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Effects of Harvest Period and Mixture Proportion on the Quality of Corn-alfalfa Silages

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Abstract

Corn silage is a worldwide forage for feeding livestock because it is high in energy and digestibility perfectly adapted to modern crop mechanization. Many dairy producers, however, include small amounts of alfalfa in corn silages to improve feed intake and milk production. The effects of the harvesting stage and the proportion of corn and alfalfa mix on the nutritional value of the feed have not been investigated yet. In this work, we studied the effects of mixed silage of whole-plant corn and alfalfa at different harvest stages on the nutritional value and quality of feed. The whole corn and alfalfa harvested at milk and wax stages were mixed and silaged in the proportions of 4:0, 3:1, 2:2, 1:3 and 0:4 of corn-alfalfa respectively, with 3 replicates for each ratio. The fermentation quality and nutrient content of mixed silage were determined 60 days after silage. The results showed that the sensory score of alfalfa silage was low, the content of ash was 10.61%, the content of crude fibre was 33.46%, the pH value was 6.73, the content of ammonium nitrogen / total nitrogen was 3.48%, the content of neutral and acid detergent fibre was 48.06%, 38.10%, the content of acid detergent lignin was 13.07%, Which was significantly higher than that of mixed silage.

Although the crude protein content was high (23.58%), the overall silage quality was poor. In mixed silage, whether corn is at the milk stage or wax stage, the pH value, ash, crude fibre and detergent fibre contents of silage decreased significantly with the increase of the proportion of whole corn, and the fermentation quality of silage improved gradually ($p<0.05$). The sensory quality of the feed was good when the corn was in the milk stage and mixed with alfalfa in the ratio of 2:2. The content of crude protein decreased slightly to 19.62%, but the content of water-soluble carbohydrates increased significantly to

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7.47%; The pH value decreased significantly to 3.62 as the contents of neutral and acid detergent fibres (37.78% and 24.77% respectively) and the content of acid detergent lignin (6.56%). Hence, the use of 2:2 corn-alfalfa ratios when corn is in the milk stage improves the nutritional quality and silage to feed dairy cows.

Keywords: Dairy Cows Nutrition; Mixed Silage; Harvest Period; Nutritional Composition; Fermentation Quality

Introduction

Medicago sativa L. is the perennial legume forage with the largest cultivated area in the world. It is known as "The king of forage" because of its strong adaptability, high grass yield, excellent quality and good palatability [1].

In the early 18th century, some European and American countries had already conducted preliminary experiments on alfalfa silage. In the mid-19th century, alfalfa silage technology entered the stage of promotion and application. In China, it was not until the 21st century that the alfalfa industry began to develop rapidly, with a sharp increase in planting area and a gradual emphasis on alfalfa silage technology. At present, alfalfa forage is mainly used to prepare hay and process grass powder. However, due to the combined effects of rain and heat in the same season in the second, third and fourth cutting periods in most of the alfalfa-producing areas, there is a nutrient impoverishment of alfalfa in the process of drying hampering the storage of high--quality hay restricting the development of alfalfa industrialization [2-3].Therefore, the current methods of alfalfa silage mostly use semi dry silage, stretch film wrapped silage, mixed silage, and additive silage to make up for the above shortcomings, while mixed silage and additive silage technology represent the future development direction of alfalfa silage.

China is a major aquaculture and planting country, and corn plays an important role in China's crop economy. It not only provides food for the vast population but also serves as an important source of feed in the animal husbandry industry. The harvesting period of corn silage directly affects its feeding value and the content of nutrients such as starch, crude fiber, and crude protein. The maturity of corn at harvest is closely related to biological yield, nutritional quality, and digestibility. As the harvest period extends, the starch content increases, while the crude protein, neutral detergent fiber, and crude ash content correspondingly decrease [4].

On the other hand alfalfa has high crude protein content, high buffer energy value and low water-soluble carbohydrate content, so it is difficult to silage alone [5]. The crude protein content of whole corn is low and the water--soluble carbohydrate content is high. The mixed silage with alfalfa can improve the nutritional value of silage and solve the problems of large nutritional loss and poor feed quality in the promotion and application of mixed feed.

