



Acoustic features of vocalization during different phases of estrous cycle in Murrah buffaloes

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Received: 5 February 2016; Accepted: 17 May 2016

ABSTRACT

The present study was conducted to investigate the changes in acoustic features of vocal signals articulated by Murrah buffaloes during different phases of estrous cycle. Data (voice) were collected from 20 Murrah buffaloes, maintained at Livestock Research Centre, NDRI, Karnal, during 4 phases of estrous cycle. Following acoustic features (call duration, formants, intensity, pitch, noise/harmonic (N/H) ratio, harmonic/noise ratio, number of periods, pulses, jitter, shimmer and unvoiced frame) were extracted from vocal signals of Murrah buffaloes for analysis purpose. The acoustic features, viz. call duration, fourth formant (F4), shimmer and intensity were significantly higher during proestrus phase as compared to other phases of estrous cycle. Whereas, F1, F5, noise to harmonic ratio, per cent unvoiced frames and pitch were significantly higher during estrus phase. All these features have significant correlation with estradiol hormone during proestrus and estrus phase. The other features namely number of periods and pulses were significantly correlated with progesterone hormone during diestrus phase. Therefore, the acoustic features, viz. F1, F5, N/H ratio and unvoiced frame can be utilized for identification of estrus phase in Murrah buffaloes.

Key words: Acoustic features, Buffalo, Phases of estrous cycle, Vocalization

The buffalo is considered to be the backbone of the farmer's economy in India, South-Asia and several European and American countries, benefiting nearly half of humanity in over 40 countries. Buffalo (*Bubalus bubalis*) is known as the world's second most important milch animal because it shares more than 95% of the milk produced in South Asia (Javaid *et al.* 2009). India is having 108.7 million buffaloes (DAHD&F 2012) and their contribution in total milk production of India is about 51% (DAHD&F 2013–14). According to the bulletin of World Dairy Situation (2013), India's buffalo milk contribution to the worldwide milk production is 67%. Buffaloes are also valued for meat and draught purposes (Bandyopadhyay *et al.* 2003). Presently, the buffalo contributes 1,534,989 metric tonne of meat to the meat pool of India, which comes out to be 45.74% of global buffalo meat and ranks first in the world (FAO 2012). The buffaloes are much known as 'peculiar shy breeder'. According to majority of scientific and research communities, buffaloes tend to have delayed puberty, silent estrus, long post-partum ovarian inactivity

and poor fertility. The buffalo is considered as a difficult breeder because of its inherent susceptibility to environmental stress, which causes anoestrus and subestrus. These conditions are responsible for a prolonged intercalving period resulting in great economic losses for the dairy industry. The productive and reproductive efficiency of animals are complementary to each other. Estrus detection in these species is difficult due to the lack of expert personnel, high expression of silent heat, poor expression of estrous behaviour, and also due to high variation in duration of estrous cycle (Thakur *et al.* 2013). Low reproductive efficiency, especially in buffaloes remains a major economic problem globally and its incidence is higher in our country. The main interest of the animal breeder is to achieve one calf/year from dairy animals. To achieve this goal, normal reproductive tools are needed to overcome problems like silent heat. The present experiment is an attempt to find out the changes in different acoustic features of vocal signals articulated during various phases of estrous cycles (proestrus, estrus, metestrus and diestrus) in Murrah buffaloes.

MATERIALS AND METHODS

Selection of animals: Murrah buffaloes (20) belonging to second and third parity in their early stages of lactation, with normal reproductive cycle, maintained at the institute, were selected as experimental animals in present

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investigation. Recording of vocalizations of these experimental animals was done only after confirming their heat/estrus condition by behavioural observations and per-rectal examinations by experts. Although the buffaloes were managed in loose housing system in a group of 50 buffaloes, but recording for each and every buffalo was done only after separating them from the herd.

