Investigation of body secretions as bioindicators in cattle estrus detection

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Abstract: The accurate determination of estrus has a fundamental role in cattle reproduction management. The determination of volatile chemical compounds (pheromones) secreted only during estrus in all body fluids can be used for determining accurate estrus time and artificial insemination time and have a potential role in technological animal reproduction control. In this study, Holstein cows were synchronized and their sudor, urine, feces, milk, saliva, vaginal secretions, and blood samples were taken in the preestrus, estrus, and postestrus periods and analyzed by gas chromatography-mass spectrometry for determination of volatile odor compounds. A total of 531 volatile compounds were detected in the preestrus period, 538 in the estrus period, and 494 in the postestrus period. Among these, 8 compounds were found to be common in all body fluids and the ratio of these compounds to those detected in all body fluids was 2.6%. Especially in the estrus, 3-methyl pentane, hexanal, 4-methylphenol (p-cresol), phenylacetaldehyde, 3-phenylpropiononitrile, 1H-indole, cyclotetrasiloxane octamethyl and pentane 2-methyl were detected. Biotechnology devices such as artificial nose with sensors can be developed, recognizing estrus-specific volatile compounds detected from all body fluids only in estrus period.

Keywords: Cow, estrus body fluids, volatile odor compounds, gas chromatography-mass spectrometry

1. Introduction
The low reproductive performance in the livestock sector, which plays a role in meeting important needs, is an serious problem. Reproductive performances of dairy farms can be defined as the number of calves for cows in a year. If meat and milk production is to be increased, cow productivity, i.e. the number of calves produced lifetime, must be improved and increased [1]. Considering that the increase in animal production depends on the reproductive performance of the herds, it is of great importance to maintain estrus detection and high pregnancy rate in the establishments. Nebel and Jobst [2] reported that the detection rate of estrus was less than 50% in many herds. Nebel [3] argues that experienced and talented business employees achieve an estrus detection efficiency of 65% to 75%. Different methods have been developed for detecting estrus and artificial insemination at the right time. One of the methods that can be used as an alternative to detect estrus in animals is to determine volatile chemical compounds (pheromones). Various attempts have been made to control and regulate reproduction in cattle through the application of biological agents and the use of hormones. The pheromones play an important role in animal behavior and reproductive processes [4]. It is accepted that volatile chemical compounds emitted by some substances contained in body fluids of estrus animals help detect animals in estrus [5].

Odors play an important role in signaling that the cow is in the estrus period. Volatile odor molecules, which are released only by the female during the estrus period, can generate important signals for the bull and coordinate reproductive activities [6]. To determine whether or not the female is estrus, the bull uses a combination of visual, tactile, auditory, and odor stimulating factors exhibited by the female [7]. Visual and auditory stimulation in cows is important for the detection of estrus by a bull [8]. In recent years, technological developments based on estrus detection have provided significant benefits to cattle reproduction management. In this respect, the aim of this study was to investigate the presence of pheromones, which are indicative of estrus in milk, blood, vaginal secretion, urine, feces, saliva, and sudor secretions taken from different stages of estrus in Holstein dairy cows.

2. Materials and methods
The present study was conducted at a research and experimental farm located at the Faculty of Agriculture, Çukurova University, Adana, Turkey. This study was approved by Çukurova University Animal Experiments Local Ethics Board (Approval no: 26.02.2018/2). There
were 160 dairy cows in the farm, of which 40 cows were fresh and were waiting for artificial insemination. Considering that the smallest sample size to represent this population is 5% in the 5% confidence interval, the number of dairy cows is 10% of fresh cows (= 40 / 0.10), thus 4 cows are enough to sample. However, in order to obtain more reliable results within the available possibilities, 15 cows were synchronized and samples were taken from 6 cows (3–4 years old) that were approved by other control methods. Body fluid samples (sudor, milk, blood, feces, urine, vaginal secretion, saliva) were taken in 3 estrus periods of a total of 6 cows and analyzed in duplicate. In total, 252 samples were analyzed for volatile compounds during estrus.

The Ovsynch protocol, which was developed for use in dairy cattle, was applied for grouping the estrus. The cows were injected with 2 mL i.m. GnRH (100 µg as 2 mL of Cystorelin i.m., Merial, Athens, GA) on any day of the cycle (day 0) and with 5 mL i.m. PGE2a (25 mg as 5 mL of Lutalyse i.m., Pharmacia Animal Health, Kalamazoo, MI) on day 7, and a second injection of 2 mL i.m. GnRH was performed on day 9 [9]. With this application, ovulations were synchronized, and samples were taken in order to determine pheromones in the secretions of milk, blood, vaginal secretion, urine, feces, saliva, and sudor at different stages of estrus (preestrus, estrus, postestrus). Ramesh Kumar et al. [10] collected all samples from preestrus animals 3–5 days before estrus and from postestrus animals 2–4 days after estrus period. With the help of veterinarians, the stages of the estrous cycle (preestrus, estrus, postestrus) were carefully determined by rectal palpation throughout the cycle. In addition, the diameter and development of follicles and ovaries in the ovary were determined by ultrasound (HASVET 838 ultrasound, Hasvet Medikal Ltd. Şti., Antalya, Turkey). The cows were fed ad libitum total mixed ration diet and fresh water.  

2.1. Data collection conditions

Urine samples, feces, vaginal fluids, saliva, milk, blood, and sudor samples were taken by the methods of Kumar et al. [11], Sankar and Archunan [12], Sankar and Archunan [13], Saliva Sankar et al. [14], Bendall [15], Klemm et al. [16], and Kennedy [17], respectively. 

Urine samples were taken by applying a urinary catheter (Female urinary catheter 31 cm Kruuse, Medical Gross MEDIBIL Medikal Bilşim Teknolojileri, İzmir, Turkey) to the cows that did not urinate in order to avoid any loss of time and stress. Vaginal secretions were collected with tampon. The tampons were disinfected and dried before use. The perineum and vulva area were disinfected and dried with clean water before sampling. The tampon was gently released into the vagina for 30 min, and then the tampon was removed and placed into screw cap bottles (Teflon-coated) (N24 Vials, Turkey). Udder were cleaned and dried with paper towel before milk samples were collected. Blood samples were taken from vena jugularis. Sweat samples were taken from the nasal regions of animals. Nasal region was cleaned with water prior to sampling and dried with paper towels. Liquid samples were 15 mL and feces were 30 g. All the samples were stored in a freezer at –30 °C (Uğur UED 280, Uğur Soğutma A.Ş., Aydın, Turkey). One day before the analysis, the samples were allowed to thaw in the refrigerator overnight.

2.2. Gas chromatography and mass spectrometry analysis of all samples

For the analysis, 2 mL of sudor, saliva, milk, blood, urine, and vaginal secretion samples and 2 g of fecal sample were placed in the gas chromatography vial and 1 mL of CaCl_2_ was added to each sample. The samples were placed in an automatic HS-40 head space autosampler (Perkin Almer GC with split splitless inlet MSD system). Volatile compounds were analyzed on an automatic HS-40 head space autosampler. Needle temperature was 120 °C, thermostatic time was 30 min, and thermostatic degree was 35 °C during the extraction in the headspace autosampler. HP-5 MS (30 m × 0.25 mm × 0.25 µm), fused-silica capillary column was used. Helium (1 mL/min) was used as the carrier gas. The injector temperature was 250 °C, set for splitless injection. The oven conditions were set to 50 °C for 1 min and then the temperature was increased to 200 °C at a rate of 4 °C/min. Thermal desorption was allowed for 1.5 min. The detector temperature was 280 °C. The components were identified by the comparison of mass spectra and retention time data with those of authentic samples and complemented by means of performing a NIST, Wiley, Flavor library search of the acquired mass spectral data.

2.3. Statistical analysis

Experimental data were used to estimate the means and standard error [18]. The data were subjected to logarithmic transformation according to base 10 in the randomized design plan. However, the average and standard errors of experimental data were given in the tables and in the text. Duncan post hoc test was used to compare the means of the groups (preestrus, estrus and postestrus). All the statistical analyses were performed in the Statistical Package for the Social Sciences software version 16.0 (SPSS Inc., Chicago, IL, USA). A difference in the mean values of P < 0.05 was used to establish the statistical significance.

