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# The Evaluation of Forage Silage Related Traits Between Maize and Hybrid Giant Napier (Pennisetum Hydridum)

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## Abstract

Selecting elite silage specific variety was important for modern animal husbandry development and agricultural production. In this paper, six forage quality related physiology traits were evaluated by using chemical detection method, the difference between maize silage and hybrid giant napier (*pennisetum hydridum*) were analyzed. Results showed that, maize showed better performance than that of hybrid giant napier, with average protein content of 8.54% VS 7.48%, starch content of 11.18% VS 7.90%, soluble sugar content of 4.09% VS 2.90%, and IVOMD of 35.23% VS 34.94%, respectively. No significant differences were found for NDF content and ADF content between maize and hybrid giant napier. In addition, QQ446 showed better performance than that of JY818, with higher IVOMD of 35.50% VS 34.95%, higher protein content of 8.60% VS 8.48%, higher starch content of 11.82% VS 10.53%, and lower ADF content of 32.86% VS 37.43%. These results proved that, QQ446 was the elite silage specific variety, it contained much more forage advantages, and could be widely used in future animal husbandry development and agricultural production.

## Introduction

Maize (*Zea may* L.) and hybrid giant napier (*pennisetum hydridum*) are always planted as feeding forage for animals because of its higher biomass yield, excellent quality, and better palatability [7]. Fresh forage grass could offer better nutritional ingredient and mouth feeling for animals, but which was impacted by seasonal temperature change during the growth period. Therefore, silage is important for animals' feeding, which could increase the feeding period and maintain the quality of fresh forage per se. Reports showed that silage grass can be used as sustainably feeding, without affected by the seasonal climate changed, which were commonly used in the ration of dairy cow, sheep, and horse over the last few decades [5]. It's also reported that silage can increased dry matter (DM) intake without affecting the milk yield (Warner *et al*, 2013). However, during the ensiling, field harvest and feeding process, DM intake decreasing and quality losses always occur, and factors affecting DM intake decreasing and quality losses are variation in terms of grass and maize, as well as for different varieties [1].

Traditionally, because of the higher biological yield and perennial characteristics, *pennisetum hydridum* silage was thought to be one of the major forage components in the ration of animals, during the winter period in tropical and subtropical regions. However, *pennisetum hydridum* silage always results in a relatively lower DM intake, lower nutritive value, lower ensiling quality and lower energy intake potential [8], which tends to reduced its usefulness in the diets of high-producing dairy cattle. Comparing analysis showed that maize silage was better than feeding dairy cows by only grass silage, which could increase feed intake, milk yield, and milk protein contents [4, 6, 9]. Over the past few decades, the incorporation of maize and grass silage together with some given rations was increased during the feeding of dairy cows, which can improve the productivity of dairy cows. Otherwise, when the grass was replaced by maize silage, DM intake and milk production were improved [2]. In addition, the maturity of harvest maize can also affect the meat quality and potential concentrated [4]. Most researches revealed that feeding related traits were most important for silage maize and grass. But knowledge about the variation of silage related traits between pair grass and maize, between pair-silage maize varieties are still limited.

Over the word, for grass silage and maize silage, releasing of excellent varieties has been proved to be one of the effect ways, which can improve silage relevant traits of carbohydrates content and composition [11]. Plant digestibility of silage maize presents significant influence on nutrition intake of animal feeding. Improving forage quality would enhance the utilization efficiency and feeding value of forage maize. He and colleagues collected 26 silage maize inbreds and analyzed silage related agronomic traits [3]. Results revealed that, different silage maize varieties always showed variant performances of straw yield, seed yield, and plant height [3]. They clarified that index of plant height was important during the silage maize selection [3]. However, most researches always focused on the agronomic traits, which often be affected by the genotype and environment interaction. Base on this, some silage maize varieties, i.e. Jinyu818 and Qianqing446, have been widely used as silage in China, during animal husbandry's development, which also possessing lager straw yield, seed yield, and plant height, but knowledge about relevant physiological properties of silage are still unknown.

