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The effect of fibrolytic enzyme, *Lactobacillus plantarum* and two food antioxidants on the fermentation quality, alpha-tocopherol and beta-carotene of high moisture napier grass silage ensiled at different temperatures

Q.H. Liu[a], T. Shao[a,*], Y.F. Bai[b]

[a] *Institute of Ensiling and Processing of Grass, Nanjing Agricultural University, Weigang 1, Nanjing, 210095, China*
[b] *Jiangsu Academy of Agricultural Science, Zhong Linjie 50, Nanjing, 210014, China*

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ABSTRACT

Alpha-tocopherol and beta-carotene of forage are antioxidants and play important roles in the immune system of ruminants. Fermentation quality, alpha-tocopherol and beta-carotene of high moisture napier grass (*Pennisetum purpureum* cv. Mott) ensiled without or with additives (fibrolytic enzyme, EN; *Lactobacillus plantarum*, LP; tert-butylhydroquinone, TBHQ; tea polyphenols, TP) at 15 °C, 30 °C and 45 °C were examined in the present study. After ensiling for 70 days, untreated silages had lower ($P < 0.05$) pH, butyric acid and ammonia-N content at 15 °C and 45 °C than at 30 °C. Compared with untreated silage, EN and LP lowered ($P < 0.05$) pH and increased ($P < 0.05$) lactic acid content at 15 °C, 30 °C and 45 °C; TBHQ lowered ammonia-N content ($P < 0.05$) at 15 °C, lowered ($P < 0.05$) pH and butyric acid content at 30 °C, and lowered ($P < 0.05$) acetic acid and ammonia-N content at 45 °C; TP lowered ($P < 0.05$) pH at 15 °C and lowered ($P < 0.05$) lactic acid content at 30 °C. Especially at 30 °C, TP had the highest ($P < 0.05$) acetic acid and butyric acid content among all silages. Napier grass ensiled at 30 °C had higher ($P < 0.05$) alpha-tocopherol content than before ensiling. Napier grass ensiled at 30 °C and 45 °C had higher ($P < 0.05$) alpha-tocopherol content than ensiled at 15 °C. EN and TP had lower ($P < 0.05$) alpha-tocopherol content than untreated silage at 15 °C, 30 °C and 45 °C. Napier grass ensiled at 15 °C, 30 °C and 45 °C had lower ($P < 0.05$) beta-carotene content than it before ensiling. Napier grass ensiled at 30 °C had higher ($P < 0.05$) beta-carotene content than ensiled at 15 °C and 45 °C. Additives-treated silages had lower ($P < 0.05$) beta-carotekne contents than untreated silage at 15 °C, 30 °C and 45 °C, except for TBHQ at 45 °C ($P < 0.05$). In conclusion, fermentation qualities of untreated and treated silages were better at 15 °C and 45 °C (V-score > 99) than at 30 °C (V-score < 96). Compared with untreated silage (V-score = 78.5) at 30 °C, EN, LP and TBHQ improved fermentation

Abbreviations: aNDF, neutral detergent fiber assayed with a heat stable amylase and expressed inclusive of residual ash; ADF, acid detergent fiber expressed inclusive of residual ash; CFU, colony forming units; DM, dry matter; EN, fibrolytic enzyme; FM, fresh matter; FW, fresh weight; LAB, lactic acid bacteria; LP, *Lactobacillus plantarum*; mEq, milliequivalent; N, nitrogen; n.d., not detected; SE, standard error; TBHQ, tert butylhydroquinone; TP, tea polyphenols; WSC, water-soluble carbohydrates.

* Corresponding author at: Institute of Ensiling and Processing of Grass, Nanjing Agricultural University, Weigang 1, Nanjing, 210095, China.
E-mail address: taoshaolan@163.com (T. Shao).

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quality (V-score > 83), and TP lowered fermentation quality (V-score = 74.5). Silage ensiled at 30 °C increased alpha-tocopherol content and lost beta-carotene slightly. Additives did not reduce alpha-tocopherol and beta-carotene loss at any temperature, except for TBHQ ensiled at 45 °C. The interactions between additives and preservation temperatures indicated that TP ensiled at 30 °C had the poorest fermentation quality, LP and TBHQ ensiled at 30 °C had lower alpha-tocopherol loss, and TBHQ and TP ensiled at 30 °C had lower beta-carotene loss. TBHQ might be a valuable additive to ensile napier grass at 15 °C, 30 °C and 45 °C, and to reduce beta-carotene loss of napier grass silage at 45 °C.

