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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, 1 H-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 Cukurova University Animal Experiments Local Ethics Board (Approval no: 26.02.2018/2). There

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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. PGF2α (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 Bilişim 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 washed 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

Table 1. Volatile odor compounds that were detected in feces during the estrus period (%).

Compound name*	Preestrus ($\bar{x} \pm S\bar{x}$)	Oestrus ($\bar{x} \pm S\bar{x}$)	Postestrus ($\bar{x} \pm S\bar{x}$)	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 - Fluoropropene	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 carbonyloxymethyl	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 – Benzisothiazol – 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 – Cholestan e- 3α.7α.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		0.12 ± 0.00	0.10 ± 0.00	0.618
1 – Penten – 3 - one. 2 – methyl -	0.09 ± 0.00	0.15 ± 0.00	0.55 ± 0.40	0.400
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 - Benzenetrikarboksilik 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 - Fluoropropene	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

Table 1. (Continued).

n - Heptane	0.07 ± 0.00	0.42 ± 0.24	0.17 ± 0.10	0.310
Pentanenitrile. 4 – methyl -		0.34 ± 0.00	0.29 ± 0.00	0.619
Phenyl acetaldehyde		0.34 ± 0.00	0.35 ± 0.20	0.496
2 - Octene. 2 – methyl – 6 – methylene -		0.32 ± 0.19	0.30 ± 0.18	0.281
1 H - İndole		0.41 ± 0.00	0.75 ± 0.43	0.345
1.1 – bis (4 - methylcyclohexyl) dodecane	0.05 ± 0.00		0.13 ± 0.00	0.578
Phosphine. 1.2 – ethanediylbis [bis (1 -methylethyl) -	0.16 ± 0.00		0.11 ± 0.00	0.607
Ethene. Methoxy -	0.44 ± 0.00	0.14 ± 0.00		0.516
2.5 - Dihydroxyacetophenone. Bis (trimethylsilyl) ether	0.06 ± 0.00	0.36 ± 0.00		0.459
Octadecane.3 – ethyl – 5 - (2 - ethylbutyl) -	0.07 ± 0.00	0.05 ± 0.00		0.600
2 – Methyl - 1H - pyrrole		0.07 ± 0.00	0.22 ± 0.00	0.517
Cyclotetrasiloxane. Octamethyl -	0.46 ± 0.00	0.05 ± 0.00	0.19 ± 0.11	0.588
4 – Imino - 6.6 – dimethy l- 5.8 – dihydro - 4H – thio pyrano [4'.3':4.5] furo [2.3 - d] pyrimidin - 3 (6H) - amine #		0.11 ± 0.00	0.06 ± 0.00	0.577
2.3 - Pentanedione		0.11 ± 0.00	0.11 ± 0.00	0.622

* 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.

analysis of variance, 6-methylhept-5-en-2-one compound was determined to be important (P < 0.05) in terms of interperiod differences. This compound was detected in both estrus period (0.05 ± 0.00) and postestrus period (0.39 ± 0.14) and was calculated as P = 0.021.

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- compound was determined as important (P < 0.05) in terms of interperiod differences. This compound was detected in the preestrus period (0.99 ± 0.93), the estrus period (0.47 ± 0.26), and the postestrus period (3.54 ± 1.01) and was calculated as P = 0.043.

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

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

Compound name*	Preestrus ($\bar{x} \pm S\bar{x}$)	Oestrus ($\bar{x} \pm SX\bar{x}$)	Postestrus ($X\bar{x} \pm SX\bar{x}$)	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		0.083
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		0.544
Pentane, 2 - methyl-	0.23 ± 0.14	1.38 ± 1.01	0.26 ± 0.00	0.383
Acetic acid, isocyanato -, butyl ester	1.58 ± 0.00	0.93 ± 0.38	0.40 ± 0.00	0.429
Dodecanoic acid, tricosafluoro -	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	0.457
3,5 – Di – t - butylbenzoic acid	0.65 ± 0.56	0.40 ± 0.00	2.50 ± 2.34	0.538
Phosphine, 1,2 – ethanediylbis [bis (1 - methylethyl) -	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 - propylphenyl) 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 - İndole	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, ethy l -		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 – methy l		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 '- [thiobis (methylene)] bis[2,6 – bis (1,1 - dimethylethyl) -		1.55 ± 0.00	10.50 ± 7.20	0.223

Table 2. (Continued).