Recording and editing of voice signals: Buffaloes were housed in individual sheds at the time of recording only to avoid any overlapping of sound signals. The dimension of the individual shed was 6 m × 3 m with open and covered space in equal ratio. The buffaloes were kept loose in these sheds at the time of recording so that their natural behaviour was not disturbed. Recording of sound signals was done for a sufficient period of time so that at least 30 complete vocal signals could be received from each buffalo during different phases of estrous cycle. Sound recording was performed by using a video camera equipped with a good quality microphone. The microphone was fixed in the front wall of the recording shed at a height of 150 cm from floor. The camera was kept on a tripod at a suitable height and distance in such a position so that it could capture each and every activity of the she-buffalo under recording. The buffaloes were not disturbed with these recording devices and environment during the experiment. Since the weather under which experiments were conducted was clear, the background noise was only the low level sound produced due to rubbing of the body of animals. The recorded sound signals were then transferred to computer and superimposed sound signals were detected and eliminated manually by observing the spectrogram of each and every signal during editing. Numbers of clips of complete voice signals for each buffaloes were prepared from these recordings, by using audio-visual editing software package. Sound signals were resampled at a sampling frequency of 48 kHz and 16 bit. The various acoustic features were extracted with the help of software package (Boersma and Weenink 2010).

Sample collection and hormones analysis: During the experimental period, blood samples (5 ml) were collected on day 0 (day on which estrus was observed for the first time), day 1 (day next to day 0), day 2 and from day 14th to 24th of estrous cycle, after voice recording. Voice signals for these animals were also recorded on the same days. The day of estrus and different phases of estrous cycle were determined based on the distinct changes in estrogen (E₂) and progesterone (P₄) secretion during the whole cycle. The hormones were estimated by using E₂ and P₄ ELISA test kits.

Statistical analysis of acoustic data: The data were analyzed by using least squares technique (Harvey 1987) and significance of difference among various subclasses was examined by using Duncan's Multiple Range Test as modified by Kramer (1957).

Following least squares model was used to examine the significant differences between the different acoustic features of voice signals uttered by Murrah buffaloes during their different phases of estrus cycle:

$$Y_{ij} = \mu + P_i + e_{ij}$$

where, Y_{ij} is the acoustic feature of voice signals articulated during ith phase of estrus cycle of Murrah buffaloes; μ is the overall mean; P_i is the effect of ith phase of estrus cycle of Murrah buffaloes and e_{ij} is the residual error ~ (μ, σ^2).

Similarly following least square model was used to find out the effect of various phases of estrous cycle on blood level of estrogen and progesterone hormones in Murrah buffaloes:

$$Y_{ij} = \mu + P_i + e_{ij}$$

where, Y_{ij} is the blood level of estrogen and progesterone hormones during ith phase of estrus cycle of Murrah buffaloes; μ is the overall mean; P_i is the effect of ith phase of estrous cycle of Murrah buffaloes and e_{ij} is the residual error ~ (μ, σ^2).

RESULTS AND DISCUSSION

The spectrogram and waveform for a single clip from sound signals articulated during proestrus, estrus, metestrus and diestrus phases of a Murrah buffalo reflecting the values of various acoustic features, viz. call duration, amplitude (maximum and minimum), intensity, pitch, formants, pulse and periods are depicted in Fig. 1a-d, respectively. Bioacoustics features extracted from voice samples of Murrah buffaloes during different phases of their estrus cycle were subjected to one-way analysis of variance to examine the significant effect of these phases of estrous cycle on their vocalization. Moreover, the least squares mean values for various acoustic features, viz. call duration, formants (F1, F2, F3, F4 and F5), intensity, pitch, noise to harmonic ratio (%), harmonic to noise ratio (dB), number of periods, number of pulses, jitter (%), shimmer (%) and percent of unvoiced frames for vocal signals from Murrah buffaloes during their different phases of estrous cycle are presented in Table 1, whereas, the least squares mean values for blood hormone levels of estrogen (pg/ml) and progesterone (ng/ml) during these phases of estrus cycle are presented in Table 2.

Call duration: Call duration (Table 1) of vocal signals during proestrus and estrus phases of estrous cycle in buffaloes was significantly (P<0.001) longer than other phases. This variation in call duration among different phases of estrous cycle might be due to significant (P<0.001) differences observed for blood estrogen and progesterone level in Murrah buffaloes during different phases of estrous cycle (Table 2). Similarly, others (Deputte and Goustard 1980, O'Connell and Cowlishaw 1994) reported longer call duration in females during and before copulation. Analogous findings were also reported in African elephant (Leong *et al.* 2003), Korean native cows (Yeon *et al.* 2006) and heifers (Scheon *et al.* 2007).