Materials and Methods

Test Materials

Corn (QuChen No. 9) and alfalfa are planted in the teaching practice base and herbage specimen Park of the College of Grassland Agriculture, Northwest A&F University, Yangling , Xianyang City, Shaanxi Province. Milk mature corn and wax mature corn were cut respectively, and alfalfa was cut at the same time. After cutting, take the fresh samples back to the laboratory for treatment.

Experimental Design

Timely harvesting is the key to obtaining the maximum biological yield and highest nutritional value of the entire maize plant. The nutritional quality of the entire maize plant is higher when harvested from the milk ripening stage to the early wax ripening stage [6]. Wang Yang et al. [7] found that the quality of mixed silage was better when corn and peanut seedlings were mixed in weight ratios of 10:0, 4:1, 3:2, 1:1, 2:3, 1:4, and 0:10. Xin Pengcheng et al. [8] found that 40% alfalfa+60% corn was more suitable for silage when mixed with 20% alfalfa+80% corn, 40% alfalfa+60% corn, and 50% alfalfa+50% corn.

Therefore, in this experiment, we mixed corn and alfalfa in two ways: milk ripe corn: alfalfa and wax ripe corn:

alfalfa. They were mixed uniformly according to the five mass ratios of 4:0, 3:1, 2:2, 1:3 and 0:4. Each mixing ratio was repeated for 3 times.

Silage Preparation

Cut the harvested whole corn and alfalfa to 1-2cm, mix them well, mix them again according to the experimental design, and fill them into a 500ml cylindrical plastic wide mouth bottle in time. After compaction, the inner and outer covers of the jar were double sealed and fermented at room temperature for 60 days in the dark.

Sample Pretreatment

After 60 days of silage, open the plastic wide mouth bottle, discard the silage 3 cm above the bottle mouth, and sample the rest for the determination of silage quality and nutritional components.

Sensory Evaluation

Sensory evaluation of the samples was carried out according to Deutche Lan Dwirtschafts Geseutschaft scoring method [9]. According to the three indicators of smell, texture and color, 20 points are full marks, 16~20 points are excellent, 10~15 points are good, 5~9 points are medium, and 0~4 points are corruption.

Determination Items and Methods

- pH value: measured by pH meter;
- Ammonia nitrogen: determined by phenol sodium hypochlorite colorimetry;
- Water-soluble carbohydrates: determined by anthrone sulfuric acid colorimetry;
- Dry matter, Moisture content, crude protein and Ether extract were determined by Soxhlet extraction method;
- The Ash and nitrogen-free extract were determined by the method in Yangsheng (1998) feed analysis and feed quality detection technology [10].
- Crude fiber, Neutral detergent fiber, Acid detergent fiber, etc. were determined by the method of Soest, etc (1991) [11].

Data Statistical Analysis

The test data were calculated by Excel, analyzed by SPSS23.0 for significant variance, and analyzed by Duncan's method for multiple analysis ($p<0.05$). The results were expressed by "mean ± standard deviation".

Results and Analysis

Nutrient Content of Raw Materials

It can be seen from table 1 that the contents of crude protein, ether extract, ash, crude fiber; acid detergent fiber and acid detergent lignin of both milk stage and wax stage corn are lower than those of alfalfa. However, the dry matter content of milk stage corn and neutral detergent fiber content of wax stage corn were higher than that of alfalfa.

Analysis of Conventional Nutrients in Mixed Silage

Crude Protein

With the increase of alfalfa content in mixed silage, the crude protein content shows an increasing trend no matter whether the corn is in milk stage or wax stage. Except alfalfa silage alone, it was mainly mixed proportion, and the crude protein content of silage at different harvest stages reached the highest when the mixed proportion was 2:2; The content of crude protein was the highest when the ratio of harvesting and mixed storage was 2:2 in milk stage, which was significantly higher than that in wax stage ($p>0.05$).

Ether Extract

After a certain amount of corn and alfalfa mixed silage, the ether extract content increased significantly. The ether extract content of silage in different harvest periods reached the highest when the proportion of mixed silage was 3:1; The corn harvest period was the main proportion, except that the mixing proportion was 1:3, the difference among other proportions was not significant ($p>0.05$). And with the increase of alfalfa, the ether extract showed a downward trend, indicating that the appropriate addition of alfalfa can significantly improve the ether extract content of si-

lage (p>0.05), but adding more alfalfa is not conducive to improve the ether extract content of silage.

