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Characterization of Weight Loss, Nutrient Composition, and Fermentation Quality of King Grass Silage Under Different Temperatures and Ensiling Periods

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Abstract

To characterize the trends in the fermentation weight loss (FWL), nutrient composition, and fermentation quality of king grass silage under different ensiling temperatures and storage periods, chopped and wilted king grass was ensiled at 15 °C, 20 °C, 25 °C, 30 °C and 35 °C for 7, 30, 60 and 120 days, respectively. Nutritional and fermentation-related parameters were predicted by near-infrared reflectance spectroscopy (NIRS) using the general Dairy One forage database. FWL was determined gravimetrically, and correlation and cluster analyses were performed for comprehensive data evaluation. The results showed that ensiling temperature, storage period, and their interaction significantly affected the FWL, nutrient composition (except for the soluble protein-to-crude protein ratio, SP/CP), and fermentation quality (except for the butyric acid, BA) of king grass silage ($p < 0.05$). FWL tended to increase with rising temperature and prolonged ensiling duration. With extended ensiling time, NIRS-predicted acid detergent fiber (ADF), neutral detergent fiber (NDF), ash, BA, ammonia, and SP/CP exhibited increasing trends. By contrast, NIRS-predicted dry matter, water-soluble carbohydrates (WSCs) and pH decreased significantly from day 7 to day 30 ($p < 0.05$). The 15 °C treatment favored the preservation of NIRS-predicted WSCs and crude protein over long-term ensiling (120 d). The NIRS-predicted ammonia under 25 °C remained at a relatively low level across the whole ensiling period; lactic acid was highest at days 7 and 60, whereas no significant difference was observed among temperatures at day 120 ($p > 0.05$). Both low temperature (15 °C) and high temperature (35 °C) were unfavorable for rapid pH decline. At most sampling time points, NIRS-predicted ADF and NDF contents under 30 °C and 35 °C were significantly higher than those under 15 °C ($p < 0.05$). Correlation analysis showed that ADF, NDF, ash, ammonia, and BA were positively correlated with each other; WSCs were negatively correlated with SP/CP, ADF, NDF, ash and acetic acid but positively correlated with pH and the lactic acid-to-acetic acid ratio. Cluster analysis classified the 13 silage quality indicators into three clusters and the 60 treatment samples into five clusters. This work offers preliminary references for developing differentiated king-grass-ensiling strategies adapted to diverse climatic regions.

Keywords: king grass; silage; ensiling temperature; ensiling period; nutrient composition; fermentation quality

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Academic Editor: Nelson P. Guerra

Received: 27 August 2026
Revised: 28 October 2026
Accepted: 28 October 2026
Published: 1 November 2026

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1. Introduction

Silage is an indispensable basal feed in ruminant diets. High-quality silage not only provides optimal nutrition for livestock but also reduces the input of concentrate feed [1], thereby improving the economic efficiency of animal production and lowering feeding costs. However, ensiling fermentation is inherently a complex biochemical process driven by microorganisms. Silage quality is co-determined by multiple factors, including raw material characteristics, environmental conditions, and the species and population of lactic acid bacteria (LAB) [2]. Among these, ensiling time and temperature are two critical factors affecting silage quality. Low temperature inhibits LAB proliferation and prolongs the ensiling process, leading to increased nutrient losses and poor fermentation quality [3]. Conversely, high temperature reduces lactic acid (LA) content, elevates pH and dry matter (DM) loss, decreases aerobic stability, and ultimately lowers the feeding value of silage [4].

Previous studies have shown that during ensiling, the contents of LA, acetic acid (AA) and propionic acid in oat silage continuously accumulated, while the contents of water-soluble carbohydrates (WSCs), acid detergent fiber (ADF), neutral detergent fiber (NDF) and pH decreased during the initial 0–30 days [5]. For alfalfa ensiled for 30–90 days, pH and the contents of AA, butyric acid (BA) and ammonia nitrogen (NH_3-N) increased, whereas the WSC and LA contents decreased [6]. Ensiling oat at 5 °C reduced LAB activity, slowed acidification, and increased pH and NH_3-N levels, indicating poor fermentation efficiency [7]. In contrast, high-moisture Napier grass ensiled at 15 °C exhibited lower pH, AA and NH_3-N levels [8]. Silage of *Moringa oleifera* leaves at 30 °C showed higher DM loss, AA and NH_3-N levels [9], whereas cauliflower waste silage was optimal at 30–35 °C [10]. Whole-plant corn silage at 21–30 °C benefited from higher acid production, a rapid pH decline, and lower DM loss [11]. These findings suggest that the optimal ensiling temperature and storage time vary depending on the forage material.

It is noteworthy that time and temperature do not exert independent effects on silage quality; rather, their interaction plays a critical role. Studies have demonstrated that storage temperature, fermentation time, and their interaction significantly affect the fermentation quality of high-moisture alfalfa silage: with prolonged fermentation, LA content increased significantly at 15 °C but decreased significantly at 35 °C [12]. For total mixed-ration silage, fermentation intensity remained weak at 5 °C for up to 30 days, with acceptable LA production observed only by 90 days, whereas effective acidification was achieved early at 25–35 °C [13]. These studies indicate that examining the individual effects of temperature or time without considering their interaction is insufficient to fully understand the dynamic changes in silage quality. A systematic analysis of the temperature × time interaction is therefore essential for precisely regulating ensiling fermentation.

Pennisetum purpureum × *Pennisetum glaucum* cv. Reyan No.4 (hereafter referred to as king grass) is an important high-quality grass species in tropical regions, characterized by high yield and good palatability, making it an ideal green forage for cattle, sheep, pigs, and poultry [14,15]. Owing to its rapid biomass accumulation and concentrated yield in summer, king grass can be ensiled to alleviate seasonal feed shortages in winter and spring [16]. However, research on the effects of time and temperature on king grass silage remains limited. Li et al. [4] evaluated the silage quality of king grass and reported that 30 °C was more favorable than 25 °C or 35 °C for 30-day ensiling. Nevertheless, the temperature range of king grass cultivation areas spans from 10 °C to 37.9 °C [17], which leads to marked differences in ensiling temperatures depending on the geographic location. In addition, the ensiling period for king grass typically exceeds 30 days by a substantial margin. Moreover, existing studies lack multi-indicator-integrated analyses based on correlation analysis and cluster analysis, making it difficult to elucidate the networked and phased response characteristics of silage quality under the combined effects of time and temperature.

Thus, the present study evaluated the effects of four ensiling durations and five temperature levels on the fermentation weight loss (FWL), nutrient composition, and fermentation quality of king grass silage. Correlation and cluster analyses were performed to comprehensively evaluate the data and reveal the dynamic evolution pattern of king grass silage quality.

2. Materials and Methods

2.1. Silage Preparation

King grass was grown at the experimental base of the Tropical and Subtropical Cash Crops Research Institute, Yunnan Academy of Agricultural Sciences (Baoshan, China). The king grass was harvested at the vegetative stage (approximately 2.0 m in height) and chopped into about 2 cm pieces using a forage shredder (Fengcheng Huaqiang Agricultural Machinery Parts Factory, Dandong, China). The raw forage was wilted in the sun to a DM content of approximately 30%, and then thoroughly mixed to ensure homogeneity. For the characterization of the pre-ensiled forage, three subsamples were collected from different positions of the wilted forage pile after thorough mixing, and each subsample was analyzed once. Subsequently, 400 g of the wilted forage was packed into polyethylene bags and vacuum-sealed using a vacuum sealer (Deli Group Co., Ltd., Ningbo, China). A total of 60 bags (4 ensiling times × 5 ensiling temperatures × 3 replicates) were weighed and stored at 15, 20, 25, 30, and 35 °C, respectively. The FWL was determined gravimetrically by weighing the intact sealed bags at each sampling time (7, 30, 60, and 120 days) without opening them. Thereafter, these bags were opened to determine nutrient composition and fermentation quality.

