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Growth, chemical components and ensiling characteristics of king grass at different cuttings

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In order to effectively use and ensile king grass (*Pennisetum purpureum* × *Pennisetum americanum*), the present research investigated growth rate, yield, chemical components and silage fermentation quality of different cuttings. King grass was harvested four times, and the 1st and 3rd cuttings were ensiled directly or after wilting for 12 and 24 h. The results showed that the dry matter daily growth of 2nd cutting was significantly higher than that of other cuttings, and the 4th cutting was the lowest (P < 0.05). The contents of crude protein (CP), crude fat and water-soluble carbohydrates (WSC) tended to reduce, and crude ash tended to increase with the increase of cutting times. All four cuttings of king grass had higher WSC content, lower buffer capacity and much lactic acid bacteria, the silages made from unwilted 1st cutting and 3rd cutting were of good fermentation quality, indicated by low pH values and high *V*-scores. Wilting had different effects on the 1st cutting and 3rd cutting silages in pH value and NH_3-N content, the 1st cutting silage tended to increase the pH values and NH_3-N content, with moisture content reduction, while the 3rd cutting silage tended to reduce NH_3-N content and its pH value was not affected by wilting (P > 0.05). Although the 3rd cutting silage had better aerobic stability than the 1st cutting silage, they all were not stable within 6 days of aerobic exposure. Considering the contents of CP, crude fat, crude fiber, crude ash and WSC, the 1st cutting of king grass might have best nutrient value, while the 4th cutting was contrary. Different cuttings of king grass could be well preserved by natural fermentation, but their aerobic stability was poor.

Key words: Cuttings, ensiling, king grass, nutrient component, wilting.

INTRODUCTION

King grass is a hybrid of napier grass (*Pennisetum purpureum* Schum.) and bulrush millet (*Pennisetum americanum* Schum.). It has high biomass and good palatability (Kiranadi et al., 2002; Michelena et al., 2002), thus it is widely cultivated in tropical and subtropical regions. Liu et al. (2002) reported that king grass of Reyan No. 4 had an average fresh yield of 274.5 t ha^{-1} (185.3 to 375 t ha^{-1}) in the productive test at eight places of South China, which was 19.4% (12.8 to 25.0%) higher than napier grass. In addition, king grass has no head sprouting in the winter and its available period is longer than that of napier grass. In the Yunnan province of southwestern China, king grass had somewhat higher dry matter (DM) yield and crude protein (CP) yield than napier grass, they were 58.2, 4.30, 53.7 and 4.18 t ha^{-1} on average of two years, respectively (Zhong et al., 2002). Since king grass is of such merits, it has been a very important grass in tropic and subtropics as a perennial plant and in the regions of temperate zone as annual plant due to failing to survive in winter.

Wei and Jiang (1994) investigated the effects of cutting time (after growth for 28, 35, 49, 63 and 77 days) on the DM and CP yields of king grass, and found that the DM and CP yields were the highest after growth of 63 days. The different cuttings of forage crops may affect the DM yield and chemical components, and further affect their silage fermentation quality (Grabber, 2009). In the experiment of Li et al. (2008), the DM and CP yields of alfalfa reduced with cuttings increase. As of king grass,

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Abbreviations: CP, Crude protein; **WSC,** water-soluble carbohydrates; **FM,** fresh matter; **DM,** dry matter; **LAB,** lactic acid bacteria; **NDF,** neutral detergent fiber; **ADF,** acid detergent fiber.

the effect of cuttings on the DM yield and chemical components was not clear. Ensiling is a best preservation method for moist forage crops; it is based on anaerobic fermentation by epiphytic lactic acid bacteria (LAB) which converts water-soluble carbohydrates (WSC) into organic acids, mainly lactic acid (McDonald et al., 1991). The chemical components of forage crops will greatly affect their silage fermentation quality. The success of ensiling will mainly be decided by the characteristics of forage crops, such as the contents of DM and WSC, buffering capacity and amount of epiphytic LAB, while these factors may vary with the different cuttings.

