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Short communication: Effects of oral flavonoid supplementation on the metabolic and antioxidative status of newborn dairy calves

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ABSTRACT

Scientific proof for flavonoids as a health tool in calf nutrition is inconsistent. We investigated the effects of the most abundant flavonoid, quercetin, and of a green tea extract (GTE) containing various catechins on the metabolic and antioxidative traits in dairy calves to clarify their potential health-promoting effects. Male newborn German Holstein calves (n = 7 per group) received either no flavonoid (control group), 10 mg of quercetin equivalents as quercetin aglycone or as rutin/kg of body weight (BW) per day, or 10 mg/kg of BW per day of a GTE from d 2 to 26 of life. The supplements were provided with the morning and evening feeding. The calves were fed colostrum and milk replacer, and BW, feed intake, and health status were evaluated daily. Blood samples were collected from a jugular vein on d 1, 5, 12, 19, and 26 before the morning feeding to investigate the metabolic and antioxidative status of the calves. The growth performance and health status remained unchanged, but the GTE-fed calves had fewer loose feces than the controls. The plasma concentrations of quercetin changed over time and were higher in the rutin-fed group than in the control group, whereas the catechins were below the detection limit. The plasma Trolox equivalent antioxidative capacity and ferric reducing ability of plasma were measured as markers for plasma antioxidative capacity. The concentrations of Trolox equivalent antioxidative capacity increased, whereas ferric reducing ability of plasma decreased after the first day of life in all the groups. The oxidative stress markers in the plasma were measured as thiobarbituric acid reactive substances and F2-isoprostanes, but these did not indicate treatment or time effects. The plasma concentrations of total protein, albumin, urea, lactate, glucose, and nonesterified fatty acids and of insulin and cortisol varied over time, but no group differences were caused by the flavonoid supplementation. In summary, orally administered quercetin and catechins at the dosages used in the present study resulted in weak effects on health and no effects on the metabolic and antioxidative status of newborn dairy calves.

Key words: antioxidative status, calf, flavonoid, quercetin, rutin

Short Communication

Calf losses mainly occur during the first week of life, mostly because of respiratory and digestive problems (USDA, 2011). Good management, such as an early and sufficient colostrum supply, is essential for optimal postnatal development. In addition, feed supplements are a daily practice of modern dairy farms. Particularly since the ban of antibiotic growth promoters in the European Union in 2006, the call for "natural" feed additives has gained enormous popularity. In this respect, flavonoids, as secondary plant metabolites, ubiquitous in all higher plants, are of interest. Their health-promoting properties are mainly thought to be due to their strong antioxidant activity shown in vitro, associated with effects on inflammatory cells (Middleton et al., 2000), glucose and lipid metabolism (Shetty et al., 2004; Kobayashi et al., 2010), or incidence of diarrhea (di Carlo et al., 1994). One of the most abundant flavonoids is the flavonol quercetin, a pentahydroxyflavon, mostly bound in a β-glycosidic manner to at least one sugar molecule and present in high concentrations in apples and onions. Quercetin bound to rutinose is called rutin and is the major glycoside of quercetin. The predominant flavanol, another subgroup of flavonoids, is catechin, which is mainly found in high concentrations in green tea.

Almost any disease is associated with increased formation of reactive oxygen species, thus causing oxidative stress (Halliwell, 1991). In calves, enhanced oxidative stress has been seen on the first day of life (Alex-

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androvich and Antonovna, 2009), and especially if the calf was sick (Ahmed and Hassan, 2007). Because the colostrum supply for newborn calves is often impaired, this possibly affects the antioxidant system (Blum et al., 1997). Thus, improvement of the antioxidant status in newborn calves may accelerate the maturation of their own immune system, improve health status, and thus reduce calf losses. In a previous study, we have shown that quercetin in newborn calves is bio-available when fed as quercetin aglycone or as rutin, a prerequest for systemic functioning of quercetin in the body (Maciej et al., 2015). Based on that, we have tested in the present study the hypothesis that quercetin and catechin supplementation affect metabolism and the antioxidative and health status during the first 4 wk of life in dairy calves.