2.2. Weight Loss

The FWL of the silage was assessed by calculating the difference in bag weights between pre-ensiling and day x (x = 7, 30, 60, and 120) under each of the five temperature treatments, according to the method of Xu et al. [18].

2.3. Nutrient Composition and Fermentation Quality Analyses

Near-infrared spectroscopy (NIRS) was employed to determine the nutrient composition and fermentation quality of all silage samples, with measurements conducted by Ulanqab Yima Agriculture and Animal Husbandry Technology Co., Ltd. (Ulanqab, China). Prior to analysis, samples were dried at 65 °C and ground to a particle size of 1 mm. Spectral data were acquired using a FOSS DS 2500 spectrometer (FOSS, Hillerød, Denmark) scanning across the 400–2500 nm range with a resolution of 0.5 nm. The resulting spectra were interpreted against the specialized feed and forage nutritional database supplied by Dairy One (Ithaca, NY, USA), utilizing the HAYLAGE KT calibration model (Product Number: 300KT) for compositional prediction. The NIRS-predicted indicators included DM, crude protein (CP), soluble protein-to-crude protein ratio (SP/CP), ADF, NDF, WSCs, ash, pH, LA, AA, BA, and ammonia.

2.4. Statistical Analysis

All experiments were conducted in triplicate. The results are presented as means with standard error of the mean (SEM) in parentheses in tables, and as means with standard deviation (SD) in bar charts (n = 3). All statistical analyses were performed using the SPSSAU (Version 25.0 and 26.0) online platform (https://spssau.com/indexs.html, accessed on 18 October 2026). A two-way ANOVA was conducted with ensiling time, temperature, and their interaction as fixed factors. Tukey's multiple comparison test was employed to

determine significant differences among treatment means, and the significance threshold was set at $p < 0.05$.

Prior to correlation analysis, the Kolmogorov–Smirnov test was used to check the normality of the data, along with skewness and kurtosis coefficients, in order to determine whether the Pearson or the Spearman correlation coefficient should be applied. Before cluster analysis, the data were standardized using Z-score normalization. Line graphs with error bars, correlation heatmaps, and cluster heatmaps were generated using the ChiPlot online platform (https://www.chiplot.online/, accessed on 19 October 2026). Hierarchical clustering was performed using Seuclidean distance as the distance metric and the Average method as the clustering algorithm.

3. Results

3.1. Pre-Ensiling Nutrient Composition and Fermentation Parameters of King Grass

As shown in Table 1, the NIRS-predicted DM and WSC contents of king grass prior to ensiling were 30.67% and 11.93%, respectively. The initial pH of the raw material was 5.59, reflecting a low-acid condition prior to ensiling. Notably, trace amounts of LA (1.42%), AA (0.25%), and BA (0.02%) were already present in the raw material.

Table 1. Chemical characteristics of king grass prior to ensiling.

Items	King Grass Straw	SEM
DM (% FW)	30.67	0.224
CP (% DM)	4.24	0.066
SP/CP (% DM)	41.67	0.667
ADF (% DM)	41.43	0.296
NDF (% DM)	62.42	0.679
WSCs (% DM)	11.93	0.384
Ash (% DM)	7.96	0.140
pH	5.59	0.084
LA (% DM)	1.42	0.269
AA (% DM)	0.25	0.000
BA (% DM)	0.02	0.023
Ammonia (% DM)	0.10	0.000

DM, dry matter; FW, fresh weight; CP, crude protein; SP/CP, soluble protein-to-crude protein ratio; ADF, acid detergent fiber; NDF, neutral detergent fiber; WSCs, water-soluble carbohydrates; LA, lactic acid; AA, acetic acid; BA, butyric acid.

3.2. FWL During Ensiling

The dynamics of FWL during ensiling are shown in Table 2 and Figure 1. The storage time, ensiling temperature, and their interaction significantly influenced FWL ($p < 0.05$). FWL increased significantly with prolonged ensiling time across all temperature treatments. Rising ensiling temperature also led to an increasing FWL at each storage time point, with the FWL under the 35 °C treatment being significantly higher than that under the other temperature treatments ($p < 0.05$). After 120 days of ensiling at 35 °C, the FWL reached 51.71 g per bag, representing 12.93% of the pre-ensiling weight (400 g).

Table 2. Characteristics of ensiling temperatures and days on FWL during ensiling.

Ensiling Temperatures (°C)	FWL at Ensiling Days (g)				SEM	*p*-Value	Significance		
	7	30	60	120			T	D	T × D
15	1.58dC	4.28cC	7.33bE	11.26aE	1.094	<0.001			
20	2.25dBC	5.97cC	9.59bD	15.74aD	1.508	<0.001			
25	2.60dBC	6.63cC	13.32bC	23.67aC	2.416	<0.001	<0.001	<0.001	<0.001
30	3.02dB	9.93cB	16.55bB	28.18aB	2.823	<0.001			
35	4.49dA	15.55cA	27.76bA	51.71aA	5.290	<0.001			
SEM	0.274	1.085	1.919	3.779					
p-value	<0.001	<0.001	<0.001	<0.001					

Data were means of three replicates. Values with different lower-case letters show significant differences among ensiling days in the same temperature; values with different capital letters show significant differences among ensiling temperature in the same days ($p < 0.05$); SEM, standard error of means; T, temperatures; D, days; T × D, interaction between ensiling temperatures and days.

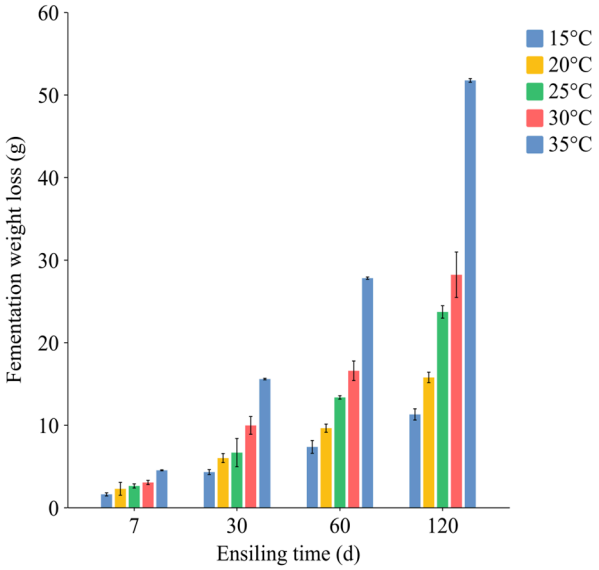


Figure 1. Changes in fermentation weight loss under different temperatures during ensiling. Values are means ± SD ($n = 3$), with error bars indicating standard deviation.

3.3. Nutrient Composition Dynamics of King Grass Silage

The dynamics of the NIRS-predicted DM, CP, ADF, NDF, WSCs, ash, and SP/CP during ensiling are shown in Table 3. The ensiling days, ensiling temperatures and their interaction significantly influenced the predicted DM, CP, ADF, NDF, WSC, ash contents, and SP/CP ($p < 0.05$), while for NIRS-predicted SP/CP, only the main effects of temperatures and days were significant ($p < 0.05$), whereas their interaction was not.

With prolonged ensiling, a marked decline in the NIRS-predicted DM content was observed during the early stage (0–30 d) across all temperature treatments (Figure 2a). Under the 15 °C, 20 °C, and 25 °C treatments, the predicted DM content rebounded significantly during the later stage (60–120 d) ($p < 0.05$). Under the 25 °C treatment, the predicted DM content during the early stage (7–60 d) was significantly lower than that at the later stage (120 d) ($p < 0.05$). Furthermore, from day 7 to day 60 of ensiling, the predicted DM content at 25 °C was significantly lower than that at other temperatures ($p < 0.05$), whereas at day 120, it became significantly higher ($p < 0.05$).

Table 3. Characteristics of ensiling temperatures and days on nutritional quality during ensiling.

