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Original scientific paper

THE INFLUENCE OF A BACTERIAL INOCULANT ON REDUCTION OF AEROBIC MICROFLORA DURING ENSILING OF ALFALFA

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ABSTRACT: Silage produced from forage crops such as grass, maize and alfalfa form the major part of the feed ration of cattle and sheep in Europe. The main principles of forage preservation by ensiling are a rapid achievement of a low pH by lactic acid production and the maintenance of anaerobic conditions. The quality of silage depends on the competition between different groups of micro-organisms. Lactic acid bacteria, responsible for the silage fermentation process, are usually present in the silage microflora. In addition, a number of undesirable micro-organisms that occur at low levels on fresh plant materials may grow during the storage of silage and lead to anaerobic or aerobic spoilage. The aim of this study was to examine the effect of added inoculant on the reduction of aerobic microflora during ensiling of alfalfa. The alfalfa was treated in laboratory conditions with a commercial silage inoculant *Bonsilage Plus*. As a control was used alfalfa without added inoculant. Both experimental and control samples were done in triplicates. The sampling was conducted on days 7, 15 and 55 and the total number of bacteria and the total number of yeasts and moulds were determined. The results showed beneficial effects of inoculant on reduction of aerobic microflora in alfalfa silage.

Keywords: *alfalfa, silage, bacterial inoculant, aerobic microflora*

INTRODUCTION

Silage is an important feed for livestock, not only in winter in cold and temperate regions, but also during the dry season in the tropics (Mannetje, 1999). Silage produced from forage crops such as grass, maize and alfalfa form the major part of the feed ration of cattle and sheep in Europe.

The main principles of forage preservation by ensiling are a rapid achievement of a low pH by lactic acid production and the maintenance of anaerobic conditions (Čabarkapa et al., 2010a).

A moist crop can support the growth of a wide range of microorganisms, most of which will degrade the crop's nutrient value to livestock. However, ensiling generally controls microbial activity by a combination of an anaerobic environment and a natural fermentation of sugars by lactic acid bacteria on the crop (Muck, 2010). The crop at ensiling contains aerobic and anaerobic microorganisms and a range of both bacteria and fungi that affect

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silage quality. The dominant populations are aerobic microorganisms or facultative aerobes and lactic acid bacteria (LAB). Often LAB, that preservation of crop is dependent on, are lower in population than other groups of microorganisms on the crop at ensiling (Pahlow et al., 2003; Dalié et al., 2010).

The genus *Lactobacillus* belongs to the large group of LAB which are all gram-positive organisms and produce lactic acid by fermentation. There are two groups of species depending on the ability to ferment sugars: homofermentative species, converting sugars mostly into lactic acid, and hetero-fermentative species, converting sugars into lactic acid, acetic acid, ethanol and CO_2 (Kandler and Weiss, 1986; Čabarkapa et al., 2010b). Because the main catabolite is lactic acid, lactobacilli prefer relatively acidic conditions (pH 5.5-6.5) (Giraffa et al., 2010). Lactobacilli are associated with food and feed production because of the preservative action due to acidification, and/or enhancement of flavor, texture and nutrition. In addition, the proteolytic activity and production of aroma compounds, bacteriocins and exopolysaccharides are relevant for the quality and nutritional value of the end product and expand the spectrum of biotechnological applications of this important group of LAB (Leroy and De Vuyst, 2004).

The number of LAB present on aflafla plants at harvest may be too low to ensure rapid and efficient preservation, and therefore silage inoculants have been developed to improve the nutritive value of silages and to reduce risks during ensiling. The aim of this study was to examine the effect of added inoculant on the reduction of aerobic microflora during ensiling of alfalfa.

MATERIALS AND METHODS

Alfalfa, harvested in May 2009, was chopped at nominal particle length of approximately 20 mm. Material was manually compacted into laboratory-scale silos (Figure 1) described by Čolovic *et al*. (2010). The alfalfa was treated in laboratory conditions with a commercial silage inoculant *Bonsilage Plus* (Schaumann, Austria) which contains *Pedio-coccus pentosaceus* (DSM 12834), *Lactobacillus brevis* (DSM 12835), *Lactobacillus buchneri* (DSM 12856), *Lactobacillus plantarum* (DSM 12836) and *Lactobacillus rhamnosus (*NCIMB 30121). As a control was used alfalfa without added inoculant. Both experimental and control samples were done in triplicates. The purpose of compaction was to minimize the presence of oxygen and ensure fast initiation of anaerobic conditions. Containers were divided in two groups. Each group consisted of three containers with bacterial inoculant and three control containers without inoculant. The containers were stored at the temperature of 20 ± 3 C°. The sampling was conducted on days 7, 15 and 55.

