



Application of an electronic nose for the diagnosis of ketosis in dairy cows

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ABSTRACT

Ketosis in dairy cows is a nutritional and metabolic disease that severely affects the milk industry and dairy cow health. An electronic nose can identify volatile organic compounds (VOCs). This study applied the electronic nose to detect VOCs in dairy cow milk, feces, and blood. Blood ketone bodies (BHBA) were measured, and 10 ketosis cows, 71 subclinical ketosis cows, and 75 healthy cows were considered the research subjects. Cow milk, feces, and blood samples were collected for electronic nose testing. The measured VOC data were processed through multivariate analysis and machine learning algorithms. The electronic nose analysis results were validated through the GC-MS analysis of the milk samples. The results unveiled spatial differences in the electronic nose VOC values of milk, feces, and blood, and the cumulative contribution rates of both principal component variables (PC) 1 and PC2 reached more than 95%. The machine learning algorithm results revealed that the accuracy of the milk probabilistic neural network (PNN) was 87.5%, the stool support vector machine was 100%, and the blood PNN was 66.7%. This result revealed that electronic nose detection can effectively differentiate healthy, subclinical ketosis, and clinical ketosis cows. According to the milk GC-MS results, significant differences were noted in the VOC content of milk samples between the different groups.

The aforementioned studies revealed that electronic nose can aid in the effective prediction and diagnosis of ketosis in dairy cows. This lays the foundation for establishing a new method for diagnosing ketosis in dairy cows.

1. Introduction

Ketosis is among the common nutritional and metabolic diseases in dairy cows occurring during the perinatal period (Baird, 1982). In ketosis, a large amount of ketone bodies is produced because the fat in the cow's body after giving birth is mobilized to compensate for the negative postpartum energy balance. When the number of these ketone bodies exceeds the threshold for hepatic catabolism, dairy cows develop ketosis (Pérez-Báez et al., 2019). During ketosis, ketone body levels are increased in the blood, milk, and urine of dairy cows. Blood obtained from dairy cows has 78% beta-hydroxybutyric acid (BHBA), 20% acetoacetic acid, and 2% acetone (Bellato et al., 2023; Serrenho et al., 2022). Clinical manifestations of ketosis include decreased appetite, body condition, and milk production. The smell of acetone is present in the exhaled breath, feces, and urine of dairy cows with ketosis (Guliński, 2021). According to statistics from the China Dairy Association,

approximately 15.0%–30.0% lactating dairy cows in China have ketosis (Jinshui et al., 2023). Clinical ketosis may lead to the animal's death. Subclinical ketosis (SK) cows may become clinical ketosis (K) cows (Kaufman et al., 2016), which may lead to the occurrence of secondary diseases (Raboisson et al., 2014). Ketogenic cows produce significantly reduced amounts of milk, which increases breeding costs (Cainzos et al., 2022; Nikkhah et al., 2021).

Currently, detecting BHBA content in blood is the main method of ketosis diagnosis in dairy cows (Duffield et al., 2009). Additionally, ELISA methods can be used to measure blood and urine BHBA concentrations. These methods require blood and urine samples. These samples are however difficult to collect and expensive to detect. Moreover, the results are not timely, and sampling causes great stress to dairy cows (Myridakis et al., 2023). Therefore, accurate, non-invasive, early, and rapid ketosis diagnosis in dairy cows is an urgent problem of the dairy farming industry requiring a solution.

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An electronic nose, also known as an odor scanner, is an instrument mimicking the olfactory organs of animals. The instrument mainly includes three functional devices: an odor sampling operator, a gas sensor array, and a signal processing system. The instrument rapidly identifies volatile organic compounds (VOCs) by using specific sensors and identification modules (Hosfield et al., 2021). The electronic nose then preprocesses the generated signals, enters a multi-dimensional response mode, rapidly evaluates different gases, generates odor fingerprints, and judges and analyzes the results (Dragonieri et al., 2017).

Gas chromatography-mass spectrometry (GC-MS) combines a gas chromatograph and a mass spectrometer in series. It is commonly used to detect and analyze VOCs and semi-volatile compounds (Ain et al., 2023; Dai et al., 2023). Following the separation of the gas or liquid in the sample, the chemical structure of the substance is determined and its different chemical information is obtained. GC-MS applied to detect milk samples serves as a basis for interpreting the VOCs present in the samples, measured using the electronic nose, and lays the foundation for additional development of odor detection equipment (Shi et al., 2021).

We here studied the feasibility of using an electronic nose for measuring dairy cow metabolites and predict dairy cow ketosis. First, the BHBA content of bovine blood was determined as a reference (Snedec et al., 2021). The VOC data of milk, feces, and blood of healthy (C), SK, and K type cows were obtained. Multivariate analysis and machine learning algorithm models were then used to establish a basis for diagnosis and prediction of ketosis. This study offers a theoretical basis for the clinical application of electronic nose to detect VOC in the milk and fecal samples of dairy cows and thus diagnose and predict ketosis in these cows on the basis of VOC data.

2. Materials and methods

2.1. Animal selection

The study with a cross-sectional experimental design has been approved by the Animal Experiment Ethics Committee of Gansu Agricultural University (GAU-Eth-VM-202-1-045). From April 1, 2023 to May 10, 2023, the trial was conducted at a large-scale TMR breeding ranch at the foot of Qilian Mountains in Zhangye City, Gansu Province. In total, 156 pregnant cows were screened from 4590 Australian Holstein cows that had no cesarean section, peripartum diseases such as milk fever or lame disease, or post-pregnancy abomasum displacement. The selected cows were housed individually in pens and examined visually and palpably to exclude those with organic, systemic, foot and gastrointestinal diseases as these diseases could reduce feed intake. We also examined the cows for the presence of mastitis (Jacobs et al., 2018).

