone of farmers. Reproductive efficiency is the primary factor affecting productivity and is hampered in female buffalo by inherent late maturity, poor estrus expression in summer, distinct seasonal reproductive patterns, long postpartum ovarian activity and prolonged intercalving intervals (Madan and Raina, 1984) [11]. They have been traditionally regarded as a poor breeder or peculiar Shy breeder having poor fertility (Barile, 2005) [3] as they have lower number of primordial follicles in their ovary varying from 10,000 to 19,000 compared with 150,000 in cattle. So, an important factor to consider in improving the productive and reproductive life in dairy buffaloes is heat detection (Sastry, 1983) [20] and it is a major problem that decreases the high reproductive efficiency in national dairy herd (Senger, 1994) [21]. The success of estrus detection not only increases conception rates, but also raises the milk production of the herd (Diskin and Srenan, 2000; Tsai *et al*., 2014) [6, 25]. Most of these problems crop up from the lack of or poor estrus detection and use of the "out of breeding season" mating technique, carried out to meet the market demand. The changes in general behavior of buffaloes is a good indicator of the reproductive status of animal. This technique neccessiates 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. When visual observation was compared with progesterone profiles of 32 cows, found an accuracy of 65.6%. A continuous observation of 24 h per day is important, because the beginning of oestrus is mostly in the early morning (Sambraus, 1978) [19] 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 two groups viz. group I and group II basing on the lactation number as there were some morphological changes in the udder and teats of buffaloes with increasing lactations. In group I, animals up to 3rd lactation were there, whereas the animals from 4th to 5th lactation were included in group II. There were 20 buffaloes under group I and 30 buffaloes under group II. 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. For this experiment a total of 50 Murrah buffaloes were followed, out of which 8 were heifers, 12 were primipara and 30 were pluripara. All behavior parameters 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. General behaviours of buffaloes like feeding time, drinking 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 all parameters. 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. All activity 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 each activity 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. The following behaviours were recorded and studied by continuous observation. Feeding was defined as the head in the manger with active ingestion and animal was considered as drinking when head in the waterer with muzzle touching the water. Total feeding time was again divided into day time feeding and night time feeding. For the estimation of P4, blood samples were collected on the day of heat. For estimation of E2, blood samples were collected from 3 days before estrus to the day of heat. After the collection of blood, samples were left to stand for some time at 4 ^{0}C, up to the oozing out of serum, and then it was centrifuged at 4 ^{0}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 0C till the assay for Progesterone hormone (P4), Estrogen (E2). 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) [28]. The data were analysed statistically by using the standard statistical methods of Snedecor and Cochran (1994) [22] and by doing two way ANOVA and post hoc test with Systat Software Inc, USA and SPSS 16.0 version software.

Results and Discussion

Feeding time

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

Mean daily feeding time of buffaloes during the reference day in heifer, primipara, pluripara and overall of buffaloes was 191.97±3.43, 262.55±3.61, 212.88±4.11 and 222.47±4.43 min, respectively (Table 1). The reference day feeding time are close to the values reported by Behera (2008) [4], who reported that that Murrah animals spend 289±5.24 min on eating. However, a higher value was reported by Yadav and Gupta (1985) [29] and Thind *et al*. (1988) [24], who reported the eating time in lactating Murrah buffaloes to be 369 min and 349 min, respectively. In general primipara fed for longer time followed by pluripara and heifers. The difference between primipara and heifer and primipara and pluripara was significant (P<0.05) on the reference day. The findings are supported by the reports of Ali *et al*. (1990) [1], who reported that eating time is related to age of lactating buffalo. The feeding time was significantly (P<0.05) lower one day before estrus by 23.33%, 19.60%, 23.15%, and 21.37% for heifer, primipara, pluripara and overall of buffaloes, respectively compared to the day of reference. However, the difference between -1d and day of estrus was not significant, but the decrease in feeding time on estrus day was by 38.88%, 28.50%, 26.39%, and 27.78% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. Similar findings were also reported by Phillips and Schofield (1990) [16], who reported that during estrus, cows spend less time in feeding and the reduction in feeding time ranged between 5 and 20% on the day of estrus. The difference between heifer, primipara and pluripara was significant (P<0.05) on the day of estrus. The findings of the present study are in agreement with the reports of Diskin and Sreenan (2000) [6], who reported a decrease in time spent for feeding been associated with estrus. The findings of the present study are also in line with Pahl *et al.* (2015) [15], who reported that feeding time was decreased on d −1 and 0. He also reported that primiparous and pluriparous cows differed in feeding behavior on normal days and so on the day of estrus and he concluded that feeding characteristics around estrus indicated their potential for useful addition in early detection of estrus. Lukas (2008) [10] reported that the feeding behavior of individual cows can be used for early detection of change in cow health or reproductive status as it is significantly affected by disease or estrus. Olsson, 2007 also reported a decreased appetite and subsequently decreased feeding time during estrus in Murrah buffaloes. The present findings are supported by the reports of Maltz *et al*. (1997) [12] and Reith *et al*. (2014) [17], they found a decrease in feeding behavior parameters on the day of heat, but it is in opposition to the findings of De Silva *et al*. (1981) [5], who reported no effect on feeding behavior parameters around estrus.