Formants (resonance frequency): The resonance frequency is defined as the frequency at which the power reaches the local maximum value. Here values for the first 5 lowest resonant frequencies or formants (F1, F2, F3, F4

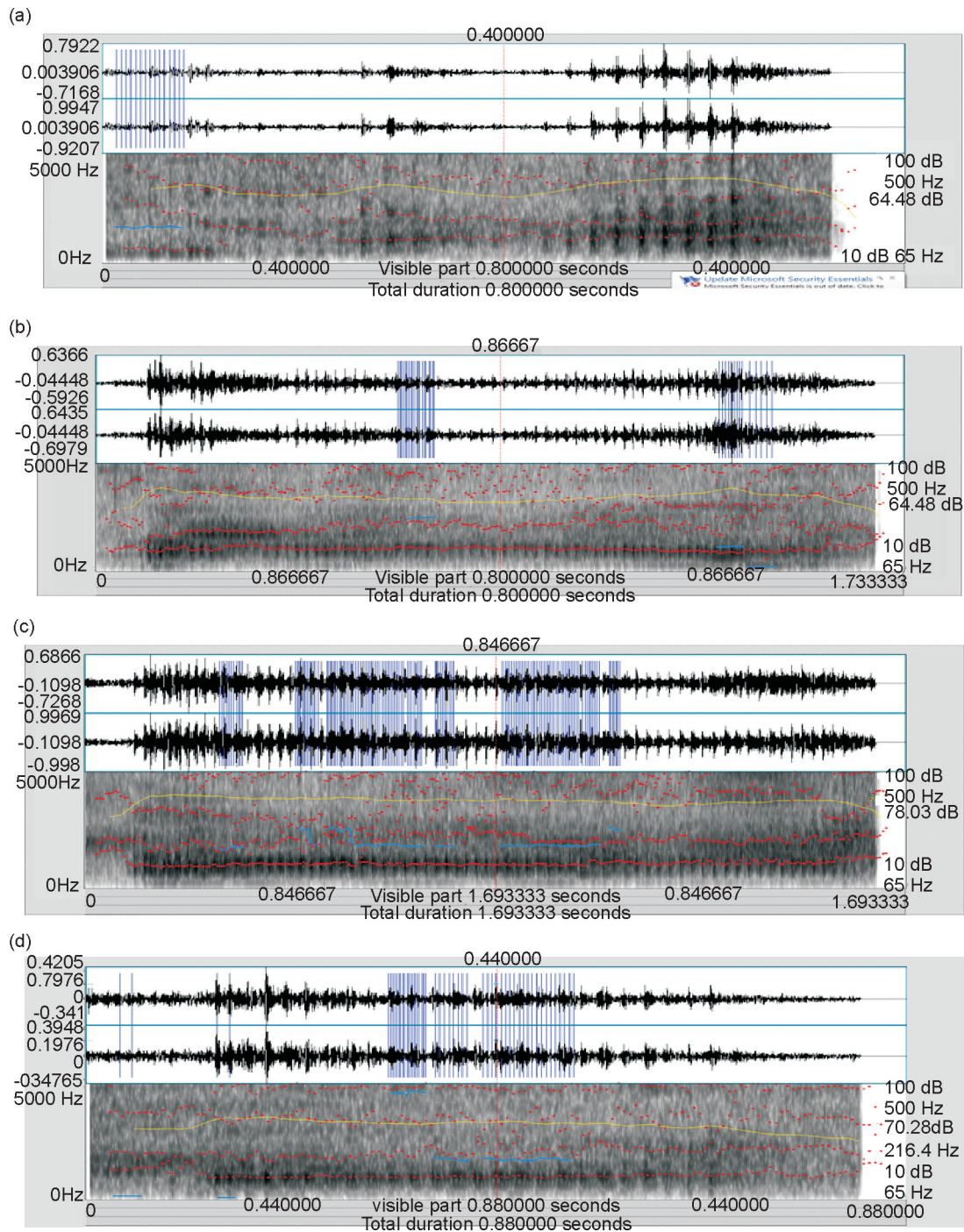


Fig. 1 (a-d). **a.** Waveform and oscillogram of a sound signal from Murrah buffalo during proestrus phase; **b.** estrus phase; **c.** metestrus phase; **d.** diestrus phase of estrous cycle showing various acoustic features, viz. pitch, intensity, formants, amplitudes, pulses etc.

and F5) were examined for vocal signals articulated by Murrah buffaloes during their different phases of estrous cycle.

