1 **Ensiling Characteristics, structural and nonstructural carbohydrate composition and Enzymatic**
2 **Digestibility of Napier grass Ensiled with Additives**

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6 Seare T. Desta¹, XianJun Yuan¹ Li Junfeng¹, Tao Shao^{1*}

7 ¹Institute of Ensiling and Processing of Grass, Nanjing Agricultural University, Nanjing, 210095,
8 China.

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12 *Corresponding author: e-mail: shaotaola@163.com Institute of Ensiling and Processing of
13 Grass, College of Animal Sciences and Technology, Nanjing Agricultural University, Weigang 1,
14 Nanjing, 210095, P. R. China

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18 Abbreviations:

19 AA, acetic acid; ADF, acid detergent fiber; ADL, acid detergent fiber; AN, ammonia nitrogen;
20 BA, butyric acid; BC, buffering capacity; DM, dry matter; FW, fresh weight; LA, Lactic acid;
21 LAB, lactic acid bacteria; NDF, neutral detergent fiber; PA, propionic acid; WSC, water soluble
22 carbohydrate.

ABSTRACT

Ensiling Characteristics, structural and nonstructural carbohydrate composition and enzymatic digestibility (ED) of Napier grass silage was examined. Napier grass ensiled with no additive control, 0.2% formic acid, 0.4% molasses, and 0.3% fibrolytic enzyme for, 7, 30, 60 and 90 days. Additives increased lactic acid, soluble carbohydrate and decreased all of lignocellulosic contents except acid detergent lignin and pH than control. The highest value of nonstructural carbohydrate and large reduction in lignocellulosic contents was observed in formic acid and fibrolytic enzyme silage respectively. The content of glucose and fructose showed rapid drop in the first 7 days of ensilage. Ensilage decreased lignocellulosic contents and increased ED compared to fresh material. The ED of formic acid and molasses silage was significantly higher than control and fibrolytic enzyme silages in all tested days. In summery the ensiling quality structural and nonstructural carbohydrate and ED value of mature Napier grass silage improved through additives.

Key words

Enzymatic digestibility; Napier grass; additives; nonstructural carbohydrate; structural carbohydrate.

1. Introduction

Napier grass (*Pennisetum purpureum.*), a monocot grass belongs to *Poaceae* family and *Pennisetum* genus, is widely distributed throughout tropical and subtropical regions. Its adaptation to grow in a wide range of soil types, fertility levels, weather condition and its high biomass production about 45 t /ha dry matter (DM) (Woodard & Sollenberger, 2008) make the grass an important crop for **biofuel** and animal **feed** production. To maximize the economic benefit from both livestock production (Gandra et al., 2016) as well as **biofuels** and bio-products(Liu et al., 2016), a continuous year round supply of biomass is very crucial. However, biomass harvest is seasonal with high accumulation of crop over short rain season, which call for proper conservation of the available resources.

Ensiling is a traditional conservation method of fresh forages and grains, which aimed at supplying year-round availability of nutritious and palatable feed for livestock. Furthermore, ensiling may function as a beneficial pretreatment for lignocellulosic materials before further processing to bio energy and **bioproduct** production(Liu et al., 2016) . However, it was reported that most tropical and subtropical grasses are very difficult to be ensiled due to their low **water soluble carbohydrate** (WSC) and high lignocellulosic content (McDonald et al., 1991). Furthermore, Phitsuwan et al. (2015) also reported that enzymatic saccharification of Napier grass is more difficult given to its natural structural complexity and heterogeneity of cellulose, hemicelluloses and lignin.

Various chemicals and biological additives have been developed to improve silage fermentation and reduce the structural complexity of the cell wall components(**Hu & Ragauskas, 2012**). Among others fermentation stimulant like fibrolytic enzymes and molasses and fermentation inhibitors like formic acid have been widely practiced as a method of preserving

and improving nutrients in silage. The application of fibrolytic enzymes during ensilage has been proposed as a means of directly enhancing fiber degradation to release fermentable substrate for lactic acid bacteria (LAB) (Dehghani et al., 2012) and improve the fermentation quality by reducing the pH. And also the addition of molasses (Guo et al., 2014) and formic acid (Ding et al., 2013) improve the fermentation quality of silage by promoting and inhibiting Lactic acid (LA) production respectively.

Even though, a number of studies have been carried out on those additives much emphases have been placed on assessing the fermentation quality and apparently, little information is available on structural and nonstructural carbohydrate contents. Furthermore, their focus were in assessing the final fermentation and chemical composition of silage; however, change in chemical composition during entire ensilage may be more important in understanding the entire fermentation process. Though it was reported that ensilage as an effective strategy for lignocellulosic biomass preservation, as well as a beneficial platform for downstream pretreatment, but to the best of our knowledge, there is shortage of reports on the effect of additive treated Napier grass silage on enzymatic saccharification. It is hypothesis that fibrolytic enzyme, molasses and formic acid affect differently the carbohydrate content and enzymatic saccharification of Napier grass silage.

