Effects of Organic Acids on Silage Quality and Nutritional Composition of Stylosanthes (Postprint)

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Abstract

To improve the silage quality of Stylosanthes guianensis, this study investigated the effects of organic acids on the silage quality and nutritional composition of Stylosanthes guianensis. A silage experiment was conducted using Stylosanthes guianensis cv. Reyan No. 2 as raw material, divided into four groups: conventional silage (control) and silage supplemented with 0.2% formic acid, acetic acid, and propionic acid, with three replicates per group. After 30 days of ensiling, the pH, lactic acid, acetic acid, propionic acid, butyric acid, and main nutritional components of Stylosanthes silage were determined. The results showed that direct silage of Stylosanthes produced poor quality. The addition of organic acids significantly reduced silage pH and butyric acid content ($P<0.05$), increased crude protein content (with the acetic acid group significantly higher than the control group ($P<0.05$)), decreased neutral detergent fiber content (with the formic acid and acetic acid groups significantly lower than the control group ($P<0.05$)), and concurrently increased relative feed value (with the formic acid and acetic acid groups significantly higher than the control group ($P<0.05$)). In conclusion, the addition of organic acids can significantly improve the silage quality and nutritional value of Stylosanthes, with 0.2% acetic acid supplementation demonstrating the best silage effect.

Full Text

Effects of Organic Acids on Quality and Nutrient Composition of Stylosanthes guianensis Silage

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Abstract

To improve the silage quality of Stylosanthes guianensis, this study investigated the effects of organic acids on silage quality and nutrient composition. Stylosanthes guianensis cv. Reyan No. 2 was used as raw material for ensiling and divided into four groups: conventional silage (control), and silage with 0.2% formic acid, acetic acid, or propionic acid as additives, with three replicates per group. After 30 days of ensiling, pH and contents of lactic acid, acetic acid, propionic acid, butyric acid, and main nutrients were measured. The results showed that direct ensiling of Stylosanthes guianensis produced poor quality silage. Organic acid addition significantly decreased pH and butyric acid content ($P<0.05$), increased crude protein content (with the acetic acid group significantly higher than control, $P<0.05$), decreased neutral detergent fiber content (with formic acid and acetic acid groups significantly lower than control, $P<0.05$), and increased relative feed value (with formic acid and acetic acid groups significantly higher than control, $P<0.05$). It is concluded that organic acid addition can significantly improve both the silage quality and nutritional value of Stylosanthes guianensis, with 0.2% acetic acid showing the best effect.

Keywords: Stylosanthes guianensis; organic acid; fermentation quality; nutrient composition

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Silage is a type of roughage obtained through anaerobic lactic acid bacteria fermentation of green forage under sealed, oxygen-deficient conditions, where soluble carbohydrates in the raw material are converted into organic acids such as lactic acid, thereby reducing pH and inhibiting the proliferation of undesirable microorganisms. Silage is characterized by its acidic aroma, green succulent appearance, rich nutrition, good palatability, and suitability for long-term storage, making it an excellent feed source for livestock. Silage additives are substances designed to inhibit undesirable microbial proliferation, reduce nutrient loss during storage, and ensure fermentation occurs within a predetermined range. Various types of silage additives exist, which can be categorized by function into inhibitory additives, promoting additives, and nutritional additives. Formic acid, acetic acid, and propionic acid all belong to the category of inhibitory additives that can suppress undesirable microbial fermentation and aerobic microbial activity during ensiling, thereby effectively preventing silage spoilage and preserving nutritional value.

Stylosanthes guianensis is an important leguminous forage in tropical and subtropical regions of China, characterized by high protein content and good palatability, making it an excellent roughage source for livestock in southern China. Stylosanthes guianensis growth exhibits distinct seasonality, with high forage yield during summer and autumn, but edible portions decrease substantially after seed maturation (around December), potentially causing feed shortages. To meet livestock nutritional demands during off-seasons, adequate feed must be stored during the peak growing season, making research on Stylosanthes guianensis silage particularly important. Li et al. studied the effects of different additives on Stylosanthes guianensis silage quality and found that adding sorbic acid and sucrose could improve silage quality. Liu et al. reported that low temperature and wilting reduced aerobic stability of Stylosanthes guianensis silage, while lactic acid bacteria addition significantly decreased pH and ammonia nitrogen content, maintained higher lactic/acetic acid ratios during ensiling, and improved silage quality.

This experiment prepared Stylosanthes guianensis silage with formic acid, acetic acid, and propionic acid additions, analyzed organic acid contents and nutrient composition, and investigated the effects of different organic acids on silage quality and nutrients to provide theoretical reference for selecting appropriate silage additives.

