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Influence of Moisture Content on the Silage Quality of *Lolium multiflorum*

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Abstract: In order to evaluate the effect of moisture content on the silage quality of Italian ryegrass (*Lolium multiflorum*) three levels of moisture content (85, 75 and 65%) were tested in the experiment. The results indicated that the value of ammonia nitrogen to total nitrogen ratio, the contents of volatile fatty acid, neutral detergent fiber and acid detergent fiber were decreased significantly with the decrease of moisture content whereas the content of dry matter, crude protein and water-soluble carbohydrate were increased significantly. It was concluded that moisture content at a level of 65% was best for Italian ryegrass silage.

Key words: *Lolium multiflorum*, silage quality, moisture content, fatty acid, crude protein

INTRODUCTION

Italian ryegrass (*Lolium multiflorum*) is known as an annual ryegrass. Owing to its strong adaptability, rapid growth, nutritious, palatability and high digestibility for cattle, Italian ryegrass is cultivated as a major forage grass (Shao *et al*., 2002). Italian ryegrass provides forage from December to May of the next year with great biomass production from March to May which exceeds the amount of cattle's requirement. However, there is not enough production in other periods. In order to solve these problems, hay and silage for Italian ryegrass are two important ways to provide forage and deal with the unbalance of forage supplying. In humid and rainy climate of Southern China, hay are difficult and a heavy loss of nutrient. Thereby, silage is the ideal way to storage the Italian ryegrass (Wright *et al*., 2000).

Italian ryegrass is rich of moisture content (>85%) which is easy to cause butyric acid fermentation and lead an unsuccessful ensiling (Fussell and McCalley, 1987). Wilting silage is drying the materials to a low moisture content before silage. Wilting inhibited undesirable microorganisms breeding and reduced the loss of nutrient by butyric acid fermentation and liquid produced during ensiling (McDonald *et al*., 1991). Furthermore, reducing the moisture content of the silage could lower the transportation cost (Borreani *et al*., 2009). However, the optimum moisture content for ryegrass silage is still controversial. High moisture content silage was more likely to fail, low moisture content silage was not easy to be compressed tightly. These might cause a restricted ferment (Woolford, 1990). Current researches about the quality of Italian ryegrass silage under different moisture contents are rare. Thus it is important to study Italian ryegrass silage to deal with the unbalance of forage supplying in Southern China. Accordingly, the experiment evaluate the effect of moisture content on ryegrass silage quality to determine the optimum moisture content which is suitable for Italian ryegrass silage in Southern China.

MATERIALS AND METHODS

Silage materials: Italian ryegrass (cv. Changjiang No. 2) was used as the silage material which was cultivated on teaching farm of Sichuan Agricultural University in September 2012. Cultivated Italian ryegrass was harvested in December 2012. And the growth and development of Italian ryegrass were good about 85% moisture content. The chemical characteristics of silage materials in this experiment are shown in Table 1.

Table 1: Chemical characteristics of the silage samples (DM)

Moisture content (%)	DM (FW %)	CP (DM %)	WSC (DM %)	ADF (DM %)	NDF (DM %)
85	14.77	17.14	10.37	28.22	46.72
75	25.30	17.16	10.35	28.35	47.91
65	34.30	17.17	10.38	28.53	50.01

DM: Dry Matter; FW; Fresh Weight; WSC: Water Soluble Carbohydrate; ADF: Acid Detergent Fiber; NDF: Neutral Detergent Fiber

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Table 2: V-score evaluation for silage (FW%)

NH_3-N/TN		AA+PA		BA		
x_N	Equations	x_A	Equations	x_B	Equations	V-score
<5	$y_N = 50$	<0.2	$y_A = 10$	0-0.5	$y_B = 40\text{-}80x_B$	$y = y_N+y_A+y_B$
5-10	$y_N = 60\text{-}2x_N$	0.2-1.5	$y_A = (150\text{-}100x_A)/13$	>0.5	$y_B = 0$	
10-20	$y_N = 80\text{-}4x_N$	>1.5	$y_A = 0$			
>20	$y_N = 0$					

y_N: The score of NH_3-N/TN; y_A: The score of acetic acid+propionic acid; y_B: The score of butyric acid. Y: The total score

Design of experiment: This experiment used single factor completely randomized design. With moisture content as variable, there were three levels, CK (85%, directly silage without wilting) W1 (75%) and W2 (65%), respectively. There were three replicates in each treatment. The Italian ryegrass was chopped into approximately 2~3 cm lengths and ensiled in jars of approximately 600±10 g L^{-1}. The jars was kept at the ambient temperature. When ensiling 42 days, 5 cm samples on the top and bottom of each jar were removed and the remaining contents were mixed thoroughly then sampled for analysis fermentation quality and chemical characteristics.