There were some studies on king grass ensiling for the past few years (Kiranadi et al., 2002; Michelena et al., 2002; Liu et al., 2009). Michelena and Molina (1990) reported that king grass silage without wilting had lower pH value and lower contents of NH_3-N and lactic acid than those wilted from 2 to 4 h, but they had higher volatile fatty acids. Therefore, he suggested that the most suitable moisture content for king grass silage should be about 70% (namely the field drying for 3 to 4 h). Increasing the DM content in king grass was more effective to improve the silage nutritive value than the addition of formic or propionic acids (Michelena et al., 2002). All the above mentioned works were carried out with any one of cuttings, there were little information about the differences in the characteristics of chemical components and silage fermentation among different cuttings of king grass. In order to effectively use and ensile king grass, the present experiment investigated the yield, chemical components and silage fermentation quality of different cuttings.

MATERIALS AND METHODS

On February 16, 2008, king grass was planted with seed stem on the experimental field of South China Agricultural University (Guangzhou, China). The experimental field is located at 23° 26′ N and 113°15′ E at an altitude of 11 m above sea level. The mean annual rainfall was about 1870 mm. The mean annual air temperature was 22°C. King grass was planted in three plots with acreage of 2 × 3 m, each plot had 20 plants. The chemical fertilizer was applied every month at 0.17 kg a plot (N: P_2O_5: K_2O = 15:6:8) during the growing period. The first cutting was harvested with a sickle on May 11, grown for 84 days after planting. Thereafter, regrowths were harvested about every two months. All the cuttings of grass were cut at a height of 10 cm above the ground, and fresh and dry yields were measured. The grasses were chopped into 2 to 3 cm lengths by a forage chopper. After thorough mixing, a part of material was taken for chemical and microbial analysis; the left materials of 1st and 3rd cutting grass were prepared for ensiling.

Silage making

The materials of 1st and 3rd cutting grass were wilted for 0, 12, and 24 h in a room at 32°C, respectively. The material of 200 g was filled into a plastic film bag (30 × 20 cm, Mingkang Packing Company Limited, Zhongshan, China) in triplicate, degassed and sealed, using a vacuum sealer (SINBO Vacuum Sealer, Hong Tai Home Electrical Appliance Company Limited, Hongkong, China). Thereafter, they were kept in the room at ambient temperature for 50 days. At the same time, the materials prior to ensiling were sampled for chemical and microbial analysis.

Chemical and microbial analyses

The plant height and the yield from the first cutting to fourth cutting were measured at five places on each plot, and their averages were the measured values of plot. DM contents were determined by oven drying at 70°C for 48 h. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were measured according to Van Soest's procedures (Van Soest et al., 1991). Hemicellulose contents were obtained by NDF minus ADF. Crude ash contents were measured after being incinerated for 3 h at 550°C. WSC content was determined using the anthrone method (Murphy, 1958). CP and crude fat were analyzed according to AOAC methods (1990). Concentration of NH_3-N was analyzed by Kjeltech analyzer according to Kjeltech method, without a digestion step. The buffering capacity of king grass was determined as described by Playne and McDonald (1966). Ten gram of king grass material was shaken well with 90 ml of sterilized saline solution (0.85% NaCl), and serial dilutions (10^{-1} to 10^{-6}) were made in sterile saline solution. LAB were counted on de Man, Rogosa and Sharpe (MRS) medium agar (Difco Laboratories, Detroit, Mich, USA.) after incubation in an anaerobic incubator (N_2: H_2: CO_2 = 85:5:10, YQX-II, CIMO Medical Instrument Manufacturing Company Limited, Shanghai, China) at 37°C for 3 days. Aerobic bacteria were counted on nutrient agar (Nissui-seiyaku Limited, Tokyo, Japan), yeasts and molds were counted on potato dextrose agar (Nissui-seiyaku Limited) acidified with sterilized tartaric acid solution (10%) to pH 3.5. These agar plates were incubated at 37°C for 3 days.

20 g sample was taken, mixed with 80 ml of distilled water and stored in a refrigerator at 4°C overnight. Then, the material was filtered, and the filtrate was used for the measurements of pH value, NH_3-N and organic acids. The pH value was measured with a glass electrode pH meter (PHS-3B, Company Limited, Shanghai, China). The organic acid contents were analyzed by high performance liquid chromatography (HPLC) (column: Sodex RS Pak KC-811, Showa Denko K.K., Kawasaki, Japan; detector: DAD, 210 nm, SPD-20A, Shimadzu Company Limited, Kyoto, Japan; eluent: 3 mm $HClO_4$, 1.0 ml min^{-1}; temperature: 40°C). *V*-score for assessing the silage fermentation quality was determined from the contents of acetic acid, propionic acid, butyric acid and NH_3-N in the silages (Association of Self-supply Feed Evaluation, 2001). The aerobic stability was studied by measuring the pH changes of the silage material exposed to air for 2, 4 and 6 days.

