RESEARCH ARTICLE

Feed safety in dairy cattle feeding: Evaluation of silage quality based on principal component analysis

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Received: June 29, 2023; **accepted:** August 16, 2023.

The quality of silage has an important impact on the growth and health of dairy cattle. Aiming at its quality evaluation, this study investigated and constructed the quality evaluation model of silage based on principal component analysis (PCA). The evaluation index system of silage quality and safety was established by using the analytic hierarchy process, and then, the comprehensive quality evaluation model of silage based on PCA was established. The results showed that cumulative variance contribution rate of 12 retained principal component feature roots reached 87.63%. According to the pH values, the superior and inferior feed samples from Inner Mongolia, China accounted for 35% and 12.3%, respectively, while superior and inferior feed samples from Beijing, China accounted for 30.48% and 12.24%, respectively. Based on the contents of ammonia nitrogen and organic acids, 72% of samples from Inner Mongolia had an excellent quality grade, while 70% of samples from Beijing had an excellent quality grade. The comprehensive silage quality evaluation model based on PCA demonstrated certain feasibility and effectiveness and was simpler than the construction method and calculation of fuzzy comprehensive evaluation, which positively affected the quality evaluation of silage, and had a certain role in promoting the long-term development of animal husbandry and feed industry.

Keywords: silage; principal component analysis; quality evaluation; hierarchical analysis; feed safety.

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Introduction

As living standards improved and health concepts changed, dairy products are becoming more popular among consumers, and food safety has always been an important issue concerning people's livelihood, while food safety of dairy products is inextricably linked to the safety of cow feeding. In addition to adequate lying down time, which is considered an important aspect of cow welfare, feed safety is crucial in their growth and health [1]. Silage has a high value of use in ruminant production and is an important source of feed for ruminants. Its primary challenge lies in preserving the feed during the fermentation process to achieve optimal nutritional and microbiological quality while minimizing fermentation losses, which is essential for maximizing its utility in ruminant nutrition [2, 3]. Silage is a class of feeds made from plant-based feeds with high moisture contents that are sealed and fermented, mainly for feeding ruminants. Ávila, *et al*. recognized that advancements in microbial identification techniques had significantly contributed to the understanding of the diversity of prokaryotes and eukaryotes in silage [4], which improved the understanding of how fermentation occurred in forage crops with diverse characteristics and how the fermentation process could be enhanced. Carvalho, *et al*.

concluded that the primary challenge in silage production was to preserve the feed while achieving high nutritional and microbial quality and minimizing fermentation losses. Many studies have focused on microbial additives, particularly lactic acid bacteria, which have been extensively studied and widely employed [5]. Zhao, *et al*. focused on addressing the existing knowledge gap regarding the relationship between yield and quality characteristics of silage maize, as well as the factors that influenced these parameters by collecting a comprehensive dataset with 5,663 observations from 196 publications across the country. Additionally, they assessed the impact of various management practices and climatic factors on the yield and overall quality of silage maize [6]. Du, *et al*. addressed the utilization of natural woody plant resources as animal feed to reveal the microbial symbiotic network and fermentation mechanism involved in silage production by using advanced PacBio single-molecule real-time sequencing technology and accurately unveiled these processes and mechanisms. The results demonstrated that their findings could be effectively employed in conjunction with wheat bran to create high-quality silage for animal production purposes [7]. In addition, Xu, *et al*. employed a comprehensive multi-omics approach to investigate the regulation of bacterial microbiota and metabolome, as well as their interactions, in whole-crop maize silage systems by inoculating the silage with either *Lactobacillus plantarum* or *Lactobacillus brucei* heterofermentative strains to gain a deeper insight into the intricate biological processes involved in silage fermentation. This approach facilitated an enhanced understanding of the complex dynamics at play, shedding light on the mechanisms underlying the fermentation process in whole-crop maize silage systems [8]. Blajman, *et al*. explored the effectiveness of lactic acid bacteria as silage inoculants for *Alfalfa* to provide a quantitative summary of published studies to shed light on this still unclear aspect. The result revealed that the inoculation of lactic acid bacteria resulted in a reduction in the concentration of acetate, propionate, ethanol, and butyrate in the silage, as well as a decrease in the number of yeasts and molds presented in the inoculum and improving aerobic stability [9].

