Pheromones in Poll Gland secretions of male dromedary camels (C*amelus dromedarius*) during the breeding season and their role in bio-stimulating sexual behavior changes in females

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Abstract

The study aimed to detect pheromones in male camel poll gland secretions during breeding and non-breeding seasons and assess their use for biostimulation in female camels. Secretions were collected from six stud males during the breeding season (PGSBS) and six cart-pulling males during the non-breeding season (PGSNBS), stored at -20°C. GC-MS analysis identified Androst-16-en-3-ol (3. alpha.5. alpha) and Androst-16-en-3-one (5. alpha) in PGSBS. For biostimulation, PGSBS, PGSNBS, or water was applied to three camel heifers (TH1, TH2, TH3) in separate trials using a Latin square design. These heifers were introduced to groups of four adult non-pregnant females to observe sexual behavior changes. The highest biostimulation responses in females were biting and sniffing the heifers, with significantly higher Flehmen and jumping responses ($P<0.001$) to PGSBS. The study concluded that male camel poll gland secretions contain pheromone-like substances during the breeding season, which evoke sexual behavior changes in female camels.

Keywords: Biostimulation; sexual behavior; Androst-16-en-3-ol; Androst-16-en-3-one; pheromone; poll gland; dromedary camel

Introduction

Biostimulation, recently redefined as the reciprocal effect of males and females on their reproductive physiology (Landaeta-Hernandez et al., 2023), is induced by pheromones present in urine, feces, or cutaneous glands. These chemical cues elicit behavioral and endocrine responses via the olfactory system (Rekwot et al., 2001). Pheromones play a crucial role in biostimulation across various livestock species (Sankarganesh et al., 2021). Pheromones can affect males and females, females and males, or females and females (Landaeta-Hernandez et al., 2023).

Dromedary camels (*Camelus dromedarius*), vital to desert ecosystems for their milk, meat, hair, manure, and energy for transportation and agriculture (Vyas & Sahani, 2000), exhibit marked seasonal reproductive activity, with females being induced ovulators (Vyas et al., 2004; Purohit et al., 2020). During the breeding season, male camels exhibit "rut" behavior, characterized by copious secretions from the poll glands, which are watery, brown, or coffee-colored with a distinctive odor (Manivannan et al., 1996; Vyas et al., 2001). Poll glands, located about 10 cm caudal to the occipital crest (Singh & Bhardwaj, 1978), show increased secretion and glandular changes during the breeding season (Ebada et al., 2012; Ibrahim & Al-Kheraije, 2021). These secretions also occur post-exercise in the non-breeding season (Singh & Bhardwaj, 1978). While previous studies suggested that poll gland secretions could be a source of sex pheromones (Tingari et al., 1984), the presence of pheromone-like substances in these secretions has not been reported. Aromatic hydrocarbons found in pregnant female camels were not similar to pheromones (El-Nadi & El-Tokri, 2007). Identifying pheromones in male camel poll gland secretions is crucial, considering the seasonal changes in secretion and testicular morphology (Purohit et al., 2022). Analytical tools like GC-MS have identified pheromonal substances in other species (Rasmussen et al., 1997; Fukuda et al., 2009;). This study evaluated the presence of pheromones in male camel poll gland secretions during breeding and non-breeding seasons and assessed their use for biostimulation in female camels.

Materials and Methods

Experimental animals

Six adult male breeding camels from the National Research Centre on Camel (NRCC), Bikaner, India, were selected based on signs of rut (Vyas et al., 2001). Bikaner is located at an altitude of 242 meters, at 27.9779° N latitude and 73.3606° E longitude, in the Thar Desert. The camels were fed dry fodder and had ad libitum water access twice daily, following NRCC standards. The study followed the guidelines of the Institute Animal Ethics Committee. Three female camel heifers and twelve adult non-pregnant female camels were maintained and fed according to farm standards.

Collection of Poll Gland secretions

During the peak breeding season (January-February), poll gland secretions (Fig.1) were aspirated from the camels using a sterile 10 mL syringe and stored in 2 mL aliquots at -20°C (PGS-BS). During the non-breeding season (June-July), scant secretions from cart-pulling male camels were collected and similarly preserved (PGS-NBS).

GC-Mass Spectrometry for evaluation of pheromones

GC-MS analysis was performed at the Institute of Pesticide Formulation Technology, Gurgaon, India (Soldin & Soldin, 2009). Samples from six breeding season camels were combined, extracted with dichloromethane, and concentrated to 1 mL. Non-breeding season samples were processed similarly. Samples were analyzed using an Agilent 7890A GC and 5975C MS with a DB-5MS column. The oven temperature was programmed from 50°C to 280°C. The injector temperature was 250°C, with a carrier gas flow rate of 1 mL/min. The mass scan range was 29–500 m/z, with MS source and quadrupole temperatures at 230°C and 150°C, respectively. Data files were subjected to a library search using NIST 2005 and Wiley 08 versions (Brakefield et al., 2009).