Fermentation quality: In laboratory, 10 g of each sample and 90 mL distilled water were added in juicer mixing about 1 min. The silage juices were filtered through 4 layers of gauze and a medium-speed qualitative filter study. The filtrates were used to determine pH. The pH of silage was measured with a Rex PHS-3C pH meter. And a portion of the filtrate were went through 4000 r min^{-1} centrifugation for 15 min then the supernatant was filtered through 0.22 μm filter study. Then the filtrate was used to determine Ammonia Nitrogen (AN) and organic acid. AN was determined spectrophotometrically according to the Method of Phenol-Sodium Hypochlorite Colorimetry (Beaufay *et al*., 1974). Organic acid was determined by high-performance liquid chromatography (Ohmomo *et al*., 1993) and analytical method had some modifications. Lactic Acid (LA), Acetic Acid (AA), Propionic Acid (PA) and Butyric Acid (BA) were analyzed by SHIMADZU-10A high-performance liquid chromatography. Chromatographic condition, chromatographic column was Sodex Rs Park KC-811 S-DVB gel column 30×8 mm, detector was SPD-M10AVP, mobile phase was 3 mmol L^{-1} perchlorate solution, flow velocity was 1 mL min^{-1}, column temperature was 50°C, wave length was 210 nm and sample size was 10 μL.

V-score value: The V-score formula scoring system is shown (Table 2). This system is based on ammonia-N/Total N (NH_3-N/TN) and Volatile Fatty Acids (VFA) to assess the quality of silage fermentation (Takahashi *et al*., 2005). And according to the scoring criteria, silage are divided into superior quality (81-100) good (80-60) and bad (<60) 3 rank.

Chemical characteristics: The remaining ensiled forage were mixed thoroughly. The 100 g sample was collected randomly and recorded weight. The sample was put into seed bags killing out at 105°C for half hour. The DM was determined by drying the sample at 60°C to a constant mass. Then, samples were smashed, over aperture 1 mm sieve put in the numbered bag and stored in a dry and dark place for later tests. Crude Protein (CP) was determined by the Kjeldahl's Method (Broderick and Kang, 1980). ADF and NDF were analyzed according to (Van Soest *et al*., 1991). WSC contents were determined by colorimetry after reaction with anthrone reagent (Wilson, 1978).

Statistical analysis: Data was used SAS (9.1) Software to variance analysis. And differences among treatments were multiply compared with Least Significant Difference (LSD).

RESULTS

Fermentation quality and V-Score value: With the decrease of moisture content in the silage materials, the pH and LA were increased significantly, the NH_3-N/TN and VFA content were decreased significantly (Table 3). The LA of W2 was significantly higher than that of CK and W1. The NH_3-N/TN of W1 and W2 were significantly lower than that of CK and the NH_3-N/TN were 18.3 and 39.3% lower compared to the control in W1 and W2, respectively. The AA, PA and BA were decreased most markedly by the treatment of W2 they were 84.8, 92.9 and 95.3% lower than those of CK, respectively followed by W1.

From the V-score with the decrease of moisture content, the scores were increased. The silage quality of CK was bad while it was superior under 65% moisture content. This indicated that decreasing moisture content can improve silage quality.