This study maintained similar pasture herd sizes (80 ± 20 cows/pen, consistent throughout the year). The animals were regularly tested for metabolic diseases. During this study, we also adhered to minimum welfare standards and comprehensive management systems for dairy farming. As the cows approached their due dates, they were moved individually into pens so the staff could monitor them closely. The farm ensured an average space margin of more than $10 \text{ m}^2/\text{head}$ and a drinking water outlet of $0.08 \text{ m}/\text{head}$. At 3 days after parturition (including day 3), the cows were housed in individual pens for care. Ketosis was not tested, and the farm veterinarian reported that ketone levels increased physiologically during this period. Cows at 3 days postpartum were kept in separate pens. The staff conducted random inspections throughout the barn to screen for cows with ketosis. The number of cows randomly inspected was more than 25% of the cows in the entire pen, and the average incidence of ketosis was within 5%. The average daily milk production of all cows was $43 \pm 11 \text{ kg}$. Based on the results of the quarterly assessments of cow health, the amount of feed to be given to the cows was determined. The feed was obtained from professional nutrition companies. The main feed raw materials were alfalfa hay, oat grass, rapeseed meal, soybean hulls, calcium propionate, Yi Kang XP, fatty acid calcium, and corn.

2.2. Sample collection

After random inspection, blood was collected from the cow tail vein and stored in a vacuum collection vessel (sodium citrate (1:9), Hongda Medical Equipment Group, Jiangxi, China). Fresh whole blood was immediately dropped on the blood ketone test paper, and the OGM-161 blood ketone meter (Aikang Biotechnology Co., Ltd., Guangdong, China) was used to measure the blood BHBA concentration. The animals were then divided into three groups based on their blood BHBA concentrations. The animals were considered C when the blood BHBA concentration was $<1.2 \text{ mmol/L}$. When the BHBA concentration was between 1.2 and 3.0 mmol/L , the animal was considered to have SK, and when it was $>3.0 \text{ mmol/L}$, the animal was considered to have K (Shen et al., 2021). In total, 156 cows (75 C cows, 71 SK cows, and 10 K cows) were included in the study. Among them, 10 cows from groups C, SK, and K were selected as samples for the electronic nose model analysis (Sailer et al., 2018). First, 2 mL blood was obtained from the selected animals, stored, and used for the VOC measurement by using the electronic nose. Then, 2 mL milk and 2 g feces of the same animal were collected and placed in a closed test tube. The samples were headspace tested within 1 h by using the electronic nose. The collected milk samples were placed in liquid nitrogen tanks for GC-MS evaluation.

2.3. Detection of the electronic nose

The samples were tested using the Syranose 320® electronic nose (Sensigent Co., CA, USA). When the 32 sensors of the instrument were exposed to the gas emitted by the sample, the resistance signal they responded to increased and produced different peaks, thereby distinguishing different ketosis types (Zhang et al., 2018). The initial conditions of the electronic nose were adjusted as follows: The power was switched on and the system was preheated for 5 min; the injection speed was adjusted to $2000 \mu\text{L/s}$, baseline clearing was purged at a medium speed for 15 s, the sample injection time was 25 s, the data acquisition time was 25 s, and the sample purge time was 15 s; the substrate was maintained at $42 \text{ }^\circ\text{C}$; KNN was selected as the method and all 32 sensors were retained; at the same time, the No. 8 needle and the No. 6 needle were inserted above the silicone caps of the blood collection tube and headspace bottle to be tested; and the No. 8 needle was connected to the electronic nose with a transparent hose. The sampling device can be used for sample testing.

2.4. Determination of GC-MS

To verify whether VOCs undergo varying degrees of changes in ketosis cows, GC-MS technology was used to analyze 10 portions of milk from the C, SK, and K groups.

2.4.1. Milk sample pretreatment

First, the sample was slowly thawed on ice. Then, 0.3 mL of 80% methanol solution and 5 μL of internal standard (ribitol, 5 mg/mL) were added to 0.1 mL of the sample. The sample was vortexed for 1 min, ground at 60 Hz for 180 s, sonicated at $4 \text{ }^\circ\text{C}$ for 30 min, and centrifuged at 13,780 g (12,000 rpm) for 10 min. Subsequently, 200 μL of the supernatant was added to a 1.5 mL centrifuge tube and concentrated through rotary evaporation and freeze-drying. Next, 35 μL of 20 mg/mL methoxamine pyridine solution was added to the concentrate, shaken vigorously for 30 s, and reacted at $37 \text{ }^\circ\text{C}$ for 90 min. The mixture was collected, and 35 μL LBSTFA (containing 1% TMCS) was added to the mixture and reacted at $70 \text{ }^\circ\text{C}$ for 60 min to achieve derivatization. After the reaction, the mixture was allowed to stand at room temperature for 30 min at $25 \pm 1 \text{ }^\circ\text{C}$ for GC-MS metabolomic detection.

2.4.2. GC-MS condition setting

For GC-MS, ISQ 7000/Trace 1300 _ 1310 (Thermo Scientific) was used as the analysis platform and DB-5 (60 m \times 0.25 mm \times 0.25 μm ;

Agilent J&W Scientific) was used as the chromatographic column. The instrument parameters were set as follows: inlet temperature, 280 °C; EI ion source temperature, 230 °C; carrier gas; high-purity helium (purity >99.999%); split ratio 30:1; injection volume, 1.0 µL; solvent delay, 5 min. The temperature program used was as follows. The initial temperature was set to 70 °C, then increased to 200 °C at 10 °C/min, further increased to 280 °C at 5 °C/min, and held at 280 °C for 10 min. The full scan mode was used for mass spectrometric detection in the 30–550 (m/z) range.

2.5. Statistical analysis

The response signal values of the electronic nose were normalized by dividing the standard deviation of each variable by centering on the mean. Principal component analysis (PCA) was performed on the electronic nose obtained data by using Metaboanalyst 5.0 (<https://www.metaboanalyst.ca/>). The 32 - sensor data of the samples measured using the electronic nose were used for modeling. OriginPro software version 2019b (OriginLab Co., MA, USA) was used for the statistical

analysis. All data were subjected to normality analysis and homoskedasticity tests, and one-way analysis of variance was performed. $P < 0.05$ was considered significant. The results were expressed with letters. Three common machine learning methods, namely random forest (RF), support vector machine (SVM), and probabilistic neural network (PNN), were applied to the obtained electronic nose data for feature training, recognition, and group classification discriminant analysis. The classification performance and the effect of electronic nose sensor signals were tested through machine learning.