3. Result

3.1. Volatile chemical compounds of feces

According to the results of feces analysis, 168 volatile compounds were detected and among these compounds, there were 72 compounds in the preestrus period, 85 compounds in the estrus period, and 55 compounds in the post-estrus period (Table 1). As a result of the
| Compound name* | Preestrus ($\bar{x} \pm Sx$) | Oestrus ($\bar{x} \pm Sx$) | Postestrus ($\bar{x} \pm Sx$) | P value ** |
|----------------|-------------------------------|----------------------------|-------------------------------|------------|
| Acetic acid. Isocyanato -. butyl ester | 2.05 ± 1.23 | 1.52 ± 1.15 | 6.54 ± 5.22 | 0.496 |
| Ethylsilane | 2.96 ± 0.00 | 2.51 ± 0.00 | 0.21 ± 0.00 | 0.662 |
| 3 - Fluoropropane | 1.11 ± 0.56 | 1.42 ± 1.11 | 0.36 ± 0.21 | 0.594 |
| Pentane. 2 – methyl - | 1.22 ± 0.60 | 0.98 ± 0.60 | 0.58 ± 0.03 | 0.665 |
| 3 - methyl pentane | 0.85 ± 0.38 | 0.69 ± 0.34 | 0.61 ± 0.06 | 0.845 |
| Benzoic acid. 2 - [(diethylamino) acetyl]amino] - 3 – methyl -. methyl ester | 0.44 ± 0.32 | 0.69 ± 0.35 | 0.13 ± 0.00 | 0.413 |
| Butanal. 4 – hydroxy – 3 – methyl - | 0.53 ± 0.36 | 0.24 ± 0.00 | 0.44 ± 0.00 | 0.848 |
| N - Pentanal | 0.055 ± 0.00 | 0.07 ± 0.00 | 0.606 |
| Oleic acid. eicosyl ester (9-Octadecenoic acid (Z) -. eicosyl ester) | 0.13 ± 0.08 | 0.23 ± 0.18 | 0.376 |
| 1 - Propanone. 1 – cyclopropyl - | 0.09 ± 0.00 | 0.02 ± 0.12 | 0.302 |
| Perfluorotributylamine | 0.15 ± 0.08 | 0.28 ± 0.16 | 0.235 |
| Pyrimidin – 2 - one. 4 - [N – methyl ureido] – 1 - [4 – methyl amino carboxyloxymethyl | 0.09 ± 0.05 | 0.05 ± 0.00 | 0.24 ± 0.15 | 0.388 |
| Hexanal | 0.41 ± 0.18 | 0.40 ± 0.00 | 14.92 ± 14.36 | 0.399 |
| 2.4.6 – Cycloheptatrien – 1 –o ne. 3.5 - bis-trimethylsilyl - | 2.14 ± 1.21 | 0.16 ± 0.00 | 0.109 |
| 1.2 – Benzosilazol – 3 – amine tbdms | 0.34 ± 0.13 | 0.12 ± 0.07 | 0.10 ± 0.00 | 0.217 |
| Dodecanoic acid. Tricosafluoro - | 0.25 ± 0.20 | 0.59 ± 0.47 | 0.05 ± 0.00 | 0.453 |
| 5β – Cholesterol e- 3a,7a,12α,24.25.26 - hexol hexa TMS | 0.29 ± 0.20 | 0.22 ± 0.13 | 0.349 |
| 4-methylphenol (p - cresol) | 1.59 ± 0.72 | 1.41 ± 0.73 | 1.11 ± 0.51 | 0.873 |
| Margaric acid. (Heptadecanoic acid. tert - butyldimethylsilyl ester) | 0.21 ± 0.13 | 0.07 ± 0.00 | 0.274 |
| Nonadecan – 1 - ol trimethylsilyl ether | 1.16 ± 0.49 | 0.60 ± 0.36 | 0.33 ± 0.00 | 0.367 |
| (S) – 2 - Methylbutan – 1 - ol | 0.09 ± 0.00 | 0.15 ± 0.00 | 0.55 ± 0.40 | 0.400 |
| 1 – Penten – 3 - onc. 2 – methyl - | 0.09 ± 0.00 | 0.12 ± 0.00 | 0.10 ± 0.00 | 0.618 |
| 8.14-Seco - 3.19 – epoxyandrostane - 8.14 - dione. 17- acetoxy - 3.beta. -methoxy - 4.4 – dimethyl - | 0.16 ± 0.00 | 0.06 ± 0.00 | 0.523 |
| Oxirane. Trimethyl - | 2.17 ± 2.04 | 0.43 ± 0.28 | 0.38 ± 0.00 | 0.515 |
| 2.3 - butanedione | 0.29 ± 0.00 | 0.30 ± 0.22 | 0.551 |
| 2 - Pentanone | 0.05 ± 0.00 | 0.15 ± 0.00 | 0.525 |
| 1.2.4 - Benzenetrikarbonsilik asit. 1.2 - dimetil ester | 0.61 ± 0.00 | 0.27 ± 0.00 | 0.555 |
| 4 - Methyl. 2.4 - bis (4’ - trimethylsilyloxyphenyl) pentene - 1 | 0.05 ± 0.00 | 0.20 ± 0.00 | 0.492 |
| 6 – Methylhept -5 - en - 2- one | 0.05 ± 0.00 | 0.39 ± 0.14 | 0.021 |
| 3 – Fluoropropane | 0.60 ± 0.00 | 0.30 ± 0.00 | 0.12 ± 0.00 | 0.691 |
| 3 - Methylbutanal | 0.21 ± 0.12 | 0.25 ± 0.17 | 0.08 ± 0.00 | 0.628 |
| 1 - Pentanol | 0.06 ± 0.00 | 0.19 ± 0.12 | 0.82 ± 0.71 | 0.430 |
| 3 – hydroxy – 3 - (1 H – indol – 3 - yl) - 1H – indol – 2 - one | 0.43 ± 0.24 | 0.06 ± 0.00 | 0.074 |
| Bicyclo [2.2.0] hex – 2 – ene – 1 - carboxylic acid. 5.5.6.6 – tetracyano - 2.3.4 – tri (1.1 - dimethylethyl) - 1.1 - dimethylethyl ester | 0.78 ± 0.00 | 0.10 ± 0.00 | 0.39 ± 0.00 | 0.643 |
| 1 - Pyridineacetamide. | 1.83 ± 1.75 | 0.08 ± 0.00 | 0.393 |
| Benzaldehyde (Benzoic acid aldehyde) | 0.05 ± 0.00 | 0.12 ± 0.00 | 0.09 ± 0.00 | 0.839 |
| Trimethyl (octadecyloxy) silane | 0.23 ± 0.14 | 0.09 ± 0.00 | 0.296 |
| Diborane (6) | 0.36 ± 0.00 | 0.16 ± 0.00 | 0.551 |
3.2. Volatile chemical compounds of urine

According to the results of GC-MS, a total of 141 volatile compounds were detected in urine and it was found that there were 76 compounds in the preestrus period, 71 compounds in the estrus period, and 66 compounds in the postestrus period (Table 2). As a result of the analysis of variance, Nonadecan-1-ol trimethylsilyl ether compound was determined to be important (P < 0.05) in terms of interperiod differences. This compound was detected in both preestrus period (0.22 ± 0.00) and estrus period (1.22 ± 0.47) and was calculated as P = 0.042.

3.3. Volatile chemical compounds of blood

As can be seen from Table 3, a total of 156 volatile compounds were detected in blood. Among these, 69 compounds in the preestrus period, 79 compounds in the estrus period, and 64 compounds in the postestrus period. As a result of the analysis of variance, pentane, 2-methyl-

3.4. Volatile chemical compounds of milk

A total of 130 volatile compounds were detected in milk and it was found that there were 56 compounds in the preestrus period, 58 compounds in the estrus period, and 91 compounds in the postestrus period (Table 4). As a result of the analysis of variance, the differences between the compounds in milk were found to be insignificant (P > 0.05).

3.5. Volatile chemical compounds of sudor

A total of 154 volatile compounds were detected in sudor and it was found that there were 84 compounds in the preestrus period, 79 compounds in the estrus period, and 81 compounds in the postestrus period (Table 5). As a result of the analysis of variance, hexanal compound was determined to be important (P < 0.05) in terms of interperiod differences. This compound was detected in the preestrus period (0.93 ± 0.19), the estrus period (0.47 ± 0.11), and the postestrus period (0.18 ± 0.11) and was calculated as P = 0.014.

3.6. Volatile chemical compounds of saliva

Table 6 shows a total of 191 volatile compounds detected in saliva, 91 compounds in the preestrus period, 89 compounds in the estrus period, and 99 compounds in the postestrus period. As a result of the analysis of variance, 3-(2-methoxyethyl)-2-(2-pyridinyl)-1 H-indole compound was determined as important (P < 0.05) in terms of interperiod differences. This compound was

* As a result of the analysis, 33 compounds in the preestrus period, 45 compounds in the estrus period and 11 compounds in the postestrus period were not included in the comparison table, since they were detected in only one period.