Items	Ensiling Temperature (°C)	Ensiling Days				SEM	*p*-Value	Significance		
		7	30	60	120			T	D	T × D
DM (% FW)	15	27.76bD	27.56cB	26.91dC	28.24aB	0.144	<0.001	<0.001	<0.001	<0.001
	20	28.55aA	26.47dD	26.55cD	27.01bD	0.253	<0.001			
	25	27.59bE	26.25cE	24.63dE	28.78aA	0.466	<0.001			
	30	28.39bC	27.61cA	28.86aA	27.25dC	0.190	<0.001			
	35	28.46aB	27.02cC	28.46aB	28.26bB	0.181	<0.001			
	SEM	0.106	0.148	0.403	0.179					
	p-value	<0.001	<0.001	<0.001	<0.001					
WSCs (% DM)	15	11.17aA	7.57bA	6.43bB	7.53bA	0.555	<0.001	<0.001	<0.001	<0.001
	20	9.40aBC	3.53cC	6.53bAB	4.10cB	0.719	<0.001			
	25	11.13aA	3.47cC	6.37bB	3.53cB	0.941	<0.001			
	30	10.57aAB	2.97cC	7.23bAB	3.33cB	0.943	<0.001			
	35	8.47aC	4.77bB	7.50aA	4.17bB	0.559	<0.001			
	SEM	0.300	0.453	0.151	0.435					
	p-value	<0.001	<0.001	0.017	<0.001					
ADF (% DM)	15	41.60cB	43.10bB	45.87aA	44.60aB	0.497	<0.001	<0.001	<0.001	<0.001
	20	42.87cA	45.03bA	45.50bAB	46.20aA	0.379	<0.001			
	25	41.47cB	44.90bA	45.10bB	46.43aA	0.560	<0.001			
	30	43.23cA	45.33bA	45.47bAB	46.57aA	0.368	<0.001			
	35	43.23cA	45.17bA	45.47bAB	46.23aA	0.338	<0.001			
	SEM	0.231	0.224	0.082	0.205					
	p-value	0.001	<0.001	0.029	<0.001					
NDF (% DM)	15	62.29dB	65.76cB	70.06aA	67.76bB	0.866	<0.001	<0.001	<0.001	<0.001
	20	61.87dB	66.83cA	69.33bAB	69.92aA	0.959	<0.001			
	25	61.81dB	65.80cB	68.66bB	69.98aA	0.948	<0.001			
	30	64.11cA	66.64bAB	69.83aA	70.10aA	0.745	<0.001			
	35	63.42cA	66.22bAB	69.89aA	70.26aA	0.849	<0.001			
	SEM	0.258	0.136	0.149	0.251					
	p-value	<0.001	0.008	0.001	<0.001					
Ash (% DM)	15	9.69cA	10.14bcA	10.60bB	11.14aA	0.170	<0.001	<0.001	<0.001	0.015
	20	9.04cB	9.97bA	10.78aAB	11.08aA	0.245	<0.001			
	25	10.16bA	10.46bA	11.22aA	11.13aA	0.143	<0.001			
	30	9.73cA	10.52bA	11.03aAB	11.23aA	0.180	<0.001			
	35	9.68cA	10.27bcA	10.80abAB	11.00aA	0.167	0.001			
	SEM	0.106	0.078	0.075	0.036					
	p-value	0.001	0.116	0.044	0.391					
CP (% DM)	15	4.45bcAB	4.57bA	4.10cC	5.18aA	0.122	<0.001	<0.001	<0.001	<0.001
	20	4.26aBC	4.36aAB	4.22aBC	4.36aB	0.028	0.216			
	25	4.63aA	4.22bB	4.59aA	4.36bB	0.054	<0.001			
	30	4.18bCD	4.53aA	4.39abAB	4.48aB	0.045	0.007			
	35	4.00cD	4.59aA	4.17bcBC	4.35bB	0.069	<0.001			
	SEM	0.061	0.045	0.051	0.089					
	p-value	<0.001	0.007	0.001	<0.001					
SP/CP (%)	15	54.00aCD	59.33aA	61.00aA	61.33aB	0.177	0.070	<0.001	<0.001	0.076
	20	53.00bD	60.00aA	63.67aA	61.67aB	1.288	<0.001			
	25	60.33cA	65.00abA	62.33bcA	67.67aA	0.878	<0.001			
	30	56.67bBC	65.00aA	68.33aA	65.33aAB	1.392	<0.001			
	35	58.33bAB	62.67abA	65.33abA	67.00aA	1.196	0.022			
	SEM	0.780	0.833	0.975	0.786					
	p-value	<0.001	0.045	0.123	0.001					

Data were means of three replicates. Values with different lower-case letters show significant differences among ensiling days in the same temperature; values with different capital letters show significant differences among ensiling temperature in the same days ($p < 0.05$); SEM, standard error of means; T, temperatures; D, days; T × D, interaction between ensiling temperatures and days. DM, dry matter; FW, fresh weight; CP, crude protein; SP/CP, soluble protein-to-crude protein ratio; ADF, acid detergent fiber; NDF, neutral detergent fiber; WSCs, water-soluble carbohydrates.