Table 1: Change in total feeding time in min in periestrus period in comparison to reference period through 24 hr videography in Murrah buffaloes

Days	Heifer	Primipara	Pluripara	Overall
Reference	191.97±3.43^{aB}	262.55±3.61^{aA}	212.88±4.11^{aB}	222.47±4.43^{a}
-2	174.33± 5.52	241.86± 5.22	200.00±3.95	208.64±4.25
-1	147.17±3.94^{bB} (23.33%)	211.07±5.75^{bA} (19.60%)	163.60±4.86^{bB} (23.15%)	174.92±4.68^{b} (21.37%)
0	117.33±3.84^{bC} (38.88%)	187.71±3.79^{bA} (28.50%)	156.70±3.90^{bB} (26.39%)	160.66±3.95^{b} (27.78%)
1	177.50±5.41	241.14±4.28	199.27±4.02	208.38±4.12
2	192.17±4.32	259.00±3.07	212.77±4.08	223.24±4.22

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 $P<0.05$

Changes in feeding behavior go along with increased physical activity and restlessness of cows in estrus (Van Eerdenburg *et al*., 1996) [27], and it is very well conceivable that they are a consequence of increased restlessness during estrus. The decrease in feeding time can be explained by the reports of Uphouse and Maswood, (1998) [26]; Mondal *et al*. (2006) [14], who reported that estrogens affect dietary behavior by reducing appetite and feed consumption. There may be diurnal variation in feeding time in realion to estrus. So the total feeding time was divided into day feeding time (Table 2) and night feeding time (Table3).

Mean day feeding time of buffaloes during the reference day was 127.25±2.72, 168.66±7.50, 139.35±2.19 and 146.10±3.26min for heifer, primipara, pluripara and overall of buffaloes, respectively. The day feeding time during reference period is lower than that reported by Behera (2008) [4], who reported that Murrah animals spent 194.07±5.24 min day feeding. The difference may be due to difference in the management practices. Primipara fed for longer time during day followed by pluripara and heifers. The difference between primipara and heifer; primipara and pluripara was significant ($P<0.05$) on the reference day. The day feeding time was significantly ($P<0.05$) lower one day before estrus by 33.07%, 25.76%, 31.99%, and 30.08% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. However, the difference between -1d and day of estrus was not significant, but the decrease in day feeding time on estrus day was by 46.16%, 36.04%, 34.60% and 36.27% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. The difference between primipara and heifer and primipara and pluripara was also significant ($P<0.05$) on the day of estrus. The change in day feeding time has potential to be used as a deciding parameter for estrus detection, if it can be capture by automatic feeding stations.

Table 2: Change in day feeding time in min in periestrus period in comparison to reference period through 24 hr videography in Murrah buffaloes

Days	Heifer	Primipara	Pluripara	Overall
Reference	127.25±2.72^{aB}	168.66±7.50^{aA}	139.35±2.19^{aB}	146.10±3.26^{a}
-2	105.67±5.13	148.14±7.44	118.53±1.89	125.28±3.24
-1	85.17±4.01^{bB} (33.07%)	125.21±6.58^{bA} (25.76%)	94.77±2.36^{bB} (31.99%)	102.14±3.17^{b} (30.08%)
0	68.50±3.25^{bB} (46.16%)	107.86±6.42^{bA} (36.04%)	91.13±2.03^{bBA} (34.60%)	93.10±2.77^{b} (36.27%)
1	105.67±2.96	148.36±7.61	117.83±1.88	124.92±3.27
2	116.67±2.40	162.86±7.18	127.67±2.19	136.20±3.43

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 $P<0.05$

Mean night feeding time of buffaloes during the reference day was 64.72±1.85, 100.95±3.29, 77.68±6.18 and 82.64±4.18 min for heifer, primipara, pluripara and overall buffaloes, respectively. Similar findings were reported by Behera (2008) [4] in Murrah buffaloes.

Table 3: Change in night feeding time in min in periestrus period in comparison to reference period through 24 hr videography in Murrah buffaloes

Days	Heifer	Primipara	Pluripara	Overall
Reference	64.72±1.85^{aB}	100.95±3.29^{aA}	77.68±6.18^{aB}	82.64±4.18^{a}
-2	68.67±2.50	100.79±2.90	80.30±1.97	84.64±2.10
-1	62.00±1.55^{aB} (4.20%)	91.71±3.40^{aA} (9.15%)	68.17±2.64^{aB} (12.24%)	74.02±2.43^{ab} (10.43%)
0	48.83±2.2^{aB} (24.55%)	86.36±2.85^{aA} (14.45%)	65.63±2.23^{aB} (15.51%)	69.42±2.30^{b} (15.99%)
1	71.83±3.46	99.79±3.21	79.80±1.85	84.44±2.03
2	75.50±2.08	103.50±2.81	83.37±1.63	88.06±1.90