**P < 0.05.
Table 2. Volatile odor compounds that were detected in urine during the estrus period (%).

| Compound name*                        | Prefestrus (X ± SX) | Oestrus (X ± SX) | Postestrus (X ± SX) | P value** |
|---------------------------------------|---------------------|-----------------|---------------------|-----------|
| 2 - Pentanone                          | 0.52 ± 0.36         | 1.15 ± 0.74     | 1.66 ± 0.87         | 0.530     |
| Dimethyl disulphide                   | 2.82 ± 2.21         | 0.06 ± 0.00     | 0.08 ± 0.00         | 0.263     |
| Hexanal                               | 1.10 ± 0.58         | 0.07 ± 0.00     | 0.08 ± 0.00         | 0.083     |
| Benzaldehyde (Benzoic acid aldehyde)  | 0.47 ± 0.28         | 0.09 ± 0.00     | 0.20 ± 0.12         | 0.364     |
| Cyclotetrasiloxane, octamethyl -      | 1.48 ± 0.54         | 0.60 ± 0.23     | 0.30 ± 0.21         | 0.110     |
| benzothiazol                          | 0.40 ± 0.00         | 0.98 ± 0.00     | 0.48 ± 0.00         | 0.544     |
| Pentane, 2 - methyl-                  | 0.23 ± 0.14         | 1.38 ± 1.01     | 0.26 ± 0.00         | 0.383     |
| Acetic acid, isocyanoato -, butyl ester | 1.58 ± 0.00       | 0.93 ± 0.38     | 0.40 ± 0.00         | 0.429     |
| Dodecanoic acid, tricosalluro -       | 0.24 ± 0.00         | 0.17 ± 0.11     | 0.09 ± 0.00         | 0.812     |
| 3 – amino – 3 - (2,4 –difluorophenyl) propanoic acid | 0.11 ± 0.00 | 0.11 ± 0.00 | 0.22 ± 0.13 | 0.743 |
| 2,4,6 – Cycloheptatrien – 1 - one, 3,5 – bis – trimethyl silyl -  | 0.19 ± 0.14         | 0.12 ± 0.00     | 0.12 ± 0.00         | 0.457     |
| 3,5 – Di – t - butylbenzoic acid       | 0.65 ± 0.56         | 0.40 ± 0.00     | 2.50 ± 2.34         | 0.538     |
| Phosphine, 1,2 – ethenediylibis [bis (1 - methylthelyl) - | 0.34 ± 0.26         | 0.44 ± 0.24     | 0.313     |
| 4 – tert – Butyl – 1 - [(3Z) – 5 –hydroxy – 3 – methyl – 3 – penten – 1 - ynyl] cyclohexanol | 0.18 ± 0.11         | 0.32 ± 0.21     | 0.314     |
| 3 – hydroxy – 3 – (1 H – indol - 3 - yl) – 1 H – indol – 2 - one | 0.15 ±0.00         | 0.37 ± 0.23     | 0.18 ± 0.11         | 0.626     |
| 1 – bromonona fluorobutane            | 12.73 ± 7.53        | 8.08 ± 0.00     | 5.28 ± 0.00         | 0.759     |
| 1,2 – Benzisothiazol – 3 - amine tbdms | 1.64 ± 0.82         | 1.00 ± 0.73     | 0.28 ± 0.17         | 0.365     |
| Phosphonic acid, (p - hydroxy phenyl) - | 0.95 ± 0.00         | 0.22 ± 0.15     | 0.478     |
| Succinic acid, di (2 - proplyphenyl) ester | 0.07 ± 0.00         | 0.11 ± 0.00     | 1.98 ± 0.00         | 0.437     |
| N - (2,6 - dimethylphenyl) – 2 - (4 –nitrophenyl) – 2 – piperidin 1 - ylacetamide | 0.09 ± 0.00         | 0.13 ± 0.07     | 0.10 ± 0.00         | 0.955     |
| Margaric acid, (Heptadecanoic acid, tert - butyl dimethylsilyl ester) | 0.11 ± 0.00         | 0.08 ± 0.00     | 0.608     |
| 1 H - Indole                          | 0.90 ± 0.73         | 0.22 ± 0.13     | 1.63 ± 0.50         | 0.214     |
| Nonadecan – 1 - ol trimethylsilyl ether | 0.22 ± 0.00         | 1.22 ± 0.47     | 0.042     |
| 1 - Propene, 2 – methoxy -            | 0.19 ± 0.00         | 0.59 ± 0.00     | 0.518     |
| Furan, 3 – methyl -                   | 0.12 ± 0.00         | 0.34 ± 0.20     | 0.248     |
| Cyclobutane, ethyl 1 -               | 0.26 ± 0.00         | 0.33 ± 0.00     | 0.615     |
| 3 - Methylbutanal                     | 0.10 ± 0.00         | 0.68 ± 0.00     | 1.92 ± 0.96         | 0.189     |
| Urea (Carbamimidic acid)              | 0.28 ± 0.00         | 0.06 ± 0.00     | 0.29 ± 0.00         | 0.748     |
| Pentanenitrile, 4 – methyl 1          | 0.15 ± 0.00         | 0.46 ± 0.00     | 0.522     |
| 1,1 – bis (4 – methyl cyclohexyl) dodecane | 0.09 ± 0.00         | 0.33 ± 0.19     | 0.197     |
| Trimethyl [4 - (1,1,3,3,- tetra methyl butyl) phenoxy] silane | 0.29 ± 0.00         | 0.22 ± 0.13     | 0.530     |
| phenyl acetaldehyde                   | 0.25 ± 0.00         | 0.15 ± 0.00     | 0.35 ± 0.20         | 0.795     |
| 3 - Phenylpropiononitrile             | 0.13 ± 0.00         | 0.10 ± 0.00     | 0.67 ± 0.21         | 0.087     |
| 2 – Buten – 1 - one, 1 - (2,6, 6 – trimethyl - 1,3 – cyclo hexadien – 1 - yl) - , (E) - | 0.09 ± 0.00         | 0.17 ± 0.10     | 0.328     |
| Methyl ethyl acetaldehyde (Butanal, 2 – methyl - ) | 0.17 ± 0.00         | 0.29 ± 0.00     | 0.587     |
| 2 – Methyl - 1H - pyrrole             | 1.02 ± 0.00         | 0.11 ± 0.00     | 0.441     |
| Pyrimidin – 2 - one, 4 - [N -methylureido] - 1 - [4 – methyl amino carbonyl oxy methyl | 0.15 ± 0.00         | 0.26 ± 0.00     | 0.582     |
| Phenol, 4,4’- [thiobiis (methylene)] bis[2,6 – bis (1,1 - dimethylethyl) - | 1.55 ± 0.00         | 10.50 ± 7.20     | 0.223     |
**Table 2. (Continued).**

| Compound name* | Preestrus $(X \pm S\%)$ | Oestrus $(X \pm S\%)$ | Postestrus $(X \pm S\%)$ | P value** |
|----------------|--------------------------|-----------------------|--------------------------|-----------|
| $5\beta$ – Cholestane - 3α,7α,12α,24,25, 26 - hexol hexa TMS | 0.67 ± 0.39 | 0.17 ± 0.11 | 0.14 ± 0.00 | 0.282 |
| 4 - methylphenol (p - cresol) | 0.07 ± 0.00 | 2.02 ± 0.00 | 3.17 ± 2.46 | 0.509 |
| Silane, 1,4 – phenyl enebis [trimethyl 1- | 0.15 ± 0.00 | 1.00 ± 0.00 | 0.14 ± 0.00 | 0.597 |
| 2 – Methoxy - 1,3 – dithiole - 4,5 - dicarboxylic acid dimethyl ester | 0.13 ± 0.08 | 0.08 ± 0.00 | 0.364 |
| 3 - (2 - Methoxyethyl) – 2 - (2 -pyridinyl) - 1H - indole | 0.87 ± 0.00 | 0.17 ± 0.00 | 0.472 |
| 2 - (Dichloromethyl) thiophene | 0.08 ± 0.00 | 0.34 ± 0.00 | 0.492 |
| Perfluorotributylamine | 0.39 ± 0.27 | 0.32 ± 0.24 | 0.413 |
| 4 - methylpentane | 0.12 ± 0.00 | 0.61 ± 0.00 | 0.475 |
| 6 – Methyl 1 - 2 – phenyl - quinoline | 0.05 ± 0.00 | 0.19 ± 0.00 | 0.493 |
| Perfluoro (dibutylmethylamine) | 0.19 ± 0.00 | 0.22 ± 0.00 | 0.620 |
| 9 – t – Butyl – 4 – iodo - 2,2 – dimethyladamantane | 0.07 ± 0.00 | 0.10 ± 0.00 | 0.610 |
| Tricyclo [2.2.1.0(2,6)] heptane, 3,5 – dioxo - | 0.05 ± 0.00 | 0.09 ± 0.00 | 0.582 |
| 4,4 – Dimethyloxadolidine – 2 - thione | 0.19 ± 0.00 | 0.16 ± 0.00 | 0.618 |

* As a result of the analysis, 36 compounds in the preestrus period, 30 compounds in the estrus period and 24 compounds in the postestrus period were not included in the comparison table, since they were detected in only one period.

**P < 0.05.

**Table 3.** Volatile odor compounds that were detected in blood during the estrus period (%).

| Compound name* | Preestrus $(X \pm S\%)$ | Oestrus $(X \pm S\%)$ | Postestrus $(X \pm S\%)$ | P value** |
|----------------|--------------------------|-----------------------|--------------------------|-----------|
| Pentane, 2 – methyl - | 0.99 ± 0.93 | 0.47 ± 0.26 | 3.54 ± 1.01 | 0.043 |
| Acetic acid, isocyanato - , butyl ester | 1.54 ± 1.10 | 2.11 ± 1.84 | 6.45 ± 4.20 | 0.414 |
| 3 - methyl pentane | 0.18 ± 0.00 | 1.35 ± 0.85 | 0.173 |
| Methyl - cyclopentane | 0.42 ± 0.00 | 0.47 ± 0.35 | 0.71 ± 0.42 | 0.858 |
| 1 - Propanamine, 3 – dibenzo [b,e]thiepin - 11(6H) – ylidene - N,N – dimethyl - , S - oxide | 0.17 ± 0.00 | 2.88 ± 0.00 | 0.425 |
| N - Heptane | 0.19 ± 0.00 | 0.52 ± 0.31 | 0.68 ± 0.28 | 0.436 |
| 1,2 – Benzisothiazol – 3 - amine tbdms | 0.22 ± 0.16 | 0.78 ± 0.67 | 0.393 |
| Dimethyl disulphide | 0.80 ± 0.68 | 0.07 ± 0.00 | 0.326 |
| [4 - (Trimethylsiloxy) phenyl] propenoic acid methyl ester | 0.74 ± 0.46 | 0.26 ± 0.00 | 0.278 |
| 5 – Bromo - 2,4 – bis (methylsulfanyl) pyrimidine | 0.79 ± 0.53 | 0.35 ± 0.23 | 0.297 |
| Hexanal | 9.24 ± 8.66 | 15.54 ± 8.86 | 11.27 ± 9.39 | 0.881 |
| 1 - Pyridineacetamide, 3 – cyano – N - (3,4 -dichlorophenyl) - 1,2 – dihydro – 4 - (methoxymethyl) – 6 – methyl – 2 – oxo - | 0.07 ± 0.00 | 0.06 ± 0.00 | 0.619 |
| 4 - (2 – Imino – 4 – oxo – thiazolidin – 3 - yl) - benzoic acid ethyl ester | 0.04 ± 0.00 | 0.06 ± 0.00 | 0.597 |
| Pyrimidin – 2 - one, 4 - [N -methylureido] – 1 - [4 – methylamino carboxyloxymethyl | 0.04 ± 0.00 | 0.12 ± 0.00 | 0.524 |
| 3 – hydroxy – 3 - (1H – indol – 3 - yl) - 1H – indol – 2 -one | 0.20 ± 0.17 | 0.15 ± 0.00 | 0.563 |
| Nonadecan – 1 - ol trimethylsilyl ether | 0.33 ± 0.00 | 2.34 ± 1.79 | 0.268 |
| Benzaldehyde (Benzoic acid aldehyde) | 0.19 ± 0.00 | 0.18 ± 0.10 | 0.514 |
| 2 – Octen – 1 - ol, (Z) - | 0.24 ± 0.00 | 0.24 ± 0.15 | 0.509 |
| 2 - n - Pentylfuran | 0.28 ± 0.00 | 0.34 ± 0.20 | 0.463 |
| Caprylic aldehyde (Octanal) | 0.17 ± 0.00 | 0.29 ± 0.17 | 0.893 |
Table 3. (Continued).