Table 1: Nutrient Contents of Corn and Alfalfa Silage Raw Materia %, DM

	Milk stage		Wax stage	
	Corn	Alfalfa	Corn	alfalfa
Dry Matter	93.36	93.04	90.62	93.04
Crude Protein	6.48	15.62	10.23	15.62
Ether Extract	1.41	1.66	1.35	1.66
Ash	6.07	9.48	5.69	9.48
Crude Fiber	18.61	31.56	25.79	31.56
Natural detergent fiber	46.60	47.64	50.77	47.64
Acid detergent fiber	23.61	36.39	26.47	36.39
Acid detergent lignin	3.30	11.33	3.51	11.33

Crude Fiber

With the increase of Alfalfa content in mixed silage, the crude fiber content shows an increasing trend, and the difference is significant, no matter whether the corn is in milk stage or wax stage (p>0.05). Except for corn silage alone, it was mainly mixed proportion, and the crude fiber content of silage at different harvest stages reached the lowest when the mixed proportion was 3:1; the crude fiber content of corn was the lowest when the ratio of harvesting and mixed storage was 3:1 at milk stage.

Ash

No matter whether the corn is in milk stage or wax stage, with the increase of alfalfa content in mixed silage, the ash content shows an increasing trend. Except alfalfa silage alone, it was mainly mixed proportion, when the mixed proportion was 1:3, the ash content reached the highest, and there was significant difference between different proportions (p>0.05). The content of ash was the highest when stage (Table 2).

Table 2: Analysis and comparison of conventional nutrients in different proportion silage % DM

Corn:Alfalfa		Milk stage	Wax stage
Crude Protein	4:0	$6.25 \pm 0.61^{a}_{a}$	$7.49 \pm 0.76^{\mathrm{a}}_{\mathrm{a}}$
	3:1	$4.79 \pm 0.42^{a}_{a}$	$12.67 \pm 2.18^{\mathrm{ab}}_{\mathrm{b}}$
	2:2	$19.62 \pm 0.88^{b}_{a}$	$15.12 \pm 1.96^{b}_{b}$
	1:3	$17.70 \pm 1.39^{b}_{a}$	$13.44 \pm 5.27^{ab}_{a}$
	0:4	$23.58 \pm 3.64^{c}_{a}$	$23.58 \pm 3.64^{c}_{a}$
Ether Extract	4:0	$2.60 \pm 0.33^{a}_{a}$	$2.66 \pm 0.08^{a}_{a}$
	3:1	$3.15 \pm 0.40^{a}_{a}$	$3.41 \pm 0.09^{b}_{a}$
	2:2	$3.07 \pm 0.14^{a}_{a}$	$3.21 \pm 0.06^{b}_{a}$
	1:3	$2.85 \pm 0.09^{a}_{a}$	$3.15 \pm 0.02^{b}_{b}$
	0:4	$2.54 \pm 0.24^{a}_{a}$	$2.54 \pm 0.24^{a}_{a}$
Crude Fiber	4:0	$21.50 \pm 1.50^{a}_{a}$	$20.03 \pm 1.47^{a}_{a}$

	3:1	$21.59 \pm 1.91_a^a$	$24.76 \pm 0.39_a^{ab}$
	2:2	$26.27 \pm 1.02_a^{ab}$	$28.98 \pm 0.08_a^{bc}$
	1:3	$30.53 \pm 1.85_a^{bc}$	$32.02 \pm 0.34_a^c$
	0:4	$33.46 \pm 2.70_a^c$	$33.46 \pm 2.70_a^c$
Ash	4:0	$5.64 \pm 0.14_a^a$	$5.29 \pm 0.29_a^a$
	3:1	$6.35 \pm 0.12_a^b$	$6.08 \pm 0.24_a^a$
	2:2	$7.33 \pm 0.11_a^c$	$7.19 \pm 0.16_a^b$
	1:3	$8.84 \pm 0.04_a^d$	$9.09 \pm 0.15_a^c$
	0:4	$10.61 \pm 0.34_a^e$	$10.61 \pm 0.34_a^e$

Note: Different superscripts in the table indicate significant differences between different proportions in the same period, and different subscripts indicate significant differences between different periods in the same proportion (P<0.05).