Effect of Poll Gland secretion on Biostimulation of female Camels

During the transition from the non-breeding to breedicg season (September-October), the behavioral effects of PGS-BS and PGS-NBS on twelve adult female camels were tested. Three camel heifers (TH1, TH2, TH3) were used as test animals, with PGS-BS, PGS-NBS, or water applied to their poll gland area in a Latin square design. Behavioral experiments were conducted in a large barn, with groups of four non-pregnant female camels each (AFC1, AFC2, AFC3). Female camels were examined by transrectal ultrasound to rule out genital abnormalities and follicular activity (Vyas et al., 2002).

In three trials, each group of four females was introduced to a test heifer with one of the test materials applied, and their behavior was recorded (Table 1). Trials were repeated at 48-hour intervals, with groups of females changed randomly in subsequent trials to rule out animal effects. The sexual behavior of the females towards the test heifers was observed and recorded manually (Table 2) by the same observer (Mahla et al., 2015) from 8:00 AM to

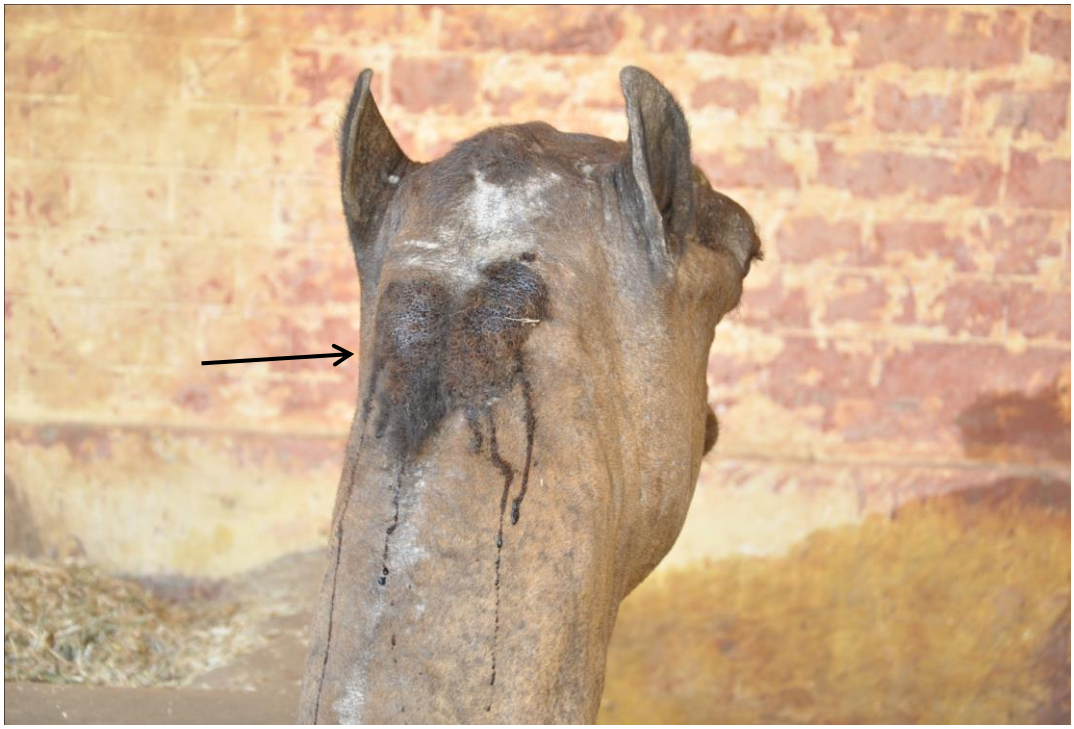

Fig. 1 Secretion from poll glands in male dromedary camel during the breeding season

Table 1: The application of test material (poll gland secretions or water) to test heifers and introduction to adult female camels for the observation of sexual behavior signs in a group of adult female camels (AFC1, 2, and 3) during Trial I.

Test Heifer	Trial I		
	Test Material/AFC		
TH1	PGSBS- AFC1	PGSNBS -AFC2	Water -AFC3
TH2	PGSNBS -AFC2	Water -AFC3	PGSBS -AFC1
TH3	Water - AFC3	PGSBS -AFC1	PGSNBS -AFC2

PGS-BS – Poll Gland Secretion in Breeding Season; PGS-NBS- Poll Gland Secretion in Non-Breeding Season; AFC- Adult non-pregnant Female Camel

Table 2: Sexual behavior responses of female camels bio-stimulated with secretions from the poll gland of males during the mating season (PGS-BS) and during the season of seasonal anestrus (PGS-NBS) or with water.