Therefore, in this paper, one *pennisetum hydridum* of Huangzucao (HZC), two silage maize varieties of Jinyu818, and Qianqing446 were selected as experimental materials, which were planted in the same points. And some physiological properties of silage were evaluated, with standard test method for neutral detergent fibers and acid detergent fibers, anthrone colorimetry for the content of starch and total soluble sugar. The objectives were to: 1) Identify the difference of physiological properties indexes between pair of HZC, Jinyu818, and Qianqing446; 2) find out the best grass varieties for silage among HZC, Jinyu818, and Qianqing446. These results would provide some scientific proof for the selection and application of superior silage varieties during future animal husbandries development.

## Materials and Methods

### *Plant Material*

Three silage varieties were used as materials. One was the *pennisetum hydridum* of HZC, which was widely used as one forage component in the ration of cattles during the winter period in Guizhou province of China. Another two silage maize varieties were released by the

Institute of Upland Food Crops, Guizhou Academy of Agricultural Sciences. Between these two maize varieties, Jinyu818 was derived from the cross of T32 (inbred selected from Suwan germplasm) and QB506 (inbred selected from temperate germplasm), Qianqing446 was derived from cross of QB1545 (inbred selected from temperate germplasm) and QQ446 (inbred selected from Suwan germplasm). These three silage varieties possess better performance of staying greed, and stronger resistances to diseases and insects.

*Field Experiment and Sampling*

In 2018, three silage varieties were planted in Huangjinshan Village, Shiqian Contry, Tongren City (TR, 108.7°N, 27.5°E, altitude of 1040 m) of Guizhou province. Each variety was planted in one field with 667 m in length and 667 m in width, with the density of 75000 plants per hectare. At the end stage of grain filling, when the milk line moved to half of kernels, 10 continuous plants were sampled to be chopped and anaerobic fermentation, three repeats for each treatment.

*Treatment and Anaerobic Fermentation*

All samples of case were treated with additive of 1 g sucrose, 0.1 g cellulase, 0.2 g lactobacillus plantarum, and 0.1 g bacillus subtilis, and samples of control were treated with additive of 1.4 g water. Then, all samples were fermented under anaerobic conditions for 0 D, 30 D, and 60 D, respectively. After treatment, all samples were taken out and sent to Baihui Biotechnology Company in Chengdu (Sichuan, China) to detect relevant silage indexes, including neutral washing fiber (NDF), acid washing fiber (ADF), starch content, total soluble sugar content, protein content, and in vitro dry matter digestibility (IVOMD).

*The Detection of Silage Related Indexes*

For the detection of NDF and ADF: One filter bag dried to constant weight ($M_0$) was selected, then some air dried samples were weighed ($M_1$) and placed into the bag. Some neutral detergent, 2 ml decalin and 0.5 g sodium sulfite anhydrous were added into this bag, it was putted on the condensing device and heated to boiling, one hour after, cool it and rinse with water. After that, this bag was placed in acetone for several minutes. Taken it out and dried it to constant weight ($M_2$). Finally, relevant NDF was calculated as follow: NDF% = $\frac{M_1-(M_2-M_0)}{M_1}\times 100\%$. After the finish of NDF detection, the remaining samples were added into acid detergent, detail detection steps were the same with the detection method of NDF described beyond. The final constant weight was named to be $M_3$. And ADF was calculated as follow: ADF% = $\frac{M_2-M_3}{M_1}\times 100\%$.

For starch content was detected by using the method of anthrone colorimetry. First, one standard curve of starch content was draw. Then starch content (SC) was detected, and calculated based on formula:

$$SC\% = \frac{C\times\frac{V}{a}}{w\times10^6}\times 100.$$

Wherein C means the content of starch read from the standard curve, with unit of microgram. V means the total amount of extract liquid, with unit of milliliters. w means the weight of sample. And a means the liquid amount when taken to develop the color.