Then this study was conducted to examine the effect of those additives on dynamic structural and nonstructural carbohydrate contents of Napier grass silage and its effect on enzymatic digestibility (ED).

2. Materials and methods

2.1. Forage harvesting and Silage preparation

Napier grass (*Pennisetum purpureum*) was cultivated and harvested at mature growth stage from experimental field of Nanjing Agricultural University (32°01'19" N, 118°51'08" E, at 25m altitude, Jiangsu, China). The grass was chopped to the theoretical length of 2-3 cm manually and mixed properly. The fresh material contains 281±0.02 g/kg DM, 718±5.03 g/kg DM neutral detergent fiber (NDF), 420±3.3 g/kg DM acid detergent fiber (ADF), 53.3±3.41 g/kg DM acid detergent fiber (ADL), 55.6±2.08 g/kg DM WSC, 22.4±3.51 g/kg DM sucrose, 13.5±1.52 g/kg DM glucose, 11.3±2.51 g/kg DM fructose, 58.7±0.82 g/kg DM crude protein and 276±4.5 mEq/kg DM buffering capacity (BC).

A 680g of Napier grass was treated with (i) no additive control (C), (ii) formic acid (F), (iii) molasses (M), (iv) fibrolytic enzyme (E). All of the additives of each treatment were mixed homogeneous, packed and compress manually into 1L (9.5 cm diameter × 18.7 cm height) laboratory silo and sealed airtight with a screw top. The additives used in this experiment were 0.2% fresh weight (FW) formic acid, 4% FW molasses and 0.3% FW fibrolytic enzyme comprises cellulase and hemicellulase (Oddfoni Biological Technology Co., Ltd., Nanjing, China) respectively. A total of 48 (4 ensiling days × 4 treatments × 3 replicate) laboratory silos were made for each treatment and kept at 25°C in incubator. Three silos from each treatment were opened after 7, 30, 60 and 90 d of ensiling, respectively.

2.2. Sampling and Chemical analyses

After the designated ensiling time the content of each silo was removed, mixed thoroughly, and divided into three silage samples. First silage sample, from each silage replicate was macerated in distilled water in the ratio of 1 to 3 for 24h. at 4°C. The extract was filtered using two layers of cheesecloth and filter paper (whatman, pore size of 11µm, Xinhua Co,

China). Immediately the pH was determined on the extract using a glass electrode pH meter (HANNA pH 211, Hanna Instruments, Italy). The extract was frozen at -20°C for subsequent analysis of ammonia nitrogen (AN) and organic acid. Ammonia nitrogen was determined by phenol-hypochlorite procedure (Kleinschmit et al., 2005). The organic acid, including lactic acid (LA), acetic acid (AA), propionic acid (PA) and butyric acid (BA) content of silage was analysed by Agilent high performance liquid chromatography (HPLC) 1260 as described in section 1.3.

The second silage sample and fresh material were used to determine DM, NDF, ADF, ADL, crude protein, BC and WSC. The DM, crude protein, WSC and BC contents were determined as described previously (Yuan et al., 2016). The NDF, ADF and ADL were determined using methods of ANKOM 2000 fiber analyzer based on the company supplied procedure. Hemicellulose and cellulose content were calculated as NDF minus ADF and ADF minus ADL respectively.

The ensilability of Napier grass was assessed as fermentation coefficient (FC) based on the following formula developed by Weissbach and Honig (1996) as described in Yitbarek and Tamir (2014):- $FC = DM (\%) + 8 WSC/BC$, where FC is the fermentation coefficient, DM is the dry matter, WSC is water soluble carbohydrate and BC buffering coefficient. To assess the quality of the silage, we calculated the V-score from the AN/Total N and volatile fatty acid concentrations (Masaki, 2001).

The third samples was freeze dried and used to determine glucose, fructose and sucrose content and enzymatic digestibility of silage and fresh material as described below.

2.3.Determination of sugars and organic acids by HPLC

Silage extracts were thawed and then centrifuged at 13 000g for 30 min at 4 °C. The resulting supernatant was pass through 0.2µm membrane into a glass vial and injected to HPLC 1260 (Agilent Technologies, Inc., Germany) by automatic sampler. To determine the LA, AA, PA, and BA the HPLC was equipped with a Carbomix® H-NP5 column (Sepax Technologies, Inc. USA) and refractive index detector (Agilent Technologies, Inc., Germany). The column was analyzed with the mobile phase and a flow rate of 2.5 mmol/l H₂SO₄ and 0.5 ml /min respectively at temperature 55°C with 30 minute run time. System calibration and integrity was monitored by preparing the standard solutions and injected periodically.

