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Improving the nutritive value, *in vitro* digestibility and aerobic stability of *Hedychium gardnerianum* silage through application of additives at ensiling time

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ABSTRACT

The present study was designed to evaluate the effect of applying lactic acid bacteria (LAB) inoculant, molasses and urea (10^6 cfu/g, 50 ml/kg and 5 g/kg on fresh basis, respectively) on ensiling characteristics of *Hedychium gardnerianum*. Eight treatments of *Hedychium* were investigated; untreated, with molasses, with urea and with molasses plus urea. Each of these was ensiled alone or after the addition of a bacterial inoculant (containing fibrolytic enzymes). The experimental design was a randomized complete block, with a $2 \times 2 \times 2$ factorial arrangement of treatments. Forages were ensiled in laboratory silos for 60 days at room temperature. Chemical composition, microbial populations and *in vitro* digestibility of forages were determined before and after ensiling. Fermentation products and aerobic stability were measured after silos opening. Addition of molasses or molasses plus urea with or without inoculation had the better ($P<0.05$) fermentation results. These silages showed lower enterobacteria, clostridial and yeast counts, the absence of molds and the higher numbers of LAB. Also in these silages, the observed pH and ammonia nitrogen were below 4 and 40 g/kg TN, respectively. The inoculated silages presented the higher lactic acid ($P<0.05$) and the lower acetic, butyric and propionic acid values. The inoculated silages had shorter periods of aerobic stability than the un-inoculated. Silages treated only with urea either inoculated or not showed the worst fermentation properties, the highest numbers of undesired microbes, the lowest digestibility values and the early aerobic instability. While, silage received molasses plus urea with inoculant, revealed the best fermentation characteristics (pH 3.70; lactic acid, 72.4 g/kg DM; ammonia N, 29.4 g/kg TN and absence of butyric acid), the highest crude protein value (183.6 g/kg DM) and the maximum digestibility (56.31% over control). So, for the better utilization of *H. gardnerianum* as an alternative feed for ruminants, the later treatment is recommended.

Abbreviations: AA, acetic acid; ADF, acid detergent fiber; ADL, acid detergent lignin; BA, Butyric acid; cfu, colony forming unit; CP, crude protein; DM, dry matter; EE, ether extract; FM, fresh material; IVDMD, *in vitro* dry matter digestibility; LA, lactic acid; LAB, lactic acid bacteria; NDF, neutral detergent fiber; NH_3-N, ammonium nitrogen; PA, propionic acid; TC, total bacterial count; TN, total nitrogen; VFA, volatile fatty acids; WSC, water soluble carbohydrates.

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1. Introduction

In Portugal as well as in Mediterranean areas, during the dry season, ruminant animals suffer from the malnutrition due to the shortage of locally produced feeds. Therefore, the better utilization of non-conventional feed resources, which do not compete as human foods, is necessary. Hence, to fill this gap, the use of fodder trees and shrub forages can be a good option (Bruno-Soares and Abreu, 2003; Alipour and Rouzbehan, 2007).

Hedychium gardnerianum Sheppard ex Ker-Gawler is a rhizomatous perennial herb of the Zingiberaceae family, it is known as the Kahili Ginger. It has a stalk which can extend up to 2 m long, with oblong leaves reaching 30 cm and several yellow-orange flowers in a spike of 20–30 cm in length. It is an aggressive invasive weed capable of spreading rapidly and dominating large areas in the Azores (Portugal). Moreover, *H. gardnerianum* out-competes many native plants and has become a significant threat to the survival of many of them (Sjögren, 1984; Medeiros et al., 2003). In the Azores, this plant is used in the ruminant feeding, in the ornamental purposes and possibly, in the protection of steep hillsides from erosion (Carvalho et al., 2003). In terms of animal feed, *H. gardnerianum* shows low levels of crude protein and dry matter digestibility (Moselhy et al., 2014).