Principal component analysis (PCA) is a statistical technique that transforms correlated variables into uncorrelated ones using orthogonal transformations. PCA is also a fundamental mathematical technique that finds extensive practical applications in various disciplines. It serves as a powerful analysis method to extract valuable insights and patterns from complex datasets and has also been used for R-peak detection [10, 11]. Schreiber established public factor models and PCA and found that PCA reduced variables [12]. PCA has been applied widely in many scientific areas including human health [13] and environmental health [14]. Privé, *et al*. stated that PCA was commonly used in various genetic analyses for inferring ancestry and for control of population structure [15]. Gewers, *et al*. reported several theoretical and practical aspects of PCA including the basic principles of PCA, data normalization, visualization of results, outlier detection, PCA-related methods, and other downscaling techniques, which were beneficial in helping researchers from the most diverse fields to use and interpret PCA [16]. Malik, *et al*. introduced an extension of the PCA transport framework by coupling PCA with Gaussian process regression in three-dimensional large eddy simulations to address the challenge of complexity in identifying low-dimensional flow patterns [17]. Another novel approach was introduced to enhance the predictability of PCA, which combined the forecasts from individual PCA subset regression models that utilized a subset of potential predictors to construct the PCA index to achieve improved predictive accuracy of PCA [18].

Although many previous studies used PCA for statistical analysis and affirmed the important value of silage for animal husbandry, there is still less research on comprehensive silage evaluation. This study established a silage quality and safety evaluation index system by using analytic hierarchy process (AHP), a systematic decision-making approach that breaks down the key elements involved in decision making into hierarchical levels, to evaluate and assess both qualitative and quantitative levels [19]. The results of this study would have important practical application value and prospect for the dairy cattle breeding industry.

Materials and Methods

Construction of silage quality and safety evaluation index system

The AHP was adopted in this study for system construction, which decomposed complex problems into several organized levels. The application steps of AHP were shown in Figure 1.

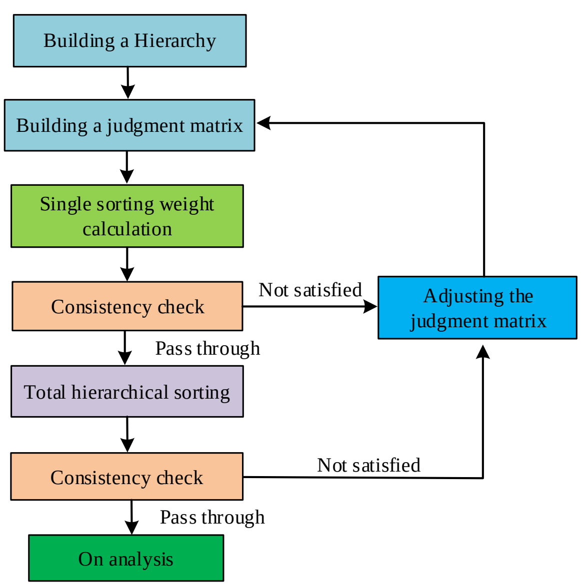

Figure 1. Application steps of AHP.

The consistent matrix method was used for two factors comparison and rating according to their importance. The matrix resulted by the comparison was judgment matrix as:

$$A = (a_{ij})_{n \times n} = \begin{pmatrix} a_{11}, a_{12}, \ldots, a_{1n} \\ a_{21}, a_{22}, \ldots, a_{2n} \\ \ldots \\ a_{n1}, a_{n2}, \ldots, a_{nn} \end{pmatrix} \tag{1}$$

where a_{ij} was the comparison result of the *i* factor compared with the *j* factor, and the matrix nature was shown in Equation (2).

$$a_{ij} = \frac{1}{a_{ji}} \tag{2}$$

The row vectors of the judgment matrix were geometrically averaged, and the eigenvectors were calculated as follows.

$$\overline{W_i} = \left(\prod_{j=1}^{n} a_{ij} \right)^{\frac{1}{n}}, (1 = 1, 2, \ldots, n) \tag{3}$$

Each feature vector was then normalized as shown in Equation (4).

$$W_i = \frac{\overline{W_i}}{\sum_{i=1}^{n} \overline{W_i}}, (i = 1, 2, \ldots, n) \tag{4}$$

