RESEARCH ARTICLE

Influence of bull exposure on the behavioural intensity of estrus in Sahiwal heifers (*Bos Indicus*)

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Received: 15 December 2018 / Accepted: 16 March 2019 / Published online: 22 June 2019
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Abstract: The present study was conducted to study the influence of the presence of a bull on the behavioural signs of estrus expressed by Sahiwal heifers. For this experiment, a total of 24 Sahiwal heifers were allotted to 3 groups (T_0 , T_1 and T_2) of 8 animals each, based on the age and body weight. In T_0 , the heifers were isolated from the bull; in T_1 group, the heifers were in contact with the bull through a fenceline exposure and in T_2 group, the heifers were exposed through a fenceline contact (24 hours) with bull along with direct contact (for 6 hours) through another bull in the heifer shed. Estrus detection was done by bulls in T_1 and T_2 heifers group whereas in T_0 group, heifers heat expectancy chart and visual observation of behavioural signs of estrus was used for estrus detection. Overall mean intensity of sniffing/licking, tail raising, micturition, chin resting and allowing mounting attempts during silent estrus in T_0 was largely weak (88.64%) and in T_1 heifers group, intensity was weak to moderate (60.05 and 34.58 %) whereas, in T_2 group, percentage of weak, moderate and intense was 38.5 %, 27.0 % and 34.4 % respectively. The per cent mean intensity of estrus behaviour in second estrus was weak to moderate (71.875 and 28.12) in T_0 , whereas in T_1 group, intensity was largely weak to moderate (42.9 % and 41.3%) and intensity of cardinal signs of estrus in T_2 was mostly moderate

to intense in nature (31.9 % and 57.7%). It can be concluded that bull biostimulation of Sahiwal heifers through direct contact plus fenceline contact was more effective in increasing estrus behaviour intensity as compared to the only fenceline contact. Most prominent cardinal symptoms of estrus in biostimulated as well as non-biostimulated Sahiwal heifers were sniffing and licking, micturition, tail raising, allowing mounting attempts and chin resting.

Keywords: Biostimulation, Estrus intensity, Silent estrus, Sahiwal

Introduction

The improper detection of estrus in heifers is the most important limiting factor of reproductive efficiency. In heifers of zebu cattle, an absence of symptoms in silent estrus leads to the poor detection rate. These problems are basic manifestations of non-detection of estrus resulting in delayed age at first service (Layek et al. 2011). The weak expression of behavioural estrus in cattle (11.05 %) and buffaloes (20.75 %) leads to insemination at the improper time (Kumaresan et al. 2001) thus, has a huge impact on the overall reproductive performance of herd (Layek et al. 2011). Therefore, efficient detection of estrus not only improve conception rates but also result in increased milk production of the herd (Diskin and Srenan, 2000; Tsai et al. 2014). Past few reports (Mattoni and Ouedraogo, 2000; Walker et al. 2008; Layek et al. 2011 and Madkar et al. 2015) have indicated that prominent difference exists between *Bos taurus* and *Bos indicus* in terms of estrus duration and intensity of expression of various estrus signs. Similarly, Layek et al. (2011) observed moderate to weak intensity of estrus behaviour in the pluriparous Sahiwal cow. Chenoweth (1983) defines the term biostimulation as stimulatory effect of the male by means of pheromonal stimulation and external cues such as visual, olfactory, tactile and auditory, which elicit both behavioural and endocrine responses in conspecifics. Roelofs et al. (2005) and Khanh et al. (2012) observed that primiparous cows exposed to bull were having a higher number of behavioural intensity. Similarly, Verma et al. (2014) reported increased intensity of estrus behaviour in the presence of the teaser bull in Murrah buffaloes. Therefore, application of

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biostimulation approaches could be an important way to improve the intensity of estrus behaviour.

As far as the indigenous cattle heifers (*Bos indicus*) are concerned, the estrus signs and their expression (intensity) during silent estrus and second estrus in presence of bull has not been studied in detail. In this regard, the present study was undertaken to investigate the effect of bull exposure through fenceline contact and direct contact on the intensity of estrus behaviour expression.

Materials and methods

Place of study and animals

The present experiment was conducted on 24 Sahiwal heifers maintained at Livestock Research Centre of National Dairy Research Institute (NDRI), Karnal, Haryana and were allocated to one of the following 3 treatments (T_0 , T_1 and T_2) with 8 animals in each group on the basis of age and body weight. In T_0 group, the heifers were not exposed to the bull; In T_1 group, the heifers were exposed to two intact bulls round the clock by housing them in a fenceline contact throughout the length of the covered area as well as open paddock of the heifer shed; In T_2 group, the heifers were housed in a fenceline contact with bull along with direct contact through another bull for a total duration of 6 hours daily (3 hours in morning from 07.00 to 10.00 am and again for 3 hours in evening from 4.00 to 7.00 pm) in the heifer shed. The experimental animals were maintained in a loose housing system under group management practice.