The least square means of first or fundamental formant (F1) for vocal signals uttered by Murrah buffaloes during proestrus, estrus, metestrus, and diestrus phases of estrous cycle were 1078.26 ± 7.86 , 1126.457 ± 9.90 , 1082.237 ± 8.58 and 1048.387 ± 5.37 Hz, respectively, and was significantly ($P < 0.001$) higher in estrus phase as compared with other

phases of estrous cycle. But no significant ($P > 0.05$) difference for second and third formant (F2 and F3) could be observed in vocal signals of Murrah buffaloes uttered during different phases of estrous cycle (Table 1).

The least square means of fourth formant (F4) for vocal signals uttered by Murrah buffaloes during proestrus, estrus, metestrus, and diestrus phases of estrous cycle were 5084.81 ± 11.44 , 5017.01 ± 16.02 , 5077.88 ± 12.68 and 5009.50 ± 11.90 Hz, respectively (Table 1). In contrast to

F1 it was significantly ($P<0.001$) higher during proestrus phase as compared to other phases of estrous cycle.

The least square means of fifth formant (F5) were 6605.61 ± 9.29 , 6620.13 ± 10.68 , 6606.36 ± 9.82 and 6585.22 ± 7.62 Hz, respectively, for vocal signals produced during proestrus, estrus, metestrus, and diestrus phases of Murrah buffaloes (Table 1), which was in accordance to the results observed for fundamental resonance frequency of vocal signals produced by Murrah buffaloes during different phases of estrous cycle and was significantly ($P<0.001$) higher in estrus phase as compared to other phases.

Thus, the higher values of F1 and F5 during estrus phase of estrous cycle might be associated with higher value of estrogen and lowest value of progesterone hormone during estrus phase. The higher values were observed for all formants (F1, F2, F3, F4 and F5) during proestrus and estrus phase of estrous cycle which may be influence of emotional state and physiological arousal of these buffaloes (caller). As salivation may interfere with resonance characteristics of vocal tract, therefore, no uniform pattern for different formants during all the phases of estrous cycle could be observed. It indicated that a significant pattern may be observed if the sample size of vocal signals from these buffaloes was much larger than used in present investigation.

Intensity (level of energy in vocalization): Intensity refers to the level of energy in vocalization. The least squares mean of intensity in the vocal signals of Murrah buffaloes during proestrus, estrus, metestrus and diestrus phases were 107.312 ± 0.29 , 79.092 ± 0.15 , 75.335 ± 0.13 and 69.431 ± 0.17 dB, respectively (Table 1). The statistical analysis revealed that intensity was significantly ($P<0.05$) higher in proestrus than other phases of estrous cycle in Murrah buffaloes. Similar results for intensity of voice signals in women were also reported by Abitbol *et al.* (1999). They also suggested a modulation in acoustic features of voice in women during different phases of menstruation cycle.

Pitch: The least squares mean of pitch for the vocal signals of Murrah buffaloes during proestrus, estrus, metestrus and diestrus phases were 129.559 ± 1.80 , 135.707 ± 2.30 , 102.411 ± 4.28 and 107.462 ± 2.01 Hz, respectively (Table 1). The pitch of voice signals from Murrah buffaloes during estrus phase was significantly ($P<0.05$) higher than other phases of estrous cycle. Bryant and Haselton (2009) reported similar findings. Moreover, Pfeifferle *et al.* (2011) reported the lowest pitch of voice signals produced during diestrus phase of menstrual cycle in women.