Then, the eigenvector was $W = [W_1, W_2, \ldots, W_N]^T$. Matrix maximum eigenvalue was calculated as:

$$\lambda_{\max} = \frac{1}{n} \sum_{i=1}^{n} \frac{[AW]_i}{W_i}, (i = 1, 2, \ldots, n) \tag{5}$$

To avoid interference of other factors, the matrix consistency test was required as Equation (6).

$$\begin{cases} CR = \dfrac{CI}{RI} \\ CI = \dfrac{\lambda_{\max} - n}{n - 1} \end{cases} \tag{6}$$

where *CI* was deviation consistency index. *RI* was an average random consistency index, whose value was related to matrix order. *CR* was consistency ratio. When it was less than 0.1, matrix passed the one-time test, and *vice versa*.

The hierarchical structure of the silage evaluation index system established by hierarchical analysis was shown in Figure 2. The silage evaluation was targeted layer, and the characteristics of fermentation quality, nutritional quality, and safety were the criterion layer. The set of indicators under the criterion layer was the indicator layer.

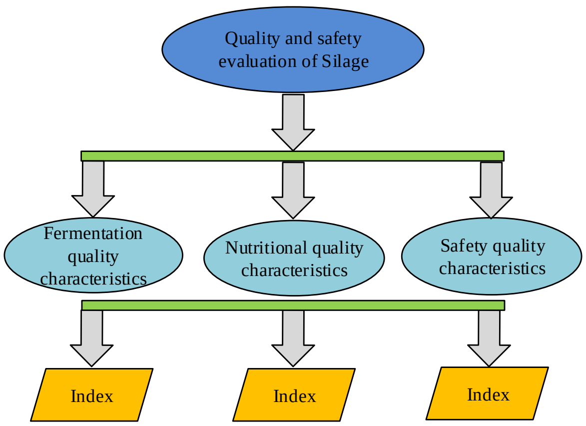

Figure 2. Hierarchical structure of silage evaluation index system.

Nutritional quality evaluation indexes included acid detergent fiber (ADF), neutral detergent fiber (NDF), and water-soluble carbohydrates, *etc*. The content of NDF in silage was calculated below.

$$NDF(\%) = \frac{m_3 - m_1}{m_2} \times 100\% \tag{7}$$

where $m1$ was the weight of the glass crucible. $m2$ was specimen's weight. $m3$ was weight of glass crucible and *NDF*. The content of ADF in silage was calculated below.

$$ADF(\%) = \frac{m_3' - m_1}{m_2} \times 100\% \tag{8}$$

where $m3'$ was the weight of the glass crucible and *ADF*. The safety characteristics of the feed then affected the animal's health and the coordination of physiological functions. The evaluation indexes of safety characteristics included natural toxic and harmful substances, exogenous toxic and harmful substances, and secondary toxic and harmful substances.

Construction of PCA-based integrated silage quality evaluation model

PCA was employed to evaluate silage comprehensive quality. A few integrated variables that reflected the main information with certain correlations were selected and transformed with more variables and different degrees of correlations into a new set of data with fewer and independent variables to search the linear relationship between the variables and simplify the data structure. The definition of PCA was shown in equation 9.

$$\begin{cases} Z_1 = l_{11}x_1 + l_{12}x_2 + \ldots + l_{1p}x_p \\ Z_2 = l_{21}x_1 + l_{22}x_2 + \ldots + l_{2p}x_p \\ \ldots \\ Z_m = l_{m1}x_1 + l_{m2}x_2 + \ldots + l_{mp}x_p \end{cases} \tag{9}$$

where Xp was the original variable indicator. Z_m was the new variable indicator. m was less than or equal to p. l_{ij} was the loadings. Zi and Zj were uncorrelated with Z_1 as the highest variance among all linear combinations of original variable indicators and Z_2 represented those that were uncorrelated with Z_1, and so on. Thus, PCA was determining the loadings of the original variables on the principal components. Assuming that there were n samples and each of which in turn had p variables, the original sample matrix constituted would be:

$$A = (x_{ij})_{n \times q} = \begin{bmatrix} x_{11}, x_{12}, \ldots, x_{1p} \\ x_{21}, x_{22}, \ldots, x_{2p} \\ \ldots \\ x_{n1}, x_{n2}, \ldots, x_{np} \end{bmatrix}, i = 1,2,\ldots,n,\ j = 1,2,\ldots,p \tag{10}$$