For the total soluble sugar content (TSSC), which was detected by using anthrone colorimetry. First we needed to construct one standard curve of sugar content, and detected the optical density of different sugar content under the wavelength of 630 nm. Then the standard curve equation was calculated. Finally, the total soluble sugar content was calculated as:

$$TSSC = \frac{\frac{Sc}{Vx}\times Vt\times Dr}{W}$$

Wherein 'TSSC' meant the total soluble sugar content. 'W' meant the dry weight of sample. 'Vx' meant the absorbed solution sample's volume. 'Sc' meant the sugar content calculated from regression equation. 'Vt' meant the extraction weight. And 'Dr' meant the dilution ratio.

Protein content was detected by using Kjeldahl method. And it was calculated as:

$$X = \frac{\frac{C\times(V-V0)\times 0.014}{\frac{m}{100}\times 10}}{} \times F\times 100$$

Wherein X meant the protein content of sample, with unit of g/100g. V meant the volume of hydrochloric acid standard solution consumed in sample titration, with unit of mL. V0 meant the volume of hydrochloric acid standard solution consumed in blank titration, with unit of mL. C meant the concentration of hydrochloric acid standard titration solution. m meant the weight of sample, with unit of g. F meant the conversion coefficient of nitrogen to protein, it was 6.25 for maize.

For in vitro dry matter digestibility (IVOMD), first acid pepsin solution was constructed to simulate colic fluid. Then sample was weighted as $W_1$ and treated with acid pepsin solution. After that, the residual sample was weighted as $W_2$. Then the IVOMD was calculated as:

$$IVOMD = \frac{W_1-W_2}{W_1}\times 100\%$$

ANOVA was performed using PROC GLM model. A Pearson correlation analysis of FT-related traits across different environments was calculated using the PROCCORR model. And all above analysis were completed using the SAS software (Release 9.3; SAS Institute, Cary, NC).

## Results

*Summary Statistic of Silage Related Indexes*

Phenotypic evaluated data was listed in Table 1, hybrid giant napier of HZC showed the lowest content of starch, protein, soluble sugar, with relevant value of 7.90%, 7.48%, 2.92%, respectively. Maize inbred of QQ446 showed the highest content of starch, protein and IVOMD, with relevant value of 11.82%, 8.60% and 35.50%, respectively. In addition, QQ446 exhibited the lowest content of ADF (32.86%) (Table 1).

*ANOVA and Correlation Analysis between Pair–Silage Related Indexes*

ANOVA analysis showed that, six silage related indexes all showed significant difference between pair-genotypes, with Pr less than 0.001 (Table 2). Correlation analysis showed that, starch content and protein content of silage forage grass showed the strongest positive correlation, with correlation coefficient of 0.66, and significance value of less than 0.0001. The second *positive correlation* was identified between protein content and soluble sugar content, with correlation coefficient of 0.46, and significance value of less than 0.0099. Except that, ADF content showed significant negative correlation with starch, protein content, soluble sugar and IVOMD, with correlation coefficient of -0.56, -0.48, -0.43, and -0.43 respectively. NDF content showed significant negative correlation with soluble sugar content, with correlation coefficient of -0.48 (Table 3).

*The Changing of IVOMD*

For the no additive treatment of 0 D, JY818 showed the lowest IVOMD than that of QQ446 and HZC, and no difference was found between HZC and QQ446. When ensiled time arrive at 60 days for treatment of without additive, JY818 showed the highest IVOMD, but QQ446 showed the lowest IVOMD value. In addition, for treatment of additive, with the ensiled time increasing, IVOMD showed increasing trend (Fig. 1), which meant that treatment of additive and increasing ensiled time also can improve the IVOMD of forage.

