



## Effects of chestnut tannins on performance and antioxidative status of transition dairy cows

H. W. Liu,\* D. W. Zhou,\*<sup>1</sup> and K. Li†

\*Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Jilin 130102, P. R. China

†Shandong Heshi Corp. Ltd., Shandong 266061, P. R. China

### ABSTRACT

This study was conducted to evaluate the effects of chestnut tannins (CT) on performance and antioxidative status of transition dairy cows. Twenty multiparous Chinese Holstein cows in late gestation were paired according to expected calving date and randomly assigned either to a diet supplemented with CT (CNT, 10 g of CT/kg of diet, dry matter basis) or to an unsupplemented control (CON) diet from 3 wk prepartum to 3 wk postpartum. Blood samples were taken on d -21, 1, 7, and 21 relative to calving for analysis of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and malondialdehyde (MDA). Liver samples were taken by puncture biopsy on d 1 and 21 relative to calving for analysis of SOD, GSH-Px, and MDA. Data were analyzed for a completely randomized block design with repeated measures. The addition of CT had no significant effects on dry matter intake, body weight, body condition score, milk yield, 3.5% fat-corrected milk yield, and milk composition but did decrease milk MDA and somatic cell score in transition dairy cows. Dry matter intake decreased from d -21 to 0 and increased from d 1 to 21 relative to calving across treatments. During the experimental period, body weight and body condition score decreased, whereas milk MDA and somatic cell score increased across treatments. A time effect was also observed for plasma MDA, which peaked on d 1 relative to calving and remained higher than that on d -21 relative to calving across treatments. Addition of CT decreased MDA concentrations in plasma and liver. Neither time nor CT × time effects were observed for SOD and T-AOC in plasma and SOD and GSH-Px in liver; a time effect was observed for plasma GSH-Px, which peaked on d 1 relative to calving and remained higher than those on d -21 relative to calving across treatments. Addition of CT increased SOD, GSH-Px, and T-AOC activities in plasma and SOD and GSH-Px

activities in liver. In conclusion, addition of CT might inhibit lipid peroxidation and increase antioxidant enzymes activities in plasma and liver of transition dairy cows. Supplementation of CT may be a feasible means to improve the antioxidative status of transition dairy cows.

**Key words:** antioxidative status, chestnut tannin, transition cow

### INTRODUCTION

During the transition period of dairy cows, the increase in oxygen requirements with increased metabolic demands results in augmented production of oxygen-derived free radicals (Weiss, 1998; Yuan et al., 2012). When generation of free radicals exceeds the body's antioxidant production capacity, oxidative stress develops (Castillo et al., 2005). Oxidative stress may contribute to an impaired immune function and an enhanced susceptibility of dairy cattle to periparturient diseases (LeBlanc et al., 2004; Sordillo and Aitken, 2009). Therefore, diets enriched with antioxidants that cope with an excess of free radicals produced upon oxidative stress could be used to reduce the incidence of health disorders in transition dairy cows (Vázquez-Añón et al., 2008). For example, vitamin E supplementation reduced oxidative damage in liver from periparturient dairy heifers (Bouwstra et al., 2008).

Tannins, a type of natural antioxidant, are a complex group of water-soluble polyphenolic compounds arising from the metabolism of plants. They consist of one or more aromatic rings with one or more hydroxyl groups, which can combine with free radicals to form resonance-stabilized phenoxyl radicals. This structure confers strong antioxidant properties (Rice-Evans et al., 1996). Barreira et al. (2008) evaluated the in vitro antioxidant activities of chestnut tannins (CT; extracted from chestnut wood, *Castanea sativa*, which is rich in hydrolysable tannins) and found that CT exerted inhibitory effects on 2,2-diphenyl-1-picrylhydrazyl radical and lipid peroxidation in pig brain tissue. Moreover, the in vivo antioxidant activities of tannins were also observed in rabbits (CT, Liu et al., 2011) and lambs

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<sup>1</sup>Corresponding author: zhoudaowei@neigae.ac.cn

(quebracho tannins, Luciano et al., 2011; López-Andrés et al., 2013). Tannins, which are present in several feed resources used for livestock feeding, have been reported to affect several aspects of ruminant nutrition and product quality (Tabacco et al., 2006; Vasta et al., 2008). However, no information on the in vivo antioxidant properties of CT in dairy cows and its potential beneficial role for transition dairy cows has been reported. Thus, we hypothesized that CT could exhibit antioxidant properties and we designed this study to investigate the effects of dietary addition of CT on performance and antioxidative status of transition dairy cows.