When p was large, dimensionality reduction was required, which meant replacing more original variable indicators with less comprehensive indicators. The raw data required in the silage evaluation system were collected, and correlation coefficient matrix was calculated as follows.

$$\begin{cases} R = (r_{ij})_{n \times q} \\ r_{ij} = \dfrac{1}{n} \sum_{i=1}^{n} \dfrac{(x_{ij} - x_i)(x_{ij} - x_j)}{S} \end{cases} \tag{11}$$

where S was the variance of the sample. r_{ij} was the correlation coefficient between X_i and X_j. Contribution margin and cumulative contribution margin were calculated as:

$$\begin{cases} d_i = \dfrac{\lambda_i}{\sum_{i=1}^{p} \lambda_i} \\ D_m = \dfrac{\sum_{i=1}^{m} \lambda_i}{\sum_{i=1}^{p} \lambda_i} \end{cases} \quad (12)$$

where d_i was the contribution rate. λ_i was the matrix eigenvalues, and $|R-\lambda_i|=0$. D_m was cumulative contribution rate. When D_m was close to 1, the first m indicator was selected as the principal component to replace variables, and then, principal component was evaluated comprehensively and calculated as:

$$Z_m = a_{mj} x_j \quad (13)$$

where a_{mj} was the feature vector. The silage evaluation system was huge. The traditional method was to determine each quality indicator of silage, add up the scores of each index of each silage sample to calculate the total score, and finally compare the quality of each silage sample, which was a tedious process with low efficiency and accuracy. The PCA-based silage quality and safety evaluation model constructed in this study consisted of three main steps (Figure 3), which included (1) the characteristic roots and contribution rates in PCA to select principal components, which retained the top 12 components with an 85% cumulative variance contribution rate or more by run results. Principal components were formalized linear combinations, and the magnitude of loading values of each indicator trait in the principal components reflected the importance of each indicator trait in the principal components; (2) the integrated principal component scores of each silage sample were calculated based on the functional expressions of the first 12 principal components, and then, the integrated quality evaluation of silage was carried out based on the principal component scores; (3) the integrated quality of silage quality and safety was clustered and analyzed based on the principal components that could express 87.62% of the integrated characteristics of the original index system traits, and the basic situation of quality and safety of different silages was analyzed.

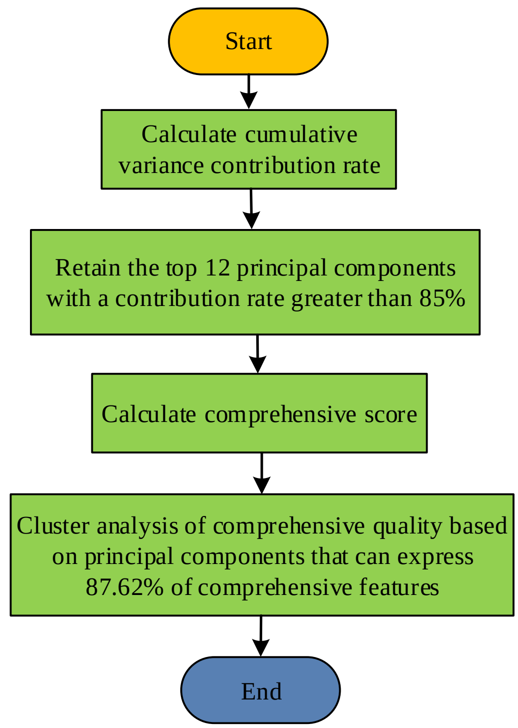

Figure 3. Flow chart of silage quality and safety assessment model based on PCA.

Applications of PCA-based silage quality and safety evaluation system

Total 200 silage samples with 100 from Beijing (Houderui Trading Co., Ltd., Beijing, China) and 100 from Inner Mongolia (Hulunbuir Mengcheng Qingcang Feed Production Co., Ltd., Hulunbuir, Inner Mongolia, China) were evaluated and compared to each other by using the PCA-based silage quality and safety evaluation system developed in this study. The correlations between the results were then investigated.

Table 1. Silage quality and safety index screening.

