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The XXI International Grassland Congress / VIII International Rangeland Congress took place in

Hohhot, China from June 29 through July 5, 2008.

Proceedings edited by Organizing Committee of 2008 IGC/IRC Conference

Published by Guangdong People's Publishing House

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The effect of polyphenol oxidase on lipolysis and proteolysis of red clover silage with and without a silage inoculant

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Key words : red clover silage ,lactic acid bacteria inoculant ,polyphenol oxidase ,proteolysis ,lipolysis

Introduction Polyphenol oxidase (PPO) has been shown to reduce both proteolysis and lipolysis in red clover either through deactivation of proteolytic and lipolytic enzymes or/and through the formation of protein-quinone-lipid complexes (Lee et al., 2004). This experiment investigated the time course of both lipolysis and proteolysis in two red clovers with different PPO activities with and without the addition of a silage inoculant (*Lactobacillus plantarum*). This will help to understand the action of PPO in the silo and hence its potential effects on protein and glycerol based lipid conservation. It will also determine the effect of a more rapid pH reduction with inoculation on PPO activity and final silage quality.

Materials and methods Four silages were prepared from high or low PPO macerated red clover in 60 micro-silos, each containing 120 g fresh weight : a) High PPO red clover without inoculation (H-), b) Low PPO red clover without inoculation (L-), c) High PPO red clover with inoculation (H⁺), and c) Low PPO red clover with inoculation (L⁺). Each treatment had 3 reps for each time course of : 1, 2, 4, 8 and 90 days. The inoculant used was *Lactobacillus plantarum* strain L54 applied at a rate of 10^6 CFU/g FM. Silage pH was determined at opening and a sample of the silage frozen for later assessment of proteolysis (free amino acid appearance) and lipolysis (loss of membrane lipid). Statistical analysis was carried out using a general ANOVA with Inoculation^{*} PPO as the treatment effect (Payne et al., 2002).

Results and discussion Silage pH was ($P \le 0.001$) reduced by inoculation (3.9 vs 4.3 at day 90) with no effect of PPO. Inoculation had no effect on either lipolysis or free amino acid (FAA) release, although more soluble protein ($P \le 0.01$) and less ammonia-N ($P \le 0.01$) were found in inoculated silages. H silages had a lower level of both proteolysis (release of FAA, $P \le 0.05$) and lipolysis (loss of membrane lipid, $P \le 0.01$) than L red clover silages (Figure 1). The release of FAA and lipolysis were affected by PPO whilst soluble protein and ammonia-N was mainly affected by inoculation. The actions of the two processes also show time differences i *e*. prior to ensiling or in the early stages of ensiling for PPO versus during ensiling for inoculum.



Figure 1 High and low PPO (H and L) red clover silage with and without (+ and-) fresh culture inoculum : a) proteolysis (release of free amino acids), b) lipolysis (loss of membrane lipid) (+ / -standard error of the mean).

Conclusion Both PPO and lactic acid bacteria inoculation offer solutions for increased protein and lipid quality in the conserved forage with potential to increase N use efficiency in the animal and the lipid profile of ruminant products .

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