The Effects of Oral Quercetin Supplementation on Splanchnic Glucose Metabolism in 1-Week-Old Calves Depend on Diet after Birth

Jeannine Gruse,4 Solvig Görs,4 Armin Tuchscherer,5 Winfried Otten,6 Joachim M Weitzel,7 Cornelia C Metges,4 Siegfried Wolffram,8 and Harald M Hammon4*

Institutes of 4Nutritional Physiology “Oskar Kellner”, 5Genetics and Biometry, 6Behavioural Physiology, and 8Reproductive Biology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany; and 7Institute of Animal Nutrition and Physiology, Faculty of Agricultural and Nutritional Sciences, Christian-Albrechts-University of Kiel, Kiel, Germany

Abstract

Background: Inadequate colostrum supply results in insufficient intake of macronutrients and bioactive factors, therefore impairing gastrointestinal development and the maturation of glucose metabolism in neonatal calves. The flavonoid quercetin has been shown to exert health-promoting properties, including effects in diabetic animals. However, quercetin interacts with intestinal glucose absorption and might therefore exert negative effects in neonates.

Objective: To evaluate the interaction between neonatal diet and quercetin feeding on splanchnic glucose metabolism in neonatal calves.

Methods: Calves (n = 28) were assigned to 4 groups and fed either colostrum or a milk-based formula on days 1 and 2 and supplemented daily with 148 mmol/kg body weight quercetin aglycone [colostrum with quercetin (CQ+)/formula with quercetin (FQ+)] or without this substance [colostrum without quercetin (CQ−)/formula with quercetin (FQ−)] from days 2–8. From day 3, all calves received milk replacer. A xylose absorption test was performed on day 3, and on day 7, blood samples were collected to study glucose first-pass uptake after [13C6]-glucose feeding and intravenous [6,6-2H2]-glucose bolus injection. Plasma concentrations of metabolites and hormones were measured by taking additional blood samples. A biopsy specimen of the liver was harvested on day 8 to measure the mRNA expression of gluconeogenic enzymes.

Results: Higher postprandial plasma concentrations of glucose, lactate, urea, adrenaline, noradrenaline, insulin, and glucagon on day 7 in colostrum-fed calves indicate that metabolic processes were stimulated. Postabsorptive xylose and glucose plasma concentrations each increased by an additional 26%, and splanchnic glucose turnover decreased by 35% in colostrum-fed calves, suggesting improved glucose absorption and lower splanchnic glucose utilization in colostrum-fed calves. Quercetin supplementation resulted in higher noradrenaline concentrations and enhanced peak absorption and oxidation of [13C6]-glucose by 10%. Liver mitochondrial phosphoenolpyruvate carboxykinase mRNA abundance was reduced by 34% in colostrum-deprived calves.

Conclusions: Feeding on colostrum during the first 2 d of life is crucial for maturation of splanchnic glucose metabolism in calves. Supplementing quercetin improves gastrointestinal absorption capacity, particularly in colostrum-deprived calves. J Nutr doi: 10.3945/jn.115.218271.

Keywords: calf, quercetin, colostrum, formula, glucose metabolism

Introduction

Flavonoids are natural polyphenols that are widespread in higher plants; flavonoids possess health-promoting properties and these compounds are often used as nutritional supplements in humans and animals, including livestock (1–3). Quercetin is one of the most abundant flavonoids; it has antioxidative, anti-inflammatory, and antimicrobial effects (4–6) that might be useful in calf health. However, quercetin is also known to affect glucose metabolism by inhibiting digestive enzymes, interfering with glucose transporters, and enhancing insulin secretion (7).
Quercetin effects might be beneficial in treating metabolic disorders, such as obesity or type 2 diabetes; however, quercetin administration might impair glucose metabolism in neonates.

Glucose is the main energy source for the fetus; birth initiates severe changes in the continuity, route, and source of glucose supply (8). Along with the formation of a functional immune response, the maturation of the gastrointestinal tract (GIT) and the adaptation of glucose metabolism are essential to ensure glucose uptake and the establishment of glucose homeostasis in neonatal calves (9). Adequate colostrum intake is crucial because colostral bioactive factors present in colostrum, such as growth factors, hormones, and antioxidants, are known to stimulate GIT development and the maturation of glucose metabolism (10–12).