Number	Index	Number	Index
1	Smell	15	Acid detergent fiber (ADF)
2	Texture	16	Crude ash
3	Color	17	Calcium
4	pH	18	Phosphorus
5	Lactic acid	19	Lead
6	Acetic Acid	20	Arsenic
7	Propionic Acid	21	Mercury
8	Butyrate	22	Chromium
9	Ammoniacal nitrogen	23	Cadmium
10	Dry matter	24	Organophosphorus
11	Crude protein	25	Nitrate
12	Crude fat	26	Nitrite
13	Water soluble carbohydrate	27	Aspergillus flavus B1
14	neutral detergent fiber (NDF)	28	Melamine

Results and discussion

Screening and analysis of silage evaluation indicators

To establish silage quality and safety evaluation system, this study used frequency statistics method analyzing existing domestic and international related reports and literatures to select the indicators with high frequency of use and high relevance. The relevant theoretical analyses were then conducted on each selected indicator for further confirmation. The expert consultation was performed to solicit professional opinions and adjust the preliminary selected evaluation indicators. The first-round screening of silage quality and safety indicators included 34 characteristics including 11 fermentation quality indicators, 10 nutritional quality indicators, and 13 safety and quality rating indicators. A number of experts in silage quality testing were invited to hold a workshop for the second round of screening, and finally 4 new indicators were added, while 10 indicators were deleted, resulting in a collection of 28 indicators (Table 1).

The silage fermentation quality and nutritional characteristics

The comprehensive quality of silage feed is influenced by fermentation quality and nutritional characteristics. After clarifying the silage evaluation indicators, the silage comprehensive characteristics could be further scientifically and reasonably evaluated through mathematical methods [20]. The evaluation indicators for silage fermentation quality include pH value, volatile fatty acids, and liquid ammonia. This study applied sensory evaluation methods to evaluate silage feed samples in three aspects including smell, color, and texture. The results showed that 52.6% of silage samples from Inner Mongolia were rated as Grade 1 with only 0.01% samples were rated as Grade 4. Among the feed samples from Beijing, 51.08% were rated as first grade, while only 0.01% were rated as fourth grade (Figure 4). Therefore, according to the results of sensory evaluation, the fermentation qualities of silages from these two regions were all good. Silage pH is an indicator reflecting the degree of decomposition of silage raw materials and the effect of nutrient preservation. It is also an important indicator for silage quality evaluation. The silage qualities were assessed as excellent, good, average, and inferior with pH values less than 4, between 4.1-4.3, between 4.4-5, and greater than 5, respectively. In this study, silage samples from Inner Mongolia consisted of 35% excellent and 12.3% inferior, while silage samples from Beijing were 30.48% excellent and 12.24% inferior (Figure 5). Therefore, there was a

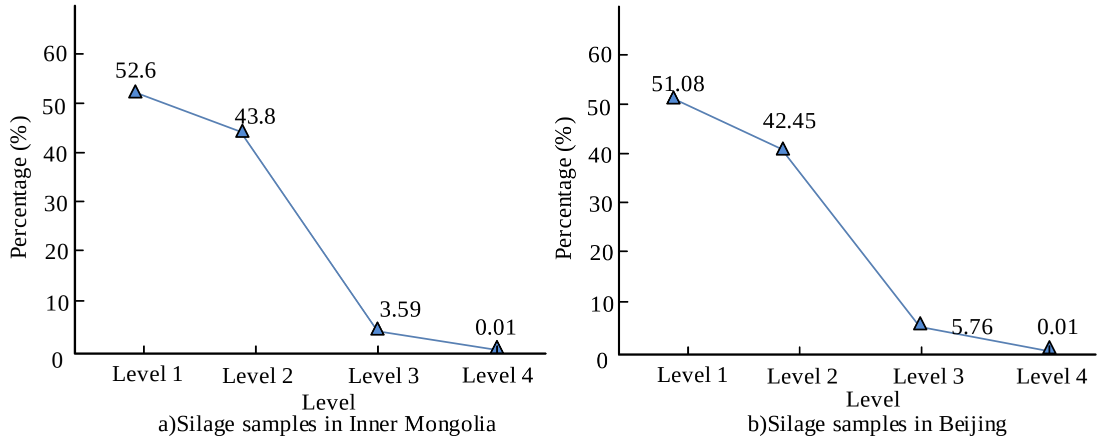

Figure 4. The fermentation qualities of silage samples from Inner Mongolia (a) and Beijing (b) based on sensory evaluation.