Data analysis

The data of agronomic traits were analyzed by one-way analysis of variance to evaluate the effects of cuttings. The silage fermentation data were analyzed by two-way analysis of variance to evaluate the effects of cuttings, wilting and their interaction on the parameters of fermentation quality and numbers of microorganisms. The means were then compared for significance by Duncan's multiple range method. All statistical procedures were performed using the statistical packages for social sciences (SPSS 13.0 for Windows).

RESULTS

Height and yield of different cuttings

King grass was harvested about every two months, and the plant height was in the range of 142 to 175 cm. There

Table 1. Agronomic traits of king grass at different cuttings.

Cut	Harvest time	Height (cm)		Fresh yield (kg ha^{-1})		DM yield (kg ha^{-1})	
		Total	Daily gain	Total	Daily gain	Total	Daily gain
First	May 11	175^{a}	2.0^{c}	90325^{a}	1075^{a}	15735^{a}	187^{b}
Second	July 5	168^{ab}	2.8^{a}	60173^{b}	1074^{a}	16078^{a}	287^{a}
Third	September 3	142^{b}	2.2^{b}	51624^{b}	860^{b}	10743^{b}	179^{b}
Fourth	October 28	156^{ab}	2.7^{a}	40502^{c}	736^{c}	8676^{c}	157^{c}

Values within the same column with different letters differ significantly from each other at $P < 0.05$.

Table 2. Chemical and microorganism compositions of fresh king grass.

Item	First cut	Second cut	Third cut	Fourth cut	SE
Dry matter (g kg^{-1})	174.2^{c}	258.6^{a}	208.1^{bc}	214.2^{b}	9.96
Crude protein (g kg^{-1} DM)	130.6^{a}	126.6^{a}	125.4^{a}	83.6^{b}	1.41
Ether extract (g kg^{-1} DM)	45.1^{a}	41.8^{a}	36.3^{ab}	32.1^{b}	2.22
Crude fiber (g kg^{-1} DM)	286.7^{c}	330.7^{a}	304.9^{b}	319.4^{a}	3.26
Crude ash (g kg^{-1} DM)	44.8^{c}	54.3^{b}	56.4^{b}	69.1^{a}	1.61
NFE (g kg^{-1} DM)	460.9^{a}	418.3^{c}	443.9^{b}	461.7^{a}	2.65
WSC (g kg^{-1}DM)	102.3^{a}	92.4^{b}	88.8^{bc}	81.3^{c}	1.93
NDF (g kg^{-1}DM)	606.0	586.8	612.5	585.5	6.69
ADF (g kg^{-1} DM)	294.2^{b}	325.1^{a}	293.4^{b}	299.8^{ab}	14.5
BC (mE kg^{-1} DM)	86.24^{b}	105.53^{a}	98.52^{ab}	100.4^{a}	8.71
LAB (lg cfu g^{-1} FM)	5.60	7.16	6.51	5.04	0.63
AB (lg cfu kg^{-1} FM)	5.10^{b}	7.62^{a}	7.22^{a}	6.24^{ab}	0.52
Yeasts (lg cfu g^{-1} FM)	6.34	6.82	6.38	5.46	0.71
Moulds (lg cfu g^{-1}FM)	3.16	4.54	3.36	3.82	0.61

Values within the same row with different letters differ significantly from each other at $P < 0.05$. DM, Dry matter; FM, fresh matter; NFE, nitrogen free extract; WSC, water-soluble carbohydrates; NDF, neutral detergent fiber; ADF, acid detergent fiber; BC, buffering capacity; LAB, lactic acid bacteria; AB, aerobic bacteria; lg, denary logarithm of the numbers; cfu, colony-forming units.

were no significant differences in the plant height among the 1st, 2nd and 4th cuttings ($P > 0.05$), but the 3rd cutting was lower than the 1st cutting ($P < 0.05$). The height daily growth of 2nd and 4th cuttings was faster than that of 1st and 3rd cuttings ($P < 0.05$). The 1st and 2nd cuttings had higher total DM yield than the 3rd and 4^{th}, while the DM daily growth of 2nd cutting was significantly higher than that of other cuttings, and the 4th cutting was the lowest ($P < 0.05$) (Table 1).