The GC-MS results of the milk samples were inputted to Chromeleon 7.0 of Thermo software for feature peak extraction. The screening conditions for differential metabolites at this time were that the VIP value in the *t*-test validation model was >1 and the *p* value was <0.05. Milk is used to identify biomarkers that may be useful for ketosis detection in dairy cows. The data were organized by generating partial least squares discriminant analysis plots (PLS-DA) and orthogonal partial least squares discriminant analysis plots (OPLS-DA) by centering the mean and dividing by the standard deviation of each variable. Hierarchical clustering heatmaps were constructed using the identified metabolites

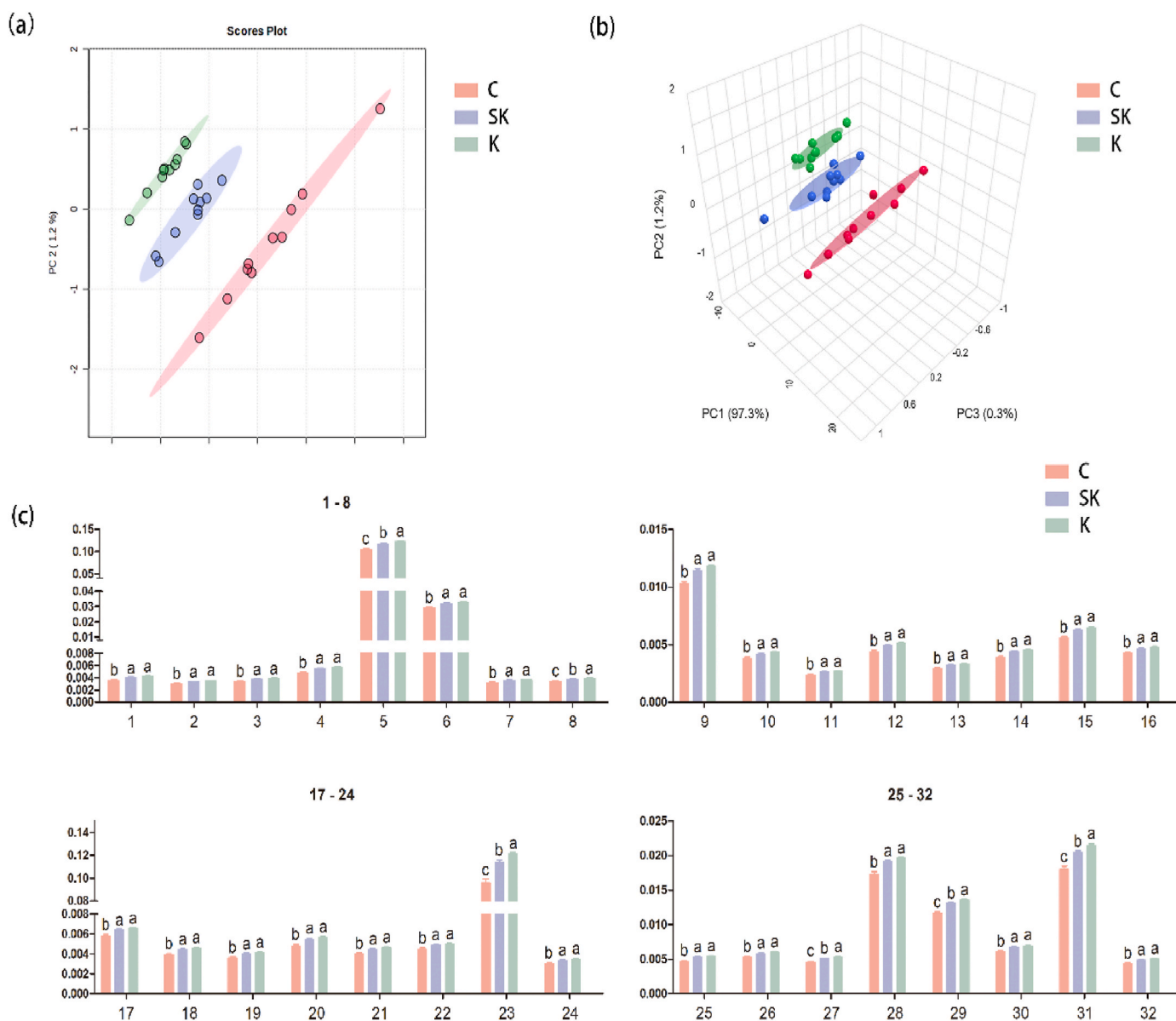


Fig. 1. VOC data obtained through electronic nose measurements of milk samples. (a) PCA of milk. (b) Three-dimensional PCA of milk. (c) The peak values and significant differences between data obtained through the 32 sensors of the electronic nose. Different letters represent significant differences ($p < 0.05$).

and employed to identify trends in crucial metabolites.

3. Results

3.1. Electronic nose detection

3.1.1. Milk electronic nose test results

After the VOC data obtained through sample monitoring by using the electronic nose were processed and analyzed, the results revealed that the contribution rates of the principal component (PC) variables PC1 and PC2 of milk reached 97.3% and 1.2%, respectively, and the cumulative contribution rate reached 98.5% (Fig. 1a). The cumulative contribution rate of three-dimensional (3D) PCA (Fig. 1b) reached 99.6%. The 32-sensor VOC data of the electronic nose were identified through linear discriminant analysis (LDA). Significant differences were observed among the three groups of sensors 5, 8, 23, 27, 29, and 31 in the milk group. The remaining sensors exhibited significant differences for C-SK and C-K (Fig. 1c). The analysis results of the measured milk data produced purified scores between the groups, which indicated that the electronic nose could effectively aid in distinguishing cows with ketosis on the basis of milk samples.