1.1 Experimental Materials

The experimental material was Stylosanthes guianensis cv. Reyan No. 2 (vegetative stage), cultivated at the Experimental Base of Team 10, Tropical Crops Genetic Resources Institute, Chinese Academy of Tropical Agricultural Sciences (19°30 N, 109°30 E, altitude 149 m). The region has a tropical monsoon climate characterized by hot, rainy summers and autumns, and cool, dry winters and springs with distinct wet and dry seasons. The experimental base soil is latosol developed from granite with poor texture and no irrigation conditions.

1.2 Organic Acids

Formic acid, acetic acid, and propionic acid were all analytical reagent grade laboratory chemicals purchased from Sinopharm Group.

1.3 Experimental Design

The experiment consisted of four treatments: conventional silage (control), and silage with 0.2% formic acid, acetic acid, or propionic acid added based on raw material weight. Each treatment had three replicates.

1.4.1 Silage Preparation

After harvesting, Stylosanthes guianensis was chopped to approximately 2 cm lengths. About 150 g was weighed into plastic bags, mixed with the appropriate amount of organic acid, sealed with a vacuum packaging machine, and stored at room temperature (approximately 25°C) for 30 days before opening for sampling and analysis.

1.4.2 Nutrient Analysis of Silage

Samples were taken before ensiling and after opening, dried at 65°C for 48 hours, ground, and sealed for storage until analysis. Dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF) contents were determined.

1.4.3 Calculation of Relative Feed Value (RFV)

RDMI = 120/NDF
DDM = 88.9 - 0.779ADF
RFV = DMI × DDM/1.29

Where: RDMI is the dry matter intake of roughage (% BW); DDM is digestible dry matter (%).

1.4.4 Silage Quality Analysis

Twenty grams of silage sample was mixed with 80 mL distilled water, soaked at 4°C for 24 hours, filtered through double-layer filter paper, left to stand for 0.5 hours, and pH was measured using a Leici PHS-3C precision pH meter. Lactic acid, acetic acid, propionic acid, and butyric acid contents were determined by high-performance liquid chromatography (Shimadzu LC-20A). Analysis conditions: column RSpak KC-811 (Showa Denko, Japan), mobile phase 3 mmol/L perchloric acid solution, flow rate 1 mL/min, column temperature 40°C, detection wavelength 210 nm.

1.5 Flieg' s Silage Grade

Flieg' s grading method is based on the contents of three main organic acids—lactic acid, acetic acid, and butyric acid—and is only applicable for evaluation of conventional silage. Silage quality is divided into five grades according to this standard: 81-100 points = "excellent" ; 61-80 points = "good" ; 41-60 points = "fair" ; 21-40 points = "poor" ; 0-20 points = "very poor" .

1.6 Data Analysis

Data were processed and statistically analyzed using SAS 9.0 and Excel 2003 software packages. Duncan's multiple range test was used for mean comparisons among treatments.

2.1 Fermentation Quality of Stylosanthes guianensis Silage

As shown in Table 1 , compared with the control group, organic acid addition significantly decreased silage pH ($P<0.05$), with the formic acid group showing the lowest pH, though differences among organic acid groups were not significant ($P>0.05$). The propionic acid group significantly decreased lactic acid content compared with the control ($P<0.05$), while formic acid and acetic acid groups showed no significant changes (P>0.05). Formic acid and acetic acid groups significantly increased acetic acid content compared with the control (P<0.05), while the propionic acid group also showed increased acetic acid content but the difference was not significant (P>0.05). The control group had significantly higher propionic acid content than other groups (P<0.05), with the acetic acid group showing the lowest propionic acid content. Organic acid addition significantly decreased butyric acid content (P<0.05), with the propionic acid group containing no butyric acid. Total acid content was also significantly decreased by organic acid addition compared with the control (P<0.05), with the propionic acid group showing the lowest total acid content, while formic acid and acetic acid groups did not differ significantly (P>0.05). Overall, organic acid addition effectively decreased pH and butyric acid content, significantly improving silage quality.

Table 1 Fermentation Quality of Stylosanthes guianensis Silage (DM basis)

Item	LA/%	AA/%	PA/%	BA/%	TA/%
Control group	5.42a	2.28a	1.09b	1.06a	9.85a
Formic acid group	4.49b	1.96ab	1.44a	0.34b	8.23b
Acetic acid group	4.61b	2.49a	1.45a	0.09c	8.64b
Propionic acid group	4.75b	1.29b	1.20ab	0.56b	7.80b

In the same column, values with the same or no letter superscripts mean no significant difference (P>0.05), while different letter superscripts mean significant difference (P<0.05). The same as below.