Chemical and microbial components prior to ensiling

Cuttings had significant effects on all the chemical components except NDF content ($P < 0.05$). There were no significant effects on the amounts of microorganisms except aerobic bacteria (Table 2). The 2nd cutting had the highest DM content beyond 25%, while the DM contents of other cuttings were all lower than 22%. There were no significant differences in CP and crude fat contents among the 1st, 2nd and 3rd cuttings, and the 4th cutting had the lowest CP content and lower crude fat content than the 1st and 2nd cuttings. The 1st cutting had the lowest crude fiber content and the highest WSC content ($P < 0.05$). Epiphytic LAB on all four cuttings was more than 10^5 cfu g^{-1} FM. The amount of aerobic bacteria on the 1st cutting was significantly lower that of 2nd and 3rd cuttings.

Fermentation quality

Cuttings and the interaction of cuttings and wilting had significant effects on DM content, pH value, butyric acid content and *V*-score ($P < 0.01$), with no significant effects on other parameters ($P > 0.05$). Wilting had significant effects on DM content, pH value, lactic acid, butyric acid and NH_3-N contents and *V*-score ($P < 0.01$), on acetic acid content and the ratio of lactic acid to acetic acid ($P < 0.05$), no significant effect on propionic acid content ($P > 0.05$) (Table 3). For the silages made from 1st cutting, the pH values significantly increased with wilting time ($P <$

Table 3. Effects of cuts and wilting on the fermentation quality and aerobic stability of king grass.

Item	First cut			Third cut			SE	Cut	Wilting	Int
	NW	SW	LW	NW	SW	LW				
DM (g kg^{-1})	17.4^{f}	27.1^{d}	35.2^{b}	20.8^{e}	30.4^{c}	39.1^{a}	7.43	**	**	**
pH	3.74^{c}	3.98^{b}	5.04^{a}	4.03^{b}	3.96^{bc}	4.11^{b}	0.07	**	**	**
Lactic acid (g kg^{-1} DM)	102.9^{a}	89.8^{ab}	25.1^{c}	86.2^{ab}	66.1^{b}	23.0^{c}	10.01	NS	**	NS
Acetic acid (g kg^{-1} DM)	18.96^{ab}	29.05^{a}	12.56^{b}	20.47^{ab}	36.61^{a}	10.81^{b}	6.16	NS	*	NS
Propionic acid (g kg^{-1} DM)	33.45	21.02	18.22	29.00	21.55	20.53	6.78	NS	NS	NS
Butyric acid (g kg^{-1} DM)	0.52^{bc}	0.20^{bc}	7.08^{a}	0.99^{b}	0.39^{bc}	0.00^{c}	0.26	**	**	**
Ratio of lactate to acetate	5.52	3.12	2.00	4.16	1.81	2.08	1.29	NS	*	NS
NH_3-N (g kg^{-1} TN)	18.5^{c}	30.9^{ab}	33.5^{a}	30.0^{ab}	20.1^{bc}	15.7^{c}	0.03	NS	**	NS
V-score	93.8^{a}	90.7^{a}	73.3^{b}	92.0^{a}	89.1^{a}	92.1^{a}	1.52	**	**	**

Values within the same row with different letters differ significantly from each other at $P < 0.05$. DM, Dry matter; TN, total nitrogen; NW, no wilting; SW, 12 h wilting; LW, 24 h wilting; Int, interaction; *significant at $P < 0.05$; **significant at $P < 0.01$; NS, not significant.