5β – 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 [trimethy l -	0.15 ± 0.00	1.00 ± 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
3 - methyl pentane	0.12 ± 0.00	0.61 ± 0.00		0.475
6 – Methy l - 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 – diiodo -	0.05 ± 0.00		0.09 ± 0.00	0.582
4,4 – Dimethyloxazolidine – 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 (%).

ompound name*	Preestrus ($\bar{x} \pm S\bar{x}$)	Oestrus ($\bar{x} \pm S\bar{x}$)	Postestrus ($\bar{x} \pm S\bar{x}$)	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 carbonyloxymethyl		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	0.22 ± 0.00	0.893

Table 3. (Continued).

4 - methylphenol (p - cresol)		0.25 ± 0.00	0.07 ± 0.00	0.496
Oxime -, methoxy – phenyl -_	0.67 ± 0.00	0.31 ± 0.18		0.521
3,5 – Di – t - butylbenzoic acid	0.05 ± 0.00		0.07 ± 0.06	0.541
Cyclotetrasiloxane, octamethyl -	0.78 ± 0.53	1.99 ± 1.77	3.92 ± 3.27	0.602
Dodecanoic acid, tricosafluoro -		0.04 ± 0.00	0.06 ± 0.00	0.595
3' – Cyano - 1beta,2beta – dihydro - 17beta – hydroxy – 3 – oxo - 3'H - cycloprop[1,2]androsta - 4,6 – diene - 3' - carboxylic acid ethyl ester	0.81 ± 0.60	0.40 ± 0.00		0.427
1 H - İndole	2.00 ± 0.00	1.34 ± 0.93		0.231
4 – Imino - 6,6 – dimethyl - 5,8 – dihydro - 4H – thiopyrano [4',3':4,5] furo[2,3 - d]pyrimidin - 3(6H) - amine #	0.20 ± 0.00	0.34 ± 0.00		0.588
Dimethylsilanediol	1.12 ± 0.00	2.72 ± 0.00		0.546
Propane, 1,3 – epoxy -		1.86 ± 0.00	2.35 ± 1.35	0.458
3' – Cyano - 1beta,2beta – dihydro - 3,17 – dioxo - 3'H –cycloprop [1,2] androsta - 4,6 – diene -3 '- carboxylic acid ethyl ester	0.22 ± 0.00	0.44 ± 0.40		0.523
Pentanamid, N - 1 H – purin – 6 – il -	0.06 ± 0.00	0.02 ± 0.00	1.05 ± 0.00	0.433
3 - methyl pentane	0.06 ± 0.00	0.86 ± 0.00	0.36 ± 0.00	0.586
Methyl ethyl acetaldehyde (Butanal, 2 – methyl -)	0.33 ± 0.00		0.35 ± 0.00	0.622
Perfluorotributylamine	0.96 ± 0.00		0.12 ± 0.00	0.449
Heptanal	0.94 ± 0.74	0.15 ± 0.00	0.14 ± 0.00	0.380
Nonanaldehyde	8.87 ± 8.71	0.15 ± 0.00	0.27 ± 0.00	0.410
Margaric acid, (Heptadecanoic acid, tert - butyldimethylsilyl ester)	0.41 ± 0.00	0.24 ± 0.00	0.06 ± 0.00	0.684
trimethylsilyloxysilyl]oxysilyl]oxysilane	1.20 ± 0.00	1.25 ± 0.00		0.622
7,12a – Dimethyl - 1,2,3,4,4a, 11,12,12a – octa hydro chrysene	0.05 ± 0.00	0.03 ± 0.00		0.459
3-methyl pentane	0.36 ± 0.00	0.21 ± 0.00	0.25 ± 0.00	0.923
2 – n - Butyl furan		0.07 ± 0.00	0.08 ± 0.00	0.621
Cyclohexane		0.09 ± 0.00	0.09 ± 0.00	0.622

* 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 name*	Preestrus ($\bar{x} \pm S\bar{x}$)	Oestrus ($\bar{x} \pm S\bar{x}$)	Postestrus ($\bar{x} \pm S\bar{x}$)	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':4,5] 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

Table 4. (Continued).

Pentanenitrile, 4 – methyl -	0.33 ± 0.00	0.15 ± 0.09		0.520
Trimethylsilyl 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 - İndole	0.62 ± 0.00	0.89 ± 0.44	0.44 ± 0.00	0.825
1,2,4 - Benzenetrikarboksilik 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 - 3α,7α,12α, 24,25,26 - hexol hexa TMS	0.04 ± 0.00		0.12 ± 0.00	0.531
1,2 – Benzisothiazol - 3- amine tbdms		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 - Oxazolidinethione,4,4 - dimethyl -	0.05 ± 0.00	0.19 ± 0.00		0.495
Pyrimidin – 2 - one, 4 - [N - methylureido] – 1 - [4 – methyl aminocarbonyloxymethyl	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 - butylbenzoic acid	1.33 ± 0.00	1.30 ± 0.00		0.560
2,2 – dimethyl – N - [2,2,2 – trichloro – 1 - (2 – methyl anilino) ethyl] propanamide	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).

