



Association of Bio-acoustic Features of Vocal Signals with Age and Semen Quality in Sahiwal Bulls

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ABSTRACT

Background: Bio-acoustic features of animal's voice can provide meaningful information about their biological and physical characteristics. The present study was conducted to get indicators of age from voice analysis and explore the relationship between voice features and seminal parameters in Sahiwal bulls.

Methods: Voice samples were collected from healthy bulls (n=20), maintained at ICAR-NDRI, Karnal. Bulls were classified into two groups i.e. young bulls and adult bulls. The voice signals were analyzed by Adobe Premium software and acoustic features were extracted by using PRAAT software.

Result: The mean of acoustic features viz. call duration (sec), mean intensity (dB), total energy (P²S), amplitude (P), pitch (Hz), unvoiced frame (%), jitter (%), bandwidth (Hz) mean N/H ratio (%) have been found significantly different while mean H/N ratio (dB), shimmer (%) and pulses were not found statistically (P>0.05) different between adult and young bulls. The seminal parameters viz. mass activity (0-5 scale), individual progressive motility (%), live sperm count (%) and total sperm abnormality (%) were found significantly different between adult and young bulls. No significant association between voice features and semen quality of bulls was observed. Hence, voice signals of male might provide some clues about their age but for semen quality, there is further need to explore the interesting relationship between voice features and fertility of breeding bulls.

Key words: Age, Auditory signals, Bio-acoustic features, Sahiwal bull.

INTRODUCTION

There are various methods available to determine age, semen quality parameters and consequently fertility of bulls, the monitoring and analysis of vocalization bio-acoustic features might be one interesting approach which is non-invasive, animal friendly, less laborious and also suitable to large commercial dairy farms. This approach has been well studied in non-human primates (Ey *et al.*, 2007), wild animals (Reby and McComb, 2003) and also in humans (Simmons *et al.*, 2011). The source filter theory has helped the researchers to understand the probable hypothesis of biological acoustic variation due to anatomical or physiological condition of the caller (Taylor and Reby, 2010). Voice signals of animals can convey useful information about age, body size and sex of caller (Briefer and McElligott, 2011), masculinity of the sender (Feinberg *et al.*, 2005), alarm calls and fighting ability (Fichtel and Kappeler, 2002), stamina and fitness (Erb *et al.*, 2013), reproductive/mating success of male animal (Saxton *et al.*, 2006) by producing context specific variation in acoustic features; which might be due to developmental changes in vocal tract anatomy. For instance, voice signals facilitated specific cues about age, sex and body size in baboons (Pfefferle and Fischer, 2006); humans (Rendall *et al.*, 2005); Chacma baboon (Ey *et al.*, 2007a); goats (Briefer and McElligott, 2011) and Simakobu (Erb *et al.*, 2013). As the animal's age increases, there is increase in size of lung's capacity, larynx, vocal folds and vocal tract due to maturity effect (Fitch and Hauser, 2002) and sex hormonal effects (Charlton *et al.*, 2011). The

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higher levels of testosterone in adult bulls (Foote *et al.*, 2013) may cause increased laryngeal descent during puberty (Fitch and Giedd, 1999) and also contribute to increased male dominance or mating success at maturity. The acoustic features like call duration, calling rate, formant and fundamental frequency have been suggested as reliable proximate indicators of age and overall body size of male animal (Ey *et al.*, 2007a; Wyman *et al.*, 2012).

Male animals produce unique calls in response to strangers, predators and aggression due to social mate or

competitors (Fischer *et al.*, 2002; Soltis *et al.*, 2005). The rate of calling is possibly indicator of class (Hinch *et al.*, 1982) and breed of animals like Sahiwal bulls vocalize more often in answer to the bellow of other mate bulls or a stranger person in the proximity. Vocal behaviour of indigenous dairy bulls is poorly understood, yet it might reflect their social and affective states. Therefore, keeping in view the above discussed hypothesis, this study was conducted to study the relationship between voice characteristics and bull's age and semen quality parameters in Sahiwal bulls.