To determine the glucose, fructose and sucrose content of silage and fresh material the freeze dried samples were extracted with 80% (v/v) ethanol following the Zhao and Oosterhuis (1998) procedure with some modification. Briefly, 200mg of ground sample (1mm) was extracted three times with a total of 10ml of 80% (v/v) ethanol by heating in an 80°C water bath for 15minute. After each extraction the mixture was centrifuged at 3000rpm for 10 minutes and the supernatants were combined. The combined supernatants were dissolved with acetonitrile (75%) and passed through 0.2µm membrane (Shao et al., 2003) into a glass vial and injected to an automatic sampler of Agilent HPLC 1260 equipped with column (Skim-pack SCR-101C, Shimadzu, Inc. Japan) and refractive index detector. The analytical column was performed at 85°C using HPLC grade water as mobile phase, with a flow rate of 1ml/min, and run time of 14 minute. Similarly, the system calibration and integrity was monitored as described above.

2.4.Enzymatic hydrolysis

Enzymatic hydrolysis of fresh and ensiled Napier grass of 30, 60 and 90 days was conducted according to a Laboratory Analytical Procedure (LAP) reported by the National

Renewable Energy Laboratory (NREL) (Selig et al., 2008). Samples were supplemented with 0.2% of cellulase of 100FPU/g (Oddfoni Biological Technology Co., Ltd., Nanjing, China) and 0.2% sodium azide to prevent microbial contamination. The hydrolysis reaction was performed at pH 4.8 using 50mM sodium citrate buffer and incubated at 50 °C for 72 h at 180 rpm. Prior to glucose analysis the saccharification process was terminated by heating the aliquot sample at 100°C for 10min. and the glucose released by enzymatic activities was determined by the HPLC described above (section 2.3) after centrifuged at 10000xg for 10 and filtered through 0.2µm membrane.

2.5. Statistic analyses

The collected data were analyzed by two-way ANOVA with the fixed effects of additives, ensilage time and additives by ensilage time interaction, using the GLM procedure of the Statistical Analysis System (SAS, 1999). Tukey's HSD test was used to compare the differences between the treatment means and the significance was declared at $P < 0.05$.

3. Results and discussion

3.1. Ensilage characteristics of fresh material

The Napier grass used in this study contains relatively low sugar and crude protein and higher lignocellulosic compounds. The concentration of WSC needed for satisfactory silage fermentation depends on the dry matter concentration and the buffer capacity of the crop (McDonald et al., 1991). According to Herrmann et al. (2015) forages with insufficient fermentable substrate or too low dry matter content have a FC of < 35 , which indicate difficulty in preservation of the biomass through ensilage. In this study, the FC of Napier grass was 30,

according Yitbarek and Tamir (2014) the satisfactory silage of such forages can only be achieved by adding sugars directly (e.g. molasses) or by adding enzymes that release extra sugars from the crop. And also formic acid found to improve the silage quality by restriction fermentation and decrease silage pH (Ding et al., 2013; McDonald et al., 1991). Then formic acid, molasses and fibrolytic enzymes in this study were added to Napier grass with the expectation of improving the fermentation quality and reducing the nutrient loss of Napier grass silage.

3.2. Fermentation characteristics and storage losses of Napier grass silage

The dynamics of LA, AA, PA and BA concentrations of Napier grass silage are presented in Table 1. The additives, storage period and their interaction significantly influenced the LA, PA and BA contents of Napier grass silage ($P < 0.05$). The addition of enzyme and molasses significantly increased ($P < 0.05$) the LA concentration, LA percentage and LA/AA, while decreased ($P < 0.05$) the concentrations of AA, PA, and BA compared to control. This could relate to the increase in soluble substrates (Fig.1) as a result of direct molasses addition or degradation of structural carbohydrates that promotes homolactic fermentation. Soluble substrates are readily used by fermentative microbes in producing lactate and thus preserving the forage by lowering the pH (Oladosu et al., 2016). Similarly, Li et al. (2014) reported that the addition of molasses and enzyme enhances LA fermentation in king grass silage.

The content of LA in F silage was significantly ($P < 0.05$) lower than control for the first 7 days and also this silage exhibit lower ($P < 0.05$) LA, AA, PA, and BA than E and M silages over the entire ensilage time. The lower organic acid production in F silage could be due to the inhibition of fermentation by restriction the growth of silage microbes due to both direct acidification and antimicrobial properties of the additive (Huhtanen et al., 2013). A summarized

report through meta-analysis by [Goeser et al. \(2015\)](#) outlined that formic acid could inhibit growth of bacteria through acidification. Similarly, [Ding et al. \(2013\)](#) reported that the addition of formic acid decreased lactic and acetic acid concentrations in alfalfa silage.