3.1.2. Fecal electronic nose test results

The PC1 and PC2 contributions to the VOC data of feces obtained using the electronic nose reached 95.6% and 1.9%, respectively, with a cumulative contribution rate of 97.5% (Fig. 2a). The cumulative contribution rate of 3D PCA (Fig. 2b) reached 98.4%. The 32-sensor VOC data of the electronic nose were identified through LDA. All 32 sensors in the fecal group exhibited significant effects on C-SK and C-K (Fig. 2c). The electronic nose distinguished cows with ketosis on the basis of fecal samples.

3.1.3. Blood electronic nose test results

PC1 and PC2 contributions to the VOC data obtained by analyzing blood samples with the electronic nose reached 91.9% and 3.7%, respectively, with a cumulative contribution rate of 95.6% (Fig. 3a). The cumulative contribution rate of 3D PCA (Fig. 3b) reached 96.6%. The 32-sensor VOC data of the electronic nose were identified through LDA. Among these sensors, 29 sensors exhibited significant effects on C-SK and SK-K. Sensor 2 exhibited no significance. Sensors 24 and 27 only displayed significance for SK-K (Fig. 3c). The analysis results of blood measurement data generated net division between the groups, indicating that blood data measured using the electronic nose effectively

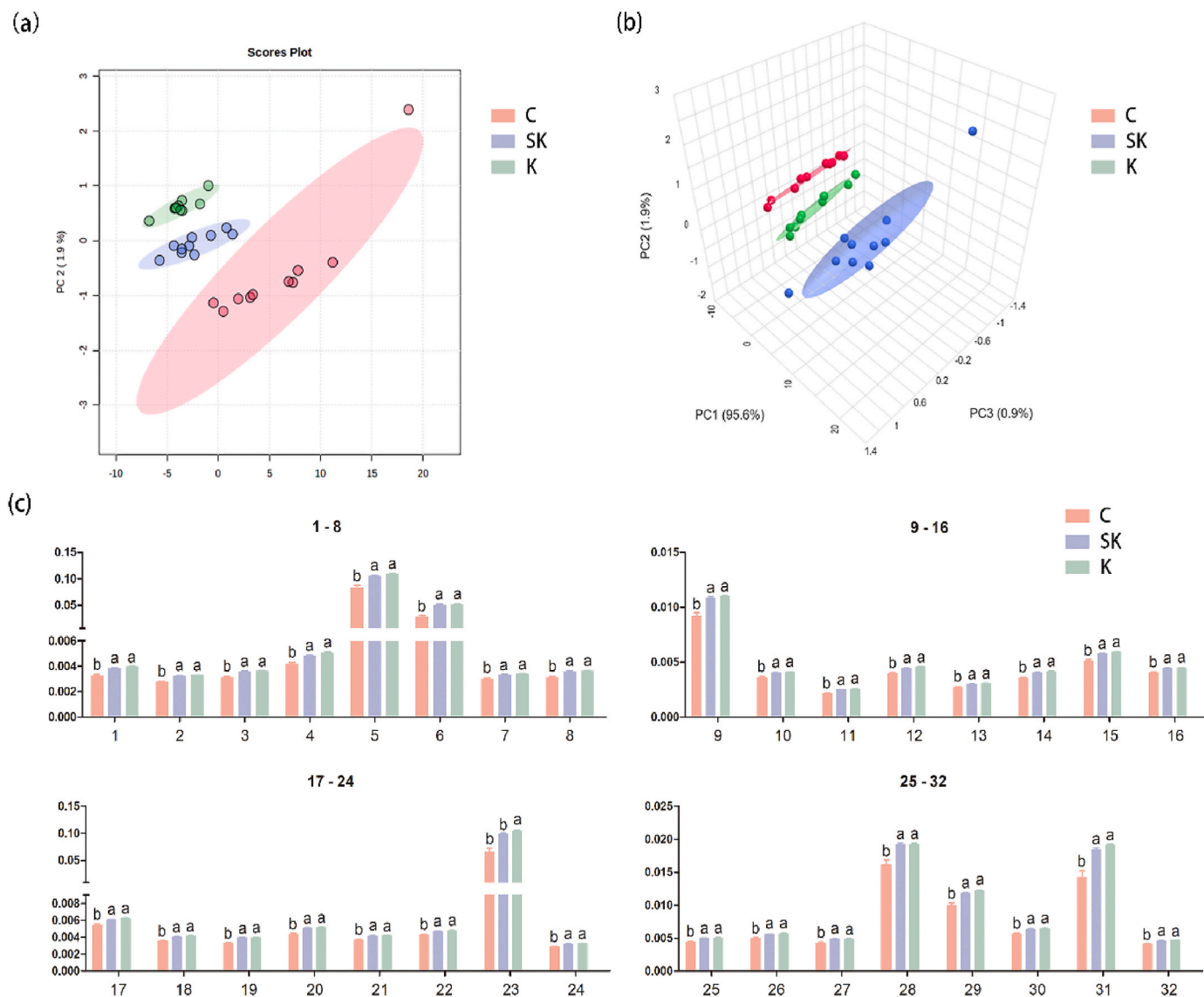


Fig. 2. VOC data obtained through electronic nose measurements of fecal samples. (a) PCA of feces. (b) Three-dimensional PCA of feces. (c) The peak values and significant differences between data obtained through the 32 sensors of the electronic nose. Different letters represent significant differences ($p < 0.05$).