Sexual Behavior
Olfactory
Sniffing camel
Sniffing poll gland region of a camel
Sniffing the vulvar region of a camel
Contact
Contact with a camel
Licking camel
Evoked behavior
Flehmen (a distinct nostril and facial movement that facilitate s chemo sensation via the vomeronasal organ)
Jumping over females
Biting the camel.

11:00 AM. Male camels were housed far from the barn to prevent disturbance. The sexual behavior data was compared by ANOVA.

Results

GC-MS Analysis of Poll Gland Secretions

GC-MS analysis showed that poll gland secretions from male camels during the breeding season (PGS-BS) contained two additional steroid compounds, Androst-16-en-3-ol (3. alpha, 5. alpha) and Androst-16-en-3-one (5. alpha), which were not present in non-breeding season secretions (PGS-NBS). These steroids appeared as peaks at RT 22.580 min and 22.839 min in PGS-BS (Fig 2).

3.2 Behavioral Response of Biostimulation

The sexual behavior responses of adult female camels towards test heifers treated with PGS-BS, PGS-NBS, or water showed that the highest biostimulation response was biting the test heifers, followed by sniffing the poll gland region. The olfactory response, particularly sniffing, was higher with PGS-BS. Significant sexual behaviors, such as Flehmen and jumping over test heifers, were significantly more frequent (P<0.001) in response to PGS-BS-treated animals (Table 3).

