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Research Article

Differential Responses in Estrus Induced Salivary Ferning Pattern Vis-A-Vis the Ambient Temperature - A Comparative Study in Water Buffaloes and Crossbred Cattle

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Abstract

A temperature-humidity index (THI) is a single value representing the combined effects of air temperature and humidity associated with the level of thermal stress. The dairy animals are thought to cope with extreme climatic conditions, especially high temperatures but fertility and milk productions are compromised during the stress period. In the present study, the mean THI values were recorded to be significantly higher during the summer than in the winter season. The present study conducted the impact of THI on salivary fern patterns of 40 Water buffaloes and 40 Crossbred Jersey cattle during winter and summer seasons. This is the first study to know the THI impact on salivary fern patterns of estrus in dairy cattle. The Water buffaloes housed at both farm and field conditions showed better estrus signs and had 55% of typical salivary ferns found positive in the winter season when compared to the summer season had only 30%. The same correlation was also found in Crossbred Jersey cattle that had better estrus symptoms and 70% of typical salivary fern patterns found in the winter season compared to the summer season had only 40% typical fern patterns. The mean salivary fern pattern of Crossbred Jersey cows housed at both farm and field conditions was 40%, whereas in Water buffaloes was 30% in the summer season, may be due to the THI impact on these animals during the summer season was high. And also studied the typical salivary fern patterns at farm and field level were 60% and 37.5% respectively in both seasons, this wide difference was due to the water buffaloes and Crossbred Jersey cows were presented to the local dispensary for artificial insemination preferably on the second day of estrus, in those animals the concentrations of Estradiol was low on second day of Estrus. In conclusion, the drastic changes in salivary fern patterns results may be due to the lowered steroidogenic capacity of follicles under thermal stress is characterized by Aromatase activity of granulosa cells and lowered Estradiol concentration in the dominant follicle effect lesser amount of chloride ions secreted into the saliva, which is one of the for fern pattern initiation.

Keywords: Estrus; Salivary Fern; Temperature Humidity Index; Comparative Study

Introduction

The temperature humidity index (THI), representing the joint effects of environmental temperature (◦ C) and relative humidity (%), is a widely used and easy way to assess the result of heat stress on dairy cattle. A negative correlation occurs in cattle on reproduction traits when the THI crosses a threshold level. Many authors have classified the THI into their choice of study and developed separate THI ranges according to either milk production or fertility traits [1]. The THI values that run over a threshold of 74 seem to have low fertility and lower milk production, while the THI threshold of 80 represented severe heat stress in Thai-Holstein crossbreds. The conception rate in dairy cows is lower with the threshold points of THI at 72-73 [2]. Another study conducted on the effect of heat stress on dairy cattle reproduction is the lower percentage of cows observed in estrus [3]. The THI can be grouped into 5 types by categorizing the effect of heat stress on cattle in terms of index as no stress (< 72), mild stress (72 - 78), severe stress (78 - 89), very severe stress (89 - 98) and dead cows (> 98) [4].

Estrus detection

One of the critical steps in the efficient reproductive management of farm animals is estrus detection. Accurate estrus detection is limited in buffaloes due to their short estrus duration (5-

27 hours) and the absence of clear behavioral signs, especially in summer seasons. Around 50% of estrus events are generally undetected with the estrus detection efficiency of 40% in dairy cattle. The study was observed that insemination at improper times in buffaloes leads to huge economical loss [5]. Presently many estrus detection methods in dairy animals are available today; one of the best non-invasive was salivary ferning to predict the fertile time in all seasons.This is the best in understanding the patho-physiology of animals, and it's an easy, simple, and effortless way for sample collection and suitable for field conditions. The highestradiol levels during the estrus stage which the LH surge for ovulation [6]. Salivary ferning is the combination of mucins and sodium chloride levels that were higher during estrus period. Unique patterns of salivary crystallization were reported in several species during estrus. In cattle, different patterns of salivary crystallisation like none, branch-like (BL), fern- like (FEL), and fir-like (FIL) were reported. Out of these, a typical clear fern pattern (FEL) was observed on the estrus day, but not on the other days of estrous cycle [7]. found that 8% of the population sample of water buffaloes had salivary fern patterns with unknown reproductive history and absence of heat symptoms [8]. Therefore, the present study has been highlighted the effect of THI on salivary fern patterns in Murrah buffaloes and Crossbred Jersey Cattle.