Chemical compositions: With the decrease of moisture content in silage materials, the chemical compositions

Table 3: Effect of different moisture content on Italian ryegrass fermentation quality and V-score

			Organic acid (DM %)					
Deals	pH	AN/TN (%)	LA	AA	PA	BA	V-score value	Rank
CK (85%)	4.01[c]	13.06[a]	4.55[b]	3.89[a]	0.42[a]	2.19[a]	57.69	Bad
W1 (75%)	4.26[b]	10.67[b]	5.33[b]	0.88[b]	0.04[b]	0.87[b]	77.34	Good
W2 (65%)	4.53[a]	7.92[c]	6.19[a]	0.59[c]	0.03[b]	0.10[c]	96.22	Superior

Values followed by different small letters are significant difference at 0.05 level

Table 4: Effect of different moisture content on Italian ryegrass chemical compositions

	Chemical characteristics				
Deals	DM (FW %)	CP (DW %)	WSC (DW %)	ADF (DW %)	NDF (DW %)
CK	9.80[c]	14.61[b]	1.15[b]	27.66[a]	46.37[a]
W1	15.08[b]	15.73[ab]	1.24[ab]	21.10[b]	43.44[ab]
W2	25.35[a]	16.55[a]	1.30[a]	20.39[b]	41.28[b]

Values followed by different small letters are significant difference at 0.05 level

changed significantly (Table 4). DM in W1 and W2 were 53.9 and 158.7% higher than that of control ($p<0.05$), respectively. The CP and WSC in W2 were 13.3 and 13.4% higher than those of CK, respectively. ADF and NDF in W2 were 26.3 and 11.0% lower compared to CK ($p<0.05$), respectively.

DISCUSSION

The pH played a key role of the standard for silage quality. The pH is mainly decreased with lactic acid fermentation. Butyric acid and putrefying bacteria fermentation can also affect the value of pH. The pH of witling silage was under 4.2 which is a standard for good silage (McDonald *et al*., 1991). However, the present study showed that pH value was increased as moisture content decreased. This may be due to DM increased and the released cytoplasm in plant was decreased with the decrease of moisture content then fermentation substrate was decreased, finally inhabit lactic acid fermentation (Meeske *et al*., 2002). On the other hand with lower moisture content, the silage materials have higher osmotic pressure which inhibited the growth of clostridium (Greenhill, 1964). All indicated that even if the pH was 4.53 in 65% moisture content, it could also be stable in fermentation (Marsh, 1979).

After 42 days of fermentation, LA was increased as moisture content decreased this is in agreement with Tyrolova and Vyborna (2011). However, it is in disagreement with Mangan *et al*. (1991) who pointed that LA content in silage was decreased with the time of wilting increased. It was likely because the LA can be produced by hetero-fermentative and homo-fermentative LA. Hetero-fermentative LA produce LA and AA. But homo-fermentative LA mainly produce LA. So, in the wilting Italian ryegrass silage, homogenous LA fermentation was mainly happened which may explain why LA was at a high level. Shao *et al*. (2005) thought that warm-season grass initially ferment through homofermentation and shifted from homofermentative to heterofermentative activity. It was likely caused by forage species and climate condition. Woolford (1978) thought that the level and compose of organic acids reflect the silage quality. The more LA in the silage, the better quality of the silage. Thus, it is concluded that 65% moisture content is better than 75 and 85% moisture content in keeping silage quality.

The ratio of NH_3-N/TN and VFA content are important indicators to silage quality. The ratio reflect the decomposition extent of protein and amino acids in silage (Zhang *et al*., 2011). A high ratio led a bad quality of silage. The results showed that with the moisture content decreased, NH_3-N/TN content significantly was decreased which can restrict bad fermentation. This is was consistent with previous experiments (Manyawu *et al*., 2003).

Results also showed that with the decrease of moisture content, DM, CP and WS content were increased while NDF-ADF were decreased. And moisture content of 65% was optimal. This was indicated that lower level moisture content had a good save of silage chemical composition. It is may be due to the silage produced more percolate under high level of moisture content. While with the decrease of moisture content, the putrefying bacteria were under a restraint condition. Thus, the degradation of chemical composition was reduced (Jaster and Moore, 1990). This was confirmed results from previous studies (Beaulieu *et al*., 1993) that wilting did not affect chemical compositions.

CONCLUSION

This experiment results indicated that the content of LA, DM, CP and WSC increased whereas the NH_3-N/TN and the content of VFA decreased with the decrease in moisture content of silage materials. It is concluded that wilting reserve the silage materials nutrient to a great content and improve the fermentation quality. Therefore, the experiment indicated that the optimum moisture content for Italian ryegrass silage was 65%.

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