With ensiling progress the concentration of LA in enzyme and molasses added silages show a dynamics increased to a plateau at day 30 and 60 respectively, the value in C and F silages significantly, continuously decreased and increased respectively. [The increase in LA in E and M silage until day 30 and 60 could be due to the availability and metabolization of soluble substrates by the epiphytic lactic acid bacteria while the continuous increase of LA in F silage might be related to regrowth of silage microorganisms with reduction in antimicrobial effect of the formic acid. Davies et al. \(1998\) confirm that acidified herbage with formic acid showed lower LAB numbers at day 1 and 4 of ensiling, then latter 90 days of ensilage.](#)

The content of DM and AN, DM lost, and pH of experimental silage are presented in [Table 2. Additives, storage period and their interaction significantly affects DM, DM lost, pH and AN contents of Napier grass silage \(\$P < 0.05\$ \). Additives increased \(\$P < 0.05\$ \) DM and decreased \(\$P < 0.05\$ \) pH, AN and DM loss as compared to control. Silage treated with molasses and formic acid showed significantly higher DM content after 7 days of ensilage, while lower DM loss and AN content in all ensilage time compared to E and C silages. The higher DM content recorded in M silages could be resulted from the higher DM content of molasses used, which is consistent with the results of \[Yuan et al. \\(2016\\)\]\(#\). The significant low DM loss reported in F and M silage could be due to inhibition of undesirable silage microbes metabolize of silage nutrient by formic acid and LA production respectively. \[Storage period gradually decreased \\(\\$P < 0.05\\$ \\) DM content of all silages, with large reduction recorded in C silage followed by E silages. This reduction could be due to microbial breakdown of silage nutrients into liquid and gases\]\(#\)](#)

(Kim et al., 2016). According to McDonald et al. (1991), heterofermentative fermentation resulted in considerable DM losses through carbon dioxide production. In this study the gradual improvement in volatile fatty acid throughout storage period (Table 1) could contribute to the reduction DM content of silages.

In all ensilage time the pH value in F silage was lower ($P < 0.05$) than E and C silages while the pH in M silage showed comparable value for the first 7 days then significantly increased to a higher level than F silage. Rapid pH decline is important in reducing the risk of early undesirable microorganism growth and nutrient loss (Ellis et al., 2016). In this study, the addition of formic acid and molasses resulted in sharp and significant drop to less than pH 4 on day 7 of ensilage however; the mechanism of action was different for both additives. Formic acid, which was attributed as carboxylic acid with 3.77 pKa could rapidly reduce the pH by direct acidifying the silage medium. Similarly, rapid drop of pH was reported by Özyurt et al. (2016) on klunzinger's ponyfish silages treated with formic acid. On the other side, the reduction in pH of molasses treated silage was resulted from enhanced LA fermentation. In this experiment the pH value recorded in E silage was higher than M and F silage. The slow response of E silage to pH might be due to lack of sufficient readily WSC availability for LAB growth as a result of the slow WSC release by the enzyme action (Fig. 1). Additives improved the fermentation quality of Napier grass silage which is indicated by higher value of V-score compared to control silage.

3.3. Nonstructural carbohydrates components of Napier grass silage

The effect of the additives on nonstructural carbohydrates, (including sucrose, glucose, fructose and WSC) compositions of Napier grass silage is given in Fig. 1. Treatments, storage

period and their interaction significantly ($P < 0.05$) affect the content of WSC, sucrose, fructose and glucose of Napier grass silage. Additives conserve significantly higher ($P < 0.05$) concentration of nonstructural carbohydrates than control over the whole ensilage.

The addition of formic acid and molasses significantly increased the concentration of all measured nonstructural carbohydrates except glucose in E silage. The higher value recorded in both F and M silages could be justified by the release of WSC and sugars by acid hydrolysis of structural carbohydrates and/ or sparing of fermentable substrates by inhibiting silage microbial growth by reducing the pH (Ding et al., 2013; Huhtanen et al., 2013). In this experiment the lowest pH was recorded in both M and F silages. The higher glucose content recorded in E than C and M ($P < 0.05$) and FA ($P > 0.05$) silages could be due to the release of glucose from lignocellulosic degradation, which were consistence with the highest reduction in NDF and ADF content in E silage. Similarly, Dehghani et al. (2012) reported that the combined addition of enzymes was most effective in increasing glucose and decreasing NDF concentration in maize stover silages. The addition of formic acid significantly increased the concentration of glucose and fructose than other silages ($P < 0.05$), which attributed to the acid hydrolysis of the biomass (Pakarinen et al., 2011). The highest sucrose recorded in M silage was consistent to the addition of molasses, which contained higher sucrose concentration.