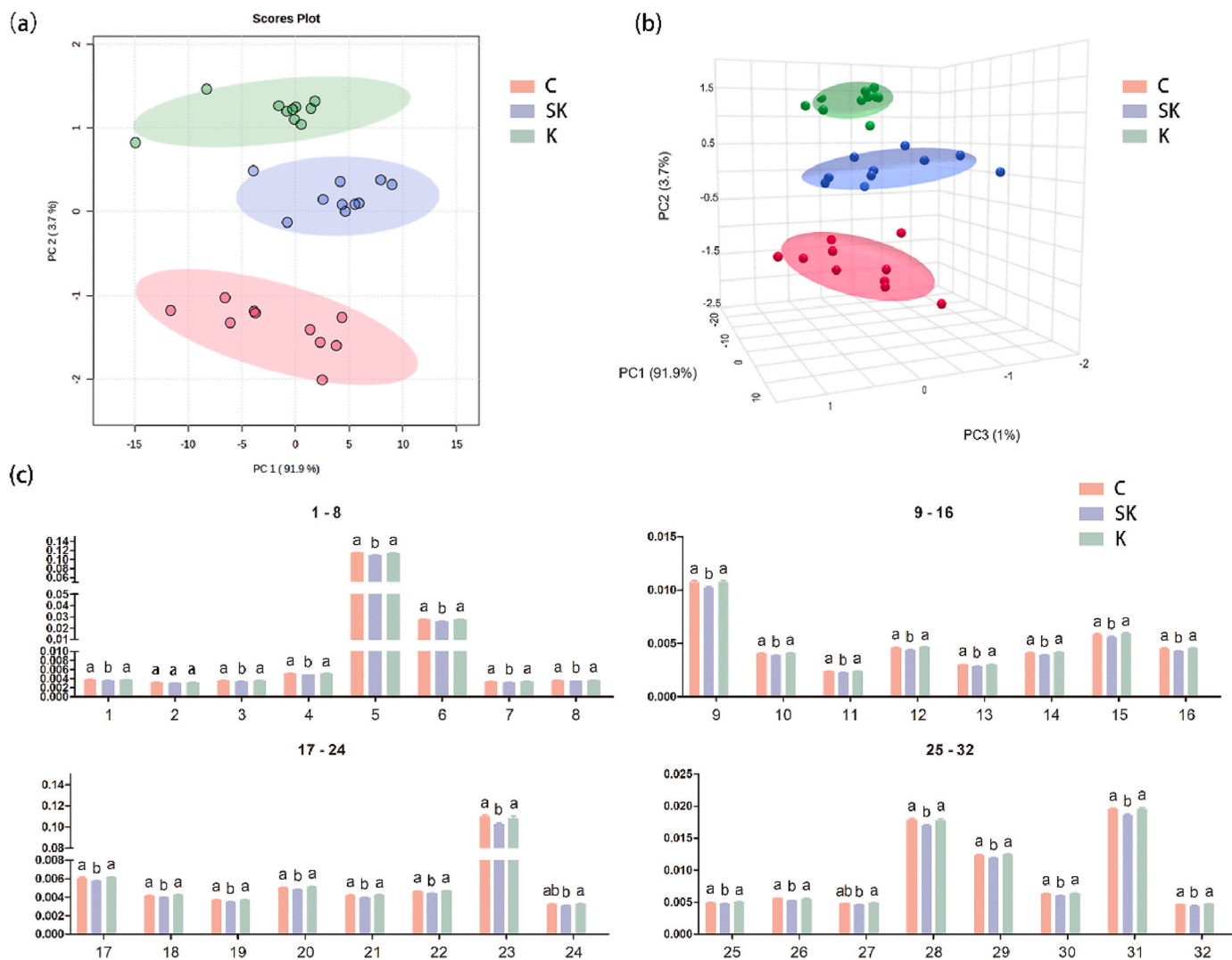


Fig. 3. VOC data obtained through electronic nose measurements of blood samples. (a) PCA of blood. (b) Three-dimensional PCA of blood. The peak values and significant differences between data obtained through the 32 sensors of the electronic nose. Different letters represent significant differences ($p < 0.05$).

distinguished and detected cows with ketosis.

3.2. Machine algorithm diagnosis and prediction of ketosis in cows

3.2.1. Results of the prediction model based on the VOC data obtained through milk sample analysis by using the electronic nose

VOC data obtained using the electronic nose sensors were trained and predicted based on machine learning algorithms. Table 1 presents the results of machine learning training of VOC data obtained using milk samples. The PNN model exhibited the highest accuracy of 87.5%. The accuracy of RF was 81.2% and that of SVM was 62.5%. Therefore, the milk data measured using the electronic nose can help establish a model for identifying ketosis in cows.

Table 1
Classification performance results of VOC data of milk samples.

Process	Accuracy/%	Precision/%	Recall/%	F1-score/%
RF	81.2	85.7	80.0	76.6
SVM	62.5	80.0	63.8	57.9
PNN	87.5	88.9	88.6	87.1

3.2.2. Results of the prediction model based on the VOC data obtained through fecal sample analysis by using the electronic nose

Table 2 presents the results of machine learning training of VOC data obtained using fecal samples. The SVM model displayed the highest accuracy and precision, all of which were 100%. The accuracy of both PNN and RF was 88.9%. Therefore, the fecal data measured using the electronic nose can help establish a model for identify ketosis in cows.

3.2.3. Results of the prediction model based on the VOC data obtained through blood sample analysis by using the electronic nose

Table 3 presents the results of machine learning training of VOC data obtained using blood samples. The PNN model exhibited the highest accuracy, precision, etc., at 66.7%. The accuracy of SVM was 66.7% and that of RF was 55.6%. Therefore, the blood data measured using the electronic nose can help establish a model for identifying ketosis in cows.

Table 2
Classification performance results of VOC data of fecal samples.

Process	Accuracy/%	Precision/%	Recall/%	F1-score/%
RF	88.9	91.7	88.9	88.6
SVM	100.0	100.0	100.0	100.0
PNN	88.9	91.7	88.9	88.6

Table 3
Classification performance results of VOC data of blood samples.

