J. Dairy Sci. 98:3906–3917
http://dx.doi.org/10.3168/jds.2015-9361

Bioavailability of the flavonol quercetin in neonatal calves after oral administration of quercetin aglycone or rutin

J. Maciej,* C. T. Schäff,* E. Kanitz,† A. Tuchscherer,‡ R. M. Bruckmaier,§ S. Wolffram,# and H. M. Hammon*[1]
*Institute of Nutritional Physiology "Oskar Kellner,"
†Institute of Behavioural Physiology, and
‡Institute of Genetics and Biometry, Leibniz Institute for Farm Animal Biology (FBN), Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
§Veterinary Physiology, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland
#Institute of Animal Nutrition and Physiology, Christian Albrechts University Kiel, 24118 Kiel, Germany

ABSTRACT

Polyphenols, such as flavonoids, are secondary plant metabolites with potentially health-promoting properties. In newborn calves flavonoids may improve health status, but little is known about the systemically availability of flavonoids in calves to exert biological effects. The aim of this study was to investigate the oral bioavailability of the flavonol quercetin, applied either as quercetin aglycone (QA) or as its glucorhamnoside rutin (RU), in newborn dairy calves. Twenty-one male newborn German Holstein calves were fed equal amounts of colostrum and milk replacer according to body weight. On d 2 and 29 of life, 9 mg of quercetin equivalents/kg of body weight, either fed as QA or as RU, or no quercetin (control group) were fed together with the morning meal. Blood samples were taken before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 12, 24, and 48 h after feed intake. Quercetin and quercetin metabolites with an intact flavonol structure (isorhamnetin, tamarixetin, and kaempferol) were analyzed in blood plasma after treatment with glucuronidase or sulfatase by HPLC with fluorescence detection. Maximum individual plasma concentration was depicted from the concentration-time-curve on d 2 and 29, respectively. Additional blood samples were taken to measure basal plasma concentrations of total protein, albumin, urea, and lactate as well as pre- and postprandial plasma concentrations of glucose, nonesterified fatty acids, insulin, and cortisol. Plasma concentrations of quercetin and its metabolites were significantly higher on d 2 than on d 29 of life, and administration of QA resulted in higher plasma concentrations of quercetin and its metabolites than RU. The relative bioavailability of total flavonols (sum of quercetin and its metabolites isorhamnetin, tamarixetin, and kaempferol) from RU was 72.5% on d 2 and 49.6% on d 29 when compared with QA (100%). Calves fed QA reached maximum plasma concentrations of total flavonols much earlier than did RU-fed calves. Plasma metabolites and hormones were barely affected by QA and RU feeding in this experiment. Taken together, orally administrated QA resulted in a greater bioavailability of quercetin than RU on d 2 and 29, respectively, and quercetin bioavailability of quercetin and its metabolites differed markedly between calves aged 2 and 29 d.
Key words: bioavailability, calf, flavonoid, quercetin, rutin

INTRODUCTION

Flavonoids are secondary plant metabolites occurring ubiquitously in all higher plants (Manach et al., 2004; Besle et al., 2010). They are known for their health-promoting properties (e.g., antioxidative and anti-inflammatory; Middleton et al., 2000; Nijveldt et al., 2001; Williams et al., 2004). Quercetin is one of the most abundant flavonoids and is present in high concentrations in onions, apples, and kale (Hertog et al., 1992; Nijveldt et al., 2001), and in low concentrations in milk (Besle et al., 2010; Bhagwat et al., 2013). In addition to their health-promoting properties, quercetin and its metabolites modulate the expression and activity of several metabolic key enzymes, and therefore might be involved in regulation of lipid and carbohydrate metabolism (Middleton et al., 2000; Gasparin et al., 2003; Kobayashi et al., 2010).

Newborn calves undergo tremendous immunological and metabolic changes after birth to adapt for extrauterine life (Blum, 2006; Chase et al., 2008; Hammon et al., 2012) and colostrum management is one of the most important factors to support neonatal health and development (Godden, 2008; Hammon et al., 2012). Nevertheless, morbidity and mortality rates are still high during first weeks of life, and calves often suffer from diarrhea and respiratory disease (McGuirk, 2008; Mee, 2008; Uetake, 2013) as well as high levels of oxidative stress (Inanami et al., 1999; Gaál et al.,

Received January 20, 2015.
Accepted February 6, 2015.
[1]Corresponding author: hammon@fbn-dummerstorf.de

2006). Frequent problems on farms are the lack of high-quality colostrum availability and the insufficient colostrum supply (Quigley and Drewry, 1998; Godden, 2008). Colostrum and mature milk contain antioxidant agents to protect neonatal calves from oxidative stress (Lindmark-Månsson and Åkesson, 2000; Besle et al., 2010). Poor quality of colostrum is reflected by low concentrations of antioxidative substances. Thus, supplementing colostrum and milk at the beginning of lactation with the natural antioxidant agent quercetin may improve neonatal oxidative status. However, there is no information about the bioavailability (**BV**) of orally applied quercetin with colostrum and milk in newborn calves, although feeding industry already offers flavonoid-supplemented feed for dairy calves.