1. Introduction

Silage is an important way for preserving the nutrient of forage, which is a lactic fermentation process driven by epiphytic lactic acid bacteria (LAB). Once LAB ferments water-soluble carbohydrates (WSC) into sufficient lactic acid in an anaerobic silo, low pH is achieved, the activity of undesired microbes is restrained and the nutrients are well preserved. The activity of epiphytic microbe is responsive to temperature during ensiling (McDonald et al., 1991). Liu et al. (2012) and Zhang et al. (2010) have reported that roughage ensiled at 30 °C showed a weak lactic fermentation which did not inhibit butyric fermentation and protein loss, while lactic fermentation was more dominant with low nutritive losses when silage was ensiled at 20 °C. The alpha-tocopherol and beta-carotene of forage are antioxidants (Granelli et al., 1998; Calderón et al., 2007), and play important roles in the immune system of ruminants (Allison and Laven, 2000). Silage containing high contents of alpha-tocopherol and beta-carotene increases nutritive value of milk (Shingfield et al., 2005). The alpha-tocopherol and beta-carotene are destroyed by oxidation, which is enhanced by heat (Seshan and Sen, 1942). During the initial period of ensiling, air still present between the plant particles and the temperature can increase to 40 °C or higher because of continuing plant respiration and aerobic microbial activity, and silage may be subjected to high temperatures for a long time in summer (Weinberg et al., 1998). Therefore, attention should be paid to the effect of temperature on fermentation quality, nutritive characteristics, alpha-tocopherol and beta-carotene of silage.

Previous study has reported that there was no clear relationship between the silage quality and its carotene content (Kalač, 1983). Müller et al. (2007) have found that there was no clear relation between degree of fermentation and content of alpha-tocopherol or beta-carotene but a positive correlation was found between lactic acid and beta-carotene and lactic acid and alpha-tocopherol. Lindqvist et al. (2012) have found that silage with high dry matter (DM) loss did not have high alpha-tocopherol and beta-carotene loss. To our knowledge, there is little information about the mechanism responsible for the variation of the content of alpha-tocopherol and beta-carotene in silage. Previous studies have reported use of additives, *e.g.* acidic additive, LAB inoculation alone or combined with fibrolytic enzyme (EN), could affect the alpha-tocopherol and beta-carotene content of silage (Nadeau et al., 2004; Shingfield et al., 2005; Lindqvist et al., 2012), and the inconsistent results from the same additive were collected. tert-butylhydroquinone (TBHQ) and tea polyphenols (TP) are food antioxidants. Liu et al. (2015) have found that TBHQ increased the stability of beta-carotene in oil-in-water emulsions. TP protects the alpha-tocopherol against free radicals (Jia et al., 1998). Unten et al. (1997) have found that TP showed dose-dependent antidiscoloring activity of beta-carotene on both the beverage and the margarine. TBHQ and TP have been used in silage for preventing fatty acid oxidation (Han and Zhou, 2013). Napier grass (*Pennisetum purpureum* cv. Mott) is a widely planted forage in tropical and subtropical areas of China and is rich in carotene (Zhou et al., 2007). To our knowledge, there is little information about the effect of TBHQ and TP on preventing alpha-tocopherol and beta-carotene oxidation during ensiling.

The purpose of this study was to investigate the fermentation quality, alpha-tocopherol and beta-carotene of high moisture napier grass ensiled without or with additives (EN; *Lactobacillus plantarum*, LP; TBHQ; TP) at 15 °C, 30 °C and 45 °C for 70 days.

2. Materials and methods

2.1. Silage material and silage making

A LAB strain LP was isolated from corn silage and was identified as *L. plantarum* by analyzing its 16S rDNA sequence (GeneBank accession Number: JN043516). LP was stored at −80 °C and was used after culturing in deMan Rogosa and Sharp (Difco Laboratories, Detroit, MI, USA) broth at 30 °C for 30 h. EN was purchased from a company (Rueyang biotechnology Co., Ltd., Wuxi, China), and was extracted from *Trichoderma reesei*. The activity of EN was measured: cellulase 50,000 U/g. TP and TBHQ were antioxidants. TP was purchased from a company (Rueyang biotechnology Co., Ltd., Wuxi, China). TBHQ was purchased from Sigma-Aldrich (112941, Sigma-Aldrich, Shanghai, China). Napier grass was planted on July 07, 2013 in an experimental field (Humid subtropical climate, Latitude 32°01′59.81″ N, Longitude 118°50′13.63″E, Altitude above sea level 17 m) of Nanjing Agricultural University (Nanjing, China). Napier grass was harvested at the vegetative growth phase on September 27, 2013. The grass was chopped into 1–2 cm-long pieces by a forage chopper (Sh-2000, Shanghai Donxe Industrial Co., Ltd., Shanghai, China).