*Phenotypic Diversity of ADF and NDF among Different Treatments*

For no additive treatment of the three forages, HZC showed the highest content of ADF content than that of JY818 and QQ446 when ensiled for 0 and 30 days. With the increasing of ensiled time, ADF content also increased for maize inbred of JY818 and QQ446, but the converse changing trend were found for HZC. Maize inbred of JY818 and QQ446 all showed increasing of ADF content during the treatment of 0 days to 30 days, with higher changing in QQ446 than that of JY818 (Fig.2). HZC showed the highest of ADF content than that of maize during the treatment of 0 day, but it showed the lowest of ADF content than that of maize when treated with additive for 30 days. NDF content showed firstly decreasing and secondly increasing when ensiling time increasing for HZC and QQ446 treated

Table 1. Phenotypic data of silage related indexes

| Treat | IVOMD (%) | NDF % | ADF (%) | Soluble sugar content (%) | Starch content(%) | Protein content (%) |
|---|---|---|---|---|---|---|
| HZC-0-0-1 | 32.31 | 57.38 | 37.65 | 6.09 | 6.74 | 7.42 |
| HZC-0-1-1 | 36.97 | 31.06 | 38.28 | 2.23 | 7.45 | 7.95 |
| HZC-0-1-2 | 36.54 | 56.99 | 41.19 | 2.32 | 9.35 | 7.46 |
| HZC-0-2-1 | 31.45 | 52.87 | 38.02 | 2.30 | 9.68 | 7.31 |
| HZC-0-2-2 | 32.64 | 51.87 | 37.02 | 2.36 | 9.48 | 7.21 |
| HZC-1-1-1 | 31.66 | 58.50 | 29.61 | 3.63 | 9.03 | 7.93 |
| HZC-1-1-2 | 35.37 | 58.11 | 40.23 | 3.59 | 7.44 | 7.09 |
| HZC-1-2-1 | 38.23 | 56.19 | 36.79 | 1.78 | 5.47 | 7.63 |
| HZC-1-2-2 | 39.23 | 57.19 | 34.79 | 1.98 | 6.47 | 7.33 |
| **Mean value** | **34.94** | **53.35** | **37.06** | **2.92** | **7.90** | **7.48** |
| JY818-0-0-1 | 30.22 | 51.15 | 29.36 | 7.97 | 12.98 | 9.23 |
| JY818-0-1-1 | 31.58 | 54.82 | 38.48 | 4.41 | 10.15 | 8.29 |
| JY818-0-1-2 | 31.48 | 55.96 | 38.74 | 4.14 | 9.89 | 8.59 |
| JY818-0-2-1 | 42.68 | 54.34 | 39.18 | 4.52 | 10.69 | 8.36 |
| JY818-0-2-2 | 39.43 | 56.15 | 39.67 | 4.68 | 10.24 | 8.34 |
| JY818-1-1-1 | 32.82 | 50.95 | 34.99 | 4.03 | 7.88 | 8.68 |
| JY818-1-1-2 | 32.85 | 55.57 | 40.30 | 4.70 | 12.34 | 8.00 |
| JY818-1-2-1 | 36.70 | 55.38 | 34.00 | 4.54 | 10.63 | 8.50 |
| JY818-1-2-2 | 36.79 | 57.04 | 42.12 | 4.49 | 10.00 | 8.30 |
| **Mean value** | **34.95** | **54.59** | **37.43** | **4.83** | **10.53** | **8.48** |
| QQ446-0-0-1 | 32.79 | 53.40 | 21.60 | 6.96 | 15.41 | 8.95 |
| QQ446-0-1-1 | 35.29 | 52.36 | 33.54 | 4.49 | 13.80 | 8.82 |
| QQ446-0-1-2 | 35.19 | 51.36 | 32.54 | 4.59 | 12.80 | 8.42 |
| QQ446-0-2-1 | 37.25 | 55.77 | 34.53 | 2.37 | 12.05 | 8.60 |
| QQ446-0-2-2 | 37.52 | 56.62 | 38.13 | 2.04 | 9.36 | 8.19 |
| QQ446-1-1-1 | 31.08 | 57.31 | 37.31 | 2.62 | 10.63 | 8.52 |
| QQ446-1-1-2 | 30.90 | 54.77 | 33.27 | 2.56 | 11.03 | 8.71 |
| QQ446-1-2-1 | 39.84 | 49.82 | 28.71 | 2.02 | 11.84 | 8.43 |
| QQ446-1-2-2 | 39.67 | 50.50 | 36.08 | 2.46 | 9.45 | 8.79 |
| **Mean value** | **35.50** | **53.55** | **32.86** | **3.34** | **11.82** | **8.60** |