Analysis of Silage Quality of Mixed Silage

Sensory Evaluation of Mixed Silage

The whole plant corn silage alone has aromatic and sour taste, the stem and leaf structure remains good, soft and loose, the color is yellow green, and the silage effect is excellent; alfalfa silage alone has weak butyric acid odor, poor stem and leaf structure, brown color, and good silage effect. For silage mixed with whole plant corn and alfalfa in different proportions, with the increase of corn mixing proportion, the aromatic and sour taste increased, the stem and leaf structure gradually improved, and the quality of mixed silage gradually increased (Table 3).

Table 3: Sensory evaluation of mixed silage of alfalfa and whole plant corn with different mixing proportions

Corn:Alfalfa		Smell (score)	Texture (score)	Color (score)	Total score	Grade
Milk stage	4:0	13	4	2	19	Excellent
	3:1	13	4	2	19	Excellent
	2:2	12	3	2	17	Excellent
	1:3	11	3	1	15	Good
	0:4	8	3	0	11	Good
Wax stage	4:0	12	4	1	17	Excellent
	3:1	13	4	1	18	Excellent
	2:2	13	4	2	19	Excellent
	1:3	12	4	2	18	Excellent
	0:4	8	3	0	11	Good

Water-soluble Carbohydrates

When corn is harvested at milk stage and mixed silage is conducted, the content of water-soluble carbohydrates is higher than that at wax stage as a whole, and when the mixed silage ratio is 2:2, the content of water-soluble carbohydrates reaches the highest, which is significantly higher than that of other treatments (p<0.05).

pH

In mixed silage, with the increase of corn content in the whole plant, the overall pH showed a downward trend. When the mixing ratio was 4:0, 3:1 and 2:2, the pH

was below 4.2, which reached the pH standard of high-quality silage, and the difference was not significant ($p<0.05$).

Ammonium Nitrogen

When corn is harvested at milk stage and mixed silage is conducted, the content of ammonium nitrogen is lower than that at wax ripening stage as a whole. When the mixing ratio was 3:1 and 2:2, the ammonia nitrogen content of silage in wax stage was significantly higher than that in milk stage ($p<0.05$); When the ratio of mixed storage was 2:2, the content of ammonium nitrogen was the lowest, and the difference was significant ($p<0.05$) (Table 4).

Table 4: Analysis and comparison of silage quality of different proportion mixed silage % DM

Corn:Alfalfa		Milk stage	Wax stage
pH	4:0	$3.50 \pm 0.06_a^a$	$3.52 \pm 0.04_a^a$
	3:1	$3.66 \pm 0.02_a^a$	$3.86 \pm 0.08_a^{ab}$
	2:2	$3.62 \pm 0.01_a^a$	$3.98 \pm 0.13_b^b$
	1:3	$4.39 \pm 0.25_a^b$	$5.07 \pm 0.20_b^c$
	0:4	$6.73 \pm 0.15_a^c$	$6.73 \pm 0.15_a^c$
Water-soluble carbohydrates	4:0	$3.95 \pm 1.19_a^b$	$5.90 \pm 0.42_a^c$
	3:1	$2.47 \pm 0.52_a^a$	$2.28 \pm 0.03_b^a$
	2:2	$7.47 \pm 0.67_a^d$	$4.10 \pm 0.41_b^b$
	1:3	$3.86 \pm 0.74_a^b$	$1.79 \pm 0.31_b^a$
	0:4	$5.80 \pm 0.36_a^c$	$5.80 \pm 0.36_a^c$
NH_3/N	4:0	$1.80 \pm 0.17_a^c$	$1.52 \pm 0.23_a^a$
	3:1	$1.07 \pm 0.16_a^b$	$3.92 \pm 0.72_b^b$
	2:2	$0.37 \pm 0.03_a^a$	$5.27 \pm 0.66_b^c$
	1:3	$1.33 \pm 0.23_a^b$	$2.39 \pm 0.26_a^a$
	0:4	$3.48 \pm 0.33_a^d$	$3.48 \pm 0.33_a^b$

Note: Different superscripts in the table indicate significant differences between different proportions in the same period, and different subscripts indicate significant differences between different periods in the same proportion ($P<0.05$).