## MATERIALS AND METHODS

All procedures were approved by the Administration Office of Laboratory Animals, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences (Jilin, China).

### *Animals, Diets, and Experimental Design*

Twenty multiparous Chinese Holstein cows in late gestation were paired according to expected calving date and randomly assigned to either a diet supplemented with chestnut tannins (CNT, 10 g of CT/kg of diet, DM basis) or an unsupplemented control diet (CON) from 3 wk prepartum to 3 wk postpartum. No differences ( $P > 0.1$ ) were found in BW and BCS between treatment groups at the beginning of the study. To prepare the CNT diet, appropriate amounts of CT were applied into the mixer wagon to be mixed with other ingredients. Pre- and postpartum diets were formulated according to the recommended values from NRC (2001); ingredients and composition are given in Table 1. Dry matter was determined by oven drying at 105°C overnight (AOAC International, 1995; method 930.15). Ether extract was determined using a Soxhlet apparatus (AOAC International, 1995; method 945.16). Crude protein was measured by a Kjeldahl nitrogen analysis (AOAC International, 1995; method 954.01). Content of NDF was determined as described by Van Soest et al. (1991) using heat-stable amylase (A3306, Sigma, St. Louis, MO) and sodium sulfite, and expressed without residual ash. Starch was measured as described by Bach Knudsen (1997). The CT (SilvaTeam, San Michele di Mondovì, Italy) were extracted from chestnut wood by a heat and low-pressure treatment according to the manufacturer's information; only the water-soluble fraction was retained and subsequently dehydrated. The product was presented as a fine brown powder. Chemical composition of the batch used in this experiment was as follows: 76% tannins

(14% condensed tannins), 18% nontannin, 5% water, and 1% insolubles (pH 3.3, 0.1 mg/mL solution) on a fresh matter basis. The total tannin contents, expressed as tannic acid equivalents, were measured according to the Folin-Ciocalteu method (Makkar et al., 1993). The condensed tannins content was determined as described by Porter et al. (1986).

Cows were housed in tiestall barns bedded with sawdust and fed a TMR for ad libitum intake with at least 10% of daily feed refusal. All cows were individually fed twice daily at 0800 and 1500 h. Feed offered and refused was recorded daily, and daily samples were collected to determine DMI. Cows had free access to water.

Body weight and BCS (1 to 5 in 0.25-unit increments; Wildman et al., 1982) were recorded once weekly. Cows were milked twice daily at 0500 and 1600 h, and production was recorded at each milking during the trial. Milk samples were collected weekly for analysis of fat, protein, lactose, and somatic cells by infrared analysis with a Milko Scan FT6000 (Foss Electric, Hillerød, Denmark). Milk malondialdehyde (MDA) concentration was measured according to Suriyasathaporn et al. (2006). In brief, 100 mL of milk sample was mixed with 1 mL of TCA on a vortex mixer. Then, 400 mL of thio-barbituric acid (TBA) was added. The mixture was boiled for 30 min and subsequently cooled by using tap water. The solution was measured 4 times by spectrophotometer (Leng Guang SFZ1606017568, Shanghai, China) at 532 nm against its blank reaction mixture (without TBA). The MDA concentration in milk was expressed in nanograms per milliliter.

Blood samples were taken on d -21, 1, 7, and 21 relative to calving for evaluation of antioxidative status. Blood samples were taken from each cow via tail vein using heparin plasma tubes. Plasma was obtained by centrifuging (Himac CR22G2, Hitachi Koki Co. Ltd., Tokyo, Japan) at  $3,000 \times g$  for 10 min at 4°C and frozen at -20°C for subsequent analysis of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), and MDA.

### *Liver Biopsy and Biochemical Assay*

Liver samples were taken by puncture biopsy (Dann et al., 2006) under local anesthesia (10 mL of lidocaine) on d 1 and 21 relative to calving. Liver samples were rinsed in saline, frozen in liquid nitrogen, and then stored at -80°C for subsequent analysis of SOD, GSH-Px, and MDA.

Forty milligrams of frozen liver in 4 mL of homogenization buffer (0.05 M Tris-HCl, pH 7.4, 1 mM EDTA, and 0.25 M sucrose) was homogenized on ice with a Polytron PT 3000 homogenizer (Kinematica AG, Lucerne, Switzerland) for 5 s at 13,500 rpm. The

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