Materials and Methods

Temperature-Humidity Index (THI)

Microclimatic data such as dry and wet bulb temperatures, minimum and maximum temperatures, and relative humidity, were recorded throughout the experimental period in the afternoon (1:00 PM) using a digital thermo hygrometer.

Temperature humidity index (THI) was calculated using the following formula [9].

THI = 0.72 (Tdb + Twb) + 40.6

Where, Tdb = dry bulb temperature (˚C),

Twb = wet bulb temperature (˚C)

Saliva sample collection

Saliva samples were obtained from a total of 80 she buffaloes and cows at different places the 3 Kilometers radius. Primarily, all the saliva samples were employed to validate the salivary fern patterns to be helpful for estrus determination in buffaloes at the local Veterinary Dispensary of Garividi mandal, Vizianagaram District, Andhra Pradesh to be reported for artificial insemination at different seasons. 40 samples were taken both from 5 buffaloes and 5 Cows maintained with uniform feeding and management practices at Livestock Farm Complex, College of Veterinary Science, Sri Venkateswara Veterinary University, Garividi at different seasons. These animals were observed every day for estrous symptoms and the saliva samples were taken from those estrus animals on some days when the estrus is approaching according to the earlier estrous cycles duly following the same procedure as established in NDRI, Karnal, [7]. Briefly, the animals were maintained in calm stress-free environmental conditions, and the unstimulated saliva accumulated at the lower lip was obtained by using 3 ml Pasteur pipettes (Tarson) and immediately lift into microcentrifuge tubes by deflating the Pasteur pipettes carefully. The saliva samples were collected before feed intake, mostly in early morning session and obtained saliva samples were carried directly to the lab and kept on a stand for 10 minutes to settle the cells, debris, and any feed materials. The top clear cell-free supernatant was utilized for smear preparation for salivary fern patterns immediately following the validated method for field conditions [8].

To identify the estrus by salivary fern patterns, 20 µl of cell-free saliva from each sample was loaded for making the salivary smear on a glass slide similar to the standard blood smear preparation. The salivary smear was dried at room temperature for 10 minutes and the dried smears were visualized under the simple microscope at 40X or by the Foldscope in the field conditions. The images were captured by using a simple mobile camera by possessing the camera lens near to the eyepiece of either a simple microscope or the Foldscope. The salivary fern patterns were categorized according to the six major fern patterns observed [7]. The typical fern-like patterns selected as specific to the estrus stage of both Cow and buffaloes. Accordingly, the percentage of estrus determination by the salivary fern patterns was studied both in cows and Water buffaloes.

Results and Discussion

The mean values of the Temperature Humidity Index (THI) prevailing during the experimental period are presented in table 1. The mean temperature-humidity index in summer was 78.02, and in winter, the mean temperature-humidity index recorded was 67.89.

Season	Summer	Winter
Average Temperature Humidity Index	78.02	67.89

Table 1: THI observed during summer, and winter seasons of the experimental period.

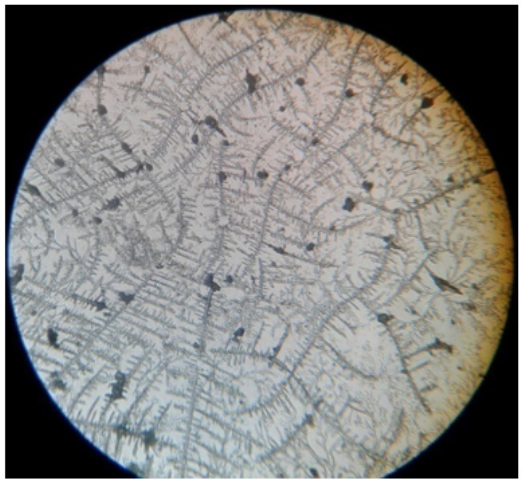

Figure 1: Typical salivary fern During Estrus with 40X.

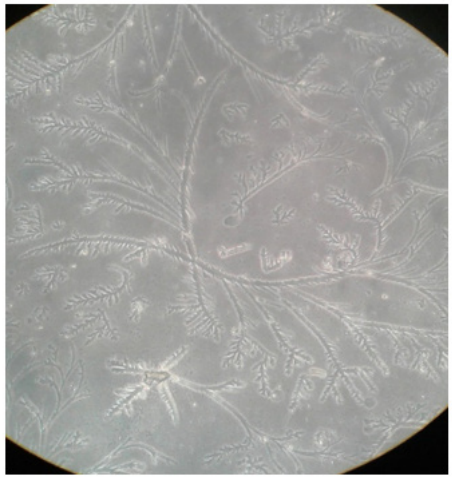

Figure 2: Salivary fern on Diestrum with 40X.

