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Ruminal degradation of quercetin and its influence on fermentation in ruminants

L. M. Berger,* R. Blank,*[1] F. Zorn,* S. Wein,* C. C. Metges,† and S. Wolffram*
*Institute of Animal Nutrition and Physiology, Christian-Albrechts-University of Kiel, 24118 Kiel, Germany
†Institute of Nutritional Physiology, Leibniz Institute for Farm Animal Biology, 18196 Dummerstorf, Germany

ABSTRACT

The aim of the present study was to investigate the ruminal degradation of the flavonol quercetin and to determine its potential antimicrobial effects on ruminal fermentation in cows. Ruminal degradation of quercetin (0 or 100 μmol/L, respectively) as well as its influence on ruminal gas production (0, 50, or 100 μmol of quercetin equivalents/L, respectively, either applied as aglycone or as its glucorhamnoside rutin) using concentrate, grass hay, and straw as substrates were investigated in vitro using the Hohenheim gas test. Additionally, the influence of quercetin on ruminal concentrations of volatile fatty acids and their molar ratio in rumen-fistulated, nonlactating cows (n = 5) after intraruminal application of quercetin as aglycone or as rutin (0, 10, or 50 mg of quercetin equivalents/kg of BW, respectively) was evaluated. Quercetin was rapidly and extensively degraded, whereby the disappearance of quercetin was accompanied by the simultaneous appearance of 2 metabolites 3,4-dihydroxyphenylacetic acid and 4-methylcatechol. In vitro total gas and methane production were not reduced by the addition of quercetin aglycone or rutin, respectively, using concentrate, grass hay, and straw as substrates. As expected, however, effects of the substrates used were detected on total gas and methane production. Highest gas production was found with concentrate, whereas values obtained with grass hay and straw were lower. Relative methane production was highest with grass hay compared with concentrate and straw (27.1 vs. 25.0 and 25.5%). After intraruminal application of the quercetin aglycone or rutin, respectively, neither total concentration nor the molar ratio of volatile fatty acids in the rumen fluid were influenced. Results of the present study show that quercetin underlies rapid ruminal degradation, whereby 3,4-dihydroxyphenylacetic acid and 4-methylcatechol are the main metabolites, whereas the latter one most likely is formed by dehydroxylation from 3,4-dihydroxyphenylacetic acid. Regarding antimicrobial effects of quercetin, results obtained indicate that fermentation processes in the forestomachs are not substantially influenced by quercetin or rutin, respectively. With regard to potential health-promoting effects of quercetin, its application in cows, especially in the form of the better available rutin, might not be accompanied by negative effects on ruminal fermentation.
Key words: antimicrobial, cow, Hohenheim gas test, quercetin, ruminal fermentation

INTRODUCTION

Flavonoids are secondary plant metabolites and ubiquitously present in feed plants of livestock (Besle et al., 2010; USDA, 2011). Among the various flavonoids, the flavonol quercetin (Figure 1) is one of the most investigated polyphenols exhibiting various health-promoting properties; for example, antioxidative, antiinflammatory, and metabolic effects (Middleton et al., 2000; Erlund, 2004). Although those findings mainly derive from in vitro studies or from studies in monogastric species, effects in ruminants such as cows are feasible. In fact, we recently have reported reduced levels of markers of liver damage in periparturient cows (Stoldt et al., 2015). Thus, quercetin might have positive effects on health especially during the stressful phase of early lactation, which is often accompanied by metabolic disorders such as fatty liver and ketoacidosis (Kehrli et al., 2006). In plants, quercetin is mainly present in form of glycosides, e.g., the glucorhamnoside rutin (Figure 1), which for example occurs in significant quantities in buckwheat. Although not commonly used as a feed plant, buckwheat might be used as natural source for quercetin in cows (Broudiscou et al., 2000; Amelchanka et al., 2010). Quercetin content in usual feed for ruminants has not been extensively investigated until now. Few data available indicate amounts of total polyphenols in grass (35.3 g/kg of DM) and maize silage (3.2 g/kg of DM), respectively (Besle et al., 2010). For cows kept on permanent pastures with polyphenol concentrations ranging between 19 and 32 g/kg of DM, Fraisse

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[1]Corresponding author: blank@aninut.uni-kiel.de