The procedures in this study were performed in accordance with the German animal protection law and approved by the relevant authorities (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei, Mecklenburg-Vorpommern, Germany). Twenty-eight male German Holstein calves were examined from d 1 to 26 of life. All the calves were spontaneously born from multiparous cows on neighboring farms and transported directly after birth to the experimental barn of the University of Rostock in Dummerstorf, where they were kept in single boxes with straw bedding. The calves had free access to water and were fed twice daily by a nipple bottle or nipple bucket. For the first 3 d of life, the calves received pooled colostrum obtained from milkings 1, 3, and 5 (d 1, 2, and 3 after parturition, respectively) in amounts of 8% of BW on d 1 and 10% of BW on d 2 and 3 (Supplemental Table S1; http://dx.doi.org/10.3168/jds.2015-9906). From d 4 until d 26, the calves received a commercial milk replacer (150 g/L; SalvaLac MiraPro 45, Salvana Tiernahrung GmbH, Klein-Offenseth Sparrieshoop, Germany) in the amount of 12% of BW/d. To ensure equal feed uptake in all the groups, the refused amounts of colostrum or milk replacer were tube fed. The milk replacer amounts were adapted to BW once per week.

From d 2 to 6, colostrum or milk replacer was supplemented with chicken-egg-derived immunoglobulins (Globigen Life Start 25%, EW Nutrition GmbH, Visbek, Germany) fed twice daily in amounts of 40, 32, 24, 16, and 8 g/d, respectively (Maciej et al., 2015). The calves had free access to pelleted concentrate (Kälber Start 18/3 Vollkraft Mischfutterwerke GmbH, Karstädt, Germany) and hay from d 4 on. Concentrate intake was measured daily after the morning milk feeding (Maciej et al., 2015).

The health status of the calves was determined daily by measuring the rectal temperature, heart and respiratory rate, nasal discharge, respiratory sounds, and by navel inspection. The fecal consistency was assessed daily by fecal consistency score according to Larson et al. (1977): normal (1), soft (2), runny (3), or watery (4). Sick calves were treated by a veterinarian accordingly.

The calves were randomly assigned to 1 of 4 feeding groups (n = 7). Control (**CTRL**) received no flavonoids, **QA** received 10 mg/(kg of BW × d) quercetin aglycone (quercetin dihydrate; Carl Roth GmbH & Co. KG, Karlsruhe, Germany), **RU** received 20 mg/(kg of BW × d) of quercetin as glucorhamnoside rutin (rutin trihydrate; Carl Roth GmbH & Co. KG), and **CA** received 10 mg/(kg of BW × d) of a green tea extract (**GTE**) containing various catechins (Polyphenon 60; Sigma-Aldrich Chemie GmbH, Steinheim, Germany). For QA and RU, the daily dose of quercetin equivalents was 10 mg/kg of BW (30 μmol/kg of BW). The GTE (70.3% total catechins) fed in CA was composed of 1.4% catechin, 0.3% catechin gallate, 5.2% gallocatechin, and 2.1% gallocatechin gallate (all as *trans*-isomers) and 6.4% epicatechin, 7.0% epicatechin gallate, 19.0% epigallocatechin, and 28.8% epigallocatechin gallate (all as *cis*-isomers). The amounts of flavonoid fed to the calves in this study were based on previous studies in pigs (Lesser et al., 2004; Lühring et al., 2011). Due to studies on the bioavailability of flavonoids, all calves received their respective daily dose with the morning feeding on d 2, and no flavonoids were fed on d 3 and 4 (Maciej et al., 2015). From d 5 on, the daily dose was equally split between the morning and evening meal until d 26. The flavonoids were suspended in water and administered with a disposable 10-mL syringe directly into the mouth during milk feeding.

Jugular blood samples were taken immediately after birth, and on d 5, 12, 19, and 26 before morning feeding using evacuated tubes (Vacuette, Greiner Bio-One GmbH, Frickenhausen, Germany). Tubes containing 15 IU/mL of lithium heparinate were used for the determination of the plasma concentrations of flavonols, catechins, and the markers for antioxidative capacity and oxidative stress; tubes containing 2.5 g/L of sodium fluoride and 1.8 g/L potassium EDTA were used for the determination of plasma protein, albumin, glucose, NEFA, urea, and lactate, and tubes containing 1.8 g/L of potassium-EDTA were used for the determination of plasma insulin and cortisol concentrations. The blood samples were immediately put on ice and centrifuged (1,500 × *g*, 4°C, 20 min). To measure the catechins, 1 mL of plasma was mixed with 20 μL of an ascorbate-EDTA solution (0.4 mol/L of NaH_2PO_4; Carl Roth GmbH & Co. KG) containing 20% ascorbic acid (Merck KGaA, Darmstadt, Germany) and 0.1% EDTA (Carl Roth GmbH & Co. KG) at pH 3.6. To measure the F2-isoprostanes, a 50 μg/mL ethanolic butylhydroxytoluene solution (50 μg/mL of ethanol, wt/vol) was added

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