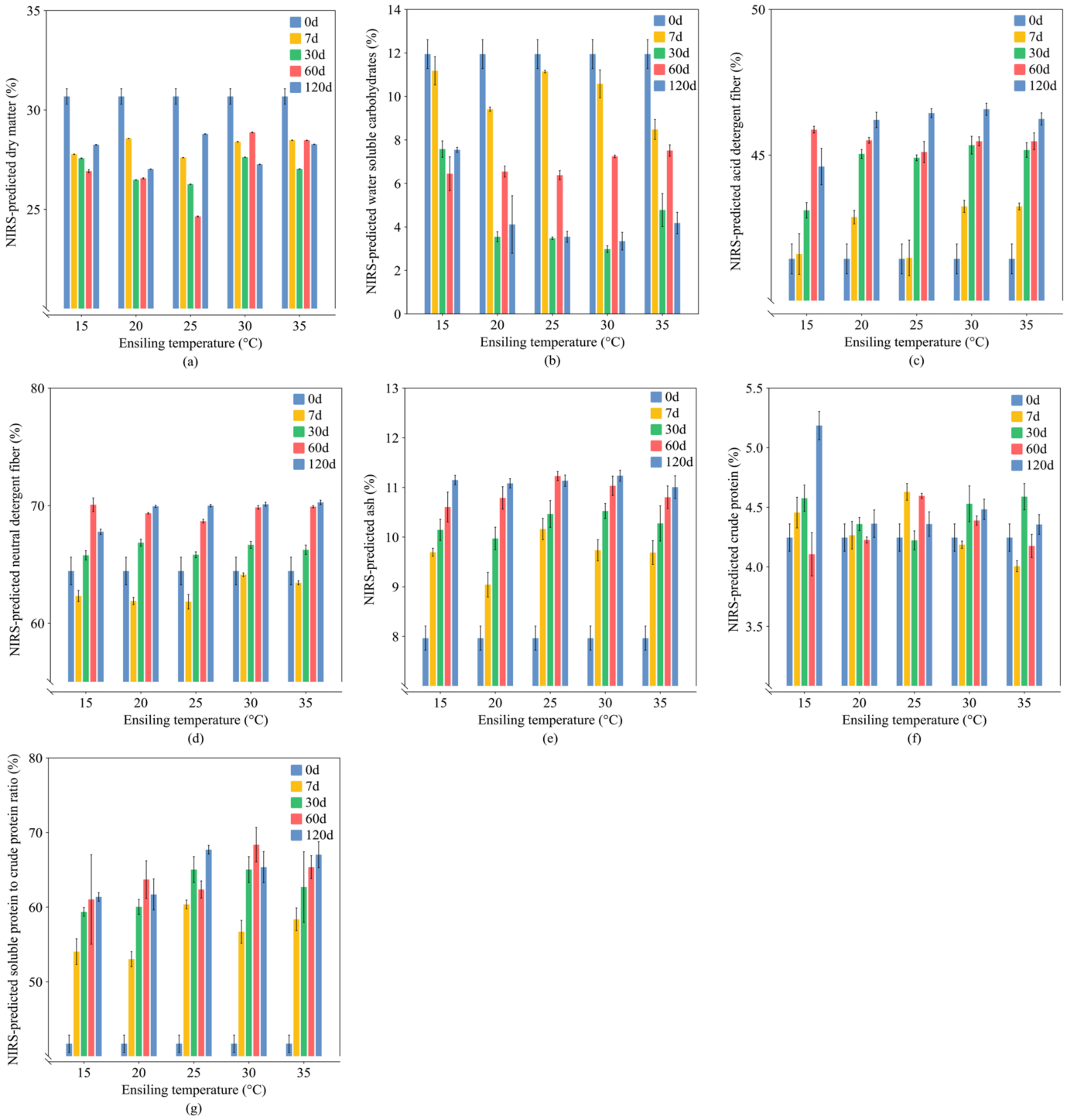


Figure 2. Changes in nutritional quality under different temperatures during ensiling. Values are means ± SD (n = 3), with error bars indicating standard deviation. (**a**) Dry matter; (**b**) water-soluble carbohydrates; (**c**) acid detergent fiber; (**d**) neutral detergent fiber; (**e**) ash; (**f**) crude protein; (**g**) soluble protein-to-crude protein ratio. Parameter (**a**) was measured in fresh silage samples; parameters (**b**–**g**) were expressed on a dry matter basis.

Across all temperature treatments, the NIRS-predicted WSC content exhibited a marked decline during the early stage of ensiling (0–30 d) (Figure 2b). Notably, a subsequent increase in the predicted WSC content was observed during the stage (30–60 d) of fermentation. The timing of the predicted WSC content increase under the 15 °C treatment lagged behind that of the other temperature treatments. Throughout the ensiling period,

the 15 °C treatment effectively preserved WSCs at all time points, with significantly higher predicted WSC contents observed at day 30 and day 120 compared to the other temperatures ($p < 0.05$).

With prolonged ensiling time, the NIRS-predicted ADF (0–120 d) and NDF (7–120 d) contents tended to increase under all temperature treatments (Figure 2c,d). At day 7 of ensiling, both predicted ADF and NDF levels were significantly lower than those at other time points ($p < 0.05$). At most sampling time points, the predicted ADF and NDF contents under the 30 °C and 35 °C treatments were significantly higher than those under the 15 °C treatment.

The NIRS-predicted ash content gradually increased across all temperature treatments over time and then leveled off (Figure 2e). Overall, the differences in the predicted ash content among temperature treatments at various storage time points were relatively small. Specifically, at day 30 and day 120 of ensiling, no significant differences were observed among temperature treatments ($p > 0.05$). These results suggest that the predicted ash content varied only slightly across different temperatures.

No consistent temporal or temperature-dependent pattern was observed for the NIRS-predicted CP content across different temperature treatments (Figure 2f). At 20 °C, no significant differences in the predicted CP content were detected among the various storage time points ($p > 0.05$). At day 120 of ensiling, the predicted CP content under 15 °C was significantly higher than that under other temperature treatments. Furthermore, low temperature (15 °C) favored the retention of CP during long-term ensiling (120 days).

Across all temperature treatments, the NIRS-predicted SP/CP increased with prolonged ensiling time (Figure 2g). However, in the present experiment, with the exception of the 25 °C treatment, no significant differences in the predicted SP/CP were observed among the remaining temperature treatments from day 30 to day 120 of ensiling ($p > 0.05$).

3.4. Fermentation Quality Dynamics of King Grass Silage

Dynamic changes in the NIRS-predicted pH, LA, AA, BA, ammonia and lactic acid-to-acetic acid ratio (LA/AA) during ensiling are shown in Table 4. The ensiling days, ensiling temperature, and their interaction significantly influenced the predicted LA, AA, ammonia, pH and LA/AA levels ($p < 0.05$), while for the predicted BA, only the main effects of days and their interaction were significant ($p < 0.05$), not the temperature.

As ensiling progressed, the NIRS-predicted pH decreased across all temperature treatments during the period from day 0 to day 30 post-ensiling (Figure 3a). Under the 20 °C and 25 °C treatments, the predicted pH continued to decline significantly from day 30 to day 60 ($p < 0.05$), whereas the remaining temperature treatments exhibited either an increasing trend or a plateau. Notably, under 25 °C, the predicted pH decreased persistently until day 120, suggesting that fermentation proceeded relatively well under this temperature condition. As temperature increased, the predicted pH at each storage time point showed a trend of first decreasing and then increasing. Among all storage time points, the predicted pH under 15 °C remained at a relatively high level. At both day 7 and day 60 of ensiling, the highest predicted pH was observed under 35 °C.

With prolonged ensiling time, the NIRS-predicted LA content exhibited slight fluctuations (Figure 3b). Under 15–30 °C, the predicted LA content reached its maximum values on day 60 of ensiling, and under 35 °C, the peak also occurred on day 30. Meanwhile, under 15–25 °C, predicted pH was at its lowest or relatively low levels on day 60. During the period from day 60 to day 120 of ensiling, the predicted LA content showed a decreasing trend. On day 7 and day 60 of ensiling, the highest predicted LA content was observed under the 25 °C. However, no significant differences in predicted LA content were observed among temperature treatments on day 120 ($p > 0.05$).

Table 4. Characteristics of ensiling temperature and days on fermentation quality during ensiling.

Items	Ensiling Temperature (°C)	Ensiling Days				SEM	*p*-Value	Significance		
		7	30	60	120			T	D	T × D
pH	15	4.78aA	4.64bcA	4.60cA	4.70bA	0.021	<0.001	<0.001	<0.001	<0.001
	20	4.77aA	4.62bA	4.44dB	4.56cB	0.036	<0.001			
	25	4.62aB	4.54bB	4.45cB	4.44cD	0.022	<0.001			
	30	4.68aB	4.53cB	4.61bA	4.39dE	0.033	<0.001			
	35	4.84aA	4.55cB	4.65bA	4.48dC	0.040	<0.001			
	SEM	0.021	0.013	0.024	0.029					
	p-value	<0.001	<0.001	<0.001	<0.001					
LA (% DM)	15	2.53bB	1.69cC	3.23aC	2.94aA	0.179	<0.001	<0.001	<0.001	<0.001
	20	0.99cD	2.56bB	3.27aBC	2.56bA	0.254	<0.001			
	25	3.34abA	2.61bB	3.95aA	2.71bA	0.185	0.007			
	30	2.78bB	2.61bB	3.84aAB	2.65bA	0.165	0.001			
	35	1.72cC	3.62aA	3.15abC	2.38bcA	0.233	<0.001			
	SEM	0.225	0.175	0.102	0.089					
	p-value	<0.001	<0.001	0.002	0.416					
AA (% DM)	15	0.25cB	1.15aA	0.78bB	0.44cB	0.109	<0.001	<0.001	<0.001	<0.001
	20	0.76bA	0.53bB	1.12bAB	1.99aA	0.182	0.001			
	25	0.25dB	0.58cB	1.40bA	1.93aA	0.200	<0.001			
	30	0.39cB	1.25bA	0.97bB	2.03aA	0.183	<0.001			
	35	0.99bA	1.20bA	0.30cC	1.77aA	0.163	<0.001			
	SEM	0.083	0.088	0.103	0.173					
	p-value	<0.001	<0.001	<0.001	<0.001					
BA (% DM)	15	0.09bA	0.20bA	0.24abA	0.41aA	0.039	0.005	0.262	<0.001	0.014
	20	0.13bA	0.02bB	0.31aA	0.40aA	0.047	<0.001			
	25	0.08bA	0.00cB	0.33aA	0.35aA	0.046	<0.001			
	30	0.08bA	0.00bB	0.14bA	0.45aA	0.056	0.001			
	35	0.11aA	0.08aAB	0.29aA	0.31aA	0.040	0.046			
	SEM	0.008	0.025	0.031	0.019					
	p-value	0.156	0.017	0.345	0.105					
LA/AA	15	10.11aA	1.47cC	4.34bcB	7.66abA	1.101	0.003	<0.001	<0.001	<0.001
	20	1.31cB	4.83aA	2.97bB	1.40cB	0.446	<0.001			
	25	13.37aA	4.49bA	2.83cB	1.41dB	1.410	<0.001			
	30	8.98aA	2.11bBC	4.06abB	1.31bB	1.064	0.014			
	35	1.78bB	3.03bB	10.79aA	1.35bB	1.181	<0.001			
	SEM	1.355	0.357	0.826	0.766					
	p-value	<0.001	<0.001	<0.001	0.003					
Ammonia (% DM)	15	0.10bA	0.10bA	0.31aB	0.39aA	0.040	<0.001	<0.001	<0.001	<0.001
	20	0.10bA	0.10bA	0.10bC	0.44aA	0.045	<0.001			
	25	0.10aA	0.10aA	0.14aC	0.15aB	0.013	0.521			
	30	0.10bA	0.10bA	0.36aB	0.31aA	0.037	<0.001			
	35	0.10bA	0.10bA	0.55aA	0.13bB	0.058	<0.001			
	SEM	0.000	0.000	0.045	0.036					
	p-value	1.000	1.000	<0.001	<0.001					