Estrus detection

All the behavioural interactions of the bull with the heifers of T_1 and T_2 group were recorded using CP Plus CCTV camera recording system. Similarly, behavioural interaction in T_0 group heifers was also recorded using the CP Plus CCTV camera recording. The observers recorded the onset of estrus based on proven cardinal signs and frequency of a particular sign over different time periods. The cardinal signs that are used were standing to be mounted by the bull, mucus discharge, swollen vulva, frequent micturition, tail raising, chin resting and flehmen reaction and sniffing/licking of the vulva. The heat expectancy chart contains the records of animals about the previous estrus. In the present study, heat expectancy chart was used to know the expected date of onset of estrus for individual animals. The estrus was detected by a bull in T_1 and T_2 groups heifers and in T_0 group heifers, the estrus was confirmed by inseminator on the basis of tumefaction of vulva, reddening of vulvar mucus membrane and uterine tonicity in all three groups. The onset and frequency of the expression of estrus signs were observed and recorded in the pre-tested estrus symptom recording chart designed for the purpose by Mangal, (2009). The midpoint time between the first observation of the cardinal signs of estrus and the previous check without any behavioural signs was considered

as the time of onset of estrus. Similarly, the end of the estrus was considered as the midpoint between first observation of animal to be off estrus and the previous check-in estrus.

Scoring of estrus signs

The scoring of the estrus behaviour was done following a scoring system developed by Dash (1980) for zebu cattle. In this scoring system, different behavioural signs observed during estrus were assigned with a score depending upon their frequency or intensity of expression (Table 1). All the frequencies or intensities of each behavioural signs were converted into a score of 1, 2 or 3. The scores obtained for each of the behavioural signs for an individual animal were added to obtain a total score. Total score for each animal observed was classified into three classes viz. weak (1–11), moderate (12–22) and intense (23–33) as developed by Layek et al. (2011).

Statistical analysis

Total Intensity of each estrus was calculated by summing up scores assigned to each value and they were classified into 3 classes viz. weak, moderate and strong. Statistical analysis was performed using SPSS computer software version 21.

Results and discussion

Intensity of estrus behaviours

In presence of bull, the estrus behaviour in cattle is expressed in a sequential pattern and the proposed sequence is sniffing/licking, tail raising, micturition and chin resting followed by mounting attempts, and finally by standing to be mounted (Roelofs et al. 2005). The data on the mean intensity of various estrus behaviours recorded on the day of estrus during silent estrus and during the second estrus in terms of percentage of animals expressing the estrus behaviour out of the total number of heifers in estrus is presented in Table 2 and Table 3.

Intensity of sniffing/licking behaviour

The intensity of sniffing/licking by a bull and other herd mates during silent estrus period in most of the heifers was weak in T_0 which is followed by moderate intensity of sniffing/licking. While half of the heifer in T_1 had exhibited a weak intensity of this behaviour and remaining heifers had displayed moderate and intense intensity, whereas the intensity of sniffing/licking in T_2 heifers was moderate to intense. The intensity of sniffing/licking by bull and other herd mates during second estrus period in T_0 was weak to moderate and per cent intensity in half of the heifers in T_1 group was weak, and remaining heifers displayed moderate and intense sniffing/licking behaviour intensity, whereas intensity in most of the heifers in T_2 group exhibited intense sniffing /licking behaviour and the rest of the heifers displayed moderate type of intensity. Our finding is in agreement with

Khanh et al. (2012) in cattle and Ahmed et al. (2006) in Mithun. They have reported a higher intensity of sniffing/licking in bull exposed females as compared to non-exposed counterparts. However, our findings are higher than reported by Parikh et al. (2018) and Layek et al. (2011) who reported weak to moderate intensity of sniffing/licking in Pluriparous Gir and Sahiwal cows without the exposure of bull. Orihuela, (2000) concluded that the expression of estrus behaviour changes with successive estrous cycles.

Intensity of tail raising

The intensity of tail raising in response to sniffing/licking by a bull and other herd mates during silent estrus in most of the heifers was weak to moderate in T_0 and T_1 heifers. Per cent intensity of tail raising in T_2 group of heifers was weak, moderate and intense. Similarly, the intensity of tail raising in response to sniffing/licking by a bull and other herd mates during the second estrus was largely weak to moderate in T_0 and T_1 and most of the heifers in T_2 exhibited intense intensity. The values of intensity observed in our study are higher than reported by Parikh et al. (2018) and Layek et al. (2011) in Gir and Sahiwal cows without bull exposure. Tail raising is very common behaviour during estrus and intensity of this behaviour increases with the approach of

the teaser bull which might be justification for intense to moderate type of intensity in the T_2 group.