MATERIALS AND METHODS

Animal selection and management

The present study was conducted at ICAR-National Dairy Research Institute, Karnal during 2018-2019. Healthy Sahiwal bulls ($n=20$) were selected and categorized into two groups *i.e.* 10 young bulls (1.5 to 3.0 years) and 10 adult bull (3.1-8.8 years). Scrotal circumference of bulls was taken at the starting of this experiment with help of a flexible cloth tape (Pant *et al.*, 2003). The bulls were maintained loose in concrete floored individual pens of 30×10 feet. with corrugated asbestos roofed shed. Feeding of green fodder and concentrate to bulls was followed as per NRC standards. Water was available *ad-libitum* throughout the day. Bulls were taken for exercise twice a week (one day before semen collection) in the morning from 5.00-6.00 AM in the rotary exerciser so as to maintain the sexual vigour of bulls and ensuring quality semen production.

Collection and processing of samples

The voice signals of bulls were recorded in their individual sheds for sufficient period of time to get at least 30 voice clips from each bull/day between 5 to 7 AM. by using a video camera (Sony HDV FX7E, handiCam). The voice of each bull was recorded twice (two days) a week for three months and total 24 days recording was done for each animal. The recorded voice signals were then transferred to computer for further editing and processing. Precautions were made so that the animals did not get disturbed by these measuring devices and environment during the experiment. All the residual sounds and noise signals were detected and eliminated manually from subsequent analysis by using Adobe Premium Pro-1.5 audio-visual editing software package at a sampling frequency of 48 KHz and 16 bits. Extraction of acoustic features was done to get best set of features which were characteristic of each individual animal and for this Mel-Frequency Cepstrum Coefficients technique (Davis and Mermelstein, 1980) was used to extract information from voice signals waveform. Finally, following acoustic features were extracted from voice samples with the help of PRAAT 5.1.36 software package developed by Boersma and Weenink (2010): Call duration (sec), Mean Intensity (dB); Total energy (P²S); Amplitude (P), Pitch (Hz); Mean Noise/Harmonic ratio (%); Mean Harmonic/Noise ratio (dB); Unvoiced frame (%); Jitter (%); Shimmer (%); Bandwidth (Hz) and Pulses.

Semen collection and evaluation

The semen was collected from bulls (out of total 20 bulls, 8 adults and 6 young bulls donated semen) at 6.00 to 7.30 AM twice a week from adult bulls and once a week from young training bulls for four weeks continuously. More than ten ejaculates from each bull were collected for evaluation of semen quality parameters. After collection, each ejaculate was placed in water bath at 35°C and following standard semen parameters *viz.* ejaculate volume (ml), mass activity (0-5 scale), individual progressive motility (%), sperm concentration (million/ml), eosinophilic (dead) and non-eosinophilic (live) and total spermatozoa morphological abnormality (%) were evaluated. To assess mass activity, Gross Swirl Rating of undiluted semen was done within one min of collection and scored on a scale of 0-5 (Tomar *et al.*, 1966). The progressive motility (0-100 %) was scored under 200X phase contrast microscope. Sperm concentration (per ml) of semen ejaculate was calculated by direct sperm count using the improved Neubauer haemocytometer (Sorenson, 1979). Eosin-nigrosine staining method was used for differentiation of live and dead spermatozoa in fresh semen samples (Campbell, 1956).

Statistical analysis

The statistical analysis of data was carried out by using SAS 9.3 software package (SAS, 2011). The acoustic data was analyzed by using least squares technique and significance of difference between subclasses was done by using DMRT. In order to assess the effect of age on the voice features of particular individual bull, following least squares model was used:

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

Where,

Y_{ij} = Voice signals of j^{th} bull of i^{th} age group

μ = Overall mean

A_i = effect of i^{th} age group

e_{ij} = random error with mean 0 and constant variance σ^2

In order to assess the effect of age, on seminal parameters of particular individual bull, following least squares model was used:

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

Where,

Y_{ij} = Seminal parameters of j^{th} bull of i^{th} age group,

μ = Overall mean

A_i = effect of i^{th} age group

e_{ij} = random error with mean 0 and variance σ^2

The Pearson's correlation coefficients (r) were calculated to find correlation between acoustic features and seminal parameters. Bonferroni correction was also applied because of multiple comparisons for correlation.