| Compound name* | Preestrus (x̄ ± Sx) | Oestrus (x̄ ± Sx) | Postestrus (x̄ ± Sx) | P value** |
|----------------|---------------------|------------------|---------------------|----------|
| Methane, isocyanato - | 0.20 ± 0.12 | 0.25 ± 0.18 | 0.356 |
| 1 - Propanamine, 3 – dibenzo [b,e]thiepin - 11(6H) – ylidene - N,N – dimethyl - , S – oxide | 1.49 ± 1.18 | 1.38 ± 0.80 | 0.399 |
| Pentane, 2 – methyl - | 2.78 ± 1.58 | 0.88 ± 0.38 | 1.31 ± 0.00 | 0.518 |
| 3 - methyl pentane | 1.24 ± 0.56 | 0.90 ± 0.62 | 3.19 ± 2.48 | 0.535 |
| 4 – Imino - 6,6 – dimethyl - 5,8 – dihydro - 4H – thiopyrano [4',3':5,4] furo[2,3 - d]pyrimidin - 3(6H) - amine # | 0.26 ± 0.00 | 0.34 ± 0.00 | 0.613 |
| Benzimidazole – 5 - carboxylic acid, 2 – methyl – 1 – phenyl - | 0.09 ± 0.00 | 0.09 ± 0.00 | 0.622 |
| Cyclobutene, 2 – propenylidene - | 0.61 ± 0.00 | 0.65 ± 0.00 | 0.442 |
| Hexanal | 0.22 ± 0.00 | 0.30 ± 0.18 | 0.08 ± 0.00 | 0.660 |

* As a result of the analysis, 37 compounds in the preestrus period, 43 compounds in the estrus period and 33 compounds in the postestrus period were not included in the comparison table, since they were detected in only one period.

**P < 0.05.

Table 4. Volatile odor compounds that were detected in milk during the estrus period (%).
| Compound                                                                 | ppm   | Standard Deviation | p Value  |
|--------------------------------------------------------------------------|-------|--------------------|----------|
| Pentanenitrile, 4 – methyl -                                             | 0.33 ± 0.00 | 0.15 ± 0.09 | 0.520    |
| Trimehtylsilyl fluoride (Silane, fluorotrimethyl - )                     | 3.12 ± 0.00 | 0.31 ± 0.00 | 0.439    |
| Oxime - , methoxy – phenyl - _                                           | 1.23 ± 0.00 | 5.81 ± 0.00 | 0.480    |
| Benzaldehyde (Benzoic acid aldehyde)                                    | 0.12 ± 0.00 | 0.15 ± 0.11 | 0.501    |
| Cyclotetrasiloxane, octamethyl                                          | 1.42 ± 0.64 | 1.04 ± 0.51 | 1.03 ± 0.66 | 0.878 |
| Fenol, 2,6 – di – tert – butil – 4 – etil -                             | 0.27 ± 0.00 | 0.56 ± 0.00 | 1.85 ± 0.00 | 0.591 |
| phenyl acetaldehyde                                                    | 0.28 ± 0.00 | 0.45 ± 0.18 | 0.18 ± 0.00 | 0.665 |
| 3 - Phenylpropiononitrile                                               | 0.25 ± 0.00 | 0.54 ± 0.22 | 0.18 ± 0.00 | 0.472 |
| 1 H - Indole                                                            | 0.62 ± 0.00 | 0.89 ± 0.44 | 0.44 ± 0.00 | 0.825 |
| 1,2,4 - Benzenetrikarbonsilik asit, 1,2 - dimetil ester                 | 0.21 ± 0.00 | 0.04 ± 0.00 | 0.42 ± 0.00 | 0.625 |
| Silane, ethoxytriethyl -                                               | 0.07 ± 0.00 | 0.20 ± 0.00 | 0.474    |
| (2 – Methyl - [1,3]dioxolan - 2 - yl) - acetic acid, phenyl ester       | 0.08 ± 0.00 | 0.17 ± 0.00 | 0.509    |
| 2 - Hexenoic acid, 5 – hydroxy - 3,4,4 – trimethyl - , (E) -             | 0.33 ± 0.00 | 0.12 ± 0.00 | 0.480    |
| Dimethyl sulfone                                                        | 1.21 ± 0.45 | 0.92 ± 0.00 | 0.386    |
| 3 – Methyl - 1,2 - cyclopentanedione                                     | 1.01 ± 8.43 | 1.29 ± 0.00 | 0.040    |
| toluene                                                                 | 1.65 ± 0.00 | 3.65 ± 2.46 | 0.337    |
| Silane, dimethyl (dimethyl (non – 5 – yn – 3 - yloxy) silyloxy) ethoxy - | 0.60 ± 0.34 | 0.93 ± 0.00 | 0.546    |
| [4 - (Trimethylsiloxy) phenyl] propenoic acid methyl ester              | 0.18 ± 0.16 | 0.17 ± 0.09 | 0.913    |
| 5β – Cholestane - 3a,7a,12a, 24,25,26 - hexol hexa TMS                  | 0.04 ± 0.00 | 0.12 ± 0.00 | 0.531    |
| 1,2 – Benzisothiazol - 3- amine tbms                                     | 0.04 ± 0.00 | 1.32 ± 1.22 | 0.366    |
| Octadecane,3 – ethyl – 5 – (2 - ethylbutyl) -                            | 10.23 ± 0.00 | 1.38 ± 1.21 | 0.382    |
| Oleic acid, eicosyl ester (9 – Octadecenoic acid (Z) -, eicosyl ester)  | 0.04 ± 0.00 | 0.12 ± 0.00 | 0.32 ± 0.12 | 0.184 |
| 4 - methylphenol (p - cresol)                                           | 0.45 ± 0.28 | 0.39 ± 0.00 | 0.487    |
| Pentanamid, N - 1 H – purin – 6 – il -                                  | 0.51 ± 0.30 | 0.13 ± 0.00 | 0.191    |
| 1,4 - Benzenedipropanol, alpha., alpha.`. gamma., gamma., gamma.`. gamma.`. hexamethyl | 0.85 ± 0.22 | 0.16 ± 0.00 | 0.371    |
| 2 - Oxaclolidimethione,4,4 - dimethyl -                                 | 0.05 ± 0.00 | 0.19 ± 0.00 | 0.495    |
| Pyrimidin – 2 - one, 4 - [N - methylureido] – 1 - [4 – methyl aminocarboxyloxymerthyl | 0.52 ± 0.28 | 0.19 ± 0.00 | 0.17 ± 0.10 | 0.440 |
| Dodecanoic acid, tricosafluoro                                          | 0.53 ± 0.23 | 0.35 ± 0.00 | 0.30 ± 0.19 | 0.819 |
| 1,1 – bis (4 – methyl cyclohexyl) dodecane                               | 0.15 ± 0.00 | 0.73 ± 0.00 | 0.569    |
| Bicyclo[2,2,0] hex – 2 – ene – 1 - carboxylic acid, 5,5,6,6 – tetra cyano - 2,3,4 – tri (1,1 – dimethylethyl) - , 1,1 - dimethylethyl ester | 0.24 ± 0.00 | 0.10 ± 0.00 | 0.17 ± 0.00 | 0.846 |
| 1 - bromononafluorobutane                                               | 0.14 ± 0.00 | 0.33 ± 0.00 | 0.08 ± 0.00 | 0.698 |
| N - (2,6 - dimethylphenyl) – 2 - (4 - nitrophenyl) -2 – piperidin – 1 - ylacetamide | 0.47 ± 0.22 | 0.05 ± 0.00 | 0.053    |
| Nonadecan – 1 - ol trimethylsilyl ether                                  | 0.58 ± 0.21 | 0.34 ± 0.00 | 0.301    |
| Acetic acid, 1 – acetoxy - 10a, 12a – dimethyl – 5 – oxo – hexa decahydro - 6 – oxabenzo [3,4 ] cyclohepta [1,2 - E] inden - 8 – yl ester | 4.81 ± 2.78 | 0.86 ± 0.69 | 2.14 ± 1.24 | 0.331 |
| 3,5 - Di - t - butylenzoic acid                                         | 1.33 ± 0.00 | 1.30 ± 0.00 | 0.560    |
| 2,2 – dimethyl – N - [2,2,2 – trichloro – 1 - (2 – methyl anilino) ethyl] propanamid | 0.40 ± 0.24 | 0.36 ± 0.21 | 0.389    |
| Perfluoro (dibutylmethylamine)                                           | 0.78 ± 0.00 | 0.90 ± 0.00 | 0.622    |
| Perfluorotributylamine                                                  | 0.45 ± 0.35 | 0.23 ± 0.00 | 1.35 ± 1.25 | 0.565 |
Table 4. (Continued).

| Compound name* | Preestrus (X ± Sx) | Oestrus (X ± Sx) | Postestrus (X ± Sx) | P value** |
|----------------|--------------------|-----------------|--------------------|----------|
| N,N’ – Dimethyl – 2 – nitro - 1,1 - ethenediamine | 0.32 ± 0.00 | 0.58 ± 0.00 | 0.578 |
| 1,3 - Bis (octylthio) propane | 0.08 ± 0.00 | 0.26 ± 0.00 | 0.512 |
| Silane, trimethyl (octadecyloxy) | | 0.31 ± 0.00 | 0.73 ± 0.00 | 0.489 |
| 1,1,1,3,5,7,9,11,11 – Decamethyl – 5 -(Trimethyl silyloxy) Hexasiloxane | 0.19 ± 0.00 | 0.22 ± 0.00 | 0.620 |
| 4 - (2 – Imino – 4 – oxo – thiazolidin – 3 - yl) - benzoic acid ethyl ester | 0.22 ± 0.00 | 1.70 ± 0.00 | 0.450 |
| 9 - Octadecenoic acid, 2 - (octadecyloxy) ethyl ester | 0.11 ± 0.00 | 0.06 ± 0.00 | 0.508 |
| Benzene, [3 - (2 – cyclo hexyl ethyl) – 6 -cyclopentylhexyl] - | 0.16 ± 0.00 | 0.78 ±0 .00 | 0.09 ± 0.00 | 0.869 |
| 8,14 – Seco - 3,19 – epoxy androstane - 8,14 - dione, 17 – acetoxy - 3.beta.- methoxy - 4,4 –dimethyl - | 0.09 ± 0.00 | 0.05 ± 0.00 | 0.21 ± 0.00 | 0.689 |
| 3 – Tert – Butylsulfanyl - 3 – fluoro – 2 –trifluoromethyl - acrylic acid ethyl ester | 0.22 ± 0.00 | 0.07 ± 0.00 | 0.519 |
| 5 (4H) - Thebenidinone | 0.12 ± 0.00 | 1.50 ± 0.00 | 0.432 |
| 1 - Pyridineacetamide, 3 – cyano – N - (3,4 - dichlorophenyl) - 1,2 – dihydro – 4 - (methoxymethyl) – 6 – methyl – 2 – oxo - | 0.30 ± 0.00 | 0.08 ± 0.00 | 0.501 |

* As a result of the analysis, 30 compounds in the preestrus period, 27 compounds in the estrus period and 49 compounds in the postestrous period were not included in the comparison table, since they were detected in only one period.