Note: HZC is the abbreviation of Huangzucao, JY818 is the abbreviation of Jinyu818. QQ446 is the abbreviation of QianQin446. The first number of 0 ment treated with no additive, 1 ment treated with additive. The second number of 0 meant ensiled 0 day, 1 meant ensiled 30 days, and 2 meant ensiled 60 days. The third number of 1 meant repeat one, and 2 meant repeat two.

Table 2. ANOVA analysis of silage related indexes

| | Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|---|
| **IVOMD** | genotypes | 14 | 1107.65 | 79.12 | 5.47 | <0.0012 |
| **NDF** | genotypes | 19 | 1283.54 | 91.68 | 71.23 | <0.0001 |
| **ADF** | genotypes | 19 | 866.31 | 61.88 | 14.04 | <0.0001 |
| **Soluble sugar** | genotypes | 19 | 113.23 | 8.09 | 128.88 | <0.0001 |
| **Starch** | genotypes | 19 | 154.58 | 11.04 | 19.52 | <0.0001 |
| **Protein** | genotypes | 19 | 19.31 | 1.38 | 9.24 | <0.0001 |

Table 3. Correlation analysis between pair-silage related indexes

| | IVOMD | NDF | ADF | Soluble sugar | Starch | Protein |
|---|---|---|---|---|---|---|
| **IVOMD** | 1.00 | 0.02 | **-0.43** | 0.08 | 0.35 | 0.30 |
| **NDF** | <0.9000 | 1.00 | -0.05 | **-0.48** | 0.05 | -0.29 |
| **ADF** | **<0.0200** | <0.7800 | 1.00 | **-0.43** | **-0.56** | **-0.48** |
| **Soluble sugar** | <0.6683 | **<0.008** | **<0.018** | 1.00 | 0.31 | **0.46** |
| **Starch** | **<0.0546** | <0.8031 | **<0.0013** | <0.0996 | 1.00 | **0.66** |
| **Protein** | <0.1018 | <0.1145 | **<0.0077** | **<0.0099** | **<0.0001** | 1.00 |

Note: Numbers in the bottom left trig table represent the significance level, and numbers in the up right trig table represent the pair-correlation coefficient.