Analysis of Detergent Fiber in Mixed Silage

Neutral Detergent Fiber

In all treatments, with the increase of the content of whole corn, the neutral detergent fiber showed a downward trend as a whole. When the corn was in milk stage and the mixing ratio was 2:2, the content of neutral detergent fiber reached the lowest, and the difference was significant ($p<0.05$).

Acid Detergent Fiber

In all treatments, with the increase of corn content in the whole plant, the acid detergent fiber showed a downward trend as a whole, and reached the lowest when the mixing ratio was 4:0. Except for the single silage, the mixed proportion is the main one. The acid detergent fiber content of the milk stage silage is the lowest when the mixed proportion is 2:2, and the wax stage silage is the lowest when the mixed proportion is 3:1, and the difference between different proportions is significant ($p<0.05$). The content of acid detergent fiber was the lowest when corn was harvested at milk stage and mixed storage ratio was 2:2.

Acid Detergent Lignin

In all treatments, with the increase of corn content in the whole plant, the acid detergent lignin content showed a downward trend as a whole, and reached the lowest when the mixing ratio was 4:0. Except for the single silage of the whole plant corn, the mixed proportion was dominant. The acid detergent lignin content of silage at different harvest stages reached the lowest when the mixed proportion was 3:1, and the difference between different mixed proportion was significant ($p<0.05$). The content of acid detergent lignin was the lowest when the ratio of waxy harvest and mixed storage was 3:1. There was no significant difference between different harvest periods and the same mixed storage ratio ($p<0.05$) (Table 5).

Table 5: Analysis and comparison of washing fiber in different proportion of mixed silage % DM

Corn:Alfalfa		Milk stage	Wax stage
Neutral detergent fiber	4:0	$44.29 \pm 2.38^{b}_{a}$	$36.94 \pm 1.42^{a}_{a}$
	3:1	$42.56 \pm 1.13^{ab}_{a}$	$44.86 \pm 0.40^{b}_{a}$
	2:2	$37.78 \pm 1.93^{a}_{a}$	$46.05 \pm 0.88^{b}_{b}$
	1:3	$46.62 \pm 1.34^{b}_{a}$	$48.29 \pm 1.12^{b}_{a}$
	0:4	$48.06 \pm 1.99^{b}_{a}$	$48.06 \pm 1.99^{b}_{a}$
Acide detergent fiber	4:0	$23.62 \pm 1.71^{a}_{a}$	$19.81 \pm 1.07^{a}_{a}$
	3:1	$25.80 \pm 1.16^{a}_{a}$	$27.16 \pm 0.39^{b}_{a}$
	2:2	$24.77 \pm 1.59^{a}_{a}$	$30.73 \pm 0.49^{c}_{b}$
	1:3	$33.97 \pm 0.79^{b}_{a}$	$36.40 \pm 0.21^{d}_{b}$
	0:4	$38.10 \pm 1.21^{b}_{a}$	$38.10 \pm 1.21^{b}_{a}$
Acid detergent lignin	4:0	$38.10 \pm 1.21^{b}_{a}$	$3.55 \pm 0.09^{a}_{a}$
	3:1	$5.49 \pm 0.86^{b}_{a}$	$5.30 \pm 0.31^{b}_{a}$
	2:2	$6.56 \pm 0.40^{b}_{a}$	$7.54 \pm 0.41^{c}_{a}$
	1:3	$9.96 \pm 0.17^{c}_{a}$	$9.78 \pm 0.37^{d}_{a}$
	0:4	$13.07 \pm 0.32^{d}_{a}$	$13.07 \pm 0.32^{d}_{a}$

Note: Different superscripts in the table indicate significant differences between different proportions in the same period, and different subscripts indicate significant differences between different periods in the same proportion ($P<0.05$).

