Analysis of cow sweat volatile chemical compounds in gas chromatography mass spectrometry (GC-MS) device and their importance in heat detection

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Abstract. Detection of estrus is very important for the profitability of dairy cattle. Because one of the main goals of cattle breeding is for each cow to give birth to a healthy calf a year. The correct determination of the artificial insemination time to be applied to the animals by the enterprise is related to the correct determination of the animal's estrus period. Knowing the signs of estrus that are specific to the estrus period of animals is very important in early and accurate detection of estrus. There are many methods for detecting estrus. One of the alternative methods to the methods developed to detect the estrus period in animals is the detection of volatile chemical compounds (pheromones) secreted in animal sweat only during the estrus period. This study will focus on the analysis of volatile chemical compounds in animal sweat using Gas Chromatography Mass Spectrometry (GC-MS) device and their importance in detecting estrus.

1 Introduction

One of the main factors affecting the economic success of a farm is reproductive performance [1]. Early and accurate detection of the estrus period of animals is very important, especially in farms. The time between two calvings is the calving interval. The calving interval is desired to be optimum 12 months (365 days) in dairy cattle [2] (Fig. 1). In other words, the main goals in cattle breeding are for each cow to give birth to a healthy calf a year, to remain dry for approximately 60 days and to be milked for an average of 305 days. The calving interval should be 360 - 385 days. Otherwise, the economic profit of the business decreases.

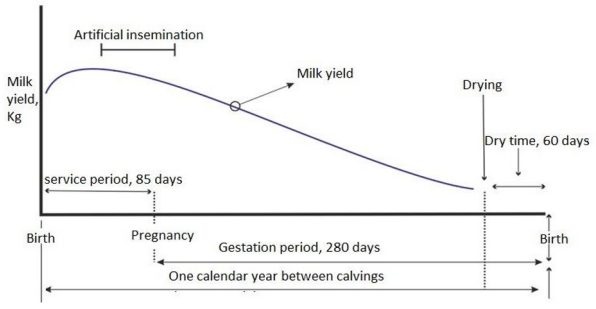

Fig. 1. Important elements of the interpartum period in cows.

In dairy cattle enterprises, in order to obtain one calf from the cows per year, the most important tasks of the enterprise are for the employees to have sufficient knowledge and to be able to detect heat correctly. Accurate oestrus detection programs in the enterprise ensure higher pregnancy rates. Early and accurate detection of heat in the animal results in greater profitability for dairy cows, longer service periods and increased pregnancy rates. A good understanding of the estrus cycle ensures the accuracy of estrus detection.

In order to determine the time of artificial insemination of animals, it is necessary to determine the estrus period well. "Estrus" is the period when an adult cow shows a desire to mate at regular intervals until she becomes pregnant or shows physiological and instinctive sexual behavior in which she accepts the male. If the animal does not become pregnant, the estrus period repeats after 21 days (18-24 days).

Animal behaviors specific to estrus:

-He lets other cows jump on him.

- He often shouts, is restless and angry.
- He stands and does not lie down while others are lying down in the barn.
- They tend to travel (wander) in groups.
- They spend less time resting than usual.
- The rump and tail head are dirty and contaminated with mucous discharge.
- Milk yield suddenly drops significantly.
- The desire to eat decreases.
- Body temperature is slightly high (about 1 °C) [3].

To detect estrus, it is necessary to observe the animal well (Fig. 2). The estrus period in cows lasts an average of 8-12 hours (but may be shorter in high milk producing cows). The short duration of estrus made it necessary to make accurate observations. In his study, Nebel [4] argues that experienced and talented business employees achieve estrus detection efficiency of 65% to 75%.

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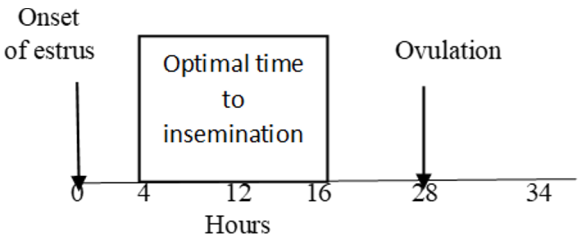

Fig. 2. Schema showing the ideal insemination time.

With the development of technology, tools have been produced to evaluate the behavior of estrus cows using computer facilities. Activity counters (pedometers), detection of vaginal temperature by radio telemetry, automatic estrus detection systems with electronic monitoring devices, vaginal pH measurement, telemetric estrus skip detectors (kamar) are some of the estrus detection methods [5].