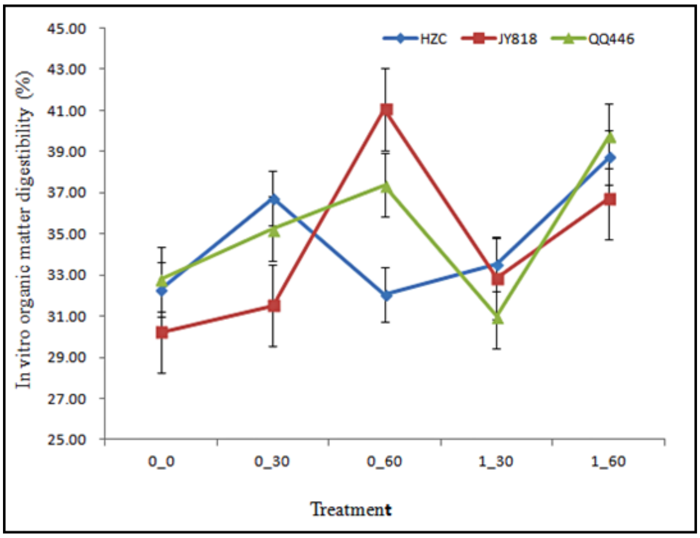


Figure 1. The comparison of IVOMD

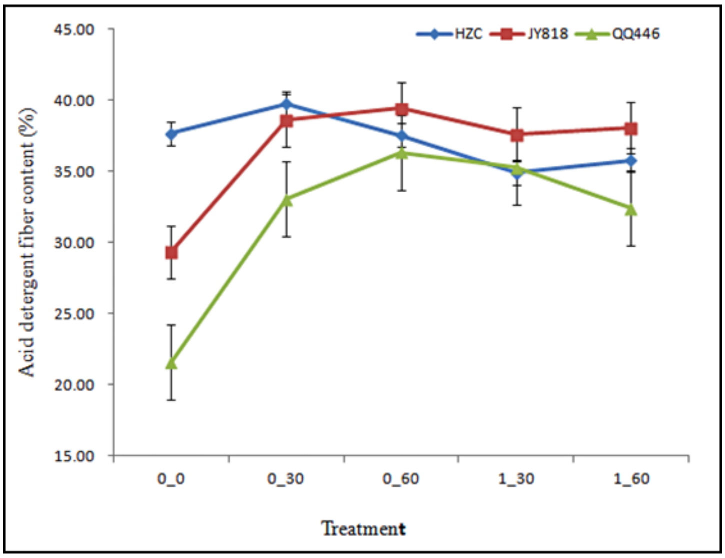


Figure 2. The comparison of ADF content

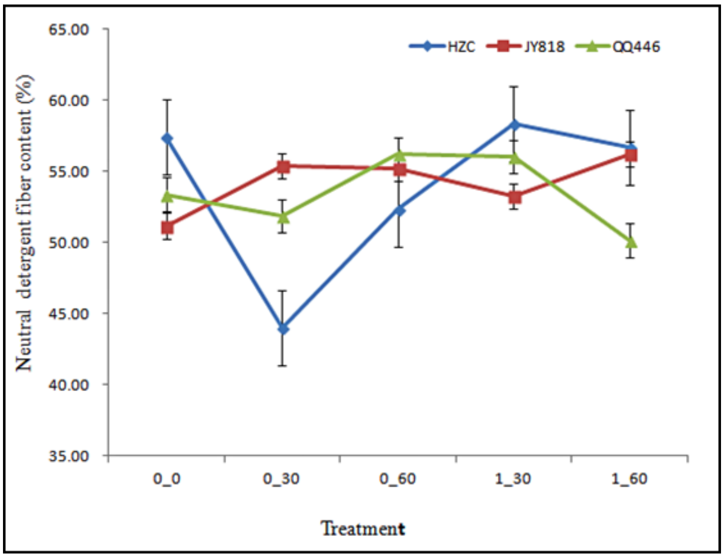


Figure 3. The comparison of NDF content

without additive, but JY818 showed reverse performance. For treatment of additive, NDF content showed increasing for JY 818, but decreasing for HZC and QQ446 when ensiling time increasing (Fig.3).

*The Changing of Soluble Sugar Content*

For no additive treatment of the three forages, the soluble sugar content decreased when ensiled from 0 day to 60 days. Wherein, QQ446 showed the highest decreasing extent. Among additive treatment of the three forages, JY818 showed the highest soluble sugar content when ensiling for 30-60 days, and QQ446 also showed higher soluble sugar content than that of HZC when ensiling for 30 days (Fig.4). These results clarified that maize always showed higher soluble sugar content than that of hybrid giant napier.

*The Comparing of Starch and Protein Content*

When ensiled time increasing, starch content showed decreased, with HZC containing the strongest changing extent (Fig. 5). Maize hybrid of QQ446 showed significant high starch content than that of JY818 and HZC when ensiled. Maize of JY818 showed the second higher starch content than that of HZC when ensiled. This proved that maize is high quality feeding grass than that of hybrid giant napier. For protein content, maize of JY818 and QQ446 all showed significant higher than that of HZC after ensiled. And QQ446 showed much higher of protein content than that of JY818 (Fig.6). These results proved that maize contained much more starch and protein content than that of HZC, which was consistent with the knowledge of practical productive forages.

