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Biochemical and Fatty Acid Analysis of Faeces in Umblachery Cattle (*Bos Indicus*) During Different Phases of Estrous Cycle

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Abstract

Mammals release different variety of molecules into the environment, either as specific chemosignals or metabolic byproducts through vaginal fluid, urine, faeces, saliva, milk, sweat and specialized scent glands. The bovine olfactory system has the ability to recognize and discriminate among thousands of structurally diverse odorants, which have access to the behavioural and physiological outputs. This chemical cues are used to pass the signals between individuals conveying information including sex, reproductive status, individual identity, ownership, competitive ability and health status. The complex mixtures of molecules sensed by bovine chemosensory systems and have important influences of behaviours such as mother-young interaction, mate choice and territorial marking. In the present study, it is planned to identify and estimate the biochemicals and fatty acids present in the faecal samples of Umblachery breed cow (Bos indicus) during different phases of estrous cycle. The biochemical estimation showed that protein, carbohydrate and lipid were higher in oestrus phase when compared to all other phases. Further, GC analysis of the faecal samples showed that 20 different fatty acids are identified in all the phases of estrous cycle. The fatty acids like valeric, caproic, myristic, gadoleic and pelargonic acids are found only in estrus not in other phases. Based on the above information, the present study suggests that the qualitative and quantitative presence of certain biochemicals and fatty acids would be reliable indicators of oestrus in bovine. The role of biochemical and fatty acids in animal behaviour must be confirmed by field study. The presence of biochemicals and specific fatty acids in estrus faeces makes the possibility to develop a biomarker for the detection of estrus in Umblachery cattle very accurately.

Keyword: Bovine, faeces, chemical cues, biochemical, fatty acids, estrous cycle.

Introduction

Cattle evolved in the Indian sub continent and only spread to other parts of Asia, Northern Africa and Europe after the great ice age, about 2, 50,000 years ago. Two distinct subtypes are distinguishable, the humped *Bos primigenius namadicus*, the forebear of today's zebu cattle, and *Bos primigenius primigenius*, which had no hump and gave rise to modern European cattle. These wild cattle or aurochs were large with big horns and powerful forequarters compared to domesticate cattle. The domesticated cattle were developed from wild cattle (*Bos primigenius*) in the Middle East, probably about 8000 – 10,000 years ago. The reason for domesticating the aurochs is not clear. Early domesticated cattle were undoubtedly used for the production of milk, meat and other purposes Phillips[1]. Milk production and reproductive performance are two major determinants of dairy cow profitability. Milk production has dramatically increased over the last several years, but the reproductive performance of umblachery breed cattle has declined worldwide Lucy[2], Royal[3] and Stevenson[4].

Based on the above information, the present work in planned to estimate various biochemical like protein, carbohydrate and lipid are the chemicals used to signalling in animal communication to improve reproductive success. The mixtures of volatile and non-volatile compounds expressed in faeces, urine, saliva, tears, sweat, breath, milk, amniotic fluid, genital secretions, and specialized glandular secretions, that underlie a complex mode of chemical signals for animal communication critical to mediating intraspecific interaction Albone[5], Brown Macdonald[6], Eisenberg and Kleiman[7], Muller Schwarze[8], Ralls[9]. Several reports regarding bio-communication including bovine faeces, vaginal fluid, urine, saliva and milk for their biological significance in relation to estrus detection. Nevertheless a simple method and inexpensive for improving detection of estrus through body fluids are still strongly needed Jezierski[10]. Among the various sources of volatile cues available in the bovine, faeces are noteworthy in intra-specific chemical communication in many vertebrates Archunan[11]. Earlier evidence provided by Kimura[12] suggested that faeces carry cues about the reproductive status of animals, especially in females of different species. Based on the above information, the present study is aimed to estimate the level of biochemicals like protein, carbohydrate, lipid and fatty acids during various estrous phases (proestrus, estrus, postestrus) of bovine. The present study suggests that the qualitative and quantitative presence of biochemicals and fatty acids would be reliable indicators of oestrus in bovine. The presence of biochemicals and specific fatty acids in estrus faeces makes

the possibility to develop a biomarker for the detection of estrus in Umblachery cattle very accurately.

Material and Methods

Experimental animals: Six sexually matured female bovine (*Bos indicus*, Umblachery breed) were selected for the present study. The animals were maintained at District Livestock Farm, Korukkai, Thiruthuraipoondi, Thiruvarur District, Tamil Nadu, India, and fed with conventional diet (cultivated forage crops, rice straw, green fodder) and water *ad libidum.*

Estrous determination: The estrous cycle (proestrus, estrous, postestrus) were determined with the help of the conventional estrous behaviours in female cow such as vaginal swelling, frequent urination, restlessness, flehmen, male mount with female, female mount with male, female-female mounting, male sniffing with female vulva, and fern pattern (saliva and vaginal fluid) also were checked for the confirmation of oestrus phase. The length of estrous cycle was 18- 21 days.