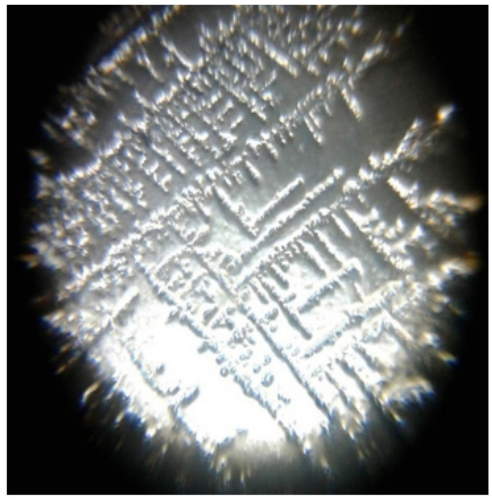

Figure 3: Typical salivary fern with Foldscope.

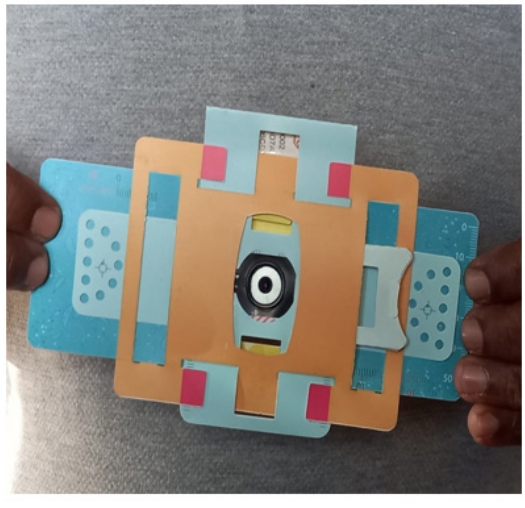

Figure 4: Use of Foldscope at Field conditions.

The percentage values of typical salivary fern patterns during different seasons in Water buffaloes and Crossbred Jersey cattle are presented in table 2 and figure 5. The Water buffaloes housed at both farm and field conditions showed better estrus signs and had 55 percent of Typical fern pattern found in the winter season when compared to the summer season had only 30 percent. The same trend was also found in Crossbred Jersey cattle that had better estrus symptoms and 70 percent of typical salivary fern patterns found in the winter season compared to the summer season

had 40 percent typical fern patterns. The dairy animals are thought to cope with extreme climatic conditions, especially high temperatures but fertility and milk productions are compromised during the stress period [10]. THI accounts for the combined effects of environmental temperature and relative humidity and is useful tool to assess the risk of heat stress in dairy cattle production. In the present study, the mean THI values were recorded to be voluminously higher during the summer than winter season were presented in table No.1. Our results were in agreement with Dash., *et al.* 2014 that the maximum depression in conception rate (-9%) of Murrah buffaloes was in June, July, and August as compared to winter months per unit increase in THI [11]. Temperature-humidity index values categorizedinto 70 or less are considered comfortable, 75-78 stressful, and values greater than 78 extreme distress and animals are unable to manage thermoregulatory mechanisms [12].

The animal body protects itself by dissipating excess heat to the environment through mechanisms that include vasodilatation and sweating. When the Water buffaloes body's temperature run over 98.6 degrees Fahrenheit (37 degrees C), vasodilatation starts as the heart pushes blood flow to microscopic vessels in the superficial layers of skin accordingly excess heat to the cooler exterior environment [2]. The summer heat stress causes many reproductive problems. A lowered steroidogenic capacity of follicles under thermal stress is characterized by aromatase activity of granulosa cells and lowered Estradiol concentration in the dominant follicle [13] results in a lesser amount of chloride ions secreted into the saliva, which is one of the major contributor fern pattern development. During winter months the dairy animals were studied both at farm level and at field conditions showed a higher percent of salivary fern patterns during estrus, which may that no stress factor influence was noticed on salivary fern formation during the winter season. The animals studied were in normal cyclical and all were in healthy conditions have direct influence of Estradiol on t [14] leads to higher amounts of Sodium and Potassium Chloride levels in the saliva [15,16]. The summer stress animals under farm conditions were showing 35% typical fern patterns, as against 62.5% in the winter season and showed little or absence of estrus signs, thereby they were not conceived during the summer period produce less milk and which led to deficit in milk production in the summer months, which results in breakdown of continuous milk production in the year.

