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Faecal chemical cues in water buffalo that facilitate estrus detection

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ABSTRACT

Chemo-signals are among the reliable non-invasive methods for estrus detection in mammals. Water buffalo is a silent heat animal and, hence, there is search for chemo-signals which would be effective non-invasive indicators of estrus state. We analyzed the faecal chemical cues during the estrous cycle in buffalo and to find the estrus-specific faecal volatile compounds adopting bull behavior assay. The faecal samples were collected at three phases of the estrous cycle (i.e., proestrus, estrus and postestrus) and subjected to gas chromatography–mass spectrometry analyses. We found 27 volatile compounds in the faeces of buffaloes, of which 4-methyl phenol (4mp) and trans-verbenol (tv) were found only in estrus faeces. The faecal samples of estrus buffaloes and the estrus-specific compound(s) (4mp + tv) at three different concentrations were tested for behavioral responses (flehmen and mounting behavior) in the bull. The bulls exhibited repeated flehmen when exposed to a combination of the two compounds (i.e., 4mp + tv) as compared to the individual compounds or raw faecal sample collected from buffalo when in estrus ($P < 0.05$). However, higher number of mounting behavior was recorded when bulls were exposed to 4mp followed by a combination of the two compounds (4mp + tv) and trans-verbenol ($P < 0.05$), in that order. By contrast, less number of mounting behavior was exhibited by bulls when exposed to the control sample (i.e., Hexadecanoic acid) ($P < 0.05$). As inferred from the bull behavior assay, the present study suggests that the two compounds, 4 methyl phenol and trans-verbenol would be reliable indicators of estrus in buffaloes.

1. Introduction

Estrus detection is a prerequisite for efficient reproductive management in farm animals (Drost, 2007). Further, artificial insemination performed in farm animals is successful only when it is done exactly during the estrus phase. Very specially, in buffalo the detection of estrus is problematic since it is a silent-heat animal in which there is no outwardly manifested clear signs of estrus (Suthar and Dhami, 2010). Realizing this problem, there have been several attempts to device methods to overcome the difficulties of estrus detection in buffaloes, but most of the methods developed till date have not yet been successful and reliable. There is, thus, pertinent need to develop an efficient estrus detection method in buffaloes in order to enhance the success rate of conception through artificial insemination.

In ungulates, volatile cues are one of the primitive communication channels (Booth and Signoret, 1992). Most often, the volatile cues are released in urine, faeces, vaginal

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fluid etc. (Epple, 1986). Among the various sources of volatile cues, faeces is noteworthy in intra-specific chemical communication in many vertebrates (Archunan, 2009). An earlier evidence provided by Kimura (2001) suggested that faeces carry cues about the reproductive status of animals, especially in females of different species. Developing on this suggestion adopting bull behavior assay we also showed that faecal constituents would provide for estrus detection in bovine (Sankar and Archunan, 2008). Further, the analysis of chemo-signals offers a promising approach to assess the reproductive status of the animal non-invasively. This has been adequately proved in bear that analysis of urinary volatiles could be one of the methods to assess the reproductive status non-invasively (Dehnhard et al., 2006).

To date, analysis of volatiles of faeces from buffaloes (*Bubalus bubalis*) belonging to different phases of estrous cycle has not been attempted, and the possibility of making use of the chemical cues in faeces in estrus detection remains unexplored. Hence, keeping in view the difficulty in estrus detection and the possibility of making use of chemical cues in faeces as reliable estrus indicator, the present study was aimed at identification of specific volatile compounds in faeces and confirmation of the estrus-specific compounds adopting bull behavior assay so as to develop estrus indicators using faeces.

2. Materials and methods

2.1. Test animals

Six sexually mature female buffaloes (*Bubalus bubalis*, Murrah breed) were used for sample collection. For behavior assay three bulls were used against the six female dummy buffaloes. The animals were maintained at exotic cattle-breeding centre, Orathanadu Livestock Farm, Tanjore District, Tamil Nadu, India, and fed with conventional diet (cultivated forage crops, supplemented with a little green fodder) and water *ad libitum*.

2.2. Determination of estrous cycle and sample collection

The phases of the estrous cycle were determined with the help of the conventional estrus behaviors in female buffaloes such as vaginal swelling and secretions, fern pattern, restlessness, frequent urination and tail wagging, and the response of the male such as exhibition of flehmen, mounting etc. (Rajanarayanan and Archunan, 2004). The trans-rectal palpation of the uterus and fern pattern also were checked for the confirmation of estrus phase. The total length of the estrous cycle was 21 days. The day on which the above behaviors were observed was considered as estrus. Two to five days prior to this day was considered as proestrus, while 2 days after estrus was taken as postestrus, and faecal samples were collected accordingly.

2.3. Sample analysis

The faecal samples (collected in two consecutive cycles from six female buffaloes) were extracted with dichloromethane (1:1) for the fractionation of volatile compounds. The extracts were filtered through cheese cloth or nylon mesh (60–120 μm) (Sankar and Archunan, 2008). Two microlitres of the extract was injected into the GC–MS system (QP-5050, Schimadzu, Japan) on a 30 m glass capillary column with a film thickness of 0.25 μm (30 mm × 0.2 i.d. coated with UCON HB 2000). The temperature regimen was as follows: initial oven temperature of 40 °C for 4 min, increasing up to 250 °C at 15 °C/min, and then held at 250 °C for 10 min. The detection accuracy of the substance was 1 ng/peak. Mass spectrometer was operated in CI mode at 70 eV, using ammonia as reagent gas. The identified compounds were matched with the library of chemical substance (NIST 6221B).

2.4. Behavioral analysis

The experimental setup consists of 3 bulls with six female dummy buffaloes. Raw faeces sample at three different concentrations (0.5%, 1.0%, 2.0%) was choked in cotton and applied on the perineal region of external genitalia of the dummy (non-estrus) buffalo (Rajanarayanan and Archunan, 2004). The bulls were allowed to sniff it for about 15 min. The flehmen and mounting behaviors in response to faeces sample were recorded.

Based on the findings from GC–MS analysis, and literature survey, two estrus-specific compounds viz., 4-methyl phenol and trans-verbenol (Sigma–Aldrich, St. Louis, MO, USA), were chosen for bull behavior assay. The methodology for preparation of samples and the assay as in previous experiment was adopted. The two compounds were tested individually and, also, in combination. The non-estrus compound, hexadecanoic acid, was used as the positive control.

2.5. Statistical analysis

Data were compiled using SPSS statistical software (Version 17, SPSS Inc., Chicago, IL, USA) and subjected to analysis of variance (ANOVA) with post hoc comparison (one-way) using Duncan's Multiple Range Test (DMRT).

3. Results

3.1. Identification of volatile compounds

GC-MS analysis revealed a total of 27 compounds, all the three phases put together (Table 1). Among these compounds 18 were present during proestrus, 21 during estrus, and 11 during postestrus. Two compounds viz., 4-methyl phenol (4mp) and trans-verbenol (tv) were present only during the estrus phase. Three compounds, 2, 4-bis cyclohexanal, 8-methyl-1-decane and 2-ethyl-1-decanol, were present during all three phases.

3.2. Behavioral assay

The flehmen and mounting behaviors exhibited by bulls towards the dummy buffalo applied with raw faecal samples differed significantly between concentrations of the faeces ($P < 0.05$). The higher flehmen was observed at 1.0% concentration whereas 0.5% was sufficient in eliciting mounting behavior ($P < 0.05$) (Table 2).