Figure 1. Mini-silo for silage study

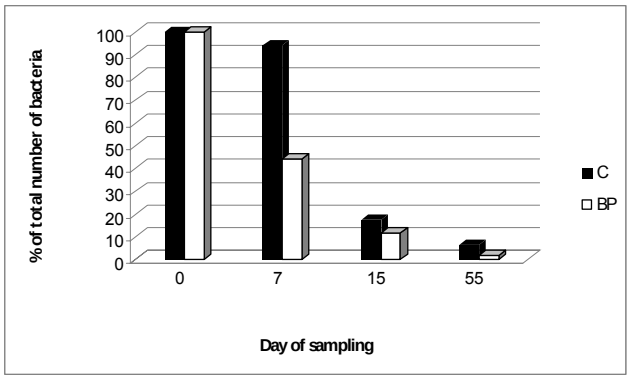
Total numberof aerobic bacteria was determined using the following procedure: 25 g of sample was transferred aseptically into individual stomacher bags, containing 225 ml of sterile Buffered Peptone Water (BPW) solution (0.1 %) and homogenized in a stomacher for 60 seconds. For each sample, appropriate serial decimal dilutions were prepared in the BPW solution. From each dilution step, 1.00 ml was transferred to a Petri dish. About 15 ml of agar tempered at 47 °C were poured into the Petri dish. Total number of all bacteria (except LAB) was determined aerobically using Plate Count Agar (PCA), after incubation for 3 days at 30 °C. LAB does not grow on this medium under aerobic conditions. Total number of yeasts and moulds was determined using Rose Bengal

Chloramphenicol agar (RBC) after aerobic incubation at 25 °C for 5 days in the dark.

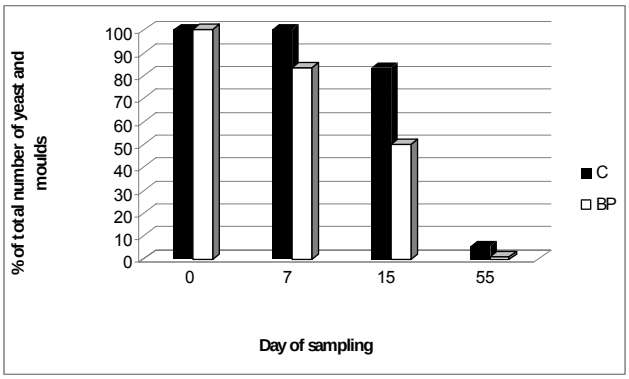
RESULTS AND DISCUSSION

The results showed that the addition of inoculant to alfalfa silage had beneficial influence on its aerobic microflora. The total number of bacteria and the total number of yeasts and moulds were lower in experimental samples than in control on all sampling days, as compared to day 0 i.e. in the fresh alfalfa.

Reduction of the total number of bacteria in silage on day 7 in the control sample was 5.9 %, whereas the silage with inoculant had reduction of 55.9 %. The reduction of the number of bacteria continued during ensiling in both experimental and control samples, but was higher in the silage with inoculant. On days 15 and 55, the control samples had reduction of 82.4 % and 88.3 %, respectively, whereas in experimental samples the reduction was 93.4 % and 98.2 %, respectively (Graph 1).



Graph 1. Reduction of total number of aerobic bacteria in alfalfa silage on different sampling days



Graph 2. Reduction of total number of yeasts and moulds in alfalfa silage on different sampling days

The control samples did not show any reduction in total number of yeasts and moulds on day 7, while that reduction in inoculated silage was 11.7 %. The reduction of number of yeasts and moulds in control and experimental samples on day 15 was 11.7 % and 50 %, respecttively, whereas on day 55 the reduction was 94.2 % in the control and 99.1 % in the inoculated sample (Graph 2).

According to Magnusson et al. (2003), three mechanisms may explain the antimicrobial efficiency of LAB: the yield of organic acid, competition for nutrients and production of antagonistic compounds. Some species of LAB are able to synthesize peptides or antimicrobial proteins known as bacteriocins, whose activity is only directed against closely taxonomically-related bacteria.

CONCLUSION

The results of this study showed beneficial effects of silage inoculant *Bonsilage Plus* with regard to reduction of aerobic microflora in alfalfa silage, thus contributing to lowering the possibility of secondary fermentation and related losses of silage nutritive value. Good preservation and stabilization of the crop in the silo is due to the combination of an anaerobic environment and the growth of lactic acid bacteria.

Note

Part of this study was presented at the 9th International symposium of animal biology and nutrition, 23-24 September, Bucharest, Romania, 2010

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