Data were means of three replicates. Values with different lower-case letters show significant differences among ensiling days in the same temperature; values with different capital letters show significant differences among ensiling temperature in the same days ($p < 0.05$); SEM, standard error of means; T, temperatures; D, days; T × D, interaction between ensiling temperatures and days. DM, dry matter; FW, fresh weight; LA, lactic acid; AA, acetic acid; BA, butyric acid; LA/AA, lactic acid-to-acetic acid ratio.

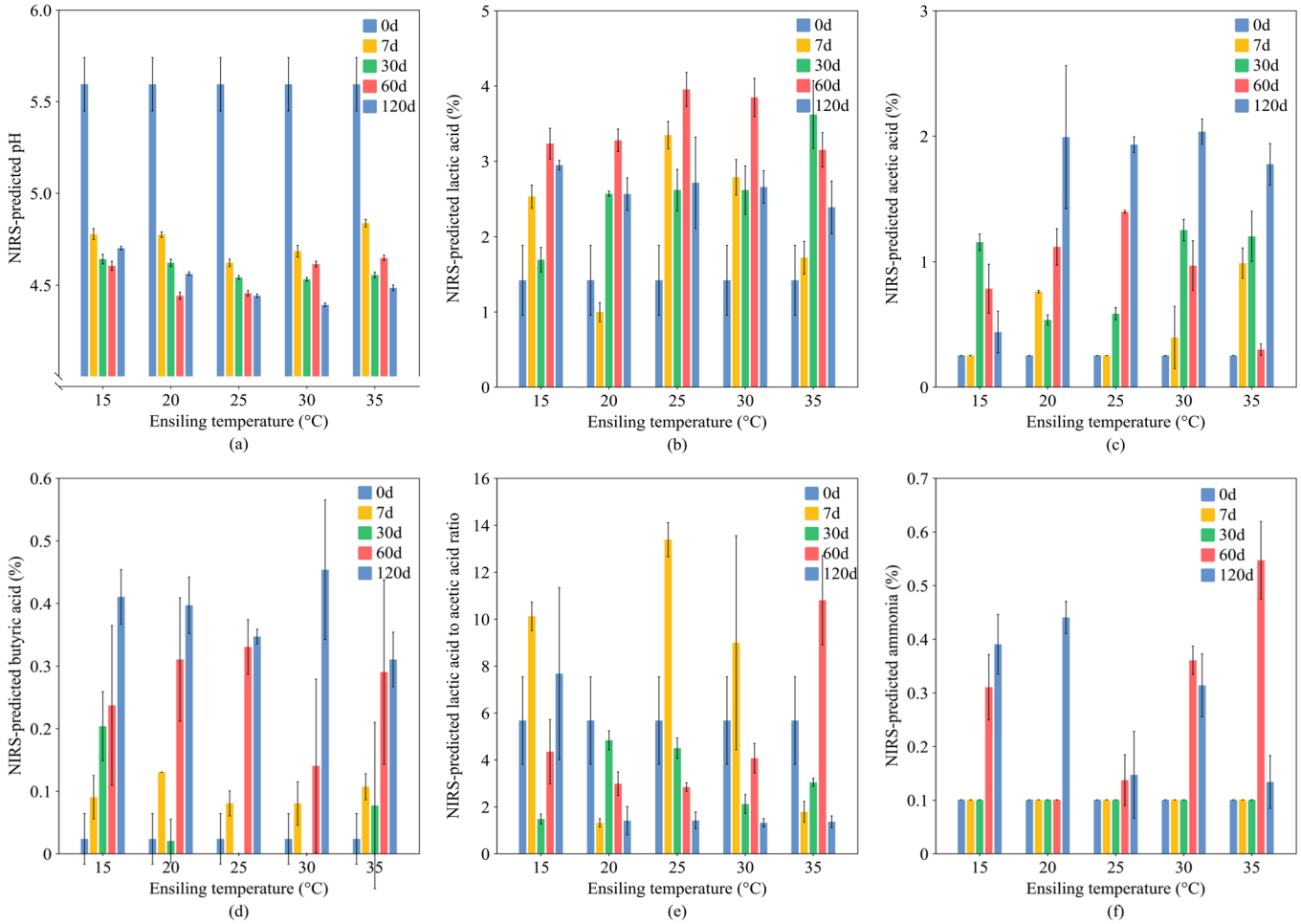


Figure 3. Changes in fermentation quality under different temperatures during ensiling. Values are means ± SD ($n = 3$), with error bars indicating standard deviation. (**a**) pH; (**b**) lactic acid; (**c**) acetic acid; (**d**) butyric acid; (**e**) lactic acid-to-acetic acid ratio; (**f**) ammonia. Parameter (**a**) was measured in fresh silage samples; parameters (**b**–**f**) were expressed on a dry matter basis.

With prolonged ensiling time, the NIRS-predicted AA content reached its maximum value on day 120 under all temperature treatments except for 15 °C (Figure 3c), and was significantly higher than at the other storage time points ($p < 0.05$). Under the 20–30 °C treatments, the predicted AA content showed an increasing trend throughout the fermentation period. Furthermore, the predicted AA content under 15 °C on day 120 was significantly lower than that under the other temperature treatments ($p < 0.05$).

With prolonged ensiling time, the NIRS-predicted BA content showed an increasing trend under the 15–30 °C treatments and reached its maximum value on day 120 across all temperature treatments (Figure 3d). During the first 30 days of ensiling, the NIRS-predicted LA/AA under the 15 °C, 25 °C, and 30 °C treatments showed an initial increase followed by a decrease, whereas under the 20 °C and 35 °C treatments, it showed an initial decrease followed by an increase (Figure 3e). During the period from day 30 to day 120, the predicted LA/AA under the 20–30 °C treatments showed either a sustained decline or remained at relatively low levels. Temperature exerted a stage-dependent regulatory effect on the predicted LA/AA. Specifically, on day 60 of ensiling, the predicted LA/AA under 35 °C was significantly higher than that under the other temperature treatments ($p < 0.05$). On day 120, the predicted LA/AA under 15 °C was significantly higher than that under the other temperature treatments ($p < 0.05$).

Across all temperature treatments, the NIRS-predicted ammonia content showed little change from day 0 to day 30 (Figure 3f). Except for the 20 °C treatment, the predicted ammonia content under the other temperature treatments rose from day 30 to day 60. The predicted ammonia content under 25 °C remained at a consistently low level.