The contents of WSC and other sugars tend to decreased with storage period ($P < 0.05$) and the reduction was very slow for additives than control. The content of glucose and fructose in all silage except E silage showed a rapid drop in the first 7 days of ensilage, while the decline in sucrose were gradually continuing till the end of the experiment. The rapid reduction in glucose and fructose than sucrose during the first 7 days of ensiling, confirmed the fact that glucose and fructose might be more favorably utilized by silage microbes than sucrose in the

initial ensilage process. Similarly, Shao et al. (2003) reported that the rate measured structural carbohydrates reduction in the initial 5 days in guinea grass silage was ranked in the order of glucose, fructose and sucrose.

3.4. Structural carbohydrates compositions of Napier grass silage

Structural carbohydrate compositions of Napier grass silages are presented in Table 3. The effects of additives and storage period were significant ($P < 0.05$) for the most of measured structural carbohydrate components of Napier grass silage. All of the treatments were effective in hydrolyzing the NDF, ADF, hemicelluloses and cellulose contents of silage compared to control, and the reduction in NDF, ADF and cellulose was higher in E silage than F, M and C silages with significant difference on day 30 and 90. Similarly, molasses addition resulted in reduction of measured lignocellulosic concentration except ADL compared to C ($P < 0.05$) and F ($P > 0.05$) silages for ADF and cellulose contents. The mechanism of structural carbohydrate degradation was different for various additives (Guo et al., 2014). The reduction in fiber fraction in M and F silage might be due to acid solubilization of the structures carbohydrates during the ensilage process (McDonald et al., 1991). The organic acids produced during ensilage and the direct acid addition could contribute to the hydrolysis of structural carbohydrate respectively. In contrast Nadeau et al. (2000) reported that no effects of formic acid addition to alfalfa silage on cell wall concentration. While the addition of fibrolytic enzyme due to their cellulase and xylanase properties they can directly hydrolysis the lignocellulosic content. Many studies reported that the cell wall content of ensiled forages were effectively hydrolyses by fibrolytic enzyme addition, upon ensilage (Dehghani et al., 2012).

The reduction in structural carbohydrates in this study was lower than other reports which

might be attributed to the over matured grass used in this experiment. Improvements in silage fermentation and fiber degradation relatively decreased with advancing crop maturity (Bakken et al., 2011), hydrolysis of the cell wall would be more difficult due to increased lignifications. Nadeau et al. (2000) reported that degradation of NDF and cellulose by cellulase enzyme (20 ml/kg) in alfalfa silage decreased by 43 and 39%, respectively, between first and second harvests, and they explained the difference to the simultaneous increase in lignin concentration by 26%.

Silage contents of all structural carbohydrates measured were lower than fresh material and with extending the storage period the value gradually decreased except for ADL content ($P < 0.05$). **Ensilage of mature Napier grass with additives in this study in general resulted in improving the nutritional characteristics of Napier grass silage to livestock, mainly due to reduction in NDF and increase in WSC reserved compared to control silage. According to Van Soest (1994) feed containing above 70% of NDF limits feed intake by the physical effect of filling the rumen and increasing time spent on chewing and rumination.** In the present study, after 7 days of ensilage, the NDF contents of all additive silages remained below the value reported by Van Soest (1994) as limiting for forage ingestion. In addition the relative higher WSC reserve in treated silage than control observed in this study could improve the energy value and utilization efficiency of the feed for milk production (Miller et al., 2001).

Lignin has been identified as primarily responsible for limiting digestibility and used as marker in digestibility study of animal research (Kanani et al., 2014). Thus, in this study, we use ADL as standard, which cannot be degraded during the ensiling (Zhang et al., 1997). Therefore, ratios of NDF/ ADL, ADF/ADL, hemicelluloses/ADL and cellulose/ADL (Fig. 2) could indicate changes of various structural carbohydrates (Guo et al., 2014). Additives decreased the average

ratio of measured structural carbohydrates to ADL compared to control and the difference was significant ($P < 0.05$) for E silage in all ensilage days and for M silage after 30 days of ensilage. Those ratios could indirectly confirm higher degradation of cell wall contents in silages treated with additives than control.

3.5. Enzymatic digestibility of fresh and ensiled Napier grass

Direct enzymatic hydrolysis of lignocellulosic materials can provide an indication of the biomass suitability for use in biofuel and bioethanol production. In this study ensilage of mature Napier grass showed improvement in ED than fresh material in all examined storage days (Fig. 3). This could be due to alteration of cell wall structural matrix resulted from the reduction in structural carbohydrates, which increase access for enzyme degradation. Nsereko et al. (2000) found the improvement in *in vitro* degradability of aNDF and they ascribed these changes to alteration of the cell wall structural matrix that increases access for rumen microbial and enzymatic activity. Similarly, Liu et al. (2016) also found that ensilage can effectively improve enzymatic digestibility of gaint reed after 90 days of ensilage than fresh material.