For all plots, HZC is the abbreviation of Huangzucao, JY818 is the abbreviation of Jinyu818. QQ446 is the abbreviation of QianQin446. 0_0 meant treatment without additive and ensiled for 0 day. 0_30 meant treatment without additive and ensiled for 30 days. 0_60 meant treatment without additive and ensiled for 60 days. 1_30 meant treatment with additive and ensiled for 30 days. 1_60 meant treatment with additive and ensiled for 60 days.

## Discussion

*Maize Showed Better Forage Quality than that of Hybrid Giant Napier*

According to the knowledge of cultivated practise, hybrid giant napier can't live through the winter at high altitudes regions, because of the lower accumulated temperature [2]. Reports showed that, maize has been widely used to be the major forage component in the ration of dairy cows over the last few decades. Khan's [5] research showed the variation of maturity at harvest played important roles for impacting grass quality, which can change the nutritive value of maize silages. He pointed that, maize silages ensiled stages would affect the dry matter (DM) intake, starch content and starch/neutral detergent fibre (NDF) ratio, and resulted in different milk yield and milk protein content. In this paper, two maize inbreds of JY818 and QQ446 all showed significant higher content of protein, starch, soluble sugar, ADF, and IVOMD, with relevant average value of 8.54, 11.18, 4.09, 35.14, and 35.23, respectively (Table 1). These results proved that maize always showed better quality for feeding animals than that of hybrid giant napier. It was common with previous reports [4]. At the same time, analysis in this paper also provide much more scientific proofs for proving that, maize can be used as ensiling forage grass for feeding animals, because which containing much more high quality of feeding related traits. Which may explain why maize has been widely used as the major forage component in the ration of dairy cows over the last few decades. In future animal husbandry development, ensiled maize would be important, which can provide natural mixed concentrated feed and roughage, resulting to the cost of feeding.

*Elite and Suitable for Silage Maize Varieties are Needed for Animal Husbandry Develop*

Better grass quality was the precondition of modern animal husbandry develop [10]. Maize varieties showed strongest variation for silage related traits[6]. Phipps proved that the changes in composition of maize silage with increasing maturity, which are associated with increased starch and reduced NDF content, resulted in