Noise to harmonic ratio: The least squares mean of noise to harmonic ratio for the vocal signals from Murrah buffaloes during proestrus, estrus, metestrus and diestrus phases were 0.548 ± 0.01 ; 0.572 ± 0.01 , 0.448 ± 0.01 and 0.434 ± 0.01 %, respectively (Table 1). The statistical analysis revealed a significantly ($P<0.05$) higher noise to harmonic ratio for voice signals during estrus phase than other phases of estrous cycle of Murrah buffaloes, which

Table 1. Mean values of acoustic features during different phases of estrous cycle in Murrah buffaloes

Acoustic features	Phases of estrous cycle			
	Proestrus	Estrus	Metestrus	Diestrus
Call duration (Sec)	2.25 ^A ± 0.03	2.21 ^A ± 0.05	1.68 ^B ± 0.02	1.61 ^C ± 0.02
F1 (Hz)	1078.63 ^B ± 7.86	1126.45 ^A ± 9.90	1082.23 ^B ± 8.58	1048.38 ^C ± 5.37
F2 (Hz)	2172.86 ± 13.85	2186.45 ± 13.40	2192.77 ± 12.82	2171.74 ± 11.06
F3 (Hz)	3579.43 ± 13.19	3541.30 ± 13.97	3544.00 ± 12.68	3560.52 ± 11.55
F4 (Hz)	5084.81 ^A ± 11.44	5017.01 ^B ± 16.02	5077.88 ^A ± 14.18	5009.50 ^B ± 11.90
F5 (Hz)	6605.61 ^{AB} ± 9.29	6620.13 ^A ± 10.68	6606.36 ^{AB} ± 9.82	6585.22 ^B ± 7.62
Intensity (dB)	107.31 ^A ± 0.29	79.09 ^B ± 0.15	75.33 ^B ± 0.13	69.43 ^B ± 0.17
Pitch (Hz)	129.55 ^B ± 1.80*	135.70 ^A ± 2.30*	102.41 ^C ± 4.28*	107.46 ^C ± 2.01*
N/H ratio (%)	0.54 ^A ± 0.01	0.57 ^A ± 0.01	0.44 ^B ± 0.01	0.43 ^B ± 0.01
H/N ratio (dB)	4.66 ^B ± 0.20	4.52 ^B ± 0.20	5.89 ^A ± 0.21	5.99 ^A ± 0.16
No of periods	109.19 ^C ± 2.79	103.12 ^C ± 3.07	135.77 ^B ± 3.79	154.33 ^A ± 2.73
No of pulses	112.99 ^C ± 2.74	98.65 ^D ± 3.26	136.16 ^B ± 6.12	150.37 ^A ± 3.26
Jitter (%)	5.12 ± 1.33	4.41 ± 0.18	4.06 ± 1.12	2.77 ± 0.10
Shimmer (%)	20.22 ^A ± 0.33	20.12 ^A ± 0.54	13.40 ^B ± 0.34	13.07 ^B ± 0.24
Unvoiced frame (%)	33.02 ^A ± 0.97	35.40 ^A ± 1.27	26.99 ^B ± 1.15	27.63 ^B ± 0.79

Values bearing different superscripts in a row differ significantly ($P<0.001$), * ($P<0.05$).

might be associated with the lowest blood progesterone level of Murrah buffaloes during estrus phase of estrous cycle. It may also be the reason for voice becoming noisy and non-harmonic during estrus than other phases of cycle. Similarly, Charlton *et al.* (2009) also reported the highest noise to harmonic ratio during estrus phase.

Harmonic to noise ratio: It is the ratio of amplitude peaks of detectable harmonics to noise threshold. The least squares mean of harmonic to noise ratio for the vocal signals from Murrah buffaloes during proestrus, estrus, metestrus and diestrus phases were 4.665 ± 0.20 , 4.528 ± 0.20 , 5.896 ± 0.21 and 5.996 ± 0.16 dB, respectively (Table 1). The statistical analysis revealed significantly ($P<0.05$) lowest harmonic to noise ratio in voice signals from Murrah buffaloes during estrus phase of their estrous cycle. The present finding is supported by the findings of Charlton *et al.* (2009), while our another finding that the noise to harmonic ratio for voice signals during estrous phase of Murrah buffaloes was higher than other phases of estrous cycle.

