Effect of Natural Antioxidants on Superoxide Dismutase and Glutathione Peroxidase mRNA Expression in Leukocytes from Periparturient Dairy Cows

M. Colitti* and B. Stefanon
Department of Scienze della Produzione Animale, Via delle Scienze, 208 – 33100 Udine, Italy
*Correspondence: E-mail: bruno.stefanon@uniud.it


ABSTRACT

During the periparturient period, high-yielding dairy cows experience metabolic stress, which alters their homeostasis and exposes the cows to illness. The aim of this study was to quantify the expression levels of genes involved in antioxidant defences during the transition period in the blood of dairy cows and to evaluate the regulative activity on these genes of natural antioxidants in the diet. Three groups of 7 heifers each, at the 7th month of pregnancy, were used. Starting from 3 weeks before the expected calving date (~22 days), the three groups were allotted to the following experimental treatments: control (CTR, basal diet); lycopene (LYC, basal diet + lycopene 540 mg/day) and grape polyphenols (POL, basal diet + grape polyphenols 10 g/day). Blood was sampled at 22 and 8 days before and 8, 15 and 22 after calving and analysed for the expression level of glutathione peroxidase (GPx) and superoxide dismutase (Cu/ZnSOD) using the real-time PCR technique with LUX (Light Upon eXension) fluorogenic primers. During the periparturient period (~22 days until + 22 days from calving), Cu/ZnSOD mRNA expression decreased (p <0.05) in the CTR and LYC groups, but increased at 15 days after calving in the POL group. No significant differences were found in GPx mRNA expression. The results suggest that grape polyphenols may have a controlling effect on peripartum metabolic stress through modulation of superoxide dismutase expression.

Keywords: antioxidants, cow, diet, glutathione peroxidase, real-time RT-PCR, superoxide dismutase

Abbreviations: Ct, threshold cycle of PCR at which amplified product is detected; CTR, control; E, PCR efficiency; GPx, glutathione peroxidase; LYC, tomato lycopene; OD, optical density; POL, grape polyphenols; RT-PCR, reverse transcription-polymerase chain reaction; SOD, superoxide dismutase

INTRODUCTION

Calving-associated immunodepression has been considered one of the main causes related to the appearance of various pathologies in dairy cows, such as retained placenta (Cai et al., 1994) or environmental mastitis (Drackley, 1999). According to Gröhn and colleagues (2003), the high-yielding dairy cow experiences at least one or more diseases after calving. This metabolic stress associated with milk yield in dairy ruminants is related to the increase in production of free radicals and reactive oxygen species (ROS) (Miller and Brzezinska-Slebodzinska, 1993; Gabai et al., 2004) and to clinically relevant pathological conditions (Kankofer, 2002).
Adequate feeding regimes and nutrient supply, on the one hand, and administration of antioxidants, on the other, are often claimed as the most appropriate strategies to reduce metabolic and oxidative stress during the peripartum period, thus controlling some of the calving-associated diseases. Many studies in humans and laboratory animals have underlined the importance of natural antioxidants in controlling reactive oxygen species (Jewell and O’Brien, 1999; Agarwal and Rao, 2000; Bagchi et al., 2000; Beatty et al., 2000; Lu and Foo, 2000), but less information is available on the role that these compounds might have in the regulation of stress and related responses in dairy ruminants (Colitti et al., 2000, 2002).

The aim of this study was to evaluate the variation, if any, of the level of expression of glutathione peroxidase (GPx) and superoxide dismutase (Cu/ZnSOD) mRNAs in blood leukocytes during the peripartum period in cows and whether natural antioxidants (lycopene or grape polyphenols) can reduce oxidative stress around calving.

MATERIAL AND METHODS

Animals

Twenty-one heifers at 7 months of pregnancy and of a similar age were selected and randomly assigned to three groups (7 cows each). All 21 animals were housed in a stall with cubicles, had free access to drinking water and were fed a total mixed ration according to their nutrient requirements (NRC, 2001). Starting from 3 weeks before the expected calving date (–22 days), the three groups were allotted to the following experimental treatments: lycopene (LYC), grape polyphenols (POL) and control (CTR).

The LYC group received a daily amount of 540 mg of tomato lycopene (LYCORed, Natural Products Industries Ltd., Beer-Sheva, Israel; 13 000 mg/kg) and the POL group a daily amount of 10 g of grape polyphenols (96% of total polyphenols; 19% of procyanidine; Naturex, Avignon, France). Natural antioxidants were mixed in pellet (ground barley, soybean meal, molasses) and administered individually until the end of the trial to each animal of the LYC or POL groups. The animals of the control (CTR) group received a similar amount of pellets without antioxidants. One animal of the CTR group was culled for calving difficulties and one animal of the LYC group refused to ingest the antioxidant-containing pellet after calving. The final number of cows used in the trial was thus 6 for the LYC and CTR groups and 7 for the POL group. Starting from 22 days before the expected calving date, blood was sampled once a week at the following sampling times: –22, –8, +8, +15 +22 days before (–) and after (+) calving. Blood was collected before the morning meal from the jugular vein in lithium-heparin vacuum tubes, kept at +4°C and immediately delivered to the laboratory for analysis. The experiment was carried out in accordance with state and local laws and ethical regulations.