The addition of formic acid and molasses significantly increased the ED of Napier grass silage compared to C and E silages in all examined storage days. The highest ED reported in F and M silage could be related to structural modification of lignocellulosic resulted from an extensive reduction in fiber content during ensilage (Table 3). According to Hendriks and Zeeman (2009) removal of hemicelluloses increases the mean pore size and the specific surface area of cellulose resulted in an increase in enzyme exposure and digestibility. Pakarinen et al. (2011) reported that the addition of formic acid increased the enzymatic hydrolysis of maize and hemp silages.

In contradiction to the highest reduction in lignocellulosic content in E silage the ED was

significantly low compared to M and F silage and comparable to C silage. Even though, the reason for the reduction in ED is not clearly known, which call for further research. The present result could be explained as follow:- the extensive reduction in structural carbohydrate form cellulose aggregates during ensilage, which increase fiber strength and resistant to enzymatic degradation (Phitsuwan et al., 2016). In addition, ensilage hydrolyze the most digestible fraction of the structural polysaccharides (Dehghani et al., 2012) and the less digestible parts left contribute to the recalcitrant of enzyme action during enzymatic sacharification.

The improvement noticed in the ED of Napier grass silage compared to fresh material and further improvement achieved by additive (molasses and formic acids), could ensure that ensilage of Napier grass using molasses and formic acids could be a good strategy to sustainable develop bioenergy because it resulted in production of substantial amount measured nonstructural carbohydrate.

4. Conclusion

Additives improved silage quality by stimulating (molasses and enzyme) and inhibiting (formic acid) fermentation. All additives increase the sugar and decreased lignocellulosic content of Napier grass silage compared to control. Higher reduction in lignocellulosic and reserve in sugar was recorded in fibrolytic enzyme and formic acid silage respectively. Additives improved enzymatic digestibility of Napier grass silage with the highest value recorded in formic acid and molasses silage. In summery ensilage of Napier grass with molasses, formic acid and enzyme improved the nutrient available for both livestock and biofuel production by reducing conservation loss, lignocellulosic fractions and improving enzymatic digestibility.

Acknowledgements

This work was supported by Independent innovation of agricultural sciences in Jiangsu province (CX(15)1003), the Fundamental Research Funds for the Central Universities (KJQN201555), Natural Science Foundation of Jiangsu Province of China (BK20140717) and China National Science Foundation (31402135).

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Fig.1. Effect of additives on (a) Water soluble carbohydrates, (b) Sucrose, (c) Glucose and (d) Fructose of Napier grass silage. Means followed by different letters shows significant difference among treatments with the same ensiling days at $p < 0.05$.

Fig.2. Effect of additives on (a) Neutral detergent fiber / Acid detergent lignin, (b) Acid detergent fiber / Acid detergent lignin, (c) hemicelluloses/ Acid detergent lignin and (d) Cellulose/ Acid detergent lignin. In each figure, means followed by different letters shows significant difference among treatments with the same ensiling days at $p < 0.05$.

Fig. 3. Enzymatic digestibility of fresh and ensiled Napier grass for 30, 60 and 90 days.

In each figure, means followed by different small letters among treatments with the same ensiling and values followed by different capital letters among ensiling days with the same treatment show significant differences at $p < 0.05$.

Table 1.

Effect of additives and storage period on organic acid composition of Napier grass silages.