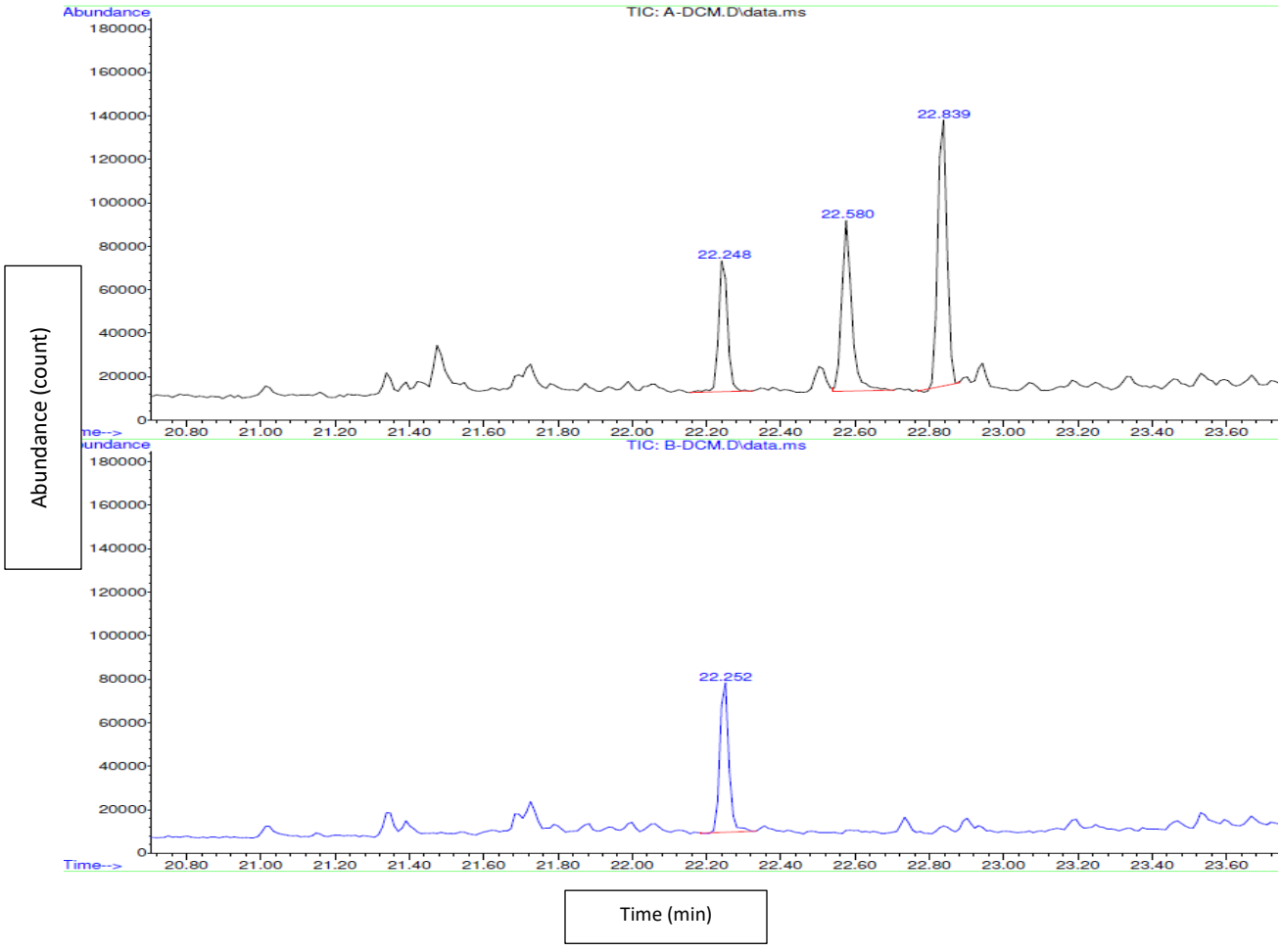

Fig. 2 The Total Ion Chromatogram (TIC) of extracted samples of camel poll gland secretion during breeding (TIC: A; PGS-BS) and non-breeding season (TIC: B; PGS-NBS).

Table 3: The result of behavior experiment with poll gland secretion collected in Breeding and non-breeding season

S.N.	Sexual behavior response of camels	PGS-BS	PGS-NBS	Water
1	Sniffing camel	7	9	5
2	Sniffing poll gland region of a camel	51	28	4
3	Sniffing the vulval region of a camel	10	5	0
4	Contact with a camel	30	19	16
5	Licking camel	3	0	0
6	Flehmen*	9	1	0
7	Jumping over camel*	12	4	0
8	Biting the camel	99	54	23

* The values are statistically different significantly at 1%. PGS-BS – Poll gland secretion in Breeding season; PGS-NBS- Poll gland secretion in non-breeding season.

Discussion

GC-MS analysis revealed the presence of Androst-16-en-3-ol (3. alpha, 5. alpha) and Androst-16-en-3-one (5. alpha) in poll gland secretions during the breeding season (PGS-BS), highlighting their role as mammalian pheromones, akin to their activity in pigs (Patterson, 1968a; Patterson, 1968b). Previous studies underscored the steroid dependence of camel poll glands, with seasonal fluctuations in testosterone and secretion levels (Tingari et al., 1984). Seasonal changes in serum testosterone and poll gland secretions have been observed in male camels (Al-Bulushi et al., 2019; Purohit et al., 2022) with reports suggesting a significant role of poll glands (Ebada et al., 2012; Ibrahim & Al-Kheraije, 2021). Histologically, the poll gland resembles endocrine glands, with secretory cells capable of apocrine and merocrine secretion, allowing hormone uptake and concentration (Taha et al., 1994). Anabolic steroids also accumulate in the poll glands of Bactrian camels (Dai et al., 2023). The present study appears to be the first to report the presence of priming sex pheromones in the poll gland secretion of camels during the breeding season.

The study demonstrated that applying PGS-BS to test heifers induced notable sexual behavior responses in female camels, including biting and sniffing the poll region, suggestive of an olfactory attraction likely mediated by these pheromones. These behaviors parallel responses observed in female camels towards rutting males (Padalino et al., 2016). Significant increases in Flehmen response and jumping over test heifers exposed to PGS-BS further support the presence of pheromone-like substances in male camel poll gland secretions during the breeding season. Studies in various species have shown that male pheromones attract females (Sankarganesh et al., 2021). One study found that female camels approached rutting males and exhibited restlessness (Mahla et al., 2015). Another study observed that female camels behaved differently in various ovarian phases in the presence of restrained males (Padalino et al., 2016). The present study is likely to be the first to evaluate the biostimulation effects of poll gland secretion from rutting males on female camel behavior.

Seasonal variations in poll gland activity, reflected in morphological and enzymatic changes, likely contribute to the absence of pheromonal substances in non-breeding season secretions (Ebada et al., 2012; Ibrahim and Al-Kheraiji, 2021). Histological insights suggest that the poll gland's alveolar region, exhibiting heightened cellular activity during the breeding season, plays a crucial role in steroidogenesis and pheromone production (Atoji et al., 1998; Soliman et al., 2006).

Male-specific olfactory signals, potentially conveyed through poll gland secretions may activate physiological responses in female camels akin to other species (Murata et al., 2009). These signals likely influence complex interactions between male and female camels, with implications for sexual behaviors and ovulation (Skidmore, 2011; Vyas et al., 2012).

This study lays the groundwork for identifying bio-stimulatory signals in camel pheromones, yet further research is warranted to validate the volatility and specific roles of these identified compounds, akin to studies in other species like elephants (Rasmussen et al., 1997). These findings hold promise for utilizing camel pheromones in biostimulation strategies, potentially enhancing reproductive efficiency in breeding programs.

Conclusions

It was concluded that poll gland secretions from male camels contain pheromone-like substances during the breeding season, and the application of these secretions evokes sexual behavior changes in female camels.

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