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
1,1,1,3,5,7,9,11,11,11 – Decamethyl – 5 -(Trımethyl sılyloxy) Hexasıloxane	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 postestrus 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 ($\bar{x} \pm S\bar{x}$)	Oestrus ($\bar{x} \pm S\bar{x}$)	Postestrus ($\bar{x} \pm S\bar{x}$)	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

Table 5. (Continued).

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 methylethyl) - , 1,1 - dimethylethyl ester		0.09 ± 0.00	0.17 ± 0.11	0.373
Pentanenitrile, 4 – methy l -	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 - İndole	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
Oxalicacid, isohexylneopentyl ester	0.27 ± 0.00	0.11 ± 0.00		0.548
Phenol, 2,6 - di-tert – buty l - 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 -	0.15 ± 0.00	0.16 ± 0.00	0.42 ± 0.00	0.730
1,4 - Benzenedipropanol, ,alpha,,,alpha,',,gamma,,,gamma,,,gamma,',,gam ma,'- hexamethyl -		0.08 ± 0.00	0.36 ± 0.00	0.482
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 acidethyl 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,6,8,8a,8b –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	0.557
1,1,1,3,5,7,9,11,11,11 – Deca methyl – 5 - (Trımethylsılyloxy) Hexasıloxane	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
Cyclopropylmethylcarbinol	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).

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 ($\bar{x} \pm S\bar{x}$)	Oestrus ($\bar{x} \pm S\bar{x}$)	Postestrus ($\bar{x} \pm S\bar{x}$)	P value**
1,2 – Dimethyl - 4,5 - bis (trimethylsilyl) benzene		0.08 ± 0.00	0.07 ± 0.00	0.613
Propanal, 2 – methyl - (Isobutyricaldehyde)		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	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
Cyclopropylmethylcarbinol	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 -aminetbdms	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 – tetrahydrooxazolo [3,2 - d] [1,4] benzodiazepin – 6 - one)		0.18 ± 0.00	0.05 ± 0.00	0.498
Benzaldehyde (Benzoicacidaldehyde)	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

Table 6. (Continued).

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 - İndole	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	030 ± 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 androstane - 8,14 - dione, 17 – acetoxy - 3.beta. – methoxy - 4,4 – dimethyl -	0.34 ± 0.23	0.18 ± 0.11		0.304
5β – Cholestane - 3α,7α,12α,24α,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, dichloronitro -		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 - dimethylethyl 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
Methylethylacetaldehyde (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 – methylethyl) -	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 – dimethylethyl) - , 1,1 - dimethylethyl 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) phenoxy] silane	0.10 ± 0.00		2.79 ± 0.00	0.417

* As a result of the analysis, 45 compounds in the preestrus period, 36 compounds in the estrus period and 48 compounds in the postestrus 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-benzisothiazol-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 isocyanato-butyl ester (except milk), 3-methylbutanal (except milk), pyrimidine-2-one 4-[N-methylureido]-1-[4-methylaminocarbonyloxymethyl (except sweat), dodecanoic acid tricosafluoro (except sweat), benzaldehyde (benzoicacidaldehyde) (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. Manikkaraja 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-iodoundecane 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 methylhydrazone 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, benzenetricarboxylic 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 ($\bar{x} \pm S\bar{x}$)	Oestrus ($\bar{x} \pm S\bar{x}$)	Postestrus ($\bar{x} \pm S\bar{x}$)	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 - 3α,7α,12α,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 – epoxyandrostane - 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 - Benzisothiazol - 3 - amine tbdms	0.61 ± 0.04		0.08 ± 0.00	0.000
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	0.33 ± 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 - 3α,7α,12α,24α,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 - (methoxymethyl) – 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 - İndole	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
Dimethylsilanediol		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

Table 7. (Continued).