Items ¹	Treatment ²	Storage days				Mean	SEM ³	Significance ⁴		
		7	30	60	90			T	S	T x S
Lactic acid (g/kg DM)	C	9.56 ^{cA}	7.56 ^{cA}	3.76 ^{cB}	2.51 ^{cB}	5.4 ^d	2.67	*	*	*
	F	8.05 ^{dC}	16.7 ^{bB}	21.7 ^{bAB}	26.0 ^{bA}	18.1 ^c				
	M	36.4 ^{aB}	50.4 ^{aA}	52.1 ^{aA}	51.1 ^{aA}	47.5 ^a				
	E	31.2 ^{bC}	48.4 ^{aA}	47.1 ^{aA}	44.1 ^{aB}	42.5 ^a				
Acetic acid (g/kg DM)	C	7.06 ^{aD}	14.3 ^{aC}	18.8 ^{aB}	25.9 ^{aA}	16.5 ^a	0.95	*	*	*
	F	2.68 ^c	3.31 ^c	3.79 ^d	4.62 ^d	3.59 ^d				
	M	5.37 ^{bD}	8.77 ^{bC}	11.0 ^{cB}	14.4 ^{cA}	9.88 ^c				
	E	6.48 ^{aD}	12.4 ^{aC}	14.2 ^{bB}	19.3 ^{bA}	13.0 ^b				
Propionic acid (g/kg DM)	C	1.88 ^a	4.01 ^a	4.24 ^a	4.86 ^a	3.74 ^a	0.2	*	*	*
	F	0.17 ^{bC}	0.56 ^{dB}	0.81 ^{cAB}	0.93 ^{cA}	0.61 ^d				
	M	0.58 ^{bC}	1.02 ^{cB}	1.40 ^{bcAB}	1.74 ^{bcA}	1.18 ^c				
	E	0.84 ^{bC}	1.47 ^{bB}	2.21 ^{bA}	2.53 ^{bA}	1.76 ^b				
Butyric acid (g/kg DM)	C	1.98 ^{aC}	6.44 ^{aB}	8.51 ^{aB}	12.0 ^{aA}	7.24 ^a	0.46	*	*	*
	F	0.55 ^{bB}	0.76 ^{cAB}	0.99 ^{bAB}	1.21 ^{bA}	0.87 ^d				
	M	0.96 ^{bC}	1.54 ^{bcBC}	2.10 ^{bAB}	2.67 ^{bA}	1.81 ^c				
	E	1.61 ^a	2.40 ^b	3.20 ^b	3.92 ^b	2.78 ^b				
Lactic acid / Acetic acid	C	1.35 ^{dA}	0.54 ^{cB}	0.20 ^{cC}	0.10 ^{bC}	0.54 ^c	0.33	*	*	*
	F	3.02 ^c	5.05 ^a	5.97 ^a	6.37 ^a	5.10 ^a				
	M	6.78 ^{aA}	5.78 ^{aAB}	4.75 ^{abB}	3.55 ^{abC}	5.21 ^a				
	E	4.82 ^{bA}	3.92 ^{bB}	3.34 ^{bB}	2.29 ^{abC}	3.59 ^b				
% Lactic acid	C	46.5 ^{dA}	23.5 ^{cB}	10.6 ^{cC}	5.55 ^{cC}	21.5 ^c	3.69	*	*	*
	F	70.3 ^{cB}	78.3 ^{abA}	79.6 ^{aA}	79.1 ^{aA}	76.8 ^a				
	M	84.0 ^{aA}	81.7 ^{aBC}	78.1 ^{aB}	73.0 ^{aC}	79.2 ^a				
	E	77.7 ^{bA}	74.8 ^{bA}	70.6 ^{bB}	63.2 ^{bC}	71.6 ^b				
V score	C	78.8 ^{cA}	49.6 ^{dB}	46.9 ^{cB}	43.8 ^{cC}	63.1 ^d	2.41	*	*	*
	F	94.9 ^{aA}	92.4 ^{aAB}	90.1 ^{aB}	87.5 ^{aC}	92.7 ^a				
	M	89.2 ^{bA}	81.7 ^{bB}	75.1 ^{bC}	68.6 ^{bD}	82.4 ^b				
	E	82.9 ^{cA}	71.6 ^{cAB}	64.3 ^{bb}	58.3 ^{bb}	74.5 ^c				

^{a-d} Means in the same column followed by different letters differ ($P < 0.05$); ^{A-D} Means in the same row followed by different letters differ ($P < 0.05$).

¹ DM, dry matter.

² C, no additive control; F, formic acid; M, molasses; E, fibrolytic enzyme.

³ SEM, standard error of means.

⁴ T, treatment; S, storage day; T x S, interaction between treatment and storage day; *, significant at $P < 0.05$.

Table 2.

Effect of additives and storage days on dry matter, dry matter loss, ammonia nitrogen and pH content of Napier grass silages.

Items ¹	Treatment ²	Storage days				Mean	SEM ³	Significance ⁴		
		7	30	60	90			T	S	T x S
Dry matter (g/kg FM)	C	268 ^{hA}	255 ^{cB}	249 ^{cBC}	245 ^{cC}	254 ^c	1.52	*	*	*
	F	279 ^{aA}	270 ^{aB}	268 ^{aC}	266 ^{aC}	270 ^a				
	M	281 ^{aA}	273 ^{aAB}	271 ^{aAB}	270 ^{aB}	273 ^a				
	E	276 ^{abA}	262 ^{bB}	258 ^{bBC}	254 ^{bC}	262 ^b				
Dry matter loss (g/kg DM)	C	36.4 ^{cC}	62.2 ^{aB}	73.7 ^{aA}	79.0 ^{aA}	62.8 ^a	2.63	*	*	*
	F	10.5 ^{dD}	24.1 ^{cC}	30.1 ^{dB}	35.0 ^{dA}	24.9 ^d				
	M	19.5 ^{cD}	32.0 ^{bC}	38.6 ^{cB}	44.4 ^{cA}	33.6 ^c				
	E	29.5 ^{bD}	37.1 ^{bC}	45.3 ^{bB}	50.3 ^{bA}	40.5 ^b				
pH	C	5.13 ^a	5.16 ^a	5.15 ^a	5.11 ^a	5.13 ^a	0.06	*	NS	NS
	F	3.93 ^{bB}	4.00 ^{cAB}	4.07 ^{cA}	4.03 ^{cAB}	4.00 ^d				
	M	3.99 ^{bB}	4.19 ^{bA}	4.26 ^{bA}	4.25 ^{bA}	4.17 ^c				
	E	4.57 ^{abA}	4.27 ^{bB}	4.35 ^{bB}	4.32 ^{bB}	4.37 ^b				
Ammonia nitrogen (g/kg total nitrogen)	C	28.6 ^{dD}	49.6 ^{aC}	65.2 ^{aB}	80.8 ^{aA}	56.0 ^a	2.85	*	*	*
	F	5.37 ^{bB}	18.6 ^{cA}	19.2 ^{cA}	24.9 ^{cA}	17.0 ^d				
	M	19.8 ^{aB}	30.2 ^{bcAB}	33.7 ^{bcAB}	38.8 ^{cA}	30.6 ^c				
	E	24.8 ^{aD}	40.4 ^{abBC}	47.6 ^{bAB}	57.9 ^{bA}	42.6 ^b				