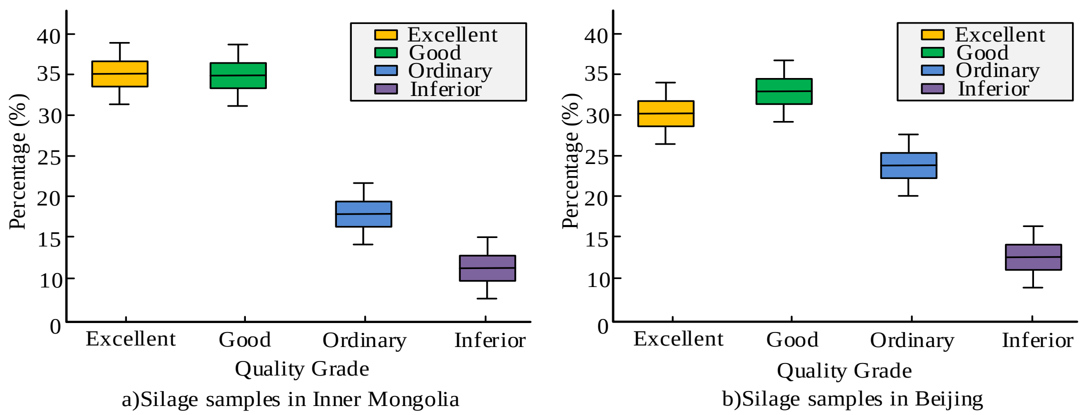

Figure 5. The fermentation qualities of silage samples from Inner Mongolia (a) and Beijing (b) based on the pH values.

significant difference between the feed grade evaluated based on the pH value and the sensory evaluation. In addition, the ammoniacal nitrogen and organic acids produced in the fermentation process of the above samples were also evaluated comprehensively. The silage samples from Inner Mongolia demonstrated 72% excellent and 1% inferior quality, while the feed samples from Beijing showed 70% excellent and 1% inferior quality (Figure 6), indicating that the compositions of ammonia nitrogen and organic acid of silages in these two different regions were reasonable, and the decompositions of protein and amino acid were not serious. Due to the significant differences in the evaluation results of the silage fermentation quality from different methods, a correlation analysis was conducted on the different evaluation methods of silage fermentation quality. The results showed that there was no correlation between sensory evaluation and the other two methods. The correlation between fermentation quality score and sensory evaluation was 0.5385, which was not significant (P > 0.05), and there was also no correlation with pH value. The correlation between pH value and fermentation quality score was 0.0001, with an extremely significant correlation (P < 0.001). The correlation with sensory score was 0.0305, with a significant correlation (P < 0.05).

Silage feed is made from plant feed that contains a large amount of water and is sealed and fermented. Therefore, attention has been paid to

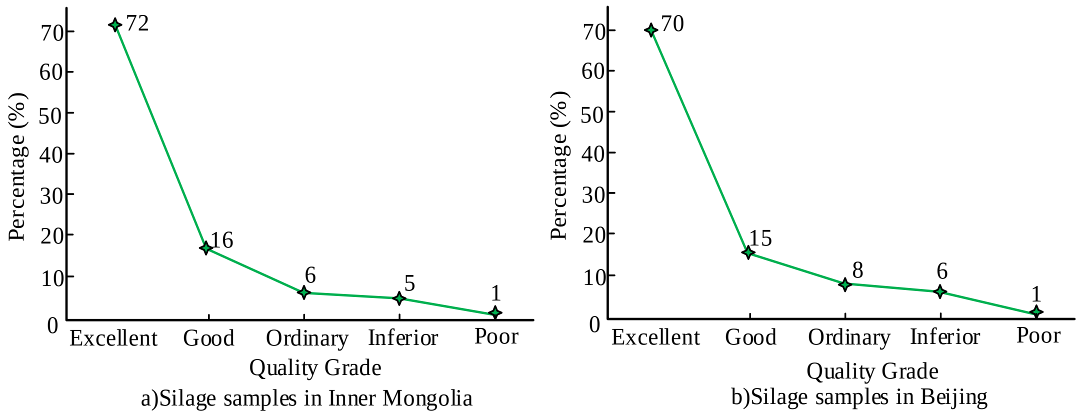

Figure 6. The fermentation qualities of silage samples from Inner Mongolia (a) and Beijing (b) based on the ammoniacal nitrogen and organic acids production in the fermentation process.