3.5. Correlation Coefficient Analysis

Prior to correlation analysis, normality was assessed using the Kolmogorov–Smirnov test, supported by skewness and kurtosis coefficients. The data were approximately normally distributed, and Pearson's correlation coefficient was therefore used. A Pearson's correlation heatmap (Figure 4) was constructed to elucidate the relationships between nutritional quality and fermentation quality. The correlation analysis revealed the following positive relationships: SP/CP correlated with ADF (r = 0.70), NDF (r = 0.70), ash (r = 0.75), LA(r = 0.44) and AA (r = 0.40); ash with NDF (r = 0.83), ADF (r = 0.73), LA (r = 0.54), AA (r = 0.51), ammonia (r = 0.51) and BA (r = 0.61); NDF with ADF (r = 0.91), LA (r = 0.42), AA (r = 0.57), BA (r = 0.62) and ammonia (r = 0.57); ADF with AA (r = 0.64), BA (r = 0.47) and ammonia (r = 0.43); and BA with AA (r = 0.52) and ammonia (r = 0.55).

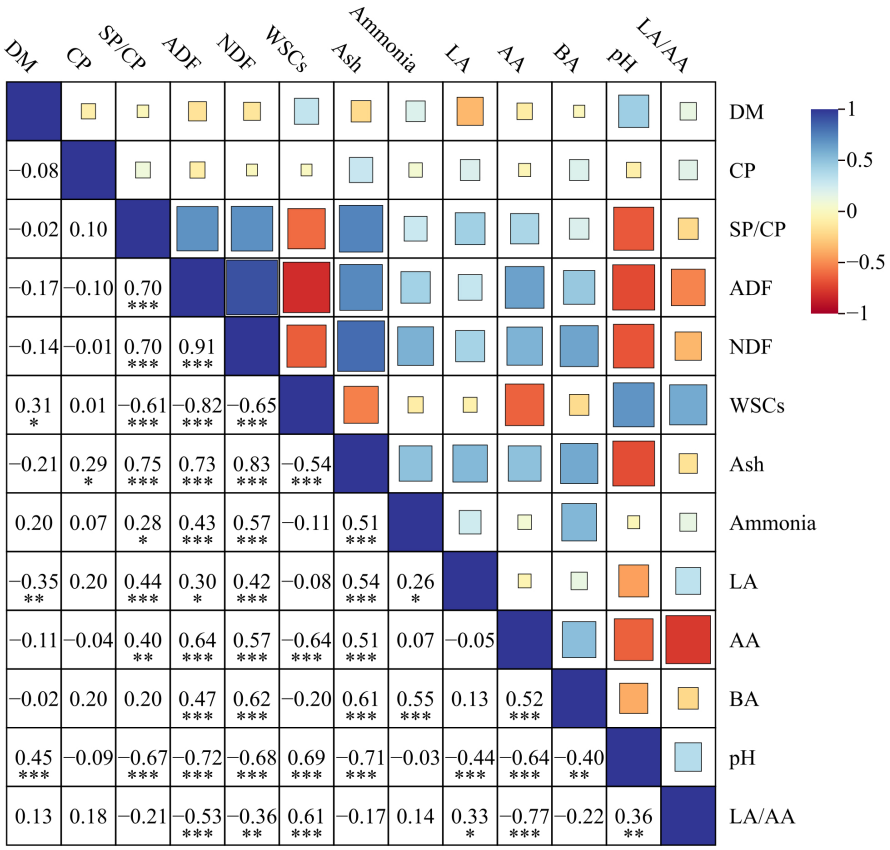


Figure 4. Pearson's correlation heatmap of nutritional quality and fermentation quality of king grass silages. Blue squares represent positive correlation, whereas red squares represent negative correlation. DM, dry matter; CP, crude protein; SP/CP, soluble protein-to-crude protein ratio; ADF, acid detergent fiber; NDF, neutral detergent fiber; WSCs, water-soluble carbohydrates; LA, lactic acid; AA, acetic acid; BA, butyric acid; LA/AA, lactic acid-to-acetic acid ratio. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

As shown in Figure 4, SP/CP (r = −0.61), ADF (r = −0.82), NDF (r = −0.65), ash (r = −0.54) and AA (r = −0.64) were negatively related to WSCs. pH (r = 0.69) and LA/AA (r = 0.61) were positively correlated with WSCs. Meanwhile, pH was negatively correlated with SP/CP (r = −0.67), ADF (r = −0.72), NDF (r = −0.68), ash (r = −0.71), LA

(r = −0.44), AA (r = −0.64) and BA (r = −0.40). LA/AA was negatively correlated with AA (r = −0.77).

3.6. Clustering Heatmap Analysis

Hierarchical cluster analysis was performed on 13 silage quality indicators and 60 treatment samples, with the clustering results presented as a heatmap (Figure 5). The dendrogram revealed that the 13 indicators could be grouped into three distinct clusters. Analyzed from left to right, the first cluster comprised NDF, ADF, ash, SP/CP, AA, LA, BA and ammonia, the second cluster contained only CP, and the third cluster included pH, WSCs, LA/AA and DM.