One of the alternative methods to the methods developed to detect estrus in animals is the investigation of volatile chemical compounds (pheromones). It is accepted that the species-specific odor emitted by some substances contained in the body fluids of the animal in estrus helps males detect the animal in estrus [6]. There were previous studies to detect the pheromones of saliva, feces, vaginal discharge, milk, blood and urine to detect the smell emitted from the bodies of animals only during the estrus period [7]. However, since there was no research on sweat, it was assumed that the detection of pheromones in animal sweat would be an important finding in the detection of estrus.

Due to the discomfort caused by taking blood with needles for diagnosis and treatment in cattle, studies have been intensified to develop alternative methods in addition to the frequently used diagnostic methods. One of these alternative methods is to determine the compounds and their ratios by analyzing sweat. It is an important advantage because animal sweat can be easily collected from body surfaces and does not cause stress like blood collection while collecting it from the animal.

In addition, since the fluid excreted from the sweat gland is obtained from the interstitial fluid, which ultimately binds to the plasma; It contains important information about the events occurring directly in the body. Therefore, compounds in sweat are expected to contain clinical biomarkers usually detected in blood. Nunome et al. [8] state that the fatty acids in human sweat are transformed into sweat through the blood as a result of being broken down in the fatty tissue, especially in stressful situations. Therefore, the concentration of fatty acids in sweat has been reported to be associated with increased levels of fatty acids in plasma. The lack of studies on the chemical compound content of sweat, especially in cattle, reveals the gap in this regard. In addition, the pheromones detected in the sweat of farm animals will provide important information about determining whether the animals are in estrus.

1.1 Roles of pheromones

Pheromones are defined as chemical signals released outside the body by an individual [9] and evoke specific endocrine or behavioral responses in another individual of the same species [10].

Pheromones can be of various types due to their interactions between different species and fulfilling various functions [11,12]. The types of pheromones according to their areas of influence are shown in Table 1 [13].

Table 1. Pheromone types and effects.

Pheromones	Effects
Secretory Pheromones	It usually elicits a direct, specific, behavioral response. They produce short-term behavioral changes and act as attractants or repellents and are also pheromones that cause a change in the recipient's behavior.
Marker Pheromones	It gives information about the individual. The marker pheromone immediately causes a behavioral response, initiating a classical stimulus-response event mediated by the central nervous system. During mating, a bull receives the chemical signal secreted from the female specific to the estrus period and then exhibits flehmen behavior.
Modulator Pheromones	It affects many (neuro)-endocrine parameters. Accordingly, it affects the mood and emotion of the individual.
Primary Pheromones	These pheromones mediate changes that proceed slowly and last longer. It affects endocrine or neuroendocrine systems related to reproductive physiology or development.

Chemical communication plays an important role in the reproductive behavior and processes of mammals [10]. Animals transmit reproductive information to other animals to coordinate their reproductive activities [14]. Recognizing the short-term (12-22 hours) estrus period is a prerequisite for the cow to be inseminated at the appropriate time [15]. To detect whether the female is in heat, the bull uses a combination of factors including visual, tactile, auditory and olfactory stimulation exhibited by the female [16] (Fig. 3).

Fig. 3. Responses of bulls to pheromones [17].

During estrus, these pheromones are found in milk, urine, blood, saliva, feces and vaginal discharge [7]. Recent research has shown that there are pheromones in sweat and that they are secreted from the body only during the estrus period [7].

1.2 Sweat

Sweating is one of the physiological (natural) functions necessary to keep body temperature constant [18]. However, in addition to the function of sweating to regulate body temperature, it has also been determined that sweat contains volatile odor compounds (pheromones) that change from time to time. Volatile organic compounds (VOCs) emitted from an animal's body sweat can provide invaluable information about the animal's physiological state and therefore constitute a chemical marker for periods such as illness, estrus, and stress of the animal.