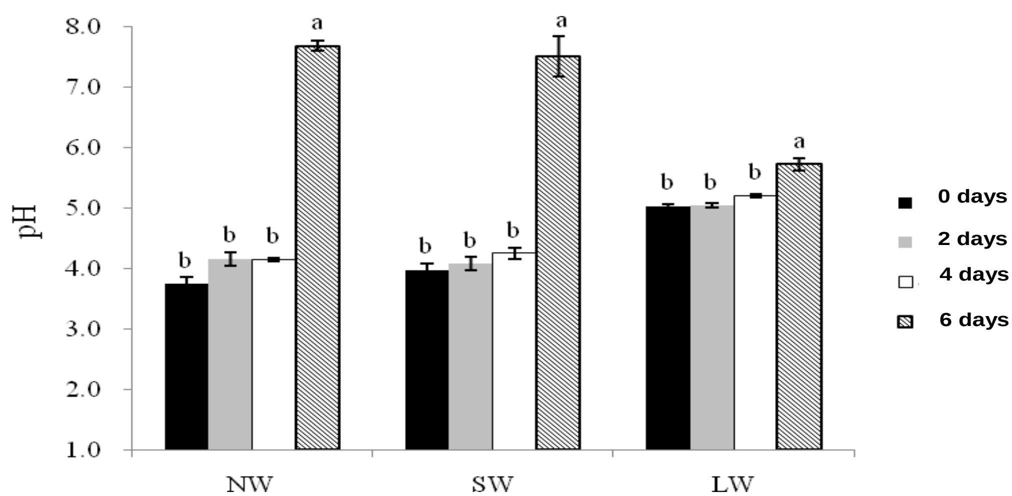

Figure 1. pH changes of 1st cut king grass silages after been exposed to the air for 6 days. Poles with different letters in the same wilting time are significantly different at the 0.05 level. NW, No wilting; SW, 12 h wilting; LW, 24 h wilting.

0.05) and NH_3-N contents tended to rise. The 24 h wilted silage had less lactic acid and more butyric acid than unwilted and 12 h wilted silages ($P < 0.05$). For the silages made from 3rd cutting, there were no significant differences in the pH values among silages ($P > 0.05$) irrespective of wilting time. However, 24 h wilted silage had lower lactic acid content than unwilted and 12 h wilted silages ($P < 0.05$). The 24 h wilted silage made from 1st cutting had lower *V*-score than other five silages ($P < 0.05$), and there were no significant differences among those five ($P > 0.05$).

Aerobic stability

After the silages were exposed to the air for 6 days, all the silages of king grass were not stable, indicated by marked pH increase. The 1st cutting silages were better than the 3rd cutting silages; their pH increase took place after the silages were exposed to air for 6 days (Figure 1), while the pH values of 3rd cutting silages began to rise within 4 days, especially for the wilted silages which began to rise within 2 days (Figure 2).

DISCUSSION

In the present experimental conditions, the fresh and DM yields of king grass were 242.5 and 51.2 t ha^{-1}, respectively. It was near to the average fresh yield of 274.5 t ha^{-1} reported by Liu et al. (2002). Since the yield in this experiment was measured in the first year after establishment, and the growth period from October to next February was not included, the total fresh yield of