Process	Accuracy/%	Precision/%	Recall/%	F1-score/%
RF	55.6	63.3	55.6	56.7
SVM	66.7	50.0	66.7	55.6
PNN	66.7	66.7	66.7	65.7

3.3. GC-MS detection results of milk

The substances corresponding to the tested C, SK, and K group samples were analyzed. PLS-DA partially overlapped (Fig. 4a), and OPLS-DA exhibited obvious clustering (Fig. 4b). Potential biomarkers found through *t*-test (Fig. 4c) included glyceryl monostearate, phosphate, and glycerol. The heatmap results of the hierarchical clustering analysis are presented in Fig. 4d. Heatmap substances include lactic acid, glycerin, glyceryl monostearate, undecane, ribonolactone, inositol, lactose, phosphoric acid, palmitic acid, stearic acid, α -D-glucose, 3-hydroxybutyric acid, 1-monopalmitin, D-lactose, N-acetyl-D-glucosamine, and maltose.

3.4. Correlation analysis between electronic nose and GC-MS

To more accurately determine the metabolites related to electronic

nose-mediated ketosis detection, a correlation analysis was conducted between significant sensors and metabolites of milk by using the Pearson correlation coefficient (PPMCC). In statistics, the PPMCC value was between -1 and 1 , and the larger the absolute value, the stronger the correlation (Akoglu, 2018). Fig. 5 present the calculation results.

The absolute values of the Pearson correlation coefficients of 19 and 23 were >0.6 , which indicated that these substances are moderately correlated with the electronic nose sensor. The absolute values of the Pearson correlation coefficients of 2, 3, 4, 5, 6, 8, 11, 14, 15, 16, 17, and 22 were greater than 0.3 , which indicated that these substances are fairly correlated with the electronic nose sensor. The absolute values of the Pearson correlation coefficients of 1, 7, 9, 10, 13, 18, 20, and 21 were >0.1 , which indicated that these substances are poorly correlated with the electronic nose sensor. The absolute value of the Pearson correlation coefficient of substance 12 was <0.1 , and so, this substance was not correlated with the electronic nose sensor.

4. Discussion

Ketosis is among the most common nutritional and metabolic diseases in dairy cows (Holtenius & Holtenius, 1996). It manifests as elevated levels of ketone bodies, such as BHBA, acetoacetate, and acetone, in blood, urine, and milk. Ketosis in dairy cows is divided into two types: SK and K based on the BHBA concentration in the blood and

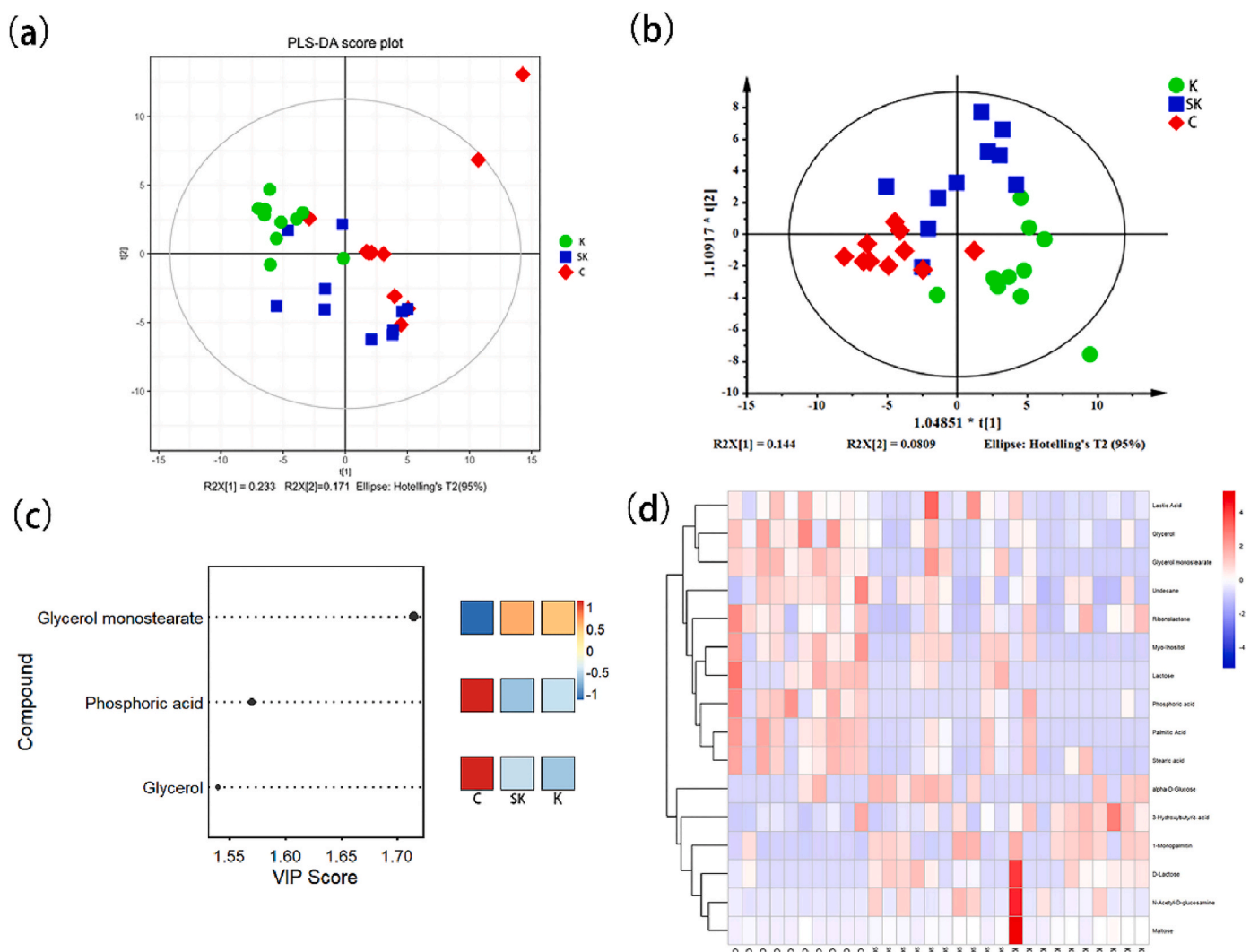


Fig. 4. Results obtained from the analysis of the measured data of the C, SK, and K groups. (a) PLS-DA chart of the C, SK, and K groups; (b) OPLS-DA chart of the C, SK, and K groups; (c) VIP score results of the C, SK, and K groups; (d) Hierarchical cluster analysis results of the C, SK, and K groups.