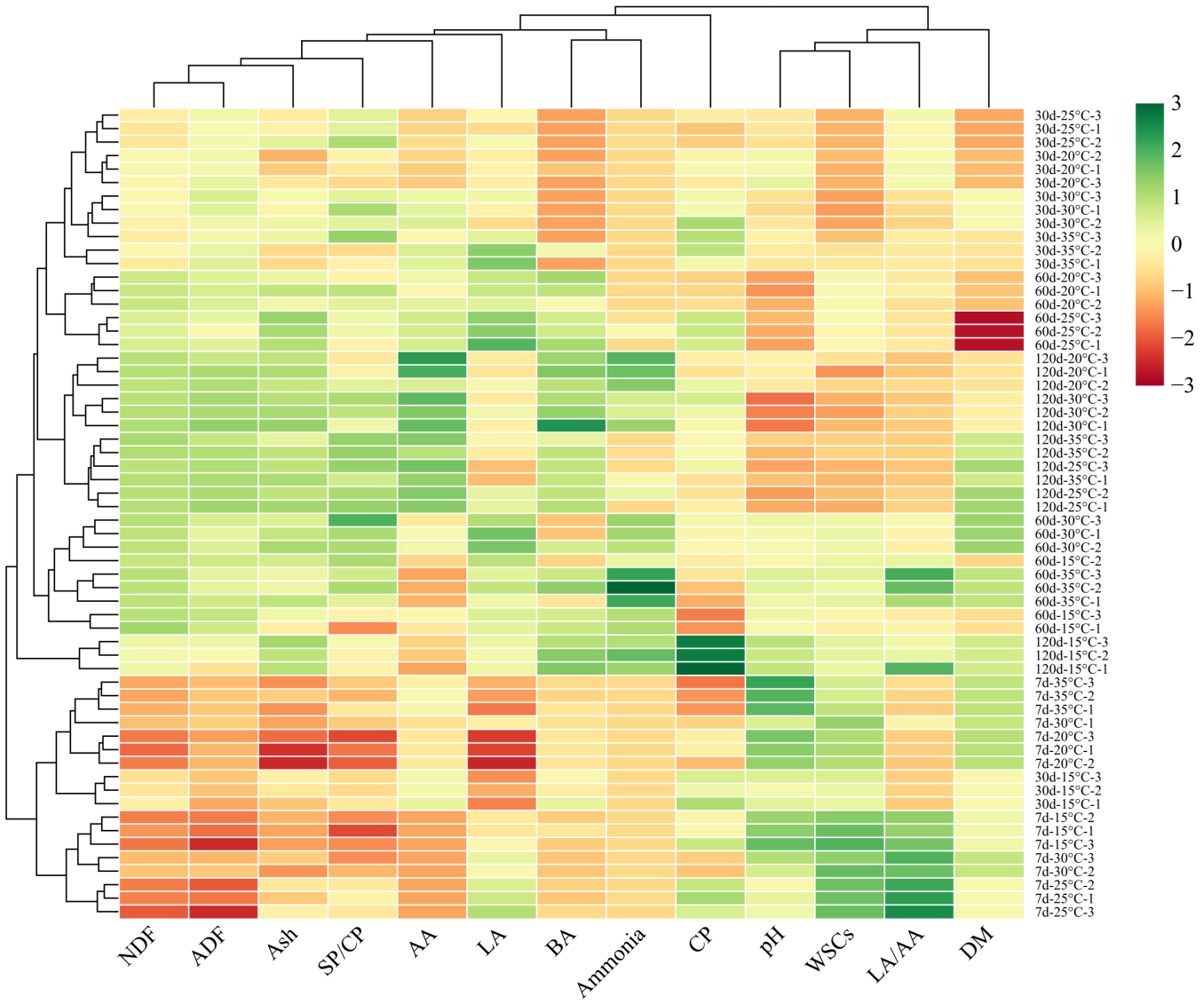


Figure 5. Clustering heatmap of 13 silage quality indicators and 60 treatment samples. Hierarchical clustering was performed based on the Seuclidean distance and Average method. The 13 silage quality indicators were classified into 3 clusters, and the 60 treatment samples were classified into 5 clusters. The color gradient from red to green represents the indicator values from low to high. DM, dry matter; CP, crude protein; SP/CP, soluble protein-to-crude protein ratio; ADF, acid detergent fiber; NDF, neutral detergent fiber; WSCs, water-soluble carbohydrates; LA, lactic acid; AA, acetic acid; BA, butyric acid; LA/AA, lactic acid-to-acetic acid ratio.

The 60 treatment samples were classified into 5 clusters (Figure 5), which showed a high degree of consistency with the experimental treatment groupings. Analyzed from top to bottom, the first cluster included samples ensiled for 30 days at 20, 25, 30, and 35 °C, as well as samples ensiled for 60 days at 20 and 25 °C. In this cluster, the NIRS-predicted contents of DM and BA were low, while the remaining indicators were at moderate levels. The second cluster comprised samples ensiled for 120 days at 20, 25, 30, and 35 °C. In this cluster, the NIRS-predicted contents of ADF, NDF, ash, AA, BA, and SP/CP were relatively high, whereas the NIRS-predicted WSC content, pH and LA/AA were relatively low. The third cluster included samples ensiled for 60 days at 15, 30 and 35 °C. In this cluster, the NIRS-predicted contents of ammonia and LA were relatively high, the predicted CP content was low, and the remaining indicators were at moderate levels. The fourth cluster included samples ensiled for 120 days at 15 °C. In this cluster, the NIRS-predicted contents of DM, CP, ash, BA, and LA/AA were relatively high. The fifth cluster included samples ensiled for 7 days at 15, 20, 25, 30, and 35 °C, as well as samples ensiled for 30 days at 15 °C. In this cluster, the NIRS-predicted contents of ADF, NDF, ash, ammonia, LA, BA, and SP/CP were all low, while the predicted WSC content was high, and the predicted pH was also high.

4. Discussion

4.1. Characteristics of King Grass Raw Material

Evaluating forage characteristics such as DM, WSC, and fiber content prior to ensiling is crucial, as it directly determines the ease of fermentation and the final quality, and serves as the primary prerequisite for producing high-quality silage. The NIRS-predicted DM content of king grass prior to ensiling was 30.67%, falling within the recommended range for silage production [19]. The availability of fermentable sugars in the raw material is a critical determinant of silage quality [20]. The NIRS-predicted WSC content of king grass prior to ensiling reached 11.93%, which not only exceeded the minimum theoretical requirement for adequate LAB fermentation (1.0–1.5%) but also met the threshold for satisfactory ensiling fermentation (>5.0%) [21,22], thereby providing an adequate substrate to support microbial activity and LA fermentation.

4.2. Characteristics of Ensiling Time and Temperature on FWL of King Grass Silage

In this experiment, FWL increased with prolonged ensiling time, a trend consistent with the findings reported by Xu et al. [23]. Dry matter loss in stored silage arises principally from two sources: anaerobic fermentation and effluent seepage [24]. In the present study, silage was prepared and sealed in polyethylene bags. As no apparent effluent loss was observed throughout the ensiling period, it was inferred that the FWL of king grass silage mainly originated from anaerobic fermentation. Previous studies have demonstrated that gases produced during anaerobic fermentation are the main cause of FWL [23]. Although polyethylene silage bags possess extremely low gas permeability, the gases generated during fermentation can still slowly permeate through the bag walls and escape into the surrounding environment [18].

4.3. Characteristics of Ensiling Time and Temperature on Nutrient Composition of King Grass Silage

Storage time played a significant role in shaping the dynamic changes in nutrient composition, with the period from day 7 to day 30 post ensiling representing the most intense stage of compositional variation. Specifically, the NIRS-predicted contents of DM and WSCs exhibited a decreasing trend, while the predicted contents of ADF, NDF, ash, and SP/CP showed an increasing tendency. With prolonged ensiling, a marked decline in predicted DM content was observed during the early stage (0–30 d) across all temperature

treatments. This decrease may be associated with CO_2 and water production from microbial fermentation [25]. Notably, under the 15 °C, 20 °C, and 25 °C treatments, the predicted DM content rebounded during the later stage (60–120 d). This phenomenon is consistent with the trend reported by Vasconcelos et al., who also observed a fluctuation in DM content from 60 to 90 days of ensiling [26]. Temperature variation modulated the degradation rate of organic matter by directly influencing microbial metabolic activity, ultimately leading to differences in DM loss across temperature regimes [7]. Under 25 °C, the NIRS-predicted DM content was the lowest across all temperature groups during the early stage (7–60 d) and the highest at the later stage (120 d), with the later stage showing significantly higher values than the early stage. These findings suggest that 25 °C may serve as a critical inflection point for the fermentation metabolism of king grass silage.

The NIRS-predicted WSC content exhibited a marked decline during the early stage of ensiling (0–30 d), which may be associated with the consumption of WSCs by LAB and other microorganisms during the fermentation process [27]. Notably, a subsequent increase in the NIRS-predicted WSC content was observed during the later stage of fermentation. This pattern aligns with the changes in WSC content reported by Diepersloot et al. for whole-plant corn silage [28]. A possible explanation is that the low pH in the silage promoted hemicellulose hydrolysis to generate sugars, thereby providing a transient supplementary source of WSCs [27]. Furthermore, the timing of the predicted WSC content increase under 15 °C lagged behind that of the other temperature treatments.

With prolonged ensiling time, the NIRS-predicted contents of ADF and NDF exhibited an increasing trend under all temperature treatments, a pattern consistent with the findings reported by Zhao et al. [20]. At day 7 of ensiling, the NDF levels were lower than those at other time points, which may be associated with the leaching of WSCs from the medium, leading to a relative increase in NDF [29]. At most sampling time points, the NIRS-predicted ADF and NDF contents under 30 °C and 35 °C were higher than those under 15 °C. This phenomenon may be associated with mild non-enzymatic browning or Maillard-type condensation reactions occurring during prolonged storage of silage at high temperatures [11].

The NIRS-predicted ash content gradually increased across all temperature treatments over time and then leveled off, which may be associated with the relative increase caused by the degradation of proteins and carbohydrates [30]. Under 20 °C, the CP content showed relatively little variation across the different storage time points, a finding consistent with the report by Duvnjak et al., who observed no significant changes in CP content in whole-plant corn silage stored at 25 ± 2 °C for up to one year [31]. Across all temperature treatments, the NIRS-predicted SP/CP increased with prolonged ensiling time, a trend consistent with the findings of Duvnjak et al. [31], which may be associated with the hydrolysis of protein during the ensiling process [28].