**P < 0.05.

Table 5. Volatile odor compounds that were detected in sudor during the estrus period (%).

| Compound name* | Preestrus (X ± Sx) | Oestrus (X ± Sx) | Postestrus (X ± Sx) | P value** |
|----------------|--------------------|-----------------|--------------------|----------|
| Aceticacid, isocyanato - ,butyl ester | 3.84 ± 2.41 | 0.41 ± 0.25 | 0.37 ± 0.18 | 0.189 |
| n - Heptane | 1.88 ± 1.26 | 2.64 ± 2.00 | 6.50 ± 3.01 | 0.329 |
| 5β – Cholestane - 3α,7α,12α,24α,25 - pentol TMS | 0.06 ± 0.00 | 0.11 ± 0.00 | 0.577 |
| 2 - methoxy - 4 - (1 – methyl - 3,6 – dihydro -2H  -pyridin – 4 - yl) phenol | 0.07 ± 0.00 | 0.27 ± 0.00 | 0.494 |
| Octadecane,3 – ethyl – 5 - (2 - ethylbutyl) - | 0.24 ± 0.15 | 0.13 ± 0.00 | 0.06 ± 0.00 | 0.596 |
| Oleicacid, eicosyl ester (9 - Octadecenoic acid (Z) -, eicosyl ester) | 0.21 ± 0.12 | 0.04 ± 0.00 | 0.28 ± 0.00 | 0.629 |
| 5β – Cholestane - 3α,7α,12α,24,25,26 - hexol hexa TMS | 0.24 ± 0.15 | 0.78 ± 0.00 | 0.56 ± 0.43 | 0.767 |
| Perfluorotributylamine | 0.28 ± 0.17 | 0.04 ± 0.00 | 0.164 |
| Hexanal | 0.93 ± 0.19 | 0.47 ± 0.11 | 0.18 ± 0.11 | 0.014 |
| Dodecanoicacid, tricosafluoro - | 0.26 ± 0.10 | 0.06 ± 0.00 | 0.061 |
| Benzaldehyde (Benzoicacidaldehyde) | 0.42 ± 0.00 | 0.09 ± 0.00 | 0.28 ± 0.10 | 0.662 |
| N,N – Diethyl – 2 - (4 - chlorophenyl) – 3 –morpholino - thioacrylamide | 0.15 ± 0.09 | 0.20 ± 0.12 | 0.274 |
| Cyclotetrasiloxane, octamethyl - | 0.68 ± 0.37 | 0.23 ± 0.07 | 0.29 ± 0.11 | 0.349 |
| Nonanaldehyde | 0.59 ± 0.34 | 0.55 ± 0.19 | 0.48 ± 10.12 | 0.939 |
| Margaricacid, (Heptadecanoicacid, tert -butyldimethylsilyl ester) | 0.23 ± 0.13 | 0.11 ± 0.00 | 0.302 |
| Nonadecan – 1 - ol trimethylsilylether | 0.37 ± 0.00 | 0.40 ± 0.00 | 0.988 |
| Pyrazole – 3 - carboxylic acid, 4 - iodo – 1 –methyl - (4 – Iodo – 1 – methyl - 1H – pyrazole - 3 - carboxylic acid #) | 0.31 ± 0.00 | 0.11 ± 0.00 | 0.06 ± 0.00 | 0.632 |
| 1 – Methyl - 2,5 – dichloro - 1,6 - diazaphenalene | 0.45 ± 0.26 | 0.20 ± 0.00 | 0.57 ± 0.00 | 0.787 |
| Dimethylbenzo (b) thiophene - 2,5 - dicarboxylate | 0.28 ± 0.00 | 0.23 ± 0.00 | 0.08 ± 0.00 | 0.798 |
| Compound                                                                 | Concentration | Concentration | Concentration | Concentration |
|--------------------------------------------------------------------------|---------------|---------------|---------------|---------------|
| Pentane, 2 - methyl - (Isohexane)                                         | 1.83 ± 1.58   | 1.62 ± 1.06   | 0.24 ± 0.14   | 0.561         |
| Ethene, methoxy -                                                        | 0.24 ± 0.00   | 0.13 ± 0.00   |               | 0.578         |
| 3 - methyl pentane                                                       | 0.13 ± 0.00   | 1.06 ± 0.44   | 0.80 ± 0.45   | 0.233         |
| 4 - Imino - 6,6 - dimethyl - 5,8 - dihydro - 4H - thiopyrano [4',3':4,5] furo [2,3 - d] pyrimidin - 3 (6H) - amine # | 0.23 ± 0.00   | 5.65 ± 0.00   | 0.23 ± 0.00   | 0.488         |
| Cyclobutene, 2 - propenylidene -                                        | 0.81 ± 0.54   | 0.29 ± 0.12   | 0.06 ± 0.00   | 0.266         |
| Trimethylsilylfluoride                                                   | 2.96 ± 0.00   | 0.45 ± 0.00   |               | 0.459         |
| Bicyclo[2,2,0]hex - 2 - ene - 1 - carboxylic acid, 5,5,6,6 - tetra cyano - 2,3,4 - tri (1,1 - di methyl) - , 1,1 - dimethylethyl ester |             | 0.09 ± 0.00   | 0.17 ± 0.11   | 0.373         |
| Pentanenitrile, 4 - methyl 1 -                                          | 0.20 ± 0.00   | 0.56 ± 0.39   | 0.64 ± 0.23   | 0.526         |
| 1,2 - Dimethyl - 4,5 - bis (trimethylsilyl) benzene                       | 0.14 ± 0.00   | 0.16 ± 0.00   |               | 0.620         |
| 4 - methylphenol (p - cresol)                                            | 0.73 ± 0.46   | 0.85 ± 0.49   | 0.47 ± 0.00   | 0.848         |
| 3 - Phenylpropiononitrile                                               | 0.56 ± 0.33   | 0.37 ± 0.25   | 0.92 ± 0.34   | 0.464         |
| Phenylacetaldehyde                                                      | 0.33 ± 0.00   | 0.38 ± 0.00   | 0.65 ± 0.39   | 0.795         |
| 1 H - Indole                                                            | 1.56 ± 0.00   | 1.74 ± 1.00   | 2.40 ± 1.46   | 0.865         |
| 1H – Indole – 3 - propanoic acid, „alpha," - hydroxy - , methyl ester    |               | 0.09 ± 0.00   | 0.09 ± 0.00   | 0.622         |
| Oxalic acid, isoxyleneopentyl ester                                      | 0.27 ± 0.00   | 0.11 ± 0.00   |               | 0.548         |
| Phenol, 2,6 - di-tert – butyl 1 - 4 - ethyl -                            | 0.07 ± 0.00   | 0.06 ± 0.00   | 0.06 ± 0.00   | 0.977         |
| 3 - hydroxy – 3 - (1H - indol - 3 - yl) - 1H – indol – 2 - one            | 0.20 ± 0.00   | 0.13 ± 0.09   | 0.04 ± 0.00   | 0.674         |
| Silane, 1,4 - phenylenebis [trimethyl - 1,4 - Benzenedipropanol, „alpha,"- „gamma”- „gamma”- „gamma”- „gamma”- „gamma” - hexamethyl - | 0.15 ± 0.00   | 0.16 ± 0.00   | 0.42 ± 0.00   | 0.730         |
| 1,2 - Benzisothiazol – 3 - aminetbdms                                    | 0.55 ± 0.32   | 0.17 ± 0.00   | 0.05 ± 0.00   | 0.266         |
| 4 - (2 - Imino – 4 - oxo – thiazolidin – 3 - yl –benzoic acid ester      | 0.09 ± 0.00   |               | 0.09 ± 0.00   | 0.621         |
| N - (2,6 - dimethylphenyl) – 2 - (4 -nitrophenyl) – 2 – piperidin – 1 - ylacetamide | 0.12 ± 0.00   | 1.50 ± 0.00   | 0.12 ± 0.00   | 0.464         |
| Toluene                                                                 | 0.13 ± 0.00   | 1.90 ± 0.00   | 0.10 ± 0.00   | 0.450         |
| 1 - Propanamine, 3 – dibenzo [b,e] thiepin -11 (6H) – ylidene - N,N – dimethyl - , S - oxide | 0.28 ± 0.00   | 2.18 ± 1.50   |               | 0.216         |
| 2 – Methyl - 1H - pyrrole                                                | 0.20 ± 0.00   |               | 0.11 ± 0.00   | 0.577         |
| 2 – t – Butyl - 6,8 - dioxo - 5,5a,6,8a,8a,b –hexahydro - 3,7 – dioxa – 1 – aza – as – indacene – 1 - carboxylic acid, methyl ester | 0.78 ± 0.00   | 0.03 ± 0.00   |               | 0.420         |
| 9,9 – Dikloro – 9 - silafluorene                                          | 0.46 ± 0.00   |               | 0.11 ± 0.00   | 0.493         |
| Pyrazolidine, 3,5 – bis (phenylimino ) -                                 | 0.25 ± 0.00   |               | 0.15 ± 0.00   | 0.594         |
| Silanediol, dimethyl -                                                  | 0.26 ± 0.00   | 3.61 ± 2.29   |               | 0.156         |
| ,psi, ,psi, - Carotene, 3,4 – didehydro - 1,2 - dihydro - 1-methoxy - (Lycopene) |               |               | 0.20 ± 0.00   | 0.09 ± 0.00   |
| 1,1,1,3,5,7,9,11,11,11 – Deca methyl – 5 - (Trimethylsilyl) Hexasloxane   | 0.40 ± 0.00   | 0.12 ± 0.00   |               | 0.508         |
| Benzimidazole – 5 - carboxylic acid, 2 – methyl - 1 – phenyl -           | 0.51 ± 0.00   | 1.41 ± 0.00   |               | 0.530         |
| Cyclopropylmethycarbinol                                                | 0.43 ± 0.00   | 0.13 ± 0.00   |               | 0.509         |
| Chloromethylpropionate                                                  | 0.65 ± 0.00   | 0.11 ± 0.00   |               | 0.465         |
| 8,14 – Seco - 3,19 – epoxy androstane – 8,14 - dione, 17 – acetoxy - 3,beta, - methoxy - 4,4 – dimethyl - | 0.09 ± 0.00   | 0.21 ± 0.00   |               | 0.544         |
Table 5. (Continued).