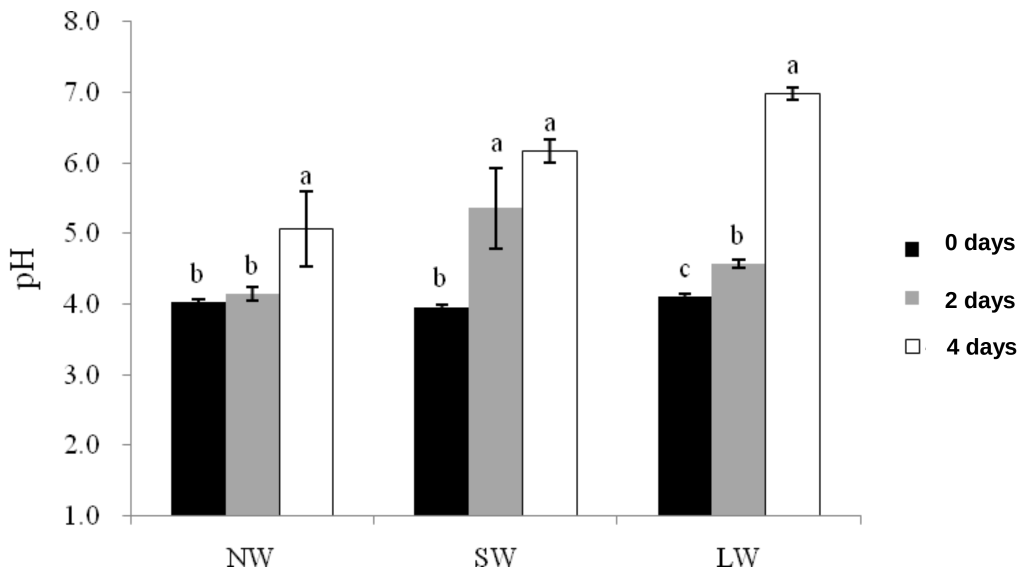

Figure 2. pH changes of 3rd cut king grass silages after been exposed to the air for 4 days. Poles with different letters in the same wilting time are significantly different at the 0.05 level. NW, No wilting; SW, 12 h wilting; LW, 24 h wilting.

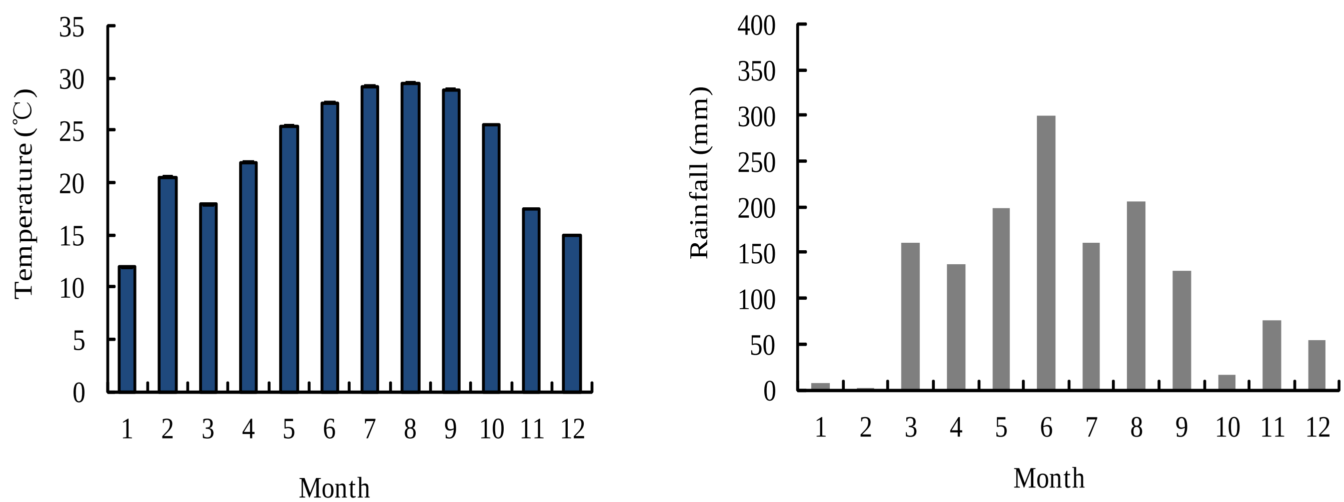

Figure 3. The air temperature and rainfall during the experimental year.

the whole year should exceed 300 t ha^{-1}. It is well known that cuttings affect the growth rate and chemical components of grass, due to the changes in air temperature, rainfall and regrowth capability for different cuttings. In this study, the FM daily growth tended to reduce with the increase in cutting times, but the 2nd cutting had the same rate as the 1st cutting. However, the DM daily growth was the largest for the 2nd cutting and the least for the 4th cutting. Although the 1st cutting had the same FM daily growth to the 2nd cutting (Table 1), the DM daily growth was significantly lower than the 2nd cutting due to high moisture content of 1st cutting (Table 2). In addition, the height daily growth of 2nd cutting was also higher that of the 1st cutting. This result might be attributed to high rainfall and suitable temperature during the growth of 2nd cutting (from May to June) (Figure 3). The DM daily growth of 4th cutting was the least, which might be attributed to the small rainfall during the growth and the reduction of stem diameter with increase in cutting times. Rengsirikul et al. (2011) reported that tiller density of napier grass decreased as the interval of cutting increased, which indicated that increasing cutting times enhanced tiller density. In our further research, the tiller density of king grass also increased, and stem diameter tended to reduce with the increase of cutting times. Therefore, the increase or reduction in the DM yield would be decided by a balance of tiller density increase and stem diameter reduction, as well as plant height.