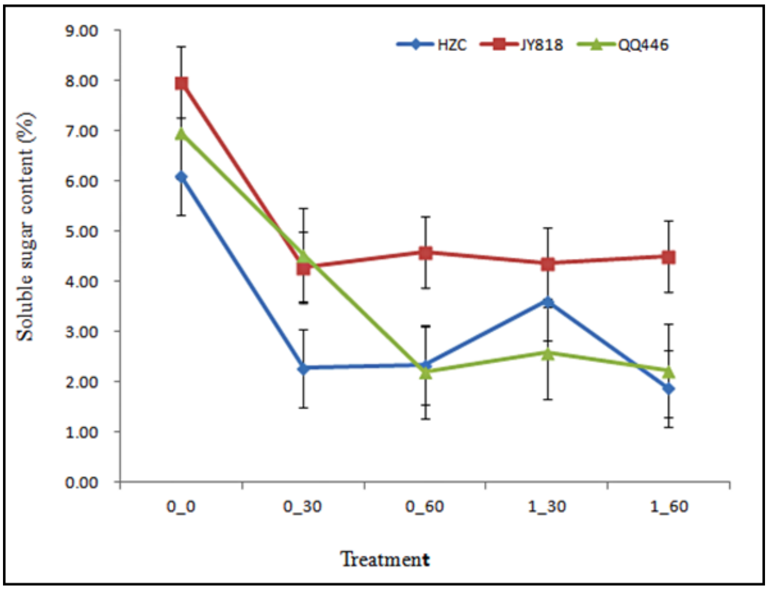


Figure 4. The variation of soluble sugar content

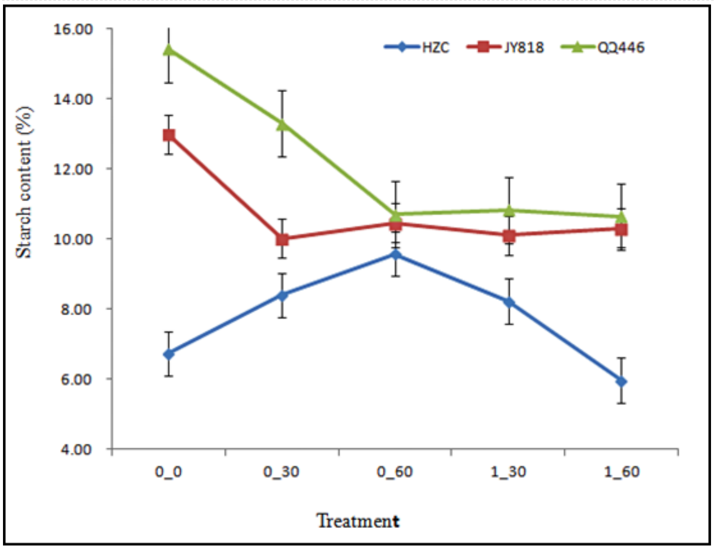


Figure 5. The plots of starch content

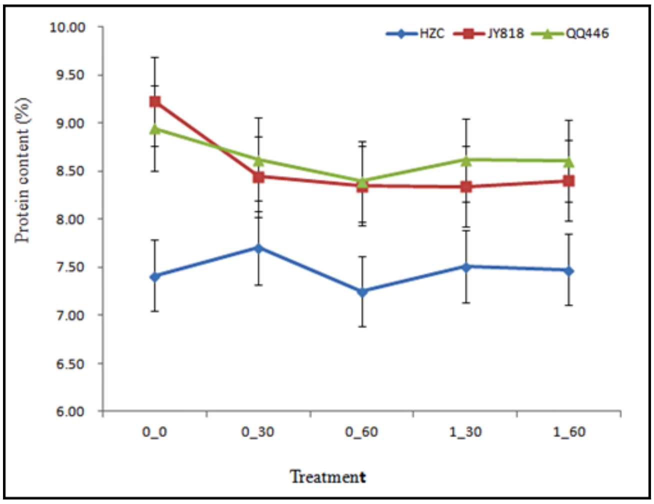


Figure 6. The plots of protein content

large increases in food intake and yield of milk and protein as crops matured from T23 to T33. However, when crop maturity increased further to T38 there was a tendency for DM intake and yield of milk and protein to decline. And these changing are variation among different varieties [8]. Our results showed that, maize silage related traits were varied between two maize hybrids of JY818 and QQ446. Wherein JY818 showed higher biological yield than that of QQ446, according our previous breeding experiment. In this paper, QQ446 showed higher content of protein, starch, and IVOMD, with relevant value of 8.60%, 11.82%, and 35.50%, respectively. But JY818 showed lower content of protein, starch, and IVOMD, with relevant value of 8.48%, 10.53%, and 34.95%, respectively (Table 1). These results were consistent with our breeding practice knowledge. JY818 was crossed by using T32 ´ QB506, it showed higher kernel yield and biomass. QQ446 was crossed by using QB1545 ´ QB446, it was silage specific maize hybrid, and were authorized by the variety certification committee of Guizhou province, which showed better formation of silage related traits, and were widely used in modern agricultural production. But the biomass of QQ446 were lower than that of JY818 according our previous planting experiment. But silage related physiology analysis of this paper showed that, QQ446 were significant better than that of JY818.

## Conclusions

In this paper, one hybrid giant napier and two silage maize were treated with or without additive, then they were ensiled for 0 day, 30 days, and 60 days, respectively. After that, six silage related traits were evaluated for each treatment. Results showed that, maize showed much more better quality than that of hybrid giant napier in silage related traits. Which proved that maize would be the elite silage grass for animal husbandry develop. In addition, stronger variations of silage related traits were also clarified between silage maize varieties.QQ446 showed much more advantages than that of JY818, which could be thought as one major silage variety in future animal husbandry development and agricultural production.


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