Discussion

Nutrient Analysis of Mixed Silage of Whole Plant Corn and Alfalfa

The results showed that the dry matter content of silage material greater than 30% could effectively control Clostridium fermentation [12]. In this experiment, the dry matter content of corn and alfalfa stored alone or mixed was more than 30%, which met the silage conditions. Water-soluble carbohydrates is the substrate for the propagation and fermentation of lactic acid bacteria in the fermentation process of silage. Sufficient water-soluble carbohydrates in raw materials can ensure that lactic acid bacteria can form lactic acid rapidly and massively, promote the rapid decline of pH, effectively inhibit bad fermentation, and improve the quality and success rate of silage [13]. In this experiment, when the ratio of mixed silage was 2:2, the content of water-soluble carbohydrates and fat were high, the content of ash and crude fiber were low, and the silage

quality was good.

Silage Quality Analysis of Whole Plant Corn and Alfalfa Mixed Silage

pH value is an important index to evaluate the quality of alfalfa silage, which can directly indicate the success of alfalfa silage [14-15]. It is generally believed that when the pH value is lower than 4.2, it can not only provide a better acidic growth environment for beneficial bacteria such as lactic acid bacteria, but also inhibit the growth and reproduction of harmful microorganisms, so as to reach the standard of high-quality silage [16-18]. When the pH value is 4.2 ~ 4.5, it is medium. If it is higher than 4.8, it indicates that the quality of silage is poor [19]. In this experiment, when the ratio of corn to alfalfa in mixed silage is 2:2, the pH of silage drops below 4.0 and the fermentation quality is good, regardless of whether the corn is in milk stage or wax stage, which indicates that the appropriate ratio of corn and alfalfa can improve the fermentation quality of alfalfa, which is consistent with previous studies [20-22]. However, with the continuous increase of corn proportion, there was no significant difference in pH value and sensory score, while the dry matter content decreased slightly [8].

As an important index to evaluate whether the ratio of crude to concentrate in livestock diet has the best effect on livestock, the content of acid detergent fiber and neutral detergent fiber plays a crucial role, especially the content of acid detergent fiber, which is negatively correlated with the digestibility of livestock [23]. In this experiment, the contents of neutral detergent fiber and acid detergent fiber showed a downward trend with the increase of the mixing ratio, whether the corn was in milk or wax stage, which showed that adding whole plant corn to the mixed silage could improve the silage quality. When the corn was in milk stage, the mixed silage ratio of 2:2 was the best, and when the corn was in wax stage, the mixed silage ratio of 3:1 was the best.

The decomposition degree of protein and amino acid in alfalfa and corn straw mixed silage can be reflected by the ratio of ammonia nitrogen to total nitrogen. The degree of protein decomposition is directly proportional to it, which indirectly indicates that there is more protein loss in silage and the quality of silage decreases [24-26]. The ratio of ammonia nitrogen to total nitrogen is less than 10%, which is considered as high-quality silage. The content of NH3-N in silage is an objective measure of the degree of CP decomposition by microorganisms in the feed. The higher the NH3-N/TH, the more NH3 produced by CP decomposition by microorganisms and the greater the loss of CP in the feed [27]. In this experiment, with the increase of silage corn content, the ammonium nitrogen content showed a downward trend, indicating that when corn and alfalfa were mixed, the silage fermentation quality could be greatly improved by appropriately increasing the corn content.

Shortcomings and Prospects

However, the corn and alfalfa varieties selected in this study are relatively limited, and many corn varieties may exhibit different physiological characteristics due to regional climate limitations. Therefore, specific experiments on different varieties still need to be studied. In addition, this study only analyzed the conventional nutritional components and fermentation quality of forage, lacking feeding experiments that combine different types of animals. Therefore, the impact on animal production activities needs further research.

Conclusion

To sum up, the whole plant corn and alfalfa mixed silage, with the increase of the proportion of the whole plant corn, the fermentation quality and nutritional value were improved. Considering the fermentation quality and nutrients, the silage quality was better when corn was in milk stage and mixed with alfalfa in the ratio of 2:2; The silage quality was better when corn was in wax ripening stage and mixed with alfalfa in the ratio of 3:1. Finally, according to the analysis of nutritional quality and silage quality, it is considered that it is more suitable to silage with alfalfa in the ratio of 2:2 when the corn is in milk stage.

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