RESULTS AND DISCUSSION

Acoustic features of adult and young bulls

Mean $\pm$ S.E of acoustic features of adult and young bull has been presented in Table 1. The acoustic features *viz.* call

duration (sec), mean intensity (dB), total energy (P^2S), amplitude (P), mean N/H ratio (%) have been found significantly higher in adult bulls while pitch (Hz), unvoiced frame (%), jitter (%) and bandwidth (Hz) were found significantly lower in adult bulls as compared to young bulls. The acoustic parameters like H/N ratio (dB), shimmer (%) and pulses were not found to be statistically different in adult and young bulls. The call duration was found significantly longer and louder in adult, large sized dominant bulls in comparison to young bulls and this finding was found in agreement with studies conducted in Chacma baboons (Ey *et al.*, 2007a), guerezas, (Blank *et al.*, 2011) and goat (Briefer and McElligott, 2011). Because as the age increases, the anatomy of voice producing vocal apparatus changed like adult and larger animals have large sized lungs and thicker air folds which would affect voice signal's features (Fitch and Hauser, 2002). Further, the mean intensity and total energy of adult bull's voice was found significantly higher than young bulls respectively because adult produce sound with more aggression and energy due to more testosterone level as intensity represents energy in vocalization or strength of vocal fold vibration (Colton and Casper, 1996). Amplitude is measurement of vocal intensity and correlate with caller's loudness (Colton and Casper, 1996) and also measured as sound pressure level (Wyman *et al.*, 2008). It can be used as a good indicator of competitive ability, dominance rank of male animal (Sanvito and Galimberti, 2003) because increased amplitude indicates increased vocal energy expenditure by males (Russell *et al.*, 1998). It was recorded that adult (dominant) bulls having larger body size produced voice with more amplitude and Sanvito and Galimberti (2003) in elephant seal; Wyman *et al.* (2008) in bison also reported similar findings. Pitch is considered as equivalent to fundamental or base frequency of voice (Hauser, 1993) and pitch of adult bulls was found significantly lower than young bulls and Hauser (1993) reported similar finding in non-human primates. It clearly indicated that voice signals of animal definitely change with increasing age in response to anatomical and hormonal change taking place

in vocal folds during growing stage, which results in lengthening and thickening of vocal folds (Fitch and Hauser, 2002). Moreover, adult male animals have larger body size and probably larger vocal tract (Reby *et al.*, 1999) and body weight is negatively correlated with pitch (Hauser, 1993). Mean N/H ratio represents ratio of detectable noisy, asymmetric structures to symmetric structures and was found significantly higher in adult bulls in comparison to young bulls. Mean H/N ratio (ratio of periodic to non-periodic structures) (Murphy and Akande, 2005) was found higher in young bull's but not statistically significant. The present study was found in agreement with Morton (1977) 'motivation-structural rules' which stated that voice frequency of an animal is negatively correlated with body size and adult, dominant animals produce noisy harsh voice with more energy bands and fearful or submissive animal produce more tonal voice. Unvoiced frame (indicator of harshness or softness in voice signal) and Jitter (cycle to cycle frequency variation of sound wave) (Zwetsch *et al.*, 2006) were found significantly higher in adults, which indicated much developed vocal cords receiving more tension resulting from strong vibrations of vocal folds. The higher levels of testosterone in adult bulls may be responsible for the differences in voice features. Bandwidth represents measure of frequency domain damping and was found significantly higher in young bulls means voice of young bulls was more heavily damped and hence vibrations of larynx died away quickly but in adults it continued for some time after the pulse has passed. Shimmer representing amplitude variation of sound wave (Zwetsch *et al.*, 2006) and pulse (short transient signal which includes a complete waveform of definite shape which is repeated at regular interval of time during vibration of vocal folds) were not found statistically different means these parameters does not much affected by maturity of vocal tracts.

Seminal Parameters of adult and young bulls

The mean $\pm$ S.E of seminal parameters has been presented in Table 2. The seminal parameters *viz* mass activity,

Table 1: Least square means ($\pm$ S.E) of acoustic features of adults and Young Sahiwal bulls.

Variables	Adult bulls	Young bulls	P-value
Call duration (sec)	0.89 ^a $\pm$ 0.37	0.45 ^b $\pm$ 0.28	0.001
Mean intensity (dB)	80.34 ^a $\pm$ 3.14	67.31 ^b $\pm$ 2.84	0.001
Total energy (P^2S)	0.44 ^a $\pm$ 0.56	0.35 ^b $\pm$ 0.34	0.009
Amplitude (P)	0.081 ^a $\pm$ 0.04	0.062 ^b $\pm$ 0.09	0.040
Pitch (Hz)	109.12 ^a $\pm$ 15.34	175.30 ^b $\pm$ 21.31	0.021
Mean N/H ratio (%)	28.47 ^a $\pm$ 0.84	33.10 ^b $\pm$ 2.12	0.017
Mean H/N ratio (dB)	12.14 $\pm$ 0.92	14.61 $\pm$ 0.98	0.074
Unvoiced frame (%)	5.75 ^a $\pm$ 0.63	8.34 ^b $\pm$ 0.42	0.001
Jitter (%)	5.50 ^a $\pm$ 0.47	3.76 ^b $\pm$ 0.52	0.002
Bandwidth (Hz)	282.04 ^b $\pm$ 50.90	440.45 ^a $\pm$ 39.33	0.001
Shimmer (%)	10.65 $\pm$ 0.69	12.54 $\pm$ 1.08	0.165
Pulses	321.36 $\pm$ 26.19	287.70 $\pm$ 38.96	0.190