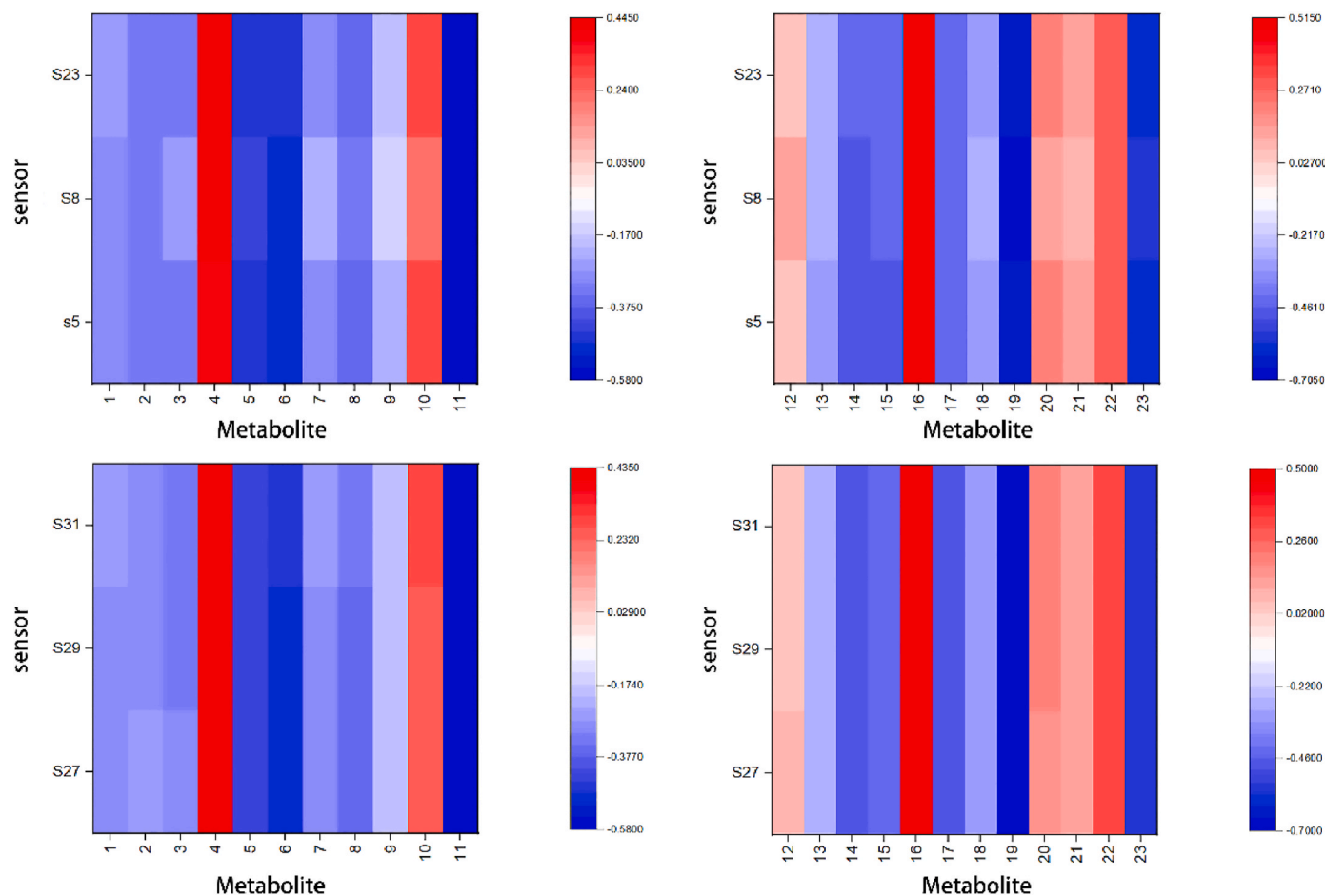


Fig. 5. Pearson correlation coefficient test results between electronic nose sensors and metabolites. 1: 2-ethylhexyl butyrate; 2: lactic acid; 3: undecane; 4: 3-hydroxybutyric acid; 5: glycerol; 6 phosphoric acid; 7: dodecyl iodide; 8: 2,4 -Di-tert-butylphenol; 9: ribose lactone; 10: N-acetyl-D-glucosamine; 11: palmitic acid; 12: palmitic acid; 13: ribose lactone; 14: inositol; 15: stearin acid; 16: 1-monopalmitin; 17: D-lactose; 18: maltose; 19: lactose; 20: melibiose; 21: α -D-glucose; 22: D-lactose; 23: glyceryl monostearyl acid ester. (The names of 11 and 12, 9 and 13, and 17 and 22 in the data are consistent. However, the numbers of the substances measured through GC-MS are different. Therefore, these substances were not eliminated from this test).

clinical characteristics in the blood (Mc Art et al., 2013). Cows with K have obvious clinical symptoms, manifesting as consumption symptoms, such as reduced appetite, weight, and milk production, or mental excitement and depression. Cows with SK have a normal appetite and no obvious clinical symptoms, but the content of ketone bodies in the blood, urine, or milk increases (Suthar et al., 2013). The SK incidence rate in China is as high as 45%. SK affects milk production performance and increases the risk of diseases such as ketosis, abomasum displacement, pleurisy, lameness, mastitis, metritis, cystic ovarian disease, and retained placenta, thereby augmenting the mortality rate of dairy cows, decreasing milk production, and reducing productivity such as calving. For example, cows with SK are 1.5, 9.5, and 5.0 times more likely to develop K, metritis, and abomasum displacement than ordinary cows, respectively (Doll et al., 2009). If the disease can be monitored in a timely manner, and warned and intervened early, the disease-related losses will be reduced. Disease monitoring is also a research hotspot on ketosis prevention and treatment in dairy cows.