Intensity of micturition

The intensity of micturition during silent estrus period in all heifers of T_0 group was weak and largely moderate in T_1 . Most of the heifers in T_2 had displayed moderate to intense micturition. During second estrus period, the intensity of micturition in T_0 heifers was weak to moderate and in T_1 intensity of micturition was moderate in few heifers and most of the heifers exhibited intense micturition and heifers in T_2 group displayed intense micturition. The results in the present study are higher due to the presence of bull (direct and fenceline contact) which is supported by Roelofs et al. (2008) and Verma et al. (2014) who reported moderate to intense micturition in bull exposed cows and buffaloes heifers.

Intensity of allowing chin resting

The intensity of allowing chin resting by a bull and other herd mates during silent estrus period in T_0 and T_1 was weak to moderate. The intensity in half of the heifers in T_2 was intense, moderate and weak which might be due to direct contact of bull.

Table 1 Scales for scoring estrus intensity

S.No.	Estrus symptoms	Degree of expression		
		Weak(Score-1)	Moderate(Score-2)	Strong(Score-3)
1	Mucus discharge	Absent	Slight	Copious
2	Swelling of vulva	Weak	Moderate	Intense
3	Standing heat	Occasional	Not frequent	Frequent
4	Mounting attempts	Occasional	Not frequent	Frequent
5	Chin resting	Infrequent	Intermittent	Frequent
6	Sniffing/licking	Occasional	Not frequent	Frequent
7	Tail raising	Infrequent	Intermittent	Frequent
8	Micturition	Infrequent	Intermittent	Frequent

Table 2 Intensity (%) of various behavioural signs during silent estrus in different groups of Sahiwal heifers

Parameters	T_0				T_1				T_2			
	Weak	Moderate	Intense	Total	Weak	Moderate	Intense	Total	Weak	Moderate	Intense	Total
Standing to be mounted	100	—	—	12.5	100	—	—	25	100	—	—	37.5
Mounting attempts with others	100	—	—	25	28.57	42.86	28.58	87.5	—	71.43	28.57	87.5
Chin resting	66.67	33.33	—	37.5	60	40	—	62.5	33.33	16.67	50.00	75
Sniffing and licking	80	20	—	62.5	50	35.79	14.29	87.5	—	14.29	85.71	87.5
Tail Raising	62.5	37.5	—	50	66.67	33.34	—	75	20.00	40.00	40.00	62.5
Micturition	100	—	—	37.5	28.58	71.43	—	87.5	—	28.57	71.43	87.5
Swollen vulva	100	—	—	25	80	20	—	62.5	83.33	16.67	—	75
Mucus discharge	100	—	—	25	66.67	33.34	—	37.5	71.43	28.57	—	87.5
Overall estrus score	88.64	11.35	—		60.05	34.58	5.35		38.51	27.02	34.46	

During second estrus period, the intensity of allowing chin resting by the bull and other herd mates in T_0 and T_1 remain weak to moderate, whereas, in T_2 heifers, the intensity of allowing chin resting was moderate to intense. The results of the present study show no difference in T_1 and T_0 heifers in the intensity of estrus behaviour. This observation agrees with the outcomes observed by Shipka and Ellis, (1998) and Roelofs et al. (2008) who reported that dairy cows are attracted by a fenceline-housed bull but fenceline bull exposure did not affect chin resting behaviour of estrus among those cows. Results observed in the T_2 group are in agreement with Verma et al. (2014) who reported higher intensity of allowing chin resting in teaser bull exposed buffalo's heifers.

Intensity of allowing mounting attempts by bull and other herd mates

The intensity of allowing mounting attempts by a bull and other herd mates during silent estrus period in all T_0 heifers was weak, whereas heifers of T_1 group exhibiting weak, moderate and intense intensity. The intensity of allowing mounting in most of the heifers was moderate and intense and none of the heifers showed weak intensity. During the second estrus intensity of this behaviour in T_0 in about two-thirds of the heifers was weak and in remaining heifers it was moderate. In contrast, the intensity of allowing mounting attempts in T_1 was moderate in most of the heifers and in remaining heifers it was intense. In the T_2 group, the intensity of allowing mounting was intense in most of the heifers showing further improvement and in the remaining small number of heifers, was moderate. Findings of the present study are supported by the earlier finding of Khanh et al. (2012) in primiparous dairy cows and Mondal et al. (2006) in multiparous dairy cows. They had reported that more intense mounting attempts were observed when cows were exposed to bulls. Further, the intensity of mounting attempts observed in the control group of heifers in the present study was found to be similar as reported by the Layek et al. (2011) in primiparous Sahiwal cows without using biostimulation.