Studies on BV of quercetin from quercetin aglycone (**QA**) or its glucorhamnoside rutin (**RU**) in different monogastric species, such as rats (Manach et al., 1997), pigs (Ader et al., 2000; Cermak et al., 2003; Lesser et al., 2004), dogs (Reinboth et al., 2010), and humans (Erlund et al., 2000; Egert et al., 2008), showed marked differences to ruminant species (e.g., cows with intraruminal QA and RU application; Berger et al., 2012). These differences may be due to differences in gastrointestinal anatomy and physiology (Arts et al., 2004; Berger et al., 2012; Gohlke et al., 2013). Conversely, in newborn calves the forestomach system is just developing, thus newborn calves are functionally monogastrics (Drackley, 2008). The aim of the present study was to investigate the relative BV of quercetin after oral administration of QA or RU in calves during first month of life. We hypothesized that BV in calves depends on the form of application (QA and RU) and changes with age due to ontogenetic development and maturation of the gastrointestinal tract during first month of life. We further tested the hypothesis that quercetin application might affect metabolic and endocrine traits, especially concerning glucose metabolism in neonatal calves, because findings in literature pointed to impaired carbohydrate digestion and glucose absorption after flavonoid intake (Cermak et al., 2004; Tadera et al., 2006).

MATERIALS AND METHODS

Animals and Feeding

The experimental procedures were carried out according to the animal care guidelines and were approved by the relevant authorities of the State Mecklenburg-West Pomerania, Germany (LVL M-V/TSD/7221.3–2.1–019/10). Twenty-one male German Holstein calves were examined on d 2 and 29 of life. All calves were spontaneously born from multiparous cows on neighboring farms and transported directly after birth to the experimental barn. Calves were kept in single boxes with straw bedding and had free access to water. Calves were fed twice daily (0700 and 1500 h) with a nipple bottle or nipple bucket. On the first 3 d of life calves received pooled colostrum obtained from milkings 1, 3, and 5 (d 1, 2, and 3 after parturition, respectively; Table 1) at amounts of 8% of BW on d 1 and 10% of BW on d 2 and 3 (Steinhoff-Wagner et al., 2011). From d 4 until 29, calves received milk replacer (150 g/L; SalvaLac MiraPro 45, Salvana Tiernahrung GmbH, Klein-Offenseth Sparrieshoop, Germany) at 12% of BW/d (Table 1). To ensure uptake of equal amounts of feed, refused amounts of colostrum or milk were tube-fed to calves. Milk intake was adapted to BW data once a week.

Colostrum or milk replacer was supplemented with chicken egg-derived immunoglobulins (Globigen Life Start 25%, EW Nutrition GmbH, Visbek, Germany) composed of 75% dextrose and 25% whole egg powder (10.75% CP, 10.50% crude fat, 0.10% crude fiber, and 2.50% ash), with high antibody titer against *Escherichia coli* type K 99, *Salmonella* Typhimurium and *Salmonella* Dublin, bovine rotavirus type G6 and G10, bovine coronavirus, *Cryptosporidium parvum*, and *Clostridium perfringens* serotype C. Immunoglobulins were added from d 2 to 6. Respective amounts of immunoglobulins fed twice daily were 40, 32, 24, 16, and 8 g/d.

From d 4 on calves had free access to pelleted concentrate (Kälber Start 18/3 pell., Vollkraft Mischfutterwerke GmbH, Karstädt, Germany; Table 1) and hay. Concentrate intake was measured daily after morning milk feeding. To avoid iron deficiency, calves received 600 mg of iron dextran subcutaneously (Ursoferran, Serumwerk Bernburg, Germany) on their first day of life. Navel disinfection was performed with 10% iodine solution (vet sept Lösung, Albrecht GmbH, Aulendorf, Germany) immediately after birth. Health status of calves was determined daily by measuring rectal temperature, heart rate, and respiratory rate, by evaluation of behavioral abnormalities, nasal discharge, respiratory sounds, fecal consistence, and by navel inspection.

Treatment and Blood Sampling

Calves were randomly assigned to 1 of 3 feeding groups (n = 7 per group) receiving either no flavonoids (control group; **CTRL**), 9 mg of QA/kg of BW (quercetin aglycone dihydrate, Carl Roth GmbH, Karlsruhe, Germany), or 18 mg of RU/kg of BW (rutin trihydrate, Carl Roth GmbH), each resulting in a dose of 9 mg of quercetin equivalents (**QE**)/kg of BW (30 μmol of QE/kg of BW) on d 2 and 29 of life. Calves received the