The VOC fingerprint technology has marker characteristics and is currently common in human medicine, food safety, quality testing, and other aspects. As early as 1964, Wilkens and other scholars found that the electronic nose simulates olfactory monitoring of gases based on the electrode oxidation-reduction reaction. The electronic nose was first used to analyze odor changes (Wilkens and Hartman, 1964). Electronic noses are typically employed to analyze physical health conditions and accurately detect lung diseases and respiratory infections, such as cystic fibrosis and asthma (Hanna et al., 2019; Lai et al., 2002). According to a

previous study, the electronic nose can directly sample the perineal area to detect estrogen levels in dairy herds (Ali et al., 2022). The electronic nose is used for early cancer screening (Machado et al., 2005). In addition, the electronic nose can detect some bacterial species such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa in vitro*. The electronic nose detects *Mycobacterium tuberculosis in vitro* with 100% accuracy (Dragonieri et al., 2023). Electronic noses can be combined with multivariate statistical analysis for machine learning. These instruments can be used as a very convenient and fast detection tool. Disease diagnoses by examining biomarkers in VOCs is a new research area. VOCs are formed through animal cell metabolism, produced in these cells, released into the blood, and excreted with respiration and body fluids through the systemic circulation. Therefore, changes in VOCs must be analyzed to diagnose diseases to a certain extent.

The milk, feces, urine, exhaled gas, and blood of cows with ketosis can smell like rotten apples. Ketosis is a typical nutritional and metabolic disease wherein VOC excretion changes. In the present study, an electronic nose with specific sensors and identification modules was used to analyze and compare the VOCs of cows in the C, SK, and K groups. When different VOCs pass through the gas sensor array of the electronic nose, the 32 different sensors produce characteristic resistance change responses to them, preprocess the generated signals, enter a multidimensional response mode, rapidly evaluate different gases, and generate odor fingerprints. The aforementioned results suggest that the six sensors measured in the milk are crucial for the electronic nose

analysis of ketosis. This study revealed that the electronic nose can rapidly identify ketosis through the blood, milk, and feces of cows. The data of sensors 5, 8, 23, 27, 29, and 31 of the electronic nose were significant for the three cow groups. The PCA and PLS-DA results unveiled that VOC data obtained using the electronic nose can completely distinguish SK and CK. At the same time, we applied machine learning algorithms to the data measured using the electronic nose for training, modeling, and evaluation. The PNN model has higher prediction accuracy in the three sample groups than the RF and SVM models. The accuracy of the PNN model in milk, feces, and blood reached 87.5%, 88.9%, and 66.7%, respectively. This further proves the feasibility of using the electronic nose in ketosis diagnosis in dairy cows.

Based on the six significant sensors measured by the electronic nose by using milk samples, we suggest that the milk of ketosis cows has differential markers. Therefore, different milk groups were tested through GC-MS in this study to provide a reference value for the clinical application of electronic nose. As expected, differences were noted in the VOC types and contents in the three milk sample groups. In this study, 23 metabolites were screened out based on the p and VIP values of metabolically different substances monitored through GC-MS. Their contents were significantly different. The correlation analysis was conducted between these metabolites and the six sensors exhibiting significant differences in milk measured using the electronic nose. Significant correlations were noted between substances 19 (lactose) and 23 (glyceryl monostearate) and the electronic nose sensors. Metabolic pathway analysis unveiled that the levels of galactose, unsaturated fatty acids, and other substances increased to varying degrees after the cows became ill (Rodrigues et al., 2017; Zhao et al., 2021). According to the literature, these substances were a result of abnormal body metabolism due to ketosis in dairy cows (Coelho et al., 2015). This also fully illustrates the feasibility of using detectable equipment, such as GC-MS set up and electronic nose, to analyze VOC-based differential diagnosis and predict ketosis in dairy cows. This is because substances that undergo changes during animal body metabolism appear in milk and other excretions.

No differences were observed in the three substances BHBA, acetoacetic acid, and acetone that deserve special attention. These three substances are all volatile. During GC-MS, the samples need to be concentrated, which will cause their loss (Benedet, et al., 2019). The GC-MS detection of milk in dairy cows with ketosis has not been reported. Compared with the 23 differential substances detected through GC-MS, short-carbon-chain VOCs such as BHBA, acetoacetate, and acetone are the key differential substances detected through monitoring with the electronic nose. This also shows that the electronic nose can detect substances that cannot be detected through GC-MS. The two inspection methods are different and complementary to each other.

Compared with BHBA monitoring in blood to diagnose ketosis in dairy cows, detecting ketosis with the proposed electronic nose by using milk and feces samples of dairy cows is easier (Schepers, et al., 2022). At the same time, the electronic nose detects the VOC fingerprint spectrum of the sample. The monitoring model established by analyzing numerous samples is more reliable. Considering funding constraints and the impact on cow welfare, the number of samples used in this experiment was low. In 1996, Dobbelaar et al. investigated how gas exhaled from cows could predict whether they had ketosis (Dobbelaar et al., 1996). Through our basic experiments, we found that compared with collecting exhaled air and urine from cows, milk and fecal samples are a more welfare-friendly sampling method in dairy farms. Developing a more convenient and accurate electronic nose sensor device is a future research goal for the diagnosis of dairy cow diseases and intelligent pasture management.

5. Conclusion

This study proves that electronic nose recognition of VOCs combined with machine learning algorithms is a feasible technology for identifying and predicting ketosis in dairy cows. This is a non-invasive, rapid, and

accurate technology for ketosis detection in dairy cows.

CRediT authorship contribution statement

Enqiu Zhang: Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Fang Wang:** Software, Formal analysis. **Chun Yin:** Software, Methodology, Formal analysis. **Haizhong Wang:** Software, Formal analysis. **Yujun Zhu:** Software, Formal analysis. **Hongwei Duan:** Software, Methodology, Formal analysis. **Shuai Yang:** Software, Methodology, Formal analysis. **Yong Zhang:** Writing – review & editing. **Junjie Hu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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