Intensity of standing to be mounted

The intensity of estrus heifer's for standing to be mounted by a bull and other herd mates during silent estrus period was weak in all the heifers (100%) among all three groups of experimental animals. However, the intensity of standing to be mount by bull and other herd mates during the second estrus period was weak in T_0 (100%) and T_1 (100%) groups, whereas the intensity of this behaviour was intense (60%) in most of the heifers in T_2 followed by moderate (20%) and weak (20%). These findings are in the agreement with the findings of Allrich, (1994), Kyle et al. (1992), Lyimo et al. (2000), Van Eerdenburg et al. (2002) who reported that the intensity of standing to be mounted was weak in Holstein Friesian heifers. The intensity of standing to be mounted in T_0 and T_1 was weak during first as well as during the second estrus due to the absence of direct contact by a bull and other estrus females in the group. Our findings are also supported by the earlier observation of Helmer and Britt (1985) and Mangal (2009) who reported that intensity of estrus increases when two or more number of estrus females are present in the group. The intensity of estrus in the majority of heifers in T_2 group was intense which is in agreement with a study of Verma et al. (2014) who reported that the presence of teaser bull increases the intensity of standing to be mounted behaviour in buffalo heifers. Lopez-Gatius et al. (2005) suggested a lower concentration of the estradiol at silent estrus may results in the weak intensity of stand to mounted behaviour, further which may support the results of the present study.

Tumefaction and swelling of vulva

There is a hyperaemia observed in the interior of the vulva in the peri-estrual period and the vulva becomes swollen and reddened (tumefaction). During silent estrus, most of the tumefaction and swelling in T_0 was weak (100%), while weak to moderate in T_1 (80% and 20% respectively) and T_2 (83.33 and 16.67% respectively). Similarly, during the second estrus, most of the

Table 3 Intensity (%) of various behavioural signs during second estrus in different groups of Sahiwal heifers

Parameters	T_0				T_1				T_2			
	Weak	Moderate	Intense	Total	Weak	Moderate	Intense	Total	Weak	Moderate	Intense	Total
Standing to be mounted	100	—	—	25	100	—	—	25	20	20	60	62.5
Mounting attempts with others	66.67	33.34	—	37.5	—	71.42	28.58	87.5	—	20.00	80.00	62.5
Chin resting	66.67	33.33	—	37.5	60	40	—	62.5	—	33.33	66.67	75
Sniffing and licking	50	50	—	25	50	35.71	14.29	87.5	—	25.00	75.00	75
Tail Raising	66.67	33.34	—	37.5	66.67	33.33	—	75	—	20.00	80.00	62.5
Micturition	25	75	—	50	—	16.67	83.33	75	—	—	100.00	50
Swollen vulva	100	—	—	25	33.34	66.67	—	75	25.00	75.00	—	75
Mucus discharge	100	—	—	25	33.34	66.67	—	37.5	37.50	62.50	—	87.5
Overall estrus score	71.87	28.12	—	—	42.91	41.30	18.03	—	10.31	31.98	57.71	—

tumefaction and swelling in T_0 was weak (100%), while weak to moderate in T_1 (33.34 and 67.66% respectively) and T_2 groups (25 and 75% respectively). The values observed in the present study are lower than reported by Layek et al. (2011). The intensity of tumefaction and swelling in the present study is weak to moderate which might be due to the fact that heifers were in the growing stage.

Mucus discharge

The intensity of mucus discharge during silent estrus in T_0 was weak (100%). However, the intensity of mucus discharge in T_1 and T_2 was weak to moderate. Similarly, during the second estrus, the intensity of mucus discharge in T_0 was also weak (100%), while intensity in T_1 and T_2 was weak to moderate. The results are in consonance with finding of Mangal, (2009) who reported intense (36.95%), moderate (34.78%) and weak (28.27%) intensity of mucus discharge in Sahiwal cow without bull exposure. In cattle, estradiol is the single most important hormone responsible for different estrus behaviours (Lyimo et al. 2000). During the time of estrus, estradiol is found to be at peak and responsible for almost all estrus behaviours.

Conclusions

From the present study, it can be concluded that intensities of various estrus behaviour were weak to moderate in silent estrus as well as in the second estrus in both non-bull-exposed and fenceline bull exposed Sahiwal heifers whereas it was mostly intense to moderate in fenceline bull exposed plus direct bull exposed Sahiwal heifers. It can be concluded from this that bull biostimulation of Sahiwal heifers through direct contact plus fenceline contact was more effective in increasing estrus behaviour intensity as compared to the only fenceline contact. Most prominent cardinal symptoms of estrus in biostimulated as well as non-biostimulated Sahiwal heifers were sniffing and licking, micturition, tail raising, allowing mounting attempts and chin resting.

Acknowledgements

First author is very grateful to Indian Council of Agricultural Research (ICAR) for providing Junior Research Fellowship (JRF). The authors are also thankful to Director, National Dairy Research Institute, Karnal for providing all necessary funds and research facilities.

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