N.B- Values bearing different superscripts (a, b) differ significantly across rows ($p < 0.05$).

Table 2: Least square means ($\pm$ S.E) of seminal and sexual behaviour parameters of adult and young Sahiwal bulls.

Variables	Adult bull	Young bull	P-value
Ejaculate volume (ml)	4.22 $\pm$ 0.38	3.91 $\pm$ 0.23	0.185
Mass activity (0-5 scale)	3.25 ^a $\pm$ 0.09	2.72 ^b $\pm$ 0.13	0.012
Individual motility (%)	75.70 ^a $\pm$ 1.28	65.19 ^b $\pm$ 0.89	0.001
Live sperm count (%)	74.75 ^a $\pm$ 0.37	67.93 ^b $\pm$ 0.31	0.001
Total sperm abnormality (%)	11.95 ^a $\pm$ 0.59	17.41 ^b $\pm$ 0.63	0.002
Sperm concentration (million/ml)	1132.37 $\pm$ 122.20	994.31 $\pm$ 31.54	0.070

N.B- Values bearing different superscripts (a, b) differ significantly across rows ($p < 0.05$).

individual progressive motility (%), live sperm count (%) and total sperm abnormality (%) were found significantly different between adult and young bulls but Seminal volume and sperm concentration (million/ml) didn't differ statistically between adult and young bull. The ejaculate volume (ml) was found higher in adult bulls but not statically and our finding was found in agreement with Ghosh (2004); but the studies of Bhakat *et al.* (2011); Argiris *et al.* (2018) found significant effect of age on ejaculate volume and breeding bulls produce maximum semen volume up to about bull maturity (5-7 years) age, afterwards, it started to decreased due to age related senile changes in bulls (Bhakat *et al.*, 2011). Moreover, ejaculate volume is probably affected by body size and weight, age, breed, health, method and frequency of collection, nutrition, season and management (Nazir, 1988; Soderquist *et al.*, 1992) and scrotal size (Ahmad *et al.*, 2011). The sperm concentration (million/ml) was found higher in adult bulls but not significant, similar results were obtained by Bhakat *et al.* (2011), but Ahmad *et al.* (2011); Argiris *et al.* (2018) reported that maximum concentration was found up to 3-5 years of age in bulls since it also affected by the maturity, sexual development, reproductive soundness of bull and accuracy in calculation of spermatozoa concentration is also crucial as it is a highly variable trait. The mass activity (0-5) and individual progressive motility (%) found significantly different between adult and young bulls and Brito *et al.* (2002) and Bhakat *et al.* (2011) confirmed that increasing age had positive effect on sperm motility especially up to 4 years of age. Live sperm count (%) and total sperm abnormality (%) of semen were found significantly different between adult and young bulls, these results were in accordance with Mandal *et al.* (2010). The live percentage of spermatozoa was affected by age of bull and frequency of semen collection (Nath *et al.*, 1991).

Correlation between acoustic features and seminal parameters of bulls

After Bonferroni correction, Pearson's correlation coefficients between acoustic features and semen quality parameters were found non-significant for all parameters. Not much literature is available regarding association between voice parameters and fertility associated seminal parameters of male animals. In humans, negative relation was reported between voice attractiveness based on low pitched quality (Apicella *et al.*, 2007) and sperm concentration (Bonde *et al.*, 1998). The present study provided the clue that adult

dominant bulls necessarily might not be high fertile although it can attract more females. In adult (dominant) bulls voice, lower pitch and high intensity was found which could be due to testosterone levels difference as reported in men (Dabbs and Mallinger, 1999).

CONCLUSION

Although this preliminary study indicated that analysis of voice features might provide some clues about age of bulls but association of voice with semen quality parameters of bulls did not provide any significant information. Therefore, we did not get any conclusive remark about relationship of bull's voice with its semen quality parameters.

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