Ensiling is a preservation method for moist forages as animal feed. It is based on lactic acid bacteria (LAB) converting water soluble carbohydrates (WSC) into organic acids, mainly lactic acid, under anaerobic conditions. As a result, pH decreases and the moist forages are preserved from spoilage microorganisms (Filya et al., 2000). In order to improve the ensiling process, various biological and chemical additives have been developed (McDonald et al., 1991). Inoculating ensiled forages with lactic acid bacteria ensures the presence of enough number of that bacteria to cause a rapid reduction in pH, which may contribute to eliminating undesirable fermentation and reduce proteolysis (Kung et al., 2003). As well, addition of cell wall degrading enzymes to forages at ensiling can improve the silage quality and chemical composition (McDonald et al., 1991; Kung et al., 2003). Moreover, molasses is often added to ensiled forages as a source of readily fermentable sugars to increase fermentation rate and feeding quality (Yunus et al., 2000; Lima et al., 2010). Since *H. gardnerianum* has low protein content, its combination with a cheap nitrogen source such as urea would be beneficial to improve its nutritive value. Besides, presence of urea would inhibit the proliferation of fungi that cause aerobic spoilage and DM losses (McDonald et al., 1991).

Although information on the effects of most of these additives on grass, maize and whole crop silages abound in the literature, little is known of their suitability for *Hedychium* silage. Therefore, the objective of the present study was to assess the effect of application of different additives (incoculant including cell wall degrading enzymes, urea and molasses) on microbial populations, fermentation quality, chemical composition, aerobic stability and *in vitro* dry matter digestibility of *H. gardnerianum* silage.

2. Materials and methods

2.1. Forage collection and preparation

The current study was conducted in the Animal nutrition lab, Department of Agricultural Sciences, University of the Azores, located in Angra do Heroísmo, Terceira, Azores, Portugal. Plant materials (*H. gardnerianum*) were collected from Serreta region (38°44′N 27°21′W, at 298 m altitude), Angra do Heroísmo. The whole-plant was manually harvested at the end of the flowering stage (May 15, 2014) about 15 cm above the soil. The herbage was chopped using a laboratory type chopper at length of 2–3 cm and wilted overnight.

2.2. Ensiling procedure

Hedychium gardnerianum was ensiled using eight treatments: (C) no additives (control); T1) with lactic acid bacteria inoculant (Sil-ALL$^{4\times4}$); T2) with molasses (50 ml/kg); T3) with molasses (50 ml/kg) + inoculant Sil-ALL$^{4\times4}$; T4) with urea (5 g/kg); T5) with urea (5 g/kg) + inoculant Sil-ALL$^{4\times4}$; T6) with molasses (50 ml/kg) + urea (5 g/kg); and T7) with molasses (50 ml/kg) + urea (5 g/kg) + inoculant Sil-ALL$^{4\times4}$. All additives were carried out on fresh weight basis. Sil-ALL$^{4\times4}$ inoculant was suspended in distilled water (5 g/l) and applied at rate 2 ml/kg of fresh material (FM), corresponding LAB of 1×10^6 colony forming unit (cfu)/g of FM. Un-inoculated treatments were sprayed with distilled water (2 ml/kg). Inoculant Sil-ALL$^{4\times4}$ (Sil-ALL$^{4\times4}$, Danstar Ferment Ag, Switzerland) formulated with four LAB: *Lactobacillus plantarum, Pediococcus acidilactici, P. pentosaceus* and *Propionibacterium acidipropionici* (>1×10^{11} cfu/g). In addition to bacteria, the inoculant contained a blend of plant cell wall-degrading enzymes: α-amylase (>9000 BAU/g), cellulase (>150 CMC/g), β-glucanase (>2500 IU/g) and xylanase (>3750 IU/g). All ingredients of each treatment were mixed homogeneously, weighed (2 kg) and packed into mini-silo bags (30 cm × 50 cm). The silos were transparent, made from polyethylene-polyamide composite with thickness of 0.14 mm and were not equipped with facility to escape neither gases nor effluent. Three replicates were conducted for each treatment. All bags were heat sealed under vacuum using a vacuum packaging machine (ECO VAC, Italy) and stored indoors at room temperature (18–25 °C) for 60 days. During the storage period, silos were divided into two groups; the silos of each were vertically arranged in a column box and a 20 kg block was placed on the top for the compaction purpose.