2.2 Silage Grading of Stylosanthes guianensis

As shown in Table 2 , the control group received a total Flieg' s score of 51, corresponding to a "fair" grade, indicating poor silage quality. Formic acid and propionic acid groups scored 61 and 77, respectively, both higher than the control and achieving a "good" grade. The acetic acid group scored 85, reaching an "excellent" grade. These results demonstrate that organic acid addition improved Stylosanthes guianensis silage quality, with acetic acid showing the best effect.

Table 2 Flieg' s Silage Grades of Stylosanthes guianensis Silage

Item	LA	AA	BA	Total value	Grade
Control group	28	20	3	51	Fair
Formic acid group	28	25	8	61	Good
Acetic acid group	28	30	27	85	Excellent
Propionic acid group	25	30	22	77	Good

2.3 Nutrient Contents of Stylosanthes guianensis Silage

As shown in Table 3 , compared with the control group, the acetic acid group significantly increased CP content (P<0.05), with no significant difference between acetic acid and propionic acid groups (P>0.05), while the formic acid group showed no significant change (P<0.05). Formic acid and acetic acid groups significantly decreased NDF content compared with the control (P<0.05), though these two groups did not differ significantly from each other (P>0.05). The propionic acid group also showed decreased NDF content, but the difference was not significant compared with the control (P>0.05). Formic acid and acetic acid groups decreased ADF content, while the propionic acid group increased it, but all changes were not significant (P>0.05). Formic acid and acetic acid groups significantly increased RFV compared with the control (P<0.05), while the propionic acid group showed no significant change (P>0.05).

Table 3 Nutrient Contents of Stylosanthes guianensis Silage (DM basis)

Item	CP/%	NDF/%	ADF/%	RFV
Control group	10.55b	32.37a	24.68ab	200.23b
Formic acid group	9.77c	24.41b	22.08b	273.24a
Acetic acid group	12.43a	25.57b	24.15ab	254.98a
Propionic acid group	11.33ab	30.65a	28.87a	201.56b

3.1 Effects of Formic Acid Addition on Stylosanthes guianensis Silage

Formic acid, also known as methanoic acid, has strong reducing capacity and functions as a fermentation-inhibiting silage additive, acting as a preservative during ensiling, particularly suitable for forages with low soluble carbohydrate and DM content. The raw material used in this experiment, Stylosanthes guianensis, is characterized by high moisture content, strong buffering capacity, low soluble carbohydrate content, and abundant aerobic microorganisms, making it difficult to ensile. Butyric acid is produced by undesirable microorganisms that decompose previously formed lactic acid or sugars in the raw material, accompanied by energy loss and protein decomposition that generates large amounts of amines or ammonia, resulting in foul odor and poor silage quality. Therefore, butyric acid can serve as an indicator of silage spoilage. After adding formic acid during Stylosanthes guianensis ensiling, silage pH decreased significantly, indicating that formic acid directly acidified the material in the early ensiling stage. Lactic acid content decreased compared with the control but not significantly, while acetic acid content increased significantly and propionic and butyric acid contents decreased significantly, demonstrating that formic acid addition provided good preservative effects but only moderate improvement in silage quality, as it failed to inhibit all microbial proliferation. Woolford reported that formic acid can inhibit all microbial proliferation only when silage pH is below 4. Randby investigated the effects of formic and acetic acids on fermentation quality of mixed silages of timothy, meadow fescue, and red clover at different ratios, finding that formic acid could inhibit lactic acid fermentation. Aksu et al. studied the effects of homofermentative lactobacilli, formic acid, and molasses on whole-plant corn silage composition, finding that formic acid addition increased acetic acid production. In this experiment, the formic acid group had the second-highest acetic acid content (not significantly different from the acetic acid group), indicating that formic acid failed to effectively inhibit heterofermentative lactic acid bacteria activity. The formic acid group received a Flieg' s score of 61, achieving a "good" grade, and although not reaching "excellent," formic acid addition inhibited undesirable microbial proliferation, reduced butyric acid production, decreased NDF and ADF contents, and could be considered a good additive if protein loss could be reduced through appropriate dosage adjustment.