| Compound name*                                      | Preestrus (x ± Sx) | Oestrus (x ± Sx) | Postestrus (x ± Sx) | P value** |
|-----------------------------------------------------|--------------------|------------------|---------------------|-----------|
| 3 – Methoxy – 19 – norpregna - 1,3,5 (10), 20-tetren – 17 - ol trifluoroacetate | 0.09 ± 0.00        | 0.13 ± 0.00      | 0.610               |           |
| 2 - [(4-hydroxy – 4 – methyloxan - 3- yl) amino] – 3 - (1H – i ndol – 2 - yl) propanoic acid | 0.19 ± 0.00        | 0.31 ± 0.00      | 0.596               |           |
| 3 - Methylbutanal                                   | 0.72 ± 0.00        | 1.48 ± 0.00      | 0.565               |           |
| Methylethylacetaldehyde (Butanal, 2 – methyl)       | 0.37 ± 0.00        | 0.29 ± 0.00      | 0.614               |           |

* As a result of the analysis, 34 compounds in the preestrus period, 31 compounds in the estrus period and 37 compounds in the postestrus period were not included in the comparison table, since they were detected in only one period.

**P < 0.05.

Table 6. Volatile odor compounds that were detected in saliva during the estrus period (%).

| Compound name*                                      | Preestrus (x ± Sx) | Oestrus (x ± Sx) | Postestrus (x ± Sx) | P value** |
|-----------------------------------------------------|--------------------|------------------|---------------------|-----------|
| 1,2 – Dimethyl - 4,5 - bis (trimethylsilyl) benzene   | 0.08 ± 0.00        | 0.07 ± 0.00      | 0.613               |           |
| Propanal, 2 – methyl - (Isobutyrylaldehyde)          | 0.74 ± 0.00        | 0.08 ± 0.00      | 0.442               |           |
| N,N – Dimethyl – 1 - (4 - [3 - (1 - piperidyl) propoxy] phenyl) – 2 - propylamine | 1.50 ± 0.65        | 4.75 ± 0.00      | 1.50 ± 0.65         | 0.650     |
| 1 - Propene, 2 – methoxy -                           | 0.32 ± 0.23        | 0.85 ± 0.00      | 0.81 ± 0.00         | 0.836     |
| Pentane, 2 – methyl -                               | 0.47 ± 0.30        | 0.98 ± 0.33      | 3.44 ± 2.31         | 0.304     |
| 3 - methyl pentane                                  | 0.71 ± 0.30        | 1.56 ± 0.53      | 4.78 ± 3.78         | 0.425     |
| Aceticacid, isocyanato -, butyl ester               | 2.03 ± 0.00        | 2.12 ± 1.67      | 2.22 ± 1.67         | 0.558     |
| Butanal, 3 – methyl -                               | 7.42 ± 6.89        | 0.96 ± 0.00      | 1.17 ± 0.55         | 0.468     |
| Cyclobutane, ethyl -                                | 0.12 ± 0.00        | 1.26 ± 0.64      | 1.51 ± 0.90         | 0.309     |
| Cyclopentylmethylcarbinol                           | 0.11 ± 0.00        | 0.44 ± 0.37      | 0.369               |           |
| n - Heptane                                         | 0.52 ± 0.32        | 0.21 ± 0.00      | 3.21 ± 1.50         | 0.078     |
| 4 – Imino - 6,6 – dimethyl - 5,8 – dihydro - 4H - thiopyrano [4’,3’;4,5] furo [2,3 - d] pyrimidin – 3 (6H ) - amine # | 0.15 ± 0.00        | 0.20 ± 0.00      | 0.612               |           |
| 1 – Amino – 4 - (phenylthio) isoquinoline            | 0.22 ± 0.00        | 0.10 ± 0.00      | 0.561               |           |
| 3 – hydroxy – 3 - (1H – indol – 3 - yl) - 1H – indol – 2 - one | 0.28 ± 0.21        | 0.22 ± 0.07      | 0.38 ± 0.25         | 0.848     |
| Spiro [3.3] hepta - 1,5 - diene                      | 0.16 ± 0.09        | 0.65 ± 0.00      | 0.480               |           |
| Pentanamid, N - 1 H – purin – 6 – il -              | 0.15 ± 0.00        | 0.14 ± 0.00      | 0.15 ± 0.09         | 0.951     |
| 1,2 – Benzisothiazol - 3 -aminetbmdms               | 1.15 ± 0.70        | 0.66 ± 0.24      | 0.38 ± 0.22         | 0.503     |
| 2,4,6 – Cycloheptatrien – 1 - one, 3,5 – bis – trimethylsilyl - | 0.11 ± 0.00        | 0.06 ± 0.00      | 0.13 ± 0.00         | 0.875     |
| 1,1 – bis (4 - methylcyclohexyl) dodecane            | 0.07 ± 0.00        | 0.25 ± 0.00      | 0.503               |           |
| 3 – Phenyl - 2H - chromene                          | 0.06 ± 0.00        | 0.09 ± 0.00      | 0.608               |           |
| 3 - (2 - Methoxyethyl) – 2 - (2 - pyridinyl) - 1H - indole | 0.41 ± 0.15        | 0.09 ± 0.00      | 0.040               |           |
| Lycopene, 3,4 – didehydro - 1,2 – dihydro – 1 –methoxy -, all - trans | 0.21 ± 0.13        | 0.26 ± 0.00      | 0.534               |           |
| Haloxazolam (10 – bromo - 11b - (2 – fluorophenyl) - 2,3,5,7 – tetrahydroxazolo [3,2 - d] [1,4] benzodiazepin – 6 - one) | 0.18 ± 0.00        | 0.05 ± 0.00      | 0.498               |           |
| Benzaldehyde (Benzocicacidaldehyde)                  | 0.22 ± 0.13        | 0.10 ± 0.00      | 0.24 ± 0.09         | 0.602     |
| Perfluoro (dibutylmethylamine)                       | 0.14 ± 0.00        | 0.30 ± 0.00      | 0.558               |           |
| Phenylacetaldehyde                                  | 0.19 ± 0.00        | 0.23 ± 0.00      | 0.62 ± 0.22         | 0.331     |
| 4 - methylphenol (p - cresol)                        | 1.16 ± 0.74        | 0.11 ± 0.00      | 2.77 ± 1.64         | 0.535     |
| Compound                                                                 | Preestrus | Estrus   | Postestrus |
|-------------------------------------------------------------------------|-----------|----------|------------|
| 3,5 – Di-t-butylbenzoic acid                                            | 0.10 ± 0.00 | 0.21 ± 0.13 | 0.312      |
| 3 - Phenylpropiononitrile                                               | 0.42 ± 0.25 | 0.51 ± 0.18 | 0.64 ± 0.24 | 0.787      |
| 1 H - Indole                                                            | 1.75 ± 1.05 | 1.01 ± 0.67 | 1.41 ± 0.72 | 0.822      |
| 4 – Methyl - 1H - indole                                                | 0.09 ± 0.00 | 0.15 ± 0.00 | 0.593      |
| Oleicacid, eicosyl ester (9-Octadecenoic acid (Z), eicosyl ester)       |           |           | 1.37 ± 1.28 | 0.08 ± 0.00 | 0.377      |
| Pentanenitrile, 4 – methyl -                                            | 0.25 ± 0.00 | 0.38 ± 0.24 | 0.47 ± 0.18 | 0.780      |
| Cyclotetrasiloxane, octamethyl -                                       | 0.08±0.00 | 0.24±0.14 | 0.43 ± 0.21 | 0.401      |
| Nonanaldehyde                                                          | 0.63 ± 0.38 | 0.21 ±0.12 | 0.22 ± 0.14 | 0.413      |
| 1 - Pyridineacetamide, 3 – cyano – N - (3,4-dichlorophenyl) - 1,2 – dihydro – 4 - (methoxymethyl) - 6 – methyl - 2 – oxo - | 0.22 ± 0.00 | 0.08 ± 0.00 | 0.16±0.00 | 0.824      |
| Aceticacid, isocyanato -, butyl ester                                   | 0.48 ± 0.00 | 0.89 ± 0.00 |           | 0.577      |
| 1,1 – bis (4 - methylcyclohexyl)dodecane                                | 0.07 ± 0.00 | 0.07 ± 0.00 |           | 0.622      |
| Oleicacid, eicosyl ester (9 – Octa decenoic acid (Z), eicosyl ester)   | 0.07 ± 0.00 | 0.11 ± 0.00 |           | 0.586      |
| Cyclotrisiloxane, hexamethyl -                                         | 0.45 ± 0.27 | 0.41 ± 0.24 | 0.03 ± 0.20 | 0.902      |
| 3H - 1,4 - Benzodiazepine, 3 – morpholino – 5 - phenyl                  | 1.25 ± 0.00 |           | 0.06 ± 0.00 | 0.422      |
| 8,14 – Seco - 3,19 – epoxy androstone - 8,14 – dione, 17 – acetoxy – 3.beta. – methoxy - 4,4 – dimethyl - | 0.34 ± 0.23 | 0.18 ± 0.11 |           | 0.304      |
| 5β – Cholestane - 3a,7α,12α,24a,25 - pentol TMS                         | 0.24 ± 0.15 | 0.13 ± 0.00 | 0.14 ± 0.00 | 0.839      |
| Cyclotetrasiloxane, octamethyl -                                       | 0.07±0.00 | 0.09±0.00 | 0.27 ± 0.00 | 0.662      |
| Nonanaldehyde                                                          | 0.30 ± 0.00 |           | 0.10 ± 0.00 | 0.526      |
| Cyclopentasiloxane, decamethyl -                                       | 0.72 ± 0.45 | 0.17 ± 0.10 | 0.22 ± 0.13 | 0.332      |
| N - Hexane                                                              | 7.02 ± 0.00 | 4.65 ± 4.45 | 4.65 ± 4.28 | 0.939      |
| N,N – Diethyl – 2 - (4 - chlorophenyl) - 3- morpholino - thioacrylamide |           |           | 0.52 ± 0.29 | 0.19 ± 0.00 | 0.231      |
| Methane, dichloronitrito -                                              | 0.39 ± 0.00 | 0.38 ± 0.23 |           | 0.512      |
| Urea (Carbamimidicacid)                                                | 0.10 ± 0.00 | 0.05 ± 0.00 |           | 0.563      |
| Hexanal                                                                | 0.07 ± 0.00 | 0.17 ± 0.10 |           | 0.293      |
| 2 - Oxa – 4 – azabicyclo [4.2.0] octa - 3,7 – diene – 6 - carboxylic acid, 1,7,8 - tris (1,1 – dimethyl ethyl) – 3 - (2,2 - dimethylpropyl) – 5 - phenyl - , 1,1 - dimethyl ethyl ester | 0.07 ± 0.00 | 0.06 ± 0.00 | 2.55 ± 2.48 | 0.406      |
| 1,4 - Benzenediol, 2,6 - bis (1,1 -dimethylethyl) -                     |           | 0.06 ± 0.00 | 0.07 ± 0.00 | 0.622      |
| Dodecanoicacid, tricosafluoro -                                        | 0.13 ± 0.00 | 0.09 ± 0.00 | 0.07 ± 0.00 | 0.910      |
| Silane, 9 – anthracenyltrimethyl -                                     | 1.35 ± 0.00 | 1.15 ± 0.00 |           | 0.619      |
| Methylethylacetalddehyde (Butanal, 2 – methyl -)                       | 0.22 ± 0.13 | 0.58 ± 0.00 | 0.51 ± 0.00 | 0.841      |
| Aceticacid                                                             | 0.26 ± 0.00 | 0.22 ± 0.00 |           | 0.619      |
| Phosphine, 1,2 – ethanediylbis [bis (1 – methylthyl) -                  | 0.17 ± 0.00 | 0.19 ± 0.00 |           | 0.621      |
| Bicyclo [2.2.0] hex – 2 – ene – 1 - carboxylic acid, 5,5,6,6 – tetra cyano - 2,3,4 – tri (1,1 – dimethylthyl) - , 1,1 - dimethyl ethyl ester | 0.06 ± 0.00 | 0.07 ± 0.00 |           | 0.619      |
| 2 – Methyl - 1H - pyrrole                                              | 0.36 ± 0.00 | 0.61 ± 0.00 |           | 0.588      |
| Trimethyl [4 - (1,1,3,3, - tetramethylbutyl) phenoxyl] silane            | 0.10 ± 0.00 | 2.79 ± 0.00 |           | 0.417      |