Sample collection: The faecal samples were approximately 30g of faeces collected from bovine during different reproductive phases (proestrus, estrus and postestrus). The Samples were collected from the animals within five minutes immediately after excretion. The samples were maintained and stored at –20°C to –24°C for further analysis.

Biochemical estimation: The biochemical constituents such as protein Lowry[13], Carbohydrate Dubois[14] and lipids Folch[15] were estimated in the faecal samples.

Analysis of Fatty acid: The faecal lipid content was extracted by using chloroform and methanol (1:1) and lipid was estimated by Folch[15]. The extracted lipid was analysed for fatty acid using gas chromatography. Two gram of faecal sample was taken and mixed with saponification reagent in screw cap tubes along with the lipid extract. The tubes were tightly closed and kept for 30 min at 60° C in a water-bath, 2 m1 of methylation reagent was added to each tube and kept again in a water-bath at 80°C for 20 min. The tubes were brought down to the room temperature. Extracting solvent (1.25 ml) was added to each tube, closed tightly and shaked thoroughly for 10 min. About 2/3of the organic phase (upper layer) containing the fatty acid methyl esters were transferred into screw cap vials. From each vial 1µl of the fatty acid methyl ester (FAME) was injected into the gas chromatography (GC) column (DEGS) using column temperature of 180° C and injection post temperature was 200° C. Flame ionization detector (FID) was used as a detector at 230° C. Nitrogen was used as reagent gas Miller[16].

Statistical analysis: The results of the biochemicals and fatty acids in different stages are presented as mean value ± SE and were statistical analyzed with the one way ANOVA test, followed by the pos hoc Duncan's test. All the data analyses were significance was taken as $p < 0.05$ Zar[17].

Results and Discussion

The present work is aimed to evaluate the biochemicals present in bovine faeces which may be helpful for estrus detection and animal communication. The present results showed that the biochemicals analysed in bovine faeces of different estrus stages informed that protein, carbohydrate and lipid were found to be significantly higher in all stages of estrus phase when compared to other stages like proestrus and postestrus (table1). The level of carbohydrate is found high when compared to protein and lipid which is similar to Rameshkumar[18] reported that the carbohydrate level was found to be higher in prepubertal stages than other stages because the animal feed on mainly carbohydrates, it is natural to expect a higher amount of carbohydrates in the urine of young animals which need high amount of carbohydrates for their growth. Our study clearly indicates that the carbohydrate was found to be high in estrus stage because the feed intake of estrus animal is very low. The faecal mucus was increased during estrus period. The mucus contain rich amount of carbohydrate because the level of carbohydrates are increased in estrus animal faeces. Carbohydrates are one of the nutrition materials which cannot act as the signalling molecules. The present findings are in similar with Boyland[19] report that the cervical mucus of estrus cows contained mainly carbohydrates.

The present results showed that the maximum level of protein and lipid were in estrus phase when compared to other phases of estrous cycle. Since, some estrus specific compounds are released through excretion (urine, faeces, saliva, etc), the high level of protein may be required to function as carriers for the ligands and convey the chemical signals. It has been reported that excretion of major urinary protein in urine can acts as pheromone carrier, by Beynon and Hurst[20]. Lipid in the urine is not certainly a waste product and it is reported that in tiger the urinary lipids can be used as fixatives PoddarSarkar[21]. The lipids are fixing volatile molecules observed in *Tilapia beanery*. Earlier reports indicated that lipids play a crucial role in the sexual attraction in rat and vary considerably on basis of physiological status Kannan[22]. Rameshkumar and Archunan[23] revealed that there is a significant variation in the excretion of lipid content across various reproductive phases that lipids have got specific role in olfactory communication depending upon the physiological state of the individuals and responders. Lipid excreted in urine is certainly not wastage; rather it serves a useful purpose, probably to protect the volatile semiochemicals from quick dispersal by evaporation and washing out by rain Poddar Sarkar[21].

Table-1
Biochemical estimation of bovine faecal samples during estrous cycle

S. No	Stages	Protein (mg/g)	Carbohydrate (mg/g)	Lipid (mg/g)
1	Proestrus	23.65±1.43[b]	61.25±1.64[b]	40.77±1.70[b]
2	Estrus	30.22±1.87[a]	69.80±1.32[a]	47.73±2.09[a]
3	Postestrus	22.03±1.78[b]	53.38±1.64[c]	38.88±1.85[b]

Values are expressed in Mean ± SE, Dissimilar alphabets in vertical column are significantly different at P < 0.05% level

Table-2
Identification of faecal fatty acids in bovine (*Bos indicus*) during the different phases of estrous cycle