Formic acid is generally considered to inhibit protease and spoilage microorganisms from decomposing silage protein, thereby reducing CP loss. However, in this experiment, the formic acid group showed decreased CP content, possibly because low-dose formic acid promoted the growth of other harmful bacteria such as clostridia, resulting in a complex microbial environment where protein decomposition rate still exceeded that of natural fermentation. NDF and ADF contents decreased significantly, and RFV was significantly higher than the control, indicating that formic acid could improve nutritional value to some extent. Lü et al. studied the effects of molasses, Lactobacillus plantarum, and formic acid on fermentation quality of earless corn stalk silage, finding that formic acid inhibited lactic acid bacteria and fungal activity, reduced DM loss and soluble carbohydrate consumption, decreased CP content, and increased ammonia nitrogen (NH3-N) content at the end of fermentation. Zhang et al. found that formic acid treatment decreased ADF and NDF contents in sorghum-sudangrass silage, consistent with our results.

3.2 Effects of Acetic Acid Addition on Stylosanthes guianensis Silage

As a common disinfectant and preservative, acetic acid can inhibit the growth and proliferation of harmful microorganisms such as molds and yeasts, reduce substrate consumption, and provide favorable conditions for lactic acid bacteria growth. Schmidt et al. reported that acetic acid addition effectively decreased pH and inhibited aerobic microbial activity, preventing silage spoilage. In this experiment, the acetic acid group showed significantly decreased pH, increased lactic acid content (not significant), significantly increased acetic acid content, and significantly decreased propionic and butyric acid contents, achieving an "excellent" Flieg' s grade. These results indicate that acetic acid addition improved silage quality, promoted lactic acid bacteria growth, and inhibited harmful microorganisms such as butyric acid bacteria. The non-significant increase in lactic acid content may be due to insufficient acetic acid dosage. Qiu et al. added 0.3% acetic acid to fermented total mixed rations, which decreased lactic acid content but showed no significant difference from the control, with good fermentation quality and improved aerobic stability.

In this experiment, the acetic acid group showed significantly increased CP content, decreased NDF and ADF contents, and significantly increased RFV compared with the control, demonstrating that acetic acid significantly improved the nutritional value of Stylosanthes guianensis silage. This may be because acetic acid effectively inhibited aerobic microbial activity, reduced protein degradation and utilization, and decreased protein loss during ensiling. Xu et al. found that 0.2% acetic acid addition significantly decreased pH and NDF and ADF contents in corn silage, showing better improvement in fermentation quality than fermented green juice.

3.3 Effects of Propionic Acid Addition on Stylosanthes guianensis Silage

Propionic acid, also an inhibitory additive, can inhibit yeast and mold activity in silage and is a highly effective antifungal volatile fatty acid. Propionic acid can also participate in lipid and glucose metabolism in ruminants, being completely oxidized or converted to glucose and glycogen, and is harmless to animals. However, at high addition rates it can also inhibit lactic acid bacteria, so it is sometimes mixed with bacterial inoculants for better results. In this experiment, the propionic acid group showed significantly decreased pH, significantly decreased lactic and propionic acid contents, increased acetic acid content (not significant), and no butyric acid production. These results indicate that under experimental conditions, propionic acid addition inhibited butyric acid bacteria activity but failed to effectively inhibit heterofermentative lactic acid bacteria, resulting in decreased lactic acid content. This result differs from Zhang et al., possibly due to differences in addition rate and raw material. The propionic acid group achieved a "good" Flieg' s grade, indicating that propionic acid also improved silage quality, but its effect was primarily manifested in preservative properties. Mixed use with promoting additives such as lactic acid bacteria may yield better results, though specific addition rates and ratios require further research.

Propionic acid addition had minimal effects on silage nutrient composition, with increased CP and ADF contents and decreased NDF content, but none of these differences were significant compared with the control. This suggests that propionic acid can reduce CP degradation and prevent nutrient loss during ensiling. Zhang et al. found that propionic acid addition helped preserve silage nutrients. Ji et al. reported that 0.5% propionic acid addition significantly increased CP content and decreased NDF and ADF contents in sorghum-sudangrass silage. In this experiment, the propionic acid addition rate was only 0.2%, which may explain the limited improvement in nutritional quality.

Conclusions

1. Organic acid addition significantly decreased pH and butyric acid content in Stylosanthes guianensis silage, clearly improving preservative properties, reducing fiber components, increasing RFV, and enhancing nutritional value.
2. After adding 0.2% acetic acid, Stylosanthes guianensis silage showed significantly decreased pH, increased lactic and acetic acid contents, decreased propionic and butyric acid contents, achieved an "excellent" Flieg' s grade, and demonstrated significantly increased CP content and RFV with decreased NDF and ADF contents, thereby improving both silage quality and nutritional value superior to the other two organic acids.

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Note: Figure translations are in progress. See original paper for figures.