4.4. Characteristics of Ensiling Time and Temperature on Fermentation Quality of King Grass Silage

As the temperature increased, NIRS-predicted pH at each storage time point showed a trend of first decreasing and then increasing, a pattern consistent with the report by Zhang et al. [10]. Among all storage time points, predicted pH under 15 °C remained at a relatively high level, which aligns with the finding of Liu et al. [7] that pH was higher under 15 °C than under 25 °C. A slow decline in pH reduces fermentation efficiency and may promote the proliferation of undesirable microorganisms, thereby increasing the risk of silage spoilage [7]. In this experiment, both low temperature (15 °C) and high temperature (35 °C) were unfavorable for a rapid decline in pH, suggesting that the effect of temperature on pH may be primarily mediated through the regulation of microbial activity [10], although this hypothesis requires further verification through microbial enumeration.

Under 15–25 °C, the NIRS-predicted LA contents reached their maximum values on day 60 of ensiling, while the predicted pH was at its lowest or relatively low level, suggesting that a lower pH plays a positive role in improving the fermentation quality of king grass. This result is consistent with the report by Hao et al. [32]. During the period from day 60 to day 120 of ensiling, the predicted LA content showed a decreasing trend, a phenomenon similarly reported by Wang et al. [33] for whole-plant corn silage. This phenomenon could be associated with a reduced WSC content, which slows the fermentation of LAB, or it may be ascribed to the catabolism of LA into AA by certain anaerobic microorganisms, consequently decreasing LA content [34]. In addition, only small differences in LA content were observed among temperature treatments on day 120, indicating that after long-term fermentation, LA concentrations under different temperature conditions eventually reached a comparable level.

Under 20–30 °C, the NIRS-predicted AA content showed a continuous increasing trend throughout the fermentation period, consistent with the findings of Xin et al. [34], suggesting the possible presence of certain heterofermentative LAB [22]. The NIRS-predicted BA concentrations were highest among all temperature treatments on day 120, which is consistent with the report by Schumacher et al. [35]. The presence of BA in silage is undesirable, as its production represents an energy-consuming and metabolically inefficient process. A BA content exceeding 0.5% dry matter suggests extensive clostridia activity, which may lead to reduced feed intake and pose health risks to animals [25]. In the present study, the maximum NIRS-predicted BA content observed during king grass ensiling across all temperature treatments was only 0.45%.

Except for the 25 °C treatment, the NIRS-predicted ammonia content under the other temperature regimes rose from day 30 to day 120, suggesting that protein breakdown may have primarily occurred after 30 days of storage. Ammonia is a compound generated during ensiling through a series of chemical reactions catalyzed by plant or microbial enzymes, resulting in the decomposition of proteins and amino acids [36]. The higher the amount of ammonia produced by microbial decomposition of proteins, the greater the protein loss in silage [37]. Temperature played a regulatory role in protein degradation. The predicted ammonia content under 25 °C remained at a consistently low level, suggesting that this temperature appeared to be the most effective among those tested for inhibiting protein degradation.

4.5. Correlation and Clustering Heatmap Analyses of Fermentation Parameters

In the present study, ADF, NDF, ash, ammonia, and BA were positively correlated with one another. These associations may reflect common responses to prolonged ensiling duration. Correlation analysis suggested that WSCs may play an important role in the king grass ensiling system. WSCs showed a negative association with ADF and NDF. A positive correlation was found between WSCs and pH, suggesting that the fermentation process under the conditions of this experiment was subject to certain limitations—namely, when WSC content was relatively high, acid production was insufficient, resulting in a correspondingly higher pH. The positive correlation between LA/AA and WSCs, together with the negative correlation between WSCs and AA, may further suggest that adequate substrate availability favored homolactic fermentation, whereas substrate deficiency potentially promoted the conversion of LA to AA.

The clustering results of fermentation quality indicators showed that most of the eight indicators in the first cluster were positively correlated with each other, suggesting a trend among fiber components (NDF, ADF), ash, proteolytic products (SP, ammonia), and non-lactic organic acids (AA, BA). The third cluster may tentatively reflect the relationship between fermentation substrate consumption and the acidification process. These clustering

results further elucidate the intrinsic associations among various quality indicators during king grass ensiling.

The clustering results of treatment samples showed that all temperature treatments at day 7 of ensiling, together with the 15 °C treatment at day 30, were classified into cluster 5, whereas the treatment ensiled for 30 days at 20–35 °C fell into cluster 1. The 15 °C treatment at 120 d was grouped into cluster 4, and the 15 °C, 30 °C, and 35 °C treatments at 60 d were grouped into cluster 3. These results suggest that under the same ensiling duration, the fermentation process at low temperatures lagged behind that at high temperatures. Cluster 2 exhibited characteristics of low LA/AA and pH, high AA, and high BA, whereas cluster 5 showed high pH, low AA and BA, suggesting that prolonged ensiling time may be associated with a shift in fermentation type—a pattern consistent with the report by Li et al. [38].

4.6. Limitations of This Study

Several limitations of this study should be acknowledged. First, the NIRS calibrations used were based on a universal database (Dairy One) that has not been specifically validated for king grass silage. Therefore, the predicted values should be interpreted as relative comparisons among treatments rather than absolute measurements. Second, microbial communities were not directly measured during ensiling; so, the discussions concerning fermentation mechanisms remain speculative and require further investigation. Despite these limitations, this study systematically describes the changes in the silage quality of king grass under different temperatures and storage periods, and may provide basic data and a reference for future research.

5. Conclusions

The ensiling days, ensiling temperature, and their interaction significantly influenced the FWL, nutrient composition, and fermentation quality. The 25 °C treatment achieved rapid acid production, with pH continuously decreasing throughout fermentation and ammonia content remaining at consistently low levels, indicating that fermentation proceeded relatively thoroughly under this temperature. The low-temperature (15 °C) treatment, although favorable for preserving WSCs and retaining CP at 120 days, exhibited relatively high pH at all sampling time points and resulted in incomplete fermentation. The high-temperature (35 °C) treatment showed the highest pH at 7 and 60 days; the LA content peaked at 30 days under this treatment, whereas it peaked at 60 days under the other temperatures, suggesting that high temperature accelerated the fermentation process but did not facilitate effective pH reduction.Correlation analysis showed that ADF, NDF, ash, ammonia, and BA were positively correlated with one another, while WSCs were correlated with most variables, suggesting that WSCs may play an important role in the fermentation of king grass silage. Cluster analysis suggested that, over the same ensiling period, the fermentation process at low temperatures lagged behind that at higher temperatures.

Author Contributions: Conceptualization, formal analysis, and writing—original draft, T.J.; methodology and software, C.D.; validation, S.S. and L.Z. investigation, resources and data curation, Z.S. and X.X.; writing—review and editing, W.Y. and S.G.; visualization, Q.L. and M.X. supervision, project administration and funding acquisition, W.Y. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Yunnan Fundamental Research Projects (grant No. 202301BD-070001-211) and the Key Research and Development Program of Yunnan Province, China (grant No. 202503AP140048).

Data Availability Statement: The data presented in this study are contained within this article. Additional raw data that support the findings of this study are available from the corresponding author, WY, upon reasonable request.

Acknowledgments: The authors thank Ulanqab Yima Agriculture and Animal Husbandry Technology Co., Ltd. (Ulanqab, China), for providing NIRS analysis services for the determination of nutrient composition and fermentation quality of samples.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

LAB	lactic acid bacteria
LA	lactic acid
DM	dry matter
AA	acetic acid
WSCs	water-soluble carbohydrates
ADF	acid detergent fiber
NDF	neutral detergent fiber
BA	butyric acid
NH_3-N	ammonia nitrogen
FWL	fermentation weight loss
NIRS	near-infrared spectroscopy
CP	crude protein
SP/CP	soluble protein-to-crude protein ratio
FW	fresh weight
SD	standard deviation
LA/AA	lactic acid-to-acetic acid ratio

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