In general, the chemical components of a plant are affected by many factors, such as growth stages, regrowth times, environmental conditions and so on. Temperature is related to cell wall lignification, light is related to cell wall content, and water availability is related to forage quality (Wilson, 1994; Van Soest, 1996). However, the chemical components of plants are a synthetic result of various factors. The present experiment showed that cuttings had significant effects on all the chemical components except NDF content. The contents of CP, crude fat and WSC tended to reduce, and crude ash tends to increase with the increase of cutting times. Considering the contents of CP, crude fat, crude fiber, crude ash and nitrogen free extract (NFE), the 1st cutting of king grass might have best nutritive value, while the 4th cutting was contrary. It is well known that the silage fermentation quality was mainly affected by WSC content, LAB numbers, moisture content and buffer capacity (McDonald et al., 1991). Sine all four cuttings of king grass had higher WSC content, lower buffer capacity and more LAB, the silages made from unwilted 1st cutting and 3rd cutting were of good fermentation quality. Liu et al. (2009) also reported that king grass of 1st cutting had good fermentation quality even if the unwilted grass was ensiled. Reducing moisture content often increases silage pH value and decreases lactic, acetic and propionic acid yields, and produces less NH_3-N (McDonald et al., 1968; Muck, 1990). In the present research, wilting had different effects on the 1st and 3rd cutting silages in pH value and NH_3-N content. The 1st cutting silage tended to increase the pH values and NH_3-N with moisture content reduction, while the 3rd cutting silage tended to reduce NH_3-N content, and its pH value was not affected by wilting. However, all the silages except 24 h wilted 1st cutting, had good silage fermentation quality indicated by high *V*-scores. The silages made from tropical and subtropical grasses often have poor fermentation quality due to less WSC content; king grass should be an exception from the present result and other researches (Michelena and Molina, 1990; Liu et al., 2009).

Acetic acid fermentation often dominates the silages made from tropical grasses (Catchpoole and Henzell, 1971). Zhang and Kumai (2000) reported that napier grass silage by the natural fermentation mainly produced acetic acid, while lactic acid fermentation dominated when LAB or cellulases were added. In another research with guinea grass, lactic acid fermentation dominated when silage was made at 10 and 20°C, while it was dominated by acetic acid fermentation when silage was made at 30 and 40°C. Furthermore, at 30 and 40°C, adding cellulases made silage from acetic acid fermentation to lactic acid fermentation, inoculating LAB alone did not alter the fermentation type (Zhang et al.,1997). These results might indicate that WSC content was a very important factor of deciding the silage fermentation type, and its role also was affected by other factors. In the present experiment, king grass silages were dominated by lactic acid fermentation, irrespective of wilting. This result was in accordance with the report of Yokota at al. (1995) who found that napier grass mainly produced lactic acid rather than acetic acid when it was directly ensiled (moisture content 87.6%) or ensiled after wilting (moisture content 81.3 or 71.1%). The reason was probably that king grass had high WSC content and low buffer capacity, which benefited lactic acid fermentation.

In the present research, although wilting did not enhance the silage fermentation quality of king grass, proper wilting will be favorable to the animal production from the views of reducing silage effluent (Bastiman, 1976; Castle and Watson, 1973) and increasing intake and performance of animals (Marsh, 1979; Wright et al., 2000). The pH increase is a main indicator of aerobic deterioration due to lactic acid reduction (Muck, 1990). Therefore, less pH change indicates good aerobic stability of silage. In the present research, although the 1st cutting silage had better aerobic stability than the 3rd cutting silage, they all were not stable within 6 days of aerobic exposure. Generally, well preserved silages are more liable to aerobic deterioration since they contain less ammonia, butyric acid and other volatile fatty acids (Weinberg et al., 1993; Nussio, 2005).

Conclusion

Cuttings had significant effect on harvest yields and most chemical components of king grass. The first two cuttings of king grass had large yields and good quality. King grass was a good silage material due to the high WSC content and low buffering capacity, it could be well preserved by the natural fermentation, but its aerobic stability was poor. In practice, proper wilting will be favorable to animal production even if it does not enhance the silage fermentation quality of king grass.

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