Number of periods and pulses: The least squares mean

Table 2. Mean values of blood plasma hormones in different phases of estrous cycle in Murrah buffaloes

Hormones	Phases of estrous cycle			
	Proestrus	Estrus	Metestrus	Diestrus
Estrogen (pg/ml)	29.46 ^{A±} 0.65	19.35 ^{B±} 0.35	6.67 ^{C±} 0.19	5.43 ^{D±} 0.13
Progesterone (ng/ml)	1.01 ^{B±} 0.90	0.56 ^{C±} 0.20	1.24 ^{B±} 0.30	2.58 ^{A±} 0.12

Values bearing different superscripts in a row differ significantly ($P < 0.001$).

of number of periods and pulses for the vocal signals from Murrah buffaloes during proestrus, estrous, metestrus and diestrus phases were 109.190 ± 2.79 ; 103.129 ± 3.07 ; 135.778 ± 3.79 ; 154.333 ± 2.73 and 112.993 ± 2.74 ; 98.651 ± 3.26 ; 136.161 ± 6.12 ; 150.378 ± 3.26 , respectively (Table 1). The statistical analysis revealed that both numbers of periods and pulses for voice signals from Murrah buffaloes during estrus phase were significantly ($P < 0.001$) lower than other phases of estrous cycle. It may be associated with the lowest level of progesterone in blood during estrous phase. Moreover, the present finding was supported by the findings of Charlton *et al.* (2009) and the findings for noise to harmonic and harmonic to noise ratio in present investigation.

Jitter: Jitter represents cycle to cycle frequency variation of fundamental frequency. The least squares mean of jitter for the vocal signals from Murrah buffaloes during proestrus, estrus, metestrus and diestrus phases were 5.127 ± 1.33 , 4.416 ± 0.18 , 4.064 ± 1.12 and $2.775 \pm 0.10\%$, respectively (Table 1). The statistical analysis revealed that jitter did not vary significantly among the phases of estrous cycle in buffaloes.

Shimmer: Shimmer represents cycle to cycle amplitude variation of fundamental frequency. The least squares mean of shimmer for the vocal signals from Murrah buffaloes during proestrus, estrus, metestrus and diestrus phases were 20.228 ± 0.33 , 20.122 ± 0.54 , 13.402 ± 0.34 and $13.071 \pm 0.24\%$, respectively (Table 1). The statistical analysis revealed that shimmer for the vocal signals from Murrah buffalo was significantly ($P < 0.001$) higher in proestrus phase than other phases of estrous cycle.

Unvoiced frame: Unvoiced frame indicated how harsh or soft a sound signal is. The least squares mean of unvoiced frame for the vocal signals from Murrah buffaloes during proestrus, estrus, metestrus and diestrus phases were 33.023 ± 0.97 , 35.402482 ± 1.27 , 26.990 ± 1.15 and $27.632 \pm 0.79\%$, respectively (Table 1). The statistical analysis revealed that unvoiced frame was significantly ($P < 0.001$) higher during estrous phase of cycle in animals. This may be associated with higher progesterone level of blood during estrus phases of estrous cycle in Murrah buffaloes. Moreover, the present finding was supported by the findings of Charlton *et al.* (2009).

Estimation of hormones: The least squares means of

blood estradiol hormone in present investigation for Murrah buffaloes during their proestrus, estrus, metestrus and diestrus phases are given in Table 2. The level of estrogen hormone in blood was significantly ($P < 0.01$) higher during proestrus phase than other phases of estrous cycle in Murrah buffaloes. Similar findings were also reported in other studies (Shemesh *et al.* 1972, Dobson and Dean 1974, Rao and Pandey 1983, Glencross and Pope 1989).

The level of progesterone hormone (Table 2) in blood was significantly ($P < 0.01$) lower in estrus phase as compared to other phases of estrous cycle in Murrah buffaloes. Gustafsson *et al.* (1986) and Mondal and Prakash (2002b), also reported higher concentration of progesterone in blood during diestrus phase and lower during estrus phase of estrous cycle.

From our findings it can be stated that acoustic features may prove an important tool in differentiation and accurate identification of various phases of estrous cycle. Moreover, some of the acoustic features, viz. F1, F5, N/H ratio and unvoiced frame may be very useful in accurate identification of estrus condition even in buffaloes. Thus, the present investigation may be very helpful in proper and accurate identification of 'Silent heat' in buffaloes for their timed A.I.

ACKNOWLEDGEMENT

The research was supported by grants under Bioacoustics Tools for Monitoring of Dairy Animals Project, funded by Department of Biotechnology (DBT), Ministry of Science and Technology, New Delhi, Government of India.

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