The present study also observed on typical salivary fern patterns at farm and field levels were 60% and 37.5% respectively in both seasons. This wide difference was due to the water buffaloes and Crossbred Jersey cows were presented to the local dispensary for artificial insemination preferably on second day of estrus, the salivary fern patterns were predominantly reduced in both seasons due to observation of the animals studied at field conditions, history and behavior of the animals were not well [10].

	Targeted animals for Saliva collection			Typical salivary fern patterns					
Season	Animal type	No. of animals/ Estrous events tested		No. of Animals/ estrous cycles		Percent			
		At Farm	At Field	At Farm	At Field	At Farm	At Field	Mean	Average
Winter	Buffaloes	10	10	7	4	70	40	55	62.5
	Crossbred Jersey Cattle	10	10	8	6	80	60	70	
Summer	Buffaloes	10	10	4	2	40	20	30	35
	Crossbred Jersey Cattle	10	10	5	3	50	30	40	
Total number of animals tested		40	40	24	15	60	37.5		

Table 2: Showing Salivary fern patterns were affected with THI.

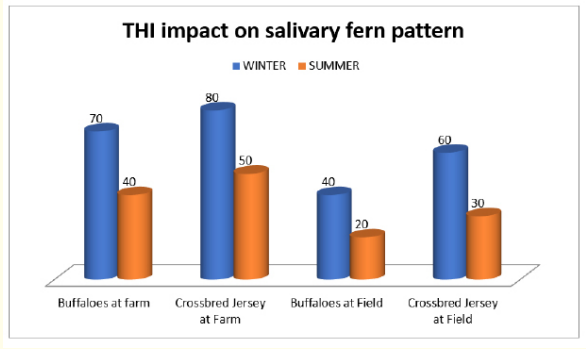

Figure 5: Salivary fern patterns of Crossbred cattle and Buffalo in different seasons.

Many studies published that the Estradiol peak occurs either before or on the day of estrus in buffaloes [17]. Specifically, a report [18] showed a high Estradiol concentration (35.8 pg/ml) a day before the estrus and this level reduced to 17.8 pg/ml when the first signs of behavioral estrus aroused in buffalo heifers.However, the typical fern-like patterns were observed during 0-1 days of estrus duration in Umblachery cattle [19]. As described above, the typical salivary fernlike patterns were representative of either late proestrus or early estrus. AI is generally advocated at 12to 18 hours after the onset of estrus in buffaloes to getmore conception rate [20].

The mean salivary fern pattern of Crossbred Jersey cows housed at both farm and field conditions was 40%, in Water buffaloes was 30% in the summer season (table No.2, figure 5), the reduction could be due to the THI impact on these animals during summer season. Crossing of the thermoregulatory zone results in adverse effects on reproduction [21]. According to data presented in table No.2, the results were supported with, that the Crossbred Jersey cows had better salivary fern patterns in all seasons and all conditions of housing and management practices compared to water buffaloes, due to that buffaloes were considered as silent estrus animal, because the lowered hormonal expressions in water buffalo [21].The consequences of summer stress one lowered Estrogen levels [22] would further lowered fertility, and another one was reduction in dry matter intake by 0.85 kg with every 1°C rise in air temperature, which causes lowered nutrient uptake through the portal system of the dairy animal [23], this may be one of the indirect cause for lowered salivary fern patterns formation during summer months. Today's cows are much more susceptible to heat stress than the cows of the 1950s due to the increased milk production and feed intake to vulnerability to stress, as a of selective breeding procedures. The temperature of the same region is an uptrend @ 0.2°C per decade [24] causes further may increase inthe difference of salivary fern in different seasons of dairy cattle. Accordingly, steps should be taken to mitigate the summer stress on fertile period prediction.

Conclusion

Summer heat stress is a significant cause of in dairy cattle from low percent of salivary fern patterns due to fewer amounts of estrogen levels during estrus period. Consequently, cows and buffaloes are unable to conceive during hot summer months, and this study requires a large population to validate the impact of THI on salivary fern patterns in dairy cattle. As far as our knowledge, this is the report on the impact of THI on estrus detection model in dairy cattle using a combination of salivary fern patterns, and estrus signs. In addition to this prepare monthly mean THI maps and seasonal maps specific to location accordingly, with the help of these maps use treatments combined with cooling methods in housing and feeding strategies in hotter months may improve fertility in dairy animals with an object of minimizing the expenditure on production costs.

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