Peaks No.	Name of the fatty acids	Molecular formula	Molecular mass (g/mol)	Proestrus	Oestrus	Postestrus
1	Succinic acid	$C_4H_6O_4$	118.09	-	-	+
2	Benzoic acid	$C_7H_6O_2$	122.12	+	+	+
3	Phthalic acid	$C_8H_6O_4$	166.14	+	+	+
4	Acetic acid	$C_2H_4O_2$	60.05	+	-	+
5	Gallic acid	$C_7H_6O_5$	170.12	+	-	+
6	Traumatic acid	$C_{12}H_{20}O_4$	228.28	+	+	+
7	Malonic acid	$C_3H_4O_4$	104.06	-	-	+
8	Carboceric acid	$C_{27}H_{54}O_2$	410.72	-	-	+
9	Formic acid	CH_2O_2	46.03	+	+	+
10	Stearic acid	$C_{18}H_{36}O_2$	284.48	+	+	+
11	Palmitic acid	$C_{16}H_{32}O_2$	256.42	+	-	+
12	Oleic acid	$C_{18}H_{34}O_2$	282.46	+	-	+
13	Behenic acid	$C_{22}H_{44}O_2$	340.58	+	-	+
14	Valeric acid	$C_5H_{10}O_2$	102.13	-	+	-
15	Caproic acid	$C_6H_{12}O_2$	116.15	-	+	-
16	Myristic acid	$C_{14}H_{28}O_2$	228.36	-	+	-
17	Gadoleic acid	$C_{20}H_{38}O_2$	310.52	-	+	-
18	Pelargonic acid	$C_9H_{18}O_2$	158.24	-	+	-
19	Maleic acid	$C_4H_4O_4$	116.07	+	-	-
20	Arachidonic acid	$C_{20}H_{32}O_2$	304.47	+	-	-

Sankar and Archunan[24] reported twenty seven compounds with consistent variation among themselves throughout estrous cycle. This is in accordance with our earlier report that bovine has significant variation in volatile compounds in its faeces across estrous cycle. In the present study, 20 different fatty acids were identified in female cow (*Bos indicus*) faeces sample. Among these fatty acids like benzoic acid, phthalic acid, traumatic acid, formic acid and stearic acid were found throughout estrous cycle (table-2). But Alagendran[25] reported that the free fatty acids such as linoleic acid, palmitic acid, lauric acid, myristic acid and butyric acid were present in all the three phases of reproductive cycle in women. Also, Rajanarayan and Archunan[26] reported that the volatile compounds such as 2-octanone, 2-methyl-*N*-phenyl-2 propenamide, decanoic acid, *N,N*-bis (2 hydroxy ethyl) dodecanamide, tetradecanoic acid and hexadecanoic acid were commonly found throughout the cycle in female buffalo urine.

In buffalo the compounds, phenol, 3-propyl phenol, and 9-octadecenal present in pro-oestrus urine suggest that these three volatiles may be considered as the pre indicators for estrus Rajanarayan and Archunan[26]. Sankar and Archunan[27] reported that the compounds, butanoic acid, 2-propenyl ester, carboxylic acid and pentanoic acid were found in pre-oestrus faeces. However, in the pro-oestrus urine, palmitic acid was present in higher quantity than all other stages. The fatty acids are act as pheromones in tigers, becasue heptanoic and isohexanoic fatty acids are present in tiger marking fluid Brahmachary[28,29]. Likewise in the present study the fatty acids likes maleic and archidonic acids were only found in pre-oestrus faecal sample. These reports are consistent with our present findings and suggest that the maleic acid and archidonic acid were pre-indicators of estrus in bovine (*Bos indicus*).

Faecal tetradecanoic acid (mysteric acid) and hexadecanoic acid (palmitic acid) signals in mares are higher in estrus than non-estrus females. Identifications of the isolated mare faecal compounds may be benefit in equine reproductive management Kimura[12]. The pheromones are actually released by the dung of cow in estrus Pheromones are volatile fatty acids i.e., acetic acid, propionic acid and 1- iodoundecane Sankar and Archunan[24].The present study revealed that the fatty acids such as valeric acid, caproic acid, myristic acid, gadoleic acid and pelargonic acid were found only in estrus not in pro-estrus and

post-estrus samples. It is based on the principle of detection of sex pheromones that are secreted by the cows, exclusively during estrus. Sex-pheromones are associated with estrus Weigerinck[30].

Conclusion

The present study concluded that the estimation of biochemicals like protein, carbohydrate, lipid and fatty acids in the faecal samples of bovine (*Bos indicus*). The fatty acids valeric, caproic, myristic, gadoleic and pelargonic fatty acids were used individually or combined to act as the faecal pheromones. Finally, the observation of biochemical variation and fatty acids during estrus period may be act as potent source for odours stimulate for bull sexual activity in order to improve the reproductive status of animals.

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