

1-Iodoundecane, an Estrus Indicating Urinary Chemo signal in Bovine (*Bos Taurus*)

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Abstract

The time of ovulation and co-ordination of sexual behaviour is important for the successful artificial insemination. In our previous studies, urine samples were collected from the bovine at pre-pubertal, pro-estrus, estrus, post-estrus, pregnant and lactating animals and a number of compounds were identified by Gas chromatography-mass spectrometry (GC-MS); 1-iodoundecane and di-n-propyl phthalate present in estrus, the bioactivity of these compounds were not tested to confirm the efficacy of their estrus-specific compounds. So, the present study was planned to confirm the nature of these compounds through testing with bull's bioactivity. The bioassay was carried out in three different groups exposed to water (Control), estrus urine and estrus-specific synthetic compounds. The behavioural study revealed that the compound 1-iodoundecane identified in the estrus urine exhibits more attraction in bull by enhanced flehmen behaviour when compared to di-n-propyl phthalate. This clearly indicates that the 1-iodoundecane in the estrus female urine is an effective estrus-specific chemical signal which may be used as marker to detect estrus in bovine.

Keywords: Estrus; Bovine; 1-Iodoundecane; GC-MS; Urine; Chemo signal

Introduction

Mammalian pheromones are involved in much reproductive behaviour including sexual attraction [1], social behaviours [2], and individual identification [3]. Urine is one of the most primitive as well as major source of mammalian pheromones [4,5]. It is apparent that pheromones carry message between animals through air which must be reasonably volatile and chemically stable during diffusion [6]. Studies on mammalian chemo communication on the laboratory species like mice, rats, golden hamsters etc., are available [7, 8], but in bovine such studies are scanty and hence, the reproductive potential of cattle is not fully exploited.

In rabbit, as many as 21 volatiles are identified in the milk but the bioassay clearly showed that the only one volatile, i.e. 2-methylbut-2-enal, attracted the rabbit pups and confirmed this volatile as pheromone molecule responsible for young one to get milk from mother [9]. As for the bioassay of identified compounds concerned, there may be several tools to test the compounds. The 'Flehmen' behaviour is one of the best indicators which are exhibited by bull when the female is in estrus. For instance, the estrus-specific volatile compound is confirmed in elephant and bovine through the 'Flehmen' response [10-12].

One of the most important problems in the cattle is the lack of effective estrus detection in large herds of cows [13]. In most of mammalian species except primates the estrous females express the desire and inform the male of their receptive state by means of chemical signals from urine and vaginal discharge [14]. The chemical signals produced from female during specific period initiate male partner to exhibit a variety of copulatory behaviour which end up with coitus. For instance, under field conditions, bulls show various behavioural responses such as sniffing, licking and repeated flehmen response to chemical cues from the urine. In our earlier studies, GC-MS studies revealed that two-estrus specific compounds viz., 1-iodoundecane and di-n-propyl phthalate have been reported [15]. However, the identified compounds need to be confirmed for their pheromonal activity through

testing with bull and this could give more information in relation to olfactory communication of bovine urinary compounds. Therefore, the present work was carried out to evaluate the bioactivity of the synthetic compounds of estrus phase through the behavioural observations with bull.

Materials and Methods

The behavioural assay was performed on the freely behaving bulls under natural conditions. The assay was carried out in 500 m² pen where mature bulls were housed. Healthy bulls (*Bos taurus*) were selected for the study. The estrus-specific compounds viz., 1-iodoundecane and di-n-propyl phthalate were procured from Aldrich chemical company, USA. The bioassay was carried out in three different groups i) Control (water), ii) estrus urine iii) estrus-specific synthetic compounds. The samples were placed in a fresh petridish and the bulls were allowed to sniff the samples. The assay was conducted when there was no rain or strong wind in order to reduce the misbehavior of the animals. The behavioural activities of bulls were observed for a period of 15 minutes. Each sample was presented to the bull in a fresh petridish. Responses were scored as neutral (no response) and aversive (positive response). During the assay a sequence of bull behaviours (i.e. attraction, salivation, urination, tongue manipulation, flehmen, vocalization, penis protrusion, mounting and copulation) was assessed in response to estrus urine, synthetic compounds (1-iodoundecane and di-n-propyl phthalate) and the control samples.

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Samples	Proportion of male behaviours							
	Attraction	Sniffing	Salivation	Urination	Tongue Manipulation	Flehmen	Vocalization	Penis Protrusion
Control	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a
Estrus Urine	4.66±0.09 ^b	3.83±0.05 ^b	3.83±0.05 ^b	3.66±0.03 ^b	3.16±0.98 ^b	3.00±0.07 ^b	2.50±0.20 ^b	2.6±0.18 ^b
1-iodoundecane	6.00±0.00 ^c	5.84±0.06 ^c	5.50±0.02 ^c	5.10±0.20 ^c	5.33±0.08 ^c	5.1±0.20 ^c	4.16±0.28 ^c	4.16±0.20 ^c
di-n-propylphthalate	1.84±0.00 ^d	0.66±0.08 ^d	1.00±0.00 ^d	0.83±0.74 ^d	0.76±0.08 ^d	0.65±0.08 ^d	0.66±0.08 ^d	0.65±0.08 ^d

Table 1: Shows the various behavioral activities of bull towards the estrus specific compounds (n=6).