Quercetin interacts with intestinal glucose absorption by various mechanisms; quercetin aglycone inhibits glucose uptake via glucose transporter–2 (GLUT2) suppression (13), whereas quercetin monoglucosides might interfere with sodium-dependent glucose transporter–1 (SGLT-1) (14). In diabetic rats and mice, treatment with quercetin decreased hyperglycemia (13, 15). Irrespective of a possible intestinal interaction with quercetin, glucose intake with milk is insufficient to meet neonate demand (8). Therefore, not only gastrointestinal maturation but also other factors influence the efficiency of hepatic gluconeogenesis is of major importance to maintain glucose homeostasis (9). In calves, glucose homeostasis is maintained by hepatic gluconeogenesis (9) and the hepatic activity of phosphoenolpyruvate carboxykinase increases with postnatal maturation (16) but is not affected by diet (12). However, studies with perfused rat liver have shown that glucose synthesis is inhibited and that glycolysis is stimulated by quercetin (17, 18). Consequently, although quercetin possibly directly promotes neonatal health (3), quercetin supplementation might negatively affect glucose supply in neonatal calves; however, data from in vivo experiments are scarce, and the extent to which quercetin feeding interferes with the developing glucose metabolism of neonates remains unknown, at least in calves.

The aim of this study was to investigate the effect of quercetin feeding on glucose metabolism in neonatal calves and to consider interactions between quercetin and diet. We hypothesized that quercetin interferes with the absorption, splanchic extraction, and hepatic metabolism of glucose and tested whether any effects depend on initial colostrum supply.

Methods

The experimental procedures were carried out according to German animal-care guidelines and were approved by the relevant authorities of the State Government of Mecklenburg-Western Pomerania, Germany (State Office for Agriculture, Food Safety, and Fisheries LALLF M-V/TSD/7221.3-1.1-044/12).

Animals, husbandry, and feeding. Twenty-eight newborn male German Holstein calves from 2 neighboring farms (Gut Dummerstorf; Landwirtschaftsgesellschaft Prisannwitz) were separated from their dams immediately after birth and transported to the experimental facility. The calves were born spontaneously as singletons via vaginal delivery by multiparous cows and were held in individual boxes on straw bedding during their first week of life.

Calves were assigned by body weight (BW) to 2 dietary groups [colostrum (C) and formula (F)] and were bucket fed twice daily. During the first 2 d of life, calves in the colostrum group received pooled colostrum from milkings 1 and 3 (days 1 and 2 after parturition, respectively), whereas calves in the formula group received milk-based formulas (Bergophor Futtermittelfabrik; Dr. Berger GmbH & Co.) for days 1 and 2 with macronutrient compositions that were comparable to the corresponding colostrum milkings (Supplemental Table 1) but that contained only trace amounts of bioactive factors (12). The amounts of colostrum or formula fed to the calves were targeted to be 10% of BW on day 1 and 12% of BW on day 2. Calves with reduced appetite were tube fed to ensure complete ingestion of colostrum or formula. From day 3 onward, all calves received a commercial milk replacer (Salvalac MiraPro 45; Salvana TIERnahrung GmbH); 12% of BW per day; 150 g powder/L water; Supplemental Table 1).

Each dietary group was subdivided into a treatment group, receiving 50 mg/kg BW·d quercetin aglycone (quercetin dihydrate ≥98%; Carl Roth) orally from days 2–8 twice daily with feeding (CQ+ and FQ+; n = 7, respectively), and a control group without any additional treatment (CQ− and FQ−, respectively; n = 7).

The navels of the calves were disinfected with povidone iodine (Vet Sept, aniMedica) to protect against infection. The calves were administered 1 g iron dextran (Ursosferran 150, Seruminum Bernburg) by mouth on day 1 and halogufonine from days 2 to 8 (Halocur; MSD Animal Health; 0.1 mg/kg BW) to protect against cryptosporidiosis. All calves were fed chicken-egg–derived immunoglobulins that exhibited high antibody titers against common enteral pathogens (2.5 g/kg BW; Globigen Life Start 25%, EW Nutrition GmbH) from days 1–5. Calves that were fed formula were administered colistin sulfate (3 mg/kg BW, intramuscular; Belacol; BelaPharm), B vitamins (100 mg nicotinamide/calf, 40 mg thiamine hydrochloride/calf, subcutaneous; vitamin B complex; subcutaneous) and bovine colostral immunoglobulins on day 1, day 3, and day 5 (2 g gammaglobulins/calf; oral, Aniserin orinject; aniMedica GmbH). Because of gastrointestinal disturbances, 2 calves (from FQ+ and CQ+) were removed from the study.

Analyses of milk. Milk samples were collected during pooling, and the chemical composition was analyzed by Qualitätspfungs- und Dienstleistungsgesellschaft Mecklenburg-Vorpommern. Dry matter (DM), crude protein, crude fat, lactose, and ash were determined according to the Weender standard procedure (19).

Xylose absorption studies. Calves received 0.5 g (i)-xylose per kg BW with the morning meal on day 3. Before and 1, 2, 3, 4, 5, 6, 7, 8, and 24 h after xylose administration, blood samples were collected by venipuncture into evacuated tubes containing K3-EDTA (1.8 g/L blood; Vacutette; Greiner Bio One International GmbH). Blood was centrifuged at 1,500 × g (2 g gammaglobulins/calf, oral; Aniserin orinject; aniMedica GmbH). Of the resulting plasma, 2 samples were taken for measurement of xylose and glucose concentrations.