Table 2. Variance and cumulative variance contribution rate of 12 principal components.

Number	Eigenvalue	Variance contribution rate	Cumulative variance contribution rate
1	4.5213	0.2263	0.2265
2	2.4007	0.1202	0.3467
3	1.8019	0.0899	0.4367
4	1.4196	0.0698	0.5071
5	1.3099	0.0662	0.5718
6	1.2485	0.0631	0.6354
7	1.0257	0.0504	0.6859
8	0.9598	0.0497	0.7336
9	0.8194	0.0406	0.7748
10	0.7083	0.0361	0.8098
11	0.6798	0.0337	0.8451
12	0.6299	0.0322	0.8759

the determination of fermentation quality, which results in certain limitations in the comprehensive silage quality evaluation. The silage fermentation quality not only directly reflects the preservation effect, but also has a great correlation with its nutritional value. Fermented silage can better maintain the nutritional composition of green forage, but it can also improve the palatability and feeding efficiency of forage and straw, thereby improving the utilization efficiency of livestock. Among fermentation quality, nutritional quality and safety quality, fermentation quality is the predominant factor being used to assess overall silage quality, while nutritional quality is the foundation of silage feed quality, which is the basic guarantee for livestock growth, development, and production performance. Along with fermentation and safety quality, it affects the value of silage feed feeding.

Verification of the PCA-based integrated silage quality evaluation system

The silage from Beijing was selected to verify the application effect of PCA-based silage comprehensive quality evaluation system. The results of the top 12 principal components whose cumulative variance contributions were above 85% were shown in Table 2. The magnitude of the loading values of each indicator trait in the principal components was shown in Table 3, which reflected the importance of each indicator trait in the principal components. Among them, principal components 1, 2, 3, 4, and 6 mainly

Table 3. Eigenvector of correlation matrix of silage quality and safety traits.

Index	1	2	3	4	5	6
Sensory score	-0.2187	0.1471	-0.2203	0.2292	-0.0637	0.3068
Propionic acid	0.2294	0.1890	-0.1840	-0.0285	0.1429	0.1301
Butyric acid	0.2479	0.2431	-0.0972	0.0042	-0.0607	-0.0829
Volatile base nitrogen /Total nitrogen	0.1995	0.2507	-0.1639	0.1407	-0.1877	-0.2302
Dry matter	0.0692	-0.2238	0.3849	0.3929	-0.1058	-0.0899
Crude protein	-0.1543	0.4053	0.3186	-0.0391	0.0859	-0.0017
Crude fat	-0.0485	0.1469	0.1250	-0.2994	-0.0462	0.6142
Neutral detergent fiber	0.2931	-0.3375	-0.0716	0.2107	-0.0763	-0.0203
Acid detergent fiber	0.3644	-0.2367	0.0441	0.2582	0.0348	-0.2148
Lead	-0.1091	0.2031	-0.1191	0.2471	0.4558	0.0691
Arsenic	0.0890	0.0853	-0.1245	-0.1903	0.4860	-0.3304
Metabolic energy	-0.0672	-0.0719	0.0733	0.2348	-0.4957	-0.1196
Index	**7**	**8**	**9**	**10**	**11**	**12**
Sensory score	0.0571	-0.1059	0.5041	-0.2730	0.0148	0.0791
Propionic acid	0.0652	0.5419	0.1018	-0.1871	0.2556	-0.1842
Butyric acid	0.4699	0.1071	-0.0579	0.3662	-0.2341	-0.3163
Volatile base nitrogen /Total nitrogen	0.1557	-0.4580	-0.2278	0.1903	0.2796	0.1746
Dry matter	0.2983	0.1451	-0.0261	-0.3369	-0.0095	0.1897
Crude protein	-0.1720	-0.0368	-0.0515	0.1072	-0.3316	0.2573
Crude fat	0.0937	-0.0392	0.1459	0.3558	0.2958	-0.0652
Neutral detergent fiber (NDF)	0.0458	0.0287	0.1696	0.0199	-0.3671	-0.0820
Acid detergent fiber (ADF)	-0.1330	0.0479	-0.1038	-0.0079	-0.0965	-0.0842
Lead	0.3622	0.2601	-0.1399	-0.0112	-0.1780	0.4497
Arsenic	-0.4645	0.0597	0.2760	0.2668	0.0491	-0.0998
Metabolic energy	-0.1879	0.5271	0.0847	0.4041	0.0159	0.3541