^{a-d} Means in the same column followed by different letters differ ($P < 0.05$); ^{A-D} Means in the same row followed by different letters differ ($P < 0.05$).

¹FM, fresh material; DM, dry matter

²C, no additive control; F, formic acid; M, molasses; E, fibrolytic enzyme.

³SEM, standard error of means.

⁴T, treatment; S, storage day; T x S, interaction between treatment and storage day; *, significant at $P < 0.05$; NS, not significant at $P < 0.05$.

Table 3.

Effect of additive and storage days on lignocelluloses composition of Napier grass silage.

Items ¹	Treatment ²	Storage days				Mean	SEM ³	Significance ⁴		
		7	30	60	90			T	S	TXS
Neutral detergent fiber (g/kg DM)	C	718 ^{aA}	712 ^{aB}	706 ^{aBC}	702 ^{aC}	710 ^a	3.53	*	*	NS
	F	688 ^{bA}	672 ^{bB}	668 ^{bB}	663 ^{bB}	673 ^b				
	M	687 ^{bA}	667 ^{bB}	660 ^{bBC}	657 ^{bC}	668 ^b				
	E	670 ^{bA}	649 ^{cB}	642 ^{cB}	639 ^{cB}	650 ^c				
Acid detergent fiber (g/kg DM)	C	417 ^{aA}	410 ^{aB}	415 ^{aB}	412 ^{aB}	414 ^a	1.93	*	*	*
	F	407 ^{bA}	395 ^{bBC}	389 ^{bC}	386 ^{bC}	394 ^b				
	M	403 ^{bCB}	395 ^{bCD}	389 ^{bCD}	386 ^{bD}	393 ^b				
	E	390 ^{cA}	382 ^{cAB}	379 ^{cB}	376 ^{cB}	382 ^c				
Acid detergent fiber (g/kg DM)	C	41.2	41.8	41.7	42.1	41.7 ^b	0.54	*	NS	NS
	F	41.2	44.3	42.2	41.6	42.3 ^b				
	M	41.2	46.9	46.8	46.5	45.4 ^a				
	E	46.3	46.7	46.6	46.5	46.5 ^a				
Hemicelluloses (g/kg DM)	C	299 ^{aA}	301 ^{aA}	290 ^{aB}	290 ^{aB}	295 ^a	1.81	*	*	NS
	F	281 ^a	276 ^b	279 ^{ab}	277 ^{ab}	279 ^b				
	M	283 ^a	273 ^b	270 ^{bc}	271 ^b	275 ^{bc}				
	E	280 ^{aA}	266 ^{bB}	263 ^{cB}	262 ^{bB}	268 ^c				
Cellulose (g/kg DM)	C	377 ^{aA}	369 ^{aB}	374 ^{aAB}	370 ^{aB}	372 ^a	2.25	*	*	NS
	F	366 ^{cabA}	351 ^{aB}	347 ^{aB}	344 ^{aB}	352 ^b				
	M	362 ^{bA}	347 ^{bB}	343 ^{bB}	339 ^{bB}	348 ^b				
	E	344 ^b	336 ^c	332 ^b	330 ^c	336 ^c				

^{a-d} Means in the same column followed by different letters differ ($P < 0.05$); ^{A-D} Means in the same row followed by different letters differ ($P < 0.05$).

¹ DM, dry matter.

² C, no additive control; F, formic acid; M, molasses; E, fibrolytic enzyme.

³ SEM, standard error of means.

⁴ T, treatment; S, storage day; T x S, interaction between treatment and storage day; *, significant at $P < 0.05$; NS, not significant at $P < 0.05$.