Bicyclo [2.2.1] heptan – 2 - ol, 1,7,7 – trimethyl - , formate, exo	0.22 ± 0.00		0.90 ± 0.00	0.491
3 - Phenylpropiononitrile	0.43 ± 0.25	0.49 ± 0.31	0.16 ± 0.00	0.635
1 – Amino – 4 - (phenylthio) isoquinoline	0.46 ± 0.00	0.70 ± 0.00		0.576
Diborane (6)	0.34 ± 0.00	0.19 ± 0.00		0.581
Dodecanoic acid, tricosafluoro -	0.13 ± 0.09	0.08 ± 0.00	0.30 ± 0.22	0.529
3,5 - Di – t - butylbenzoic acid		0.18 ± 0.00	0.02 ± 0.00	0.434
2,2 – dimethyl – N - [2,2,2 – trichloro – 1 - (2 - methylanilino) ethyl] propanamide		0.06 ± 0.00	0.10 ± 0.08	0.517
Propanoic acid,2 - [[(1-methylethylidene)amino]oxy] -, ethyl ester	0.07 ± 0.00		0.09 ± 0.00	0.405
Acetic acid ethyl ester		0.15 ± 0.00	0.15 ± 0.00	0.622
Propanoic acid, ethyl ester		0.14 ± 0.00	0.19 ± 0.00	0.609
Dimethyl disulfide		1.10 ± 0.00	3.66 ± 0.00	0.511
N - Heptanal		0.08 ± 0.00	0.10 ± 0.00	0.617
Cyclotrisiloxane, hexamethyl -	0.11 ± 0.00	0.01 ± 0.00	0.25 ± 0.00	0.563
1H - Indole, 3 - (2 - methoxyethyl) – 2 - (2 - pyridyl) -		0.35 ± 0.00	0.33 ± 0.00	0.622
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 - , methyl ester		0.14 ± 0.00	2.00 ± 0.00	0.428

* 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.

Compound name	Chemical formula	Cas number
3 - methyl pentane	C_6H_{14}	96 – 14 - 0
hexanal	$C_6H_{12}O$	66 - 25 - 1
4 - methylphenol (p -cresol)	C_7H_8O	25.5 – 44 - 5
Phenylacetaldehyde	C_8H_8O	122 – 78 - 1
3- phenylpropiononitrile	C_9H_9N	645 – 59 - 0
1 H - indole	C_8H_7N	120 – 72 - 9
Cyclotetrasiloxane octamethyl -	$C_8H_{24}O_4Si_4$	556 – 67 - 2
Pentane 2 - methyl	C_6H_{14}	107 – 83 - 5

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

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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References

1. Goncu S, Anitas Ö, Gungor C, Gokce G. New technology usage for sustaniable dairy cow reproductive perfomances. Journal of Environmental Sience and Engineering B 2017; 6: 370-379.. doi: 10.17265/2162-5263/2017.07.004
2. Nebel RL, Jobst S. Evaluation of systemic breeding programs for lactating cows. Journal of Dairy Science 1998; 81 (4): 1169-1174. doi: 10.3168/jds.S0022-0302(98)75679-6
3. Nebel RL. Components of a successful heat detection program. Advance in Dairy Technology 2003; 15: 191-203. doi: 10.1016/S0301-6226(01)00323-2
4. Kekan PM, Ingole SD, Sirsat SD, Bharucha SV, Kharde SD et al. The role of pheromones in farm animals-a review. Agricultural Reviews 2017; 38 (2): 83-93. doi: 10.18805/ag.v38i02.7939
5. Lin DY, Zhang SZ, Block E, Katz LC. Encoding social signals in the mouse main olfactory bulb. Nature 2005; 434: 470-477. doi: 10.1038/nature03414
6. Rekwot PI, Ogwu D, Oyedipe EO, Sekoni VO. The role of pheromones and biostimulation in animal reproduction. Animal Reproduction Science 2001; 65: 157-70. doi: 10.1016/S0378-4320(00)00223-2
7. Zalesky DD, Day ML, Winder MG, Imakawa K, Kittok RJ et al. Influence of exposure to bulls on resumption of estrous cycles following parturition in beef cows. Journal of Animal Science 1984; 59: 1135-1139. doi: 10.1016/S0093-691X(98)00013-2
8. Nordéus K, Båge R, Gustafsson H, Glinwood R, Söderquist L. A small expose on bovine pheromones: with special reference to modifications of the reproductive cycle. Chemical Signals in Vertebrates 2016; 13, 33-42. doi: 10.1007/978-3-319-22026-0_4
9. Aksu EH, Bozkurt T, Türk G. Farklı senkronizasyon uygulamaları ile senkronize edilen ineklerde üreme performansı üzerine vitamin E'nin etkisi. Fırat Üniversitesi Sağlık Bilimleri Dergisi 2010; 24 (2): 71-76.
10. Ramesh Kumar K, Archunan G, Jeyraman R, Narasimhan S. Chemical characterization of bovine urine with spezial reference to estrous cycle. Veterinary Research Communication 2000; 24: 445-54. doi: 10.1023/a:1006495404407
11. Kumar RK, Archunan G, Jeyaraman R, Narasimhan S. Chemical characterization of bovine urine with special reference to oestrus. Veterinary Research Communications 2000; 24 (7): 445-454. doi: 1006495404407
12. Sankar R, Archunan G. Identification of putative pheromones in bovine (Bos taurus) faeces in relation to oestrus detection. Animal Reproduction Science 2008; 103: 149-153. doi: 10.1016/j.anireprosci.2007.04.014
13. Sankar R, Archunan G. Flehmen response in bull: role of vaginal mucus and other body fluids of bovine with special reference to oestrus. Behavioural Processes 2004; 67: 81-86. doi: 10.1016/j.beproc.2004.02.007
14. Sankar R, Archunan G, Habara Y. Detection of oestrous-related odour in bovine (Bos taurus) saliva: bioassay of identified compounds. Animal 2007; 1: 1321-1327. doi: 10.1017/S1751731107000614
15. Bendall JG. Aroma compounds of fresh milk from New Zealand cows fed different diets. Journal of Agricultural and Food Chemistry 2001; 49 (10): 4825-32. doi: 10.1021/jf010334n