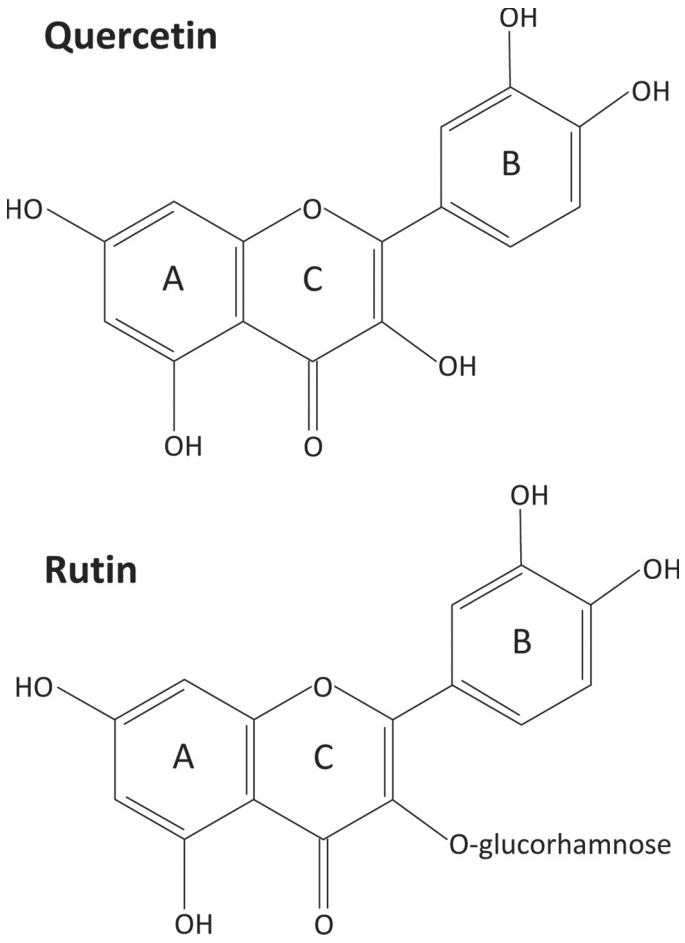

Figure 1. Structure of quercetin and its glucorhamnoside rutin, where A, B, and C are the ring structures of flavonols.

et al. (2007) assumed a daily intake of polyphenols of 500 g/d. Although flavonols including quercetin represent only a fraction of total polyphenols, the amounts consumed by ruminants with their natural feed might well be associated with biological effects.

The systemic availability of a substance is a prerequisite for any postabsorptive effect in vivo. Whereas the bioavailability of quercetin has been extensively investigated in monogastric species (Cermak et al., 2003; Chen et al., 2005; Wein and Wolffram, 2013), only few studies in cows have been performed (Berger et al., 2012; Gohlke et al., 2013). Absolute bioavailability of quercetin after intraruminal (**i.r.**) application of quercetin either as aglycone or as rutin was low (Berger et al., 2012). Nevertheless, maximal plasma concentrations after rutin application were similar to those obtained in monogastric species after application of equimolar amounts of quercetin aglycone (Cermak et al., 2003). Most interesting, rutin was a much better source of quercetin compared with quercetin aglycone when applied intraruminally (Berger et al., 2012), whereas intraduodenal application of both quercetin sources yielded results as in monogastric species (Gohlke et al., 2013). In monogastric species, quercetin bioavailability from rutin is inferior to that of quercetin aglycone (Cermak et al., 2003). A crucial point regarding the oral bioavailability of a compound is its fate within the gastrointestinal tract. In ruminants, quercetin is expected to undergo intensive microbial fermentation within the forestomachs. Several studies investigated the microbial degradation of quercetin and its glycosides using ruminal and fecal inocula in vitro (Cheng et al., 1969; Krishnamurty et al., 1970; Lin et al., 2003; Labib et al., 2004). Based on those findings, an extensive microbial degradation of quercetin can be assumed, accompanied by the appearance of its degradation products 3,4-dihydroxyphenylacetic acid (**3,4-DHPAA**); phloroglucinol (**PG**); and some minor metabolites identified in humans, cows, and pigs (Aura et al., 2002; Labib et al., 2004; Rechner et al., 2004).

Despite the promising health-promoting properties of quercetin, it has to be kept in mind that quercetin might also reveal some negative effects in ruminants due to its antimicrobial effects. Thus, quercetin might negatively influence fermentation processes in the forestomachs and in turn feed efficiency and animal performance. Hence, application of quercetin in ruminants would only be acceptable if positive effects dominate possible negative effects. Several in vitro studies have already investigated the influence of quercetin or quercetin-containing plant extracts, respectively, on microbial fermentation (Broudiscou et al., 2000, 2002; Bodas et al., 2008; Leiber et al., 2012). Those studies, however, yielded inconsistent results.

In the present study, we investigated ruminal degradation of quercetin in vitro. Beside the disappearance of quercetin over time, we also have monitored appearance of its degradation products 3,4-DHPAA, PG, and 4-methylcatechol (**4-MC**). To gather information on effects of quercetin on fermentation processes, we applied quercetin either as aglycone or as rutin to determine in vitro total gas as well as methane production using the Hohenheim gas test (**HGT**) together with various substrates (concentrate, grass hay, and straw). In addition, we investigated the influence of quercetin (as aglycone and as rutin, respectively) on concentrations and molar ratios of VFA in vivo after i.r. application in cows.

MATERIALS AND METHODS

Experimental Design

All animal experiments were approved by the Ministry of Agriculture, Environment and Rural Areas of