To determine plasma xylose concentration, 0.3 mL of plasma or blank solution (saturated benzoic acid) were mixed with 0.6 mL trichloroacetic acid, incubated for 5 min, and then centrifuged at 1,500 × g for 20 min. The samples were then measured spectrophotometrically at 515 nm. Glucose concentration was analyzed as described below.

Plasma flavonols, metabolites, and hormones. On day 7, blood samples were collected before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 24 h after the morning meal to determine the concentrations of quercetin and its metabolites with intact flavonol structures (isorhamnetin, tamarixetin, kaempferol) in plasma (Li-heparin tubes; Sarstedt AG & Co.). Further blood samples were collected hourly until 10 h after the morning meal, and the plasma concentrations of glucose, lactate, urea, nonesterified fatty acids (NEFA), insulin, glucagon (K3-EDTA; Sarstedt

9 Abbreviations used: BW, body weight; C, colostrum; CQ−, colostrum without quercetin; CQ+, colostrum with quercetin; D, diet; DM, dry matter; F, formula; FPU, first pass uptake of glucose; FQ−, formula without quercetin; FQ+, formula with quercetin; GBPC, glucose-6-phosphatase; GIP, glucose-dependent insulinotropic polypeptide; GIT, gastrointestinal tract; GLUT2, glucose transporter–2; HPCL1, hippocalcin-like 1; LRPI0, low-density lipoprotein; LSM, least squares mean; NEFA, non-esterified fatty acids; PC, pyruvate carboxylase; PCK1, phosphoenolpyruvate carboxykinase (cytosolic); PCK2, phosphoenolpyruvate carboxykinase (mitochondrial); POLA2A, RNA polymerase II; PYGL, glycogen phosphorylase; Q, quercetin; Ra, rate of appearance; SGLT-1, sodium-dependent glucose transporter–1; T, time.
AG & Co.), and catecholamines (Li-heparin, Sarstedt AG & Co.) were measured. The tubes that were used to collect the samples for catecholamine (adrenaline, noradrenaline) measurement were supplemented with 1 mol/L semicarbazide (10 μL/mL whole blood) before centrifugation to prevent enzymatic breakdown. The collected blood samples were immediately put on ice and centrifuged (1500 × g, 4°C, 20 min). The supernatants were then portioned and stored at −20°C until analysis. Flavonol and catecholamine aliquots were stored at −80°C.

Plasma flavonol concentrations were measured, as previously described in the literature (20). The detection limit for flavonols was 2 nmol/L, and the recovery mean ± SE rate was 92 ± 2%. Inter- and intra-assay CV for flavonols were 7.2% and 0.5%, respectively. The term total flavonols refers to the sum of the concentrations of individual flavonols (quercetin, isorhamnetin, tamarixetin, and kaempferol). Plasma metabolites were measured with the use of an automatic spectrophotometer (ABX Pentra 400; HORIBA Ltd.) and the following appropriate kits: glucose (#A11A01667) and lactate (#A11A01721) from HORIBA Ltd.; urea (#LT-UR 0010) from Labor+Technik, Eberhard Lehmann GmbH; and NEFA (#434–91795, #436–91995) from Wako Pure Chemical Industries Ltd.

Plasma concentrations of insulin (#RIA-1257) and glucagon (#RIA-1258) were determined by RIA with the use of kits obtained from DRG International, Inc. (21). Plasma concentrations of adrenaline and noradrenaline were measured in duplicate with the use of HPLC (22). The CV were 4.6% and 8.5% for adrenaline and 3.3% and 1.9% for noradrenaline were measured in duplicate with the use of HPLC (22). The morning meal (equivalent to 4% BW) was fed to ensure complete tracer ingestion. Simultaneously, [6,6-2H2]-glucose (5 mg/kg BW; 99 atom% 2H, Campro Scientific GmbH; dissolved in 10 mL sterile 0.9% saline) was injected as an intravenous bolus into the jugular vein by catheter (Cavafix Certo 338, B. Braun Vet Care GmbH).

Blood samples were collected from the jugular catheter (Li-heparin, Sarstedt AG & Co.) twice before and 5, 10, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, 300, 360, 480, 600, and 1440 min after tracer application to measure 13C and 2H enrichment in plasma glucose (12, 23). The limits of quantitation and detection were 12 ng/L and 3 ng/L for glucose, and 15 ng/L and 4 ng/L for noradrenaline. Intra- and interassay CV were 4.6% and 8.5% for adrenaline and 3.3% and 1.9% for noradrenaline, respectively.