1.3 Presence of pheromones in sweat

Water, electrolytes, fatty acids, lactic acid and nitrogen metabolites such as ammonia, urea and uric acid have been analyzed as the main components of sweat [19,20]. Fatty acids (FAs), one of the main components that make up sweat, are biological molecules used primarily as metabolic fuel and involved in important metabolic processes. Hodson [21] stated that fats, fatty acids and metabolic products formed after the use of fatty acids in the body have important roles in metabolism. These functions of fatty acids include creating resistance to stress and damage that may come from external factors, providing the body with the necessary energy, and being the precursor of hormone-like eicosanoid compounds such as thromboxane, leukotrienes and prostaglandins. Volatile fatty acids (organic volatile fatty acids; acetic, butyric and propionic) are released in the rumen after fermentation caused by different microorganisms to break down the feed for microbial digestion [22]. In addition, short-chain fatty acids such as acetic, propionic, butyric, isobutyric, valeric, isovaleric, 2-methylbutyric, hexanoic and heptanoic acid, produced in various parts of the gastrointestinal tract, are also called volatile fatty acids (SCFA) [23]. Ruminants meet 80% of their body's energy requirements from SCFAs [24]. These volatile fatty acids easily enter the bloodstream and are transported to body tissues where they are used for hepatic gluconeogenesis, lipogenesis in peripheral tissues and milk synthesis. Its transport across the plasma membrane in most cells shows how essential it is for the body. Volatile fatty acids, which have such an important place, are also found in sweat. Volatile odor compounds in sweat are formed as a result of the conversion of short-chain fatty acids into volatile short-chain fatty acids (VFAs) [25]. This task is catalyzed by lipases secreted from sweat glands in the skin [26]. It is known that these compounds are released into sweat from the fat tissue through the blood as an energy source when the body needs more energy [8]. Since the fluid excreted from the sweat gland is obtained from the interstitial fluid, which ultimately binds to the plasma; It contains important information about the events occurring directly in the body. Therefore, compounds in sweat are expected to contain clinical biomarkers usually detected in blood [8]. In addition, the detection of volatile odor compounds (pheromones) in the sweat of farm animals, especially compounds belonging to the estrus period, will provide important information to show that the animals are in the estrus period.

These important volatile odor compounds, which will indicate that the animals are in heat, are detected by analyzing the sweat in the Gas Chromatography Mass Spectrophotometer device. Among these volatile odor compounds, those that are detected only during the estrus period and not in other periods are determined and called estrus-specific pheromones [7]. Some authors, in their studies, have detected volatile odor compounds in the sweat of cow that belong only to the estrus period. In studies, aldehyde (dihydrobenzaldehyde, Methylethylacetaldehyde), alkane (dimethyl-1',3'-dioxane, Cyclopropane), alkene (Diazene, 1,3,7,11-Cyclotetradecatetrene, Benzene) ester (methyl ester, carboxylic acidethyl ester, 2-Propenoic acid, 2-methyl-, dodecyl ester) alkyne (2-Myristynoyl pantetheine, 6-Hydroxyundulatine), acid (10,12,14-Nonacosatriynoic acid, 9,12,15-Octadecatrienoic acid) alcohol (Phenol(carbolicacid), 4 -tert-Butyl-2,6-diisopropylphenol) group volatile odor compounds are among the compounds detected only during the estrus period [7,27,28]. Since these volatile odor compounds are not found in animal sweat samples during the pre-estrus and post-estrus periods, they can be considered as estrus-specific pheromones. These compounds, which belong only to the estrus period, spread with the animal's sweat and explain how the bull recognizes the estrous animal through smell.

2 Conclusion

As an alternative to the methods developed to detect oestrus, volatile compounds were detected from sweat in the pre-oestrus, oestrus and post-oestrus periods to determine the chemical composition of the pheromones emitted from the body during the oestrus period and the ratios of these compounds.

With this study, it can be said that detecting volatile compounds that belong only to the oestrus period will be an important indicator in the detection of oestrus and will provide significant economic advantages to the cattle industry as it will allow artificial insemination at the right time by detecting oestrus correctly.

In this study, it was concluded that identifying the chemical compounds (pheromones) secreted during oestrus can be used as a reliable alternative method to detect cows' oestrus. In addition, taking sweat samples causes less stress to the animal than taking blood and urine samples. Recently, more attention has been paid to animal welfare and health. The use of sweat becomes important in terms of animal welfare.

Using the compounds obtained from this study, an electronic device can be developed to detect whether animals are in oestrus. A set of chemical sensors can be designed to electronically analyse volatile chemical compounds that are detected only during the oestrus period but not during other periods. By developing a "device" that is practical and easy to apply in the enterprise, containing sensors that will enable the recognition of these compounds, it will enable research to be carried out to recognize the animal in oestrus and perform artificial insemination at the right time, thus increasing the fertility of the enterprise.

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