Attraction					
	Sum of squares	df	Mean squares	F	P
Between groups	128.5797101	3	42.85990338	110.9251208	.0000***
Within the group	1.333333333	19	0.0701754386		
Total	129.9130434	22			
Sniffing					
	Sum of squares	df	Mean squares	F	P
Between groups	132.350724	3	44.11690821	292.4027637	.0000***
Within the group	2.86666666	19	0.150877193		
Total	135.2173913	22			
Salivation					
	Sum of squares	df	Mean squares	F	P
Between groups	112.884058	3	37.62801932	3066.3995859	.0000***
Within the group	2.333333333	19	0.1228070176		
Total	115.2173913	22			
Urination					
	Sum of squares	df	Mean squares	F	P
Between groups	102.7724638	3	34.25748792	131.052135	.0000***
Within the group	4.966666667	19	0.2614034088		
Total	107.7391305	22			

Table 2: Analysis of variance (ANOVA) with post hoc comparison (one-way), Homogeneous subsets (Attraction), Homogeneous subsets (Sniffing), Homogeneous subsets (Salivation), Homogeneous subsets (Urination) in different stages of Bovine (*Bos taurus*) urine.

Tongue Manipulation					
	Sum of squares	df	Mean squares	F	P
Between groups	101.5	3	33.83333333	225.555555	.0000***
Within the group	3	19	0.15		
Total	104.5	22			
Flehmen					
	Sum of squares	df	Mean squares	F	P
Between groups	104.3362319	3	34.77874396	260.8405797	.0000***
Within the group	2.5333333	19	0.1333333334		
Total	106.8695652	22			
Vocalization					
	Sum of squares	df	Mean squares	F	P
Between groups	961.6666667	3	32.05555556	174.8484848	.0000***
Within the group	3.66666667	20	0.1833333333		
Total	990.8333333	23			
Penis Protrusion					
	Sum of squares	df	Mean squares	F	P
Between groups	64.45942029	3	21.48647343	121.2602956	.0000***
Within the group	3.66666667	19	0.1771929825		
Total	67.82608696	22			

Table 3: Analysis of variance (ANOVA) with post hoc comparison (one-way), Homogeneous subsets (Tongue Manipulation), Homogeneous subsets (Flehmen), Homogeneous subsets (Vocalization), Homogeneous subsets (Penis Protrusion) in different stages of Bovine (*Bos taurus*) urine.

Statistical Analyses

The data were compiled using SPSS version-10.0 (SPSS-X) statistical software and subjected to analysis of variance (ANOVA)

with post hoc comparison (one-way) using Duncan's Multiple Range Test (DMRT).

Results

The behavioural activities of bulls were observed for a period of 15 minutes and the responses were scored as neutral (no response) and aversive (positive response). During the assay the bull performed a sequence of behaviours like attraction, salivation, urination, tongue manipulation, flehmen and vocalization (Table 1) towards estrus urine, 1-iodoundecane (Figure 1) and to some extent with di-n-propyl phthalate. In continuation with the previous behaviours, the bull was also stimulated to show some other reproductive behaviour like penis protrusion (Table 1) towards cow. Totally, all the behavioural activities were observed and recorded towards estrus urine and estrus-specific synthetic compounds (i.e. 1-iodoundecane) but it was totally absent in control group.

The statistical analyses showed that the compound 1-iodoundecane was significantly activated the male behaviours when compared to estrus urine, di-n-propyl phthalate, and control. The present results showed that the male behaviours were higher towards 1-iodoundecane (estrus specific compound) when compared to other three samples, but at the same time estrus urine was also attracted the male and elucidated various types of behaviours similar to 1-iodoundecane. In contrast control and di-n-propyl phthalate did not activate the male to show various reproductive behaviours as compared to estrus urine and 1-iodoundecane. The various behaviours showed by male towards 1-iodoundecane was significantly ($P<0.001$) higher than that of other samples (Tables 2 and 3).

Discussion

Among the volatile compounds identified in estrus urine, the behavioural study confirmed that the compound 1-iodoundecane is highly effective in stimulating the bulls for several sexual behavioral responses. By contrast, the compound di-n-propyl phthalate failed to demonstrate such positive responses. The reasons may be because of phthalate/esters derived from laboratory contamination or from plasticizers present in plastic containers in which the urine is frequently collected [16]. It should be noted that the identification of these high boiling compounds are based solely on the software matching algorithm and hence must be considered tentative. It is also noticed that after inhaling the urine, bull frequently lick the genital region and exhibited sexual reproductive behaviours. The bulls revealed a high frequency of flehmen response when exposed to 1-iodoundecane when compared to control and estrus urine. Likewise, the chemosensory responses and pre-mating behaviours of the adult male to the female urinary cues have been well documented [17]. It is reported that mice in the various phases of estrous cycle showed maximum olfactory sensitivity as compared to other phase's indicating the role of ovarian estrogen to olfactory sensitivity in mammals [18].

The behavioural activities exhibited by the bull in response to an

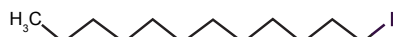


Figure 1: Structure of 1-Iodoundecane.

estrous compound (i.e. 1-iodoundecane) show similarities to that seen in the bull in response to natural estrus urine. The present results coincide with the reports that the bulls frequently mount the cows that are in estrus stage [19]. Nevertheless, it is not known whether there are some substances/compounds produced from other sources, which may act together with 1-iodoundecane of urinary source for exhibiting the mating performance. The present finding is in agreement with the earlier reports on identification of estrus-specific urinary Chemosignals in mouse [20] and buffalo [21]; their role as sex-pheromones has been confirmed by bioactivity. Based on various behaviour activities exhibited by bulls suggest that the 1-iodoundecane may be considered as a reliable indicator for estrus, probably a strong stimulator for sexual activities in bull.

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