Tracer studies. To determine the first-pass uptake of glucose (FPU), [i.e., glucose utilization in the splanchnic tissue (intestine and liver)], a tracer study was performed on day 7 according to Schönhusen et al. (23) [i.e., glucose utilization in the splanchnic tissue (intestine and liver)], a tracer study was performed on day 7 according to Schönhusen et al. (23) [i.e., glucose utilization in the splanchnic tissue (intestine and liver)], a tracer study was performed on day 7 according to Schönhusen et al. (23) [i.e., glucose utilization in the splanchnic tissue (intestine and liver)], a tracer study was performed on day 7 according to Schönhusen et al. (23) [i.e., glucose utilization in the splanchnic tissue (intestine and liver)], a tracer study was performed on day 7 according to Schönhusen et al. (23) [i.e., glucose utilization in the splanchnic tissue (intestine and liver)], a tracer study was performed on day 7 according to Schönhusen et al. (23) [i.e., glucose utilization in the splanchnic tissue (intestine and liver)], a tracer study was performed on day 7 according to Schönhusen et al. (23) [i.e., glucose utilization in the splanchnic tissue (intestine and liver)].

Plasma [13C6]- and [6,6-2H2]-glucose enrichment–time curve (mole percent excess glucose (mmol/kg BW), respectively, and AUC is the area under the curve). The morning meal (equivalent to 4% BW) was fed to ensure complete tracer ingestion. Simultaneously, [6,6-2H2]-glucose (5 mg/kg BW; 99 atom% 2H, Campro Scientific GmbH; dissolved in 10 mL sterile 0.9% saline) was injected as an intravenous bolus into the jugular vein by catheter (Cavafix Certo 338, B. Braun Vet Care GmbH). The morning meal (equivalent to 4% BW) was fed to ensure complete tracer ingestion. Simultaneously, [6,6-2H2]-glucose (5 mg/kg BW; 99 atom% 2H, Campro Scientific GmbH; dissolved in 10 mL sterile 0.9% saline) was injected as an intravenous bolus into the jugular vein by catheter (Cavafix Certo 338, B. Braun Vet Care GmbH).

Measurement of plasma enrichment of [13C6]- and [6,6-2H2]-glucose was determined by RIA with the use of kits obtained from DRG International, Inc. (21). Plasma concentrations of adrenaline and noradrenaline were measured in duplicate with the use of HPLC (22). The CV were 4.6% and 8.5% for adrenaline and 3.3% and 1.9% for noradrenaline, respectively.

Measurement of plasma enrichment of [13C6]- and [6,6-2H2]-glucose has been described elsewhere (23–26). Enrichment data were used to calculate the rate of appearance (Ra) of glucose in plasma (26):

\[
Ra_{glc}[mmol/(kg \times h)] = \frac{D_{oral}}{AUC(13C)}
\]

where \(D_{oral} \) refers to the sum of the concentrations of individual flavonols (quercetin, isorhamnetin, tamarixetin, and kaempferol). Plasma metabolites were measured with the use of an automatic spectrophotometer (ABX Pentra 400; HORIBA Ltd.) and the following appropriate kits: glucose (#A11A01667) and lactate (#A11A01721) from HORIBA Ltd.; urea (#LT-UR 0010) from Labor+Technik, Eberhard Lehmann GmbH; and NEFA (#434–91795, #436–91995) from Wako Pure Chemical Industries Ltd.

Plasma concentrations of insulin (#RIA-1257) and glucagon (#RIA-1258) were determined by RIA with the use of kits obtained from DRG International, Inc. (21). Plasma concentrations of adrenaline and noradrenaline were measured in duplicate with the use of HPLC (22). The CV were 4.6% and 8.5% for adrenaline and 3.3% and 1.9% for noradrenaline, respectively.

Liver sampling and analyses. Two hours after morning feeding on day 8, ~300 mg of liver tissue was collected from each calf by biopsy (22) with the use of a custom-made biopsy trocar (length: 400 mm; i.d.: 4 mm). Liver samples were immediately frozen in liquid nitrogen and stored at −80°C.

Liver tissue was pulverized in liquid nitrogen. Glycogen content was determined with the use of a commercial photometric kit based on amyloglucosidase-catalyzed glucose release (#10207748035, Boehringer Mannheim/R-Biopharm AG).

The relative mRNA abundance of genes that are related to glucose metabolism was quantified as described in a previous article (28). Primer sequences and PCR conditions for reference genes [hippocalin-like 1 (HPCAL1); low-density lipoprotein 10 (LRP10); and RNA polymerase II (POLR2A)] and target genes [glucose-6-phosphatase (G6PC); phosphoenolpyruvate carboxykinase (cytosolic: PCK1; mitochondrial: PCK2); pyruvate carboxylase (PC); and glycogen phosphorylase (PYGL)] are listed in Supplemental Table 2. The primer products were verified by sequencing with the use of the BigDye Terminator v1.1 Cycle Sequencing kit and an ABI 3