* As a result of the analysis, 45 compounds in the preestrous period, 36 compounds in the estrus period and 48 compounds in the postestrous period were not included in the comparison table, since they were detected in only one period.

**P < 0.05."
detected in both the preestrus period (0.41 ± 0.15) and the estrus period (0.09 ± 0.00) and was calculated as \( P = 0.040 \).

3.7. Volatile chemical compounds of vaginal secretion

According to Table 7, a total of 191 volatile compounds were detected in vaginal secretion and it was found that there were 91 compounds in the preestrus period, 89 compounds in the estrus period, and 99 compounds in the postestrus period. As a result of the analysis of variance, 1,2-benzenothiazol-3-amine tbdms compound was determined to be important (\( P < 0.05 \)) in terms of interperiod differences. This compound was detected in both the preestrus period (0.61 ± 0.04) and the postestrus period (0.08 ± 0.00) and was calculated as \( P = 0.000 \).

3.8. The volatile compounds of body fluids in estrus period

A total of 1563 compounds were detected in the body secretions during the estrus period. In all body fluids, 531 volatile compounds were detected in the preestrus period, 538 in the estrus period, and 494 in the postestrus period. Furthermore, the number of compounds found in at least one of the body fluids is 216 and the rate is 69%. However, the number of compounds found in all body fluids is 8 and the rate is 2.6% (Table 8). In addition, a total of 8 compounds are common in all body fluids. The chemical formula and the Cas number of these compounds are shown in Table 9.

In addition, the compounds detected in all other body fluids except only one body fluid during the estrus period are acetic acid isocyanoacet-butyl ester (except milk), 3-methylbutanal (except milk), pyrimidine-2-one 4-N-methylureido]-1-[4-methylaminocarbonyloxymethyl (except sweat), dodecanenitrile 4-methyl (except vaginal secretion), and pentanenitrile 4-methyl (except vaginal secretion).

4. Discussion

As a result of the analysis, 85 compounds of feces were obtained from the cows in the estrus period. Sankar and Archunan [12] detected a total of 10 different volatile compounds, and acetic acid, propionic acid, and 1-iodo undecane compounds were found in the estrus period, butanoic acid, 2-propenyl ester, carboxylic acid, and pentanoic acid compounds were found in the preestrus period, and 3-hexanol, butane, 2,2-dimethyl, and phosphonic acid compounds were found in the postestrus period. Sankar and Archunan [12] detected acetic acid, propionic acid, and 1-iodo undecane compounds in feces during the estrus, which are similar to the results of the current study. Manikkara et al. [19] evaluated pheromone metabolites in urine and feces using a colorimetric method to predict cattle sex pheromones (acetic acid and propionic acid) with high precision. They detected a total of 45 compounds during the estrus period in feces. Mozūraitis et al. [20] found a total of 31 compounds in the estrus period in feces. Of these compounds, 9 were also found in the current study (Table 1). These compounds are butanol, pentanol, acetic acid, benzaldehyde, propanoic acid, pentanoic acid, dodecanal, p-cresol, and indole. In addition, Gnanamuthu et al. [21] found 13 compounds in the estrus period in feces, and 3-methyl-tricyclo, undecane, and 2, 10 dimethyloctacosanoic acid were specifically present in the estrus stage, not in other stages. As can be seen in Table 1, 1-iodo-2-methylundecane compound was detected only in the estrus period, but 2, 10 dimethyloctacosanoic acid and 3-methyl-tricyclo compounds were not detected in feces. Kumar et al. [11] found urine pheromone compounds including ethylbenzene, 2,2-dimethylbutane, 1-iodoundecane, di-n-propyl phthalate, oxiranemethanol, and 1-iododecane. Ethylbenzene and 2,2-dimethylbutane were detected in all three periods, while 1-iiodoundecane and di-n-propyl phthalate compounds were detected only in the estrus. They indicated that these compounds may have an important role in sexual attraction. Archunan and Ramesh Kumar [22] stated that these 2 specific compounds (1-iodoundecane and di-n-propyl phthalate) reveal the flehmen behavior of the bull and thus are important in estrus detection. As given in Table 2, undecane 6-ethyl compound was detected only in the estrus period, but di-n-propyl phthalate compound was not detected in urine. In addition, Muniasamy et al. [23] found the volatile compounds of 4-methyl phenol (p-cresol) and 9-octadecanoic acid (oleic acid) only in urine during the estrus. Muthukumar et al. [24] developed a p-cresol-based test kit since p-cresol detected in urine has been proven to be a candidate pheromone peculiar to anger in various studies. Klemm et al. [16] detected acetaldehyde compound in blood pheromones in the estrus period. They also reported that sexual hormones regulate the metabolic production of acetaldehyde. Table 3 shows that phenyl acetaldehyde, trimethyl acetaldehyde, methyl ethyl acetaldehyde, and acetaldehyde methylhydrzone compounds are similar to acetaldehyde compound in the estrus. Zebari et al. [25] reported that acetic acid (\( P < 0.001 \)), valeric acid (\( P = 0.016 \)), caproic acid (\( P < 0.001 \)), and myristoleic acid (\( P = 0.035 \)) concentrations were high during the estrus period. In Table 7, volatile chemical compounds such as phenylpropionic acid, acetic acid, carboxylic acid, hexenoic acid, benzeneticarboxylic acid, carbamimidic acid, dicarboxylic acid, and pentanoic acid were determined from the acid group during the estrus period in milk. Weidong et al. [26] identified about 80 volatile compounds in milk, which belong to ester, aldehyde, ketone, alcohol, fatty acid, and lactone class. At the end of the analysis, no specific compounds of postestrus, preestrus, and estrus
Table 7. Volatile odor compounds that were detected in vaginal secretion during the estrus period (%).