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 $P<0.05$

Total drinking time

The data on the mean value of total drinking time during the reference period, on the day of estrus and during the peri-estrus period has been presented in Table 4. Mean total drinking time of buffaloes during the reference day was 4.72±0.31, 8.62±0.65, 6.65±0.81and 6.97±0.54 min for heifer, primipara, pleuripara and overall of buffaloes, respectively. The drinking time in primipara and pluripara is more than that of heifers; this may be due to the more water requirement for milk production in these two groups. The total drinking time was decreased one day before estrus by 8.12%, 37.86%, 31.32% and 31.70% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference. The difference was significant (P<0.05) for all groups except heifer. However, the difference between -1d and day of estrus was non-significant (P<0.05) for all groups except primipara and the decrease in total drinking time on estrus day was by 29.32%, 70.17%, 35.33% and 46.91% for heifer, primipara, pluripara and overall buffaloes, respectively compared to the day of reference.

Table 4: Change in drinking time in min in periestrus period in comparison to reference period through 24 hr videography in Murrah buffaloes

Days	Heifer	Primipara	Pluripara	Overall
Reference	4.72±0.31^{aB}	8.62±0.65^{aA}	6.65±0.81^{aB}	6.97±0.54^{a}
-2	3.83±0.17	5.36±0.48	5.57±0.28	5.30±0.23
-1	4.33±0.56^{aA} (8.12%)	5.36±0.46^{bA} (37.86%)	4.57±0.38^{bA} (31.32%)	4.76±0.27^{b} (31.70%)
0	3.33±0.71^{aA} (29.32%)	2.57±0.25^{cA} (70.17%)	4.30±0.28^{bA} (35.33%)	3.70± 0.22^{b} (46.91%)
1	6.00±0.68	7.21±0.47	5.97±0.31	6.32±0.25
2	5.00±0.93	4.79±0.52	4.97±0.35	4.92±0.27

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

The findings of the present study is supported by reports of who told that decreased intake of water occurs due to estrus and reproductive changes have all been associated with a decrease in feed intake. Huzzey *et al*. (2007) [8] also reported that presence of estrus was associated with increased DMI and tended to have the opposite effect on water intake.

Estrogen Conc. (pg/ml) during proestrus and estrus

Table 5: Estrogen Conc. (pg/ml) during proestrus and estrus in Murrah buffalo

-3d	-2d	-1d	0d
6.24±0.37	26.70±0.83	30.02±0.54	20.59±0.82

The findings of the present study are in agreement with that of Bachalaus *et al*. (1979) [2], who observed a peak oestradiol concentration of 35.8 pg/ml, 1 day before oestrus, with levels declining to 17.8 pg/ml when the first signs of behavioural oestrus. Samad *et al*. (1988) [18] also reported similar findings with a higher value that the concentration of oestradiol-17β increased following luteolysis and reached its peak value of 30-35 pg/ml either a day before or on the day of oestrus. Similar findings are reported by Batra and Pandey (1982), who reported that Peak concentrations of Estradiol-17β 30–35 pg/ml were detected on the day of estrus or one day before. Similar findings are also reported by Glencross and Pope (1981), who found that the estradiol concentration begin to rise from 4-5 days prior to the estrus and reach to its maximum level at the day of estrus. However, Mirmahmoudi *et al*. (2014) reported a lower value for Estrogen peak concentration, which was found to be 8.7 and 11.0 pg/mL under Heatsynch Estradoublesynch protocol respectively.

The correlation of estrogen concentration and Estrogen: Progesterone with feeding behaviour is given in the Table 5. Feeding time (r=0.252, p=0.779) was found to be positively correlated with estrogen concentration. A positive correlation was found between feeding time (r=0.289 and estrogen: progesterone concentration ratio. Similarly, in a study by Mondal *et al*., (2006) [14] estrus behavior was found to be positively correlated with the maximum peak concentration of E2 (r = 0.89, P<0.001) and total estrogen (r = 0.66, P = 0.019) during the peri-estrus period in Mithun. Reported in a study that maximum E2, levels correlate with the total amount of estrous behavior shown by a cow during an estrous period.

Table 6: Correlation of Feeding Time with Hormone profile

Parameter	p-value	Correlation coefficient
Estrogen Concentration	0.0779	0.252
Estrogen : Progesterone Concentration	0.0417	0.289

Conclusion

From the above study, it can be concluded that feeding characteristics around estrus indicated their potential for useful markers in early detection of estrus. The feeding 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 feeding behavior was correlated with Estrogen, the hormone responsible for most of the estrus signs. The more observed change in day feeding time can make the observation time limited to day time for accurate prediction of estrus. The total drinking time was decreased significantly (P<0.05) one day before estrus by 37.86%, 31.32% and 31.70% for primipara, pluripara and overall buffaloes, respectively compared to the day of reference.

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