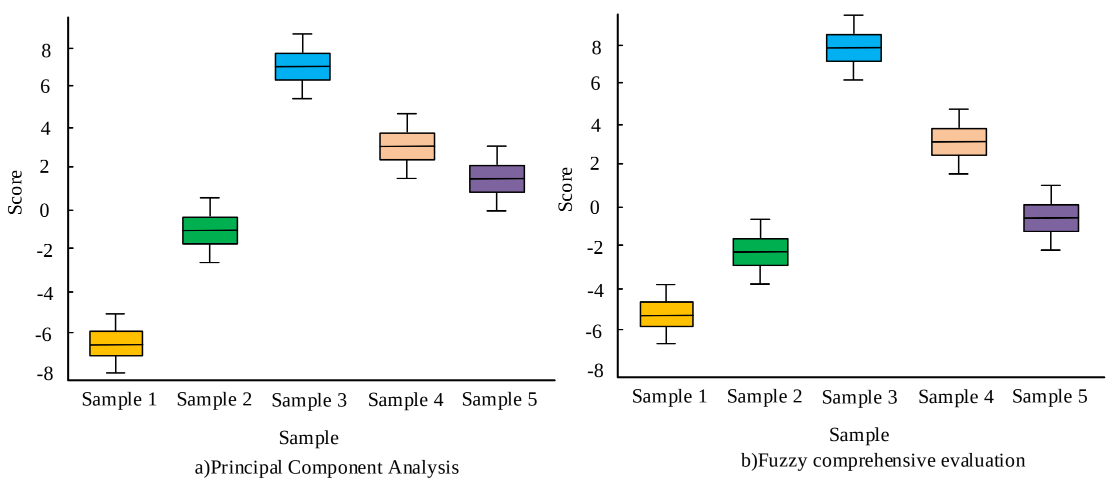

Figure 7. Sample comprehensive scores.

reflected the nutritional quality traits, while principal components 7, 8, 9, and 10 mainly reflected the fermentation quality traits and principal components 5, 11, and 12 mainly reflected the safety quality traits. In addition, the correlation coefficients of the 12 retained principal components with NDF, ADF, and melamine were large. Since cumulative variance contribution of characteristic roots of principal components retained in this study reached 87.63%, which could represent most of the information in the evaluation index system, the retained principal components were used for further analysis. The composite score of the principal components was calculated separately for each silage sample based on the functional expression of the principal components. The quality and safety of the five silages from Beijing were compared according to their scores of the composite quality, which was then compared with scores of fuzzy comprehensive evaluation method. The composite scores obtained from those two different methods were shown in Figure 7. There was a slight difference in the scores of the five samples obtained by the two methods, and the scores of the samples based on the PCA method were samples 3, 4, 5, 2, and 1 in descending order, which were consistent with the results obtained by fuzzy comprehensive evaluation method. However, the accuracy might be affected by the selection of factors and weight distribution, and the judgment matrix construction method was more complicated, and the calculation was more cumbersome. Therefore, the comprehensive quality evaluation model of silage based on PCA had certain feasibility and validity.

Conclusion

With the changes in health concepts and dietary habits, dairy products are becoming increasingly popular among consumers. Silage feed is one of the main feed materials for ruminants. Its quality not only affects the development of the feed industry itself, but also affects the growth and health status of livestock, as well as the quality of corresponding dairy and meat products. Therefore, establishing a silage feed system that meets international standards is crucial for the long-term development of the feed industry and animal husbandry. However, establishing an evaluation index system for the quality and safety of silage feed is an extremely complex process, which must comply with both objective reality and scientific statistical principles. It follows the principles of hierarchy and systematicity, integrity and correlation, applicability and universality, as well as the combination of qualitative and quantitative analysis. This study established an evaluation index system for the quality and safety of silage feed, and a comprehensive evaluation model for silage quality based on PCA to more accurately evaluate the quality and safety of silage feed. The model constructed by this study demonstrated a certain feasibility and validity. However, the samples selected for the research experiments were still not rich enough, which might affect the practical application of the model. Therefore, more silage sample data need to be collected to prove the effectiveness of the model in silage quality evaluation, so that it can be better applied to the actual feed production process and improve the safety of dairy cattle feeding.

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