16. Klemm WR, Rivard GF, Clement BA. Blood acetaldehyde fluctuates markedly during bovine estrous cycle. Animal Reproduction Science 1994; 35: 9-26. doi: 10.1016/0378-4320(94)90003-5

17. Kennedy GA. Detecting hypocalcemia using bovine nose sweat. United States Patent, No: 7.964.409, dated 21.06.2011.

18. Kayaalp G, Yıldırım N. Araştırma ve Deneme Metodları. Genel Yayın No: 282. Ders Kitapları Yayın No: A-88. Adana, Turkey: Çukurova Üniversitesi Ziraat Fakültesi; 2010.

19. Manikkaraja C, Mahboob S, Al-Ghanim KA, Rajesh D, Selvaraj K et al. A novel method to detect bovine sex pheromones using l-tyrosine-capped silver nanoparticles: special reference to nanosensor based estrus detection. Journal of Photochemistry and Photobiology B: Biology 2019; 111747. doi: 10.1016/j.jphotobiol.2019.111747

20. Mozūraitis R, Kutra J, Borg-Karlson AK, Būda V. Dynamics of putative sex pheromone components during oestrus periods in oestrus-induced cows. Journal of Dairy Science 2017; 100 (9): 7686-7695. doi: 10.3168/jds.2016-12376

21. Gnanamuthu G, Ahmed WM, Rameshkumar K. Observations of estrous behaviour and identification of faecal compounds in Umblachery cow (Bos indicus) with reference to live stock production. Global Veterinaria 2018; 20 (3): 126-138. doi: 10.5829/idosi.gv.2018.126.138

22. Archunan G, Kumar R. 1-Iodoundecane, an oestrus indicating urinary chemo signal in bovine (Bos Taurus). Journal of Veterinary Science & Technology 2012; 03: 04. doi: 10.4172/2157- 7579.1000121

23. Muniasamy S, Selvam RM, Rajanarayanan S, Saravanakumar VR, Archunan G. P-cresol and oleic acid as reliable biomarkers of estrus: evidence from synchronized Murrah buffaloes. Iranian Journal of Veterinary Research 2017; 18: 124-127. doi: 10.22099/ijvr.2017.4097

24. Muthukumar S, Muniasamy S, Srinivasan M, Ilangovan A, Satheshkumar S et al. Evaluation of pheromone-based kit: a noninvasive approach of estrus detection in buffalo. Reproduction in Domestic Animals 2018; 53 (6): 1466-1472. doi: 10.1111/rda.13281

25. Zebari HM, Rutter, SM, Bleach ECL. Fatty acid profile of milk for determining reproductive status in lactating Holstein Friesian cows. Animal Reproduction Science 2019; 202: 26-34. doi: 10.1016/j.anireprosci.2019.01.004

26. Weidong MA, Clement BA, Klemm WR. Volatile compounds of bovine milk as related to the stage of the estrous cycle. Journal of Dairy Science 1997; 80: 3227-33. doi: 10.3168/jds.S0022-0302(97)76296-9

27. Sankar R, Archunan G. Gas chromatographic/mass spectrometric analysis of volatile metabolites in bovine vaginal fluid and assessment of their bioactivity. International Journal of Analytical Chemistry 2011; 256106. doi: 10.1155/2011/256106

28. Preti G. Method for detecting bovine estrus by determining methyl heptanol concentrations in vaginal secretions. 1984. US Patent No. 4,467,814.