| Compound name* | Preestrus (X ± SX) | Oestrus (X ± SX) | Postestrus (X ± SX) | P value** |
|----------------|--------------------|-----------------|---------------------|----------|
| 2 - Propanamine, 2 – methyl - | 2.11 ± 0.00 | 2.15 ± 1.34 | 1.55 ± 0.00 | 0.962 |
| Pentane, 2 – methyl - | 0.98 ± 0.44 | 0.19 ± 0.11 | 1.21 ± 0.94 | 0.488 |
| 3 - methyl pentane | 1.58 ± 0.43 | 0.38 ± 0.17 | 0.81 ± 0.47 | 0.105 |
| Acetic acid, isocyanato - , butyl ester | 9.90 ± 8.75 | 1.22 ± 0.79 | 2.48 ± 1.95 | 0.470 |
| n - Heptane | 0.44 ± 0.07 | 0.70 ± 0.00 | 0.496 |
| 3 – hydroxy – 3 – (1H – indol – 3 - yl) - 1H – indol – 2 - one | 0.11 ± 0.07 | 0.32 ± 0.15 | 0.113 |
| N - (2,6 - dimethylphenyl) - 2 - (4 - nitrophenyl) – 2 –piperidin – 1 - ylacetamide | 0.14 ± 0.05 | 0.12 ± 0.07 | 0.153 |
| Oleic acid, eicosyl ester (9 - Octadecenoic acid (Z) –, eicosyl ester) | 0.20 ± 0.10 | 0.06 ± 0.00 | 0.06 ± 0.04 | 0.285 |
| 5β – Cholestane - 3a,7a,12a,24,25,26 - hexol hexa TMS | 0.13 ± 0.00 | 0.07 ± 0.00 | 0.18 ± 0.00 | 0.653 |
| 8,14 – Seco - 3,19 – epoxyandrostan - 8,14 - dione, 17 – acetoxy - 3.beta. – methoxy - 4,4 – dimethyl - | 0.13 ± 0.07 | 0.49 ± 0.23 | 0.06 ± 0.00 | 0.144 |
| 1,2 - Benzosothiazol - 3 - amine tbdms | 0.61 ± 0.04 | 0.08 ± 0.00 | 0.000 |
| Bicyclo [2.2.0] hex - 2 – e ne - 1 - carboxylic acid, 5,5,6,6 – tetracyano - 2,3,4 – tri (1,1 – dimethylthyl) -, 1,1 - dimethylethyl ester | 0.33 ± 0.19 | 4.14 ± 3.95 | 0.01 ± 0.00 | 0.402 |
| Pyrimidin – 2 - one, 4 - [N - methylureido] – 1 - [4 -methylaminocarbonyloxymethyl | 0.65 ± 0.34 | 0.07 ± 0.00 | 0.05 ± 0.00 | 0.096 |
| 5β – Cholestane - 3a,7a,12a,24a,25 - pentol TMS | 0.12 ± 0.07 | 0.24 ± 0.16 | 0.307 |
| 4 – tert – Butyl – 1 - [(3Z)- 5-hydroxy – 3 – methyl – 3 – penten – 1 - ynyl] cyclohexanol | 1.25 ± 0.00 | 0.05 ± 0.00 | 0.476 |
| Cyclotetrasiloxane,octamethyl - | 0.19 ± 0.07 | 0.44 ± 0.33 | 3.46 ± 3.03 | 0.383 |
| 4 - methylphenol (p - cresol) | 3.93 ± 2.82 | 1.39 ± 0.40 | 2.45 ± 0.00 | 0.664 |
| 1 - Pyridineacetamide, 3 – cyano – N - (3,4 –dichloro phenyl) - 1,2 – dihydro – 4 - (m ethoxy methyl) – 6 – methyl – 2 – oxo - | 0.15 ± 0.09 | 0.17 ± 0.10 | 0.288 |
| 16 – Methyl – heptadecane - 1,2 - diol, trimethylsilyl ether | 0.06 ± 0.00 | 0.08 ± 0.00 | 0.05 ± 0.00 | 0.953 |
| Nonadecan – 1 - ol trimethylsilyl ether | 0.60 ± 0.35 | 0.31 ± 0.00 | 8.40 ± 8.07 | 0.417 |
| 1 - Methyl - 2,5 - dichloro - 1,6 - diazaphenalene | 0.17 ± 0.00 | 0.70 ± 0.00 | 0.11 ± 0.00 | 0.566 |
| Benzo [4,5] imidazo [1,2 - a] pyridine – 4 - carbonitrile, 1 - (2 - diethylaminoethylamino) – 3 – methyl - | 0.24 ± 0.00 | 0.29 ± 0.00 | 0.618 |
| 3 - Methylbutanal | 0.55 ± 0.48 | 0.58 ± 0.31 | 0.07 ± 0.00 | 0.510 |
| 2 - Pentanone | 0.33 ± 0.21 | 0.31 ± 0.21 | 0.35 ± 0.00 | 0.995 |
| 2 – methoxy – 4 - ( 1- methyl - 3,6 – dihydro - 2H – pyridin – 4 - yl) phenol | 1.75 ± 0.00 | 0.09 ± 0.00 | 0.423 |
| 1 H - Indole | 0.78 ± 0.46 | 3.35 ± 1.50 | 0.066 |
| 1,1 – bis (4 - methylcyclohexyl) dodecane | 0.08 ± 0.00 | 0.14 ±0.07 | 0.298 |
| 2 – Methoxy - 1,3 – dithiole - 4,5 - dicarboxylic acid dimethyl ester | 0.11 ± 0.00 | 1.50 ± 0.00 | 0.430 |
| Cyclobutane, ethyl - | 0.06 ± 0.00 | 0.08 ± 0.00 | 0.609 |
| N,N – Diethyl – 2 - (4 – chloro phenyl) – 3 – morpholino - thioacrylamide | 0.10 ± 0.00 | 0.18 ± 0.10 | 0.345 |
| Dimethysilanediol | 0.18 ± 0.00 | 0.17 ± 0.00 | 0.622 |
| Phenol (carbolic acid) | 0.17 ± 0.00 | 3.15 ± 2.14 | 1.92 ± 1.82 | 0.418 |
| Phenyl acetaldehyde | 0.31 ± 0.18 | 0.36 ± 0.22 | 0.16 ± 0.00 | 0.760 |
periods were found, but there were significant differences in the concentrations of 36 compounds in 3 estrous cycles and these quantitative differences could explain the change of milk odors in the estrous cycle. Table 4 shows specific volatile compounds belonging to the preestrus, estrus, and postestrus periods in milk and a total of 27 volatile compounds were determined. There is no available information on the volatile compounds of cattle sudor in the literature and therefore no comparison was made. However, there are very few studies on the volatile compounds of bovine saliva. In the current study, although a total of 206 volatile compounds were detected in saliva, Sankar et al. [14] identified 14 volatile compounds. They

Table 7. (Continued).

| Compound name                                    | Chemical formula | Cas number |
|--------------------------------------------------|------------------|------------|
| Bicyclo[2.2.1] heptan-2-ol, 1,7,7-trimethyl-, formate, exo | C H O          | 96 – 14 - 0 |
| 3-Phenylpropiononitrile                          | C H N           | 66 – 25 - 1 |
| 1-Amino-4-(phenylthio)isoquinoline                | C H O           | 25.5 – 44 - 5 |
| Diborane(6)                                      | C H O           | 122 – 78 - 1 |
| Dodecanoic acid, tricosaiuoro                   | C H O           | 645 – 59 - 0 |
| 3,5-Di-t-butylbenzoic acid                       | C H O           | 120 – 72 - 9 |
| 2,2-dimethyl-N-[2,2,2-trichloro-1-(2-methylanilino)ethyl]propanamide | C H O       | 556 – 67 - 2 |
| Propanoicacid,2-[[1-methylethylidene]amino]oxy-, ethyl ester | C H O       | 107 – 83 - 5 |

* As a result of the analysis, 23 compounds in the preestrus period, 39 compounds in the estrus period and 32 compounds in the postestrus period were not included in the comparison table, since they were detected in only one period. **P < 0.05.

Table 8. The number of repetitions of the identified compounds in body fluids and the rate (%).

| Number of repetitions | Number of compounds | Rate (%) |
|-----------------------|---------------------|----------|
| 1.00                  | 216                 | 69.0     |
| 2.00                  | 48                  | 15.3     |
| 3.00                  | 18                  | 5.8      |
| 4.00                  | 12                  | 3.8      |
| 5.00                  | 5                   | 1.6      |
| 6.00                  | 6                   | 1.9      |
| 7.00                  | 8                   | 2.6      |
| Total                 | 313                 | 100.0    |

Table 9. Compounds common in all body fluids in estrus.
detected trimethylamine, acetic acid, phenol 4-propyl and propionic acid compounds in the period of estrus, carbonic acid, phosphonic dichloride, butanoic acid, and 2-propenyl ester compounds in the preestrus period, and 3-hexanol, butanoic acid, 2-propenyl ester, and pentanoic acid compounds in the postestrus period. Propionic acid and phenol compounds in saliva were detected only in the estrus period (Table 6), while acetic acid compound was found both in the estrus and postestrus phases. In addition, butanoic acid was observed to be a common compound in both studies. Sankar and Archunan [27] analyzed the volatile compounds in the vaginal secretion of animals in 3 estrus periods and found 8 different organic compounds. They stated that trimethylamine, acetic acid, and propionic acid compounds were present during the estrus period and also compounds having similar structures to these compounds were determined in all 3 periods. Preti [28] reported alcohol compounds such as 6-methyl-1-heptanol from methyl 1-heptanol group and 2-methyl-7-hydroxy-3-4 from methyl hydroxy-heptane group during the period of estrus only in vaginal secretion.

It was thought that estrus-specific volatile compounds detected only in the estrus period could be important indicators of the estrus status of cows and can be used to define the optimal insemination time. Therefore, a technological device will be developed by using these volatile compounds, which is more effective and easier than other methods for the most appropriate timing of artificial insemination. Timely detection of estrus depending on volatile compounds will provide the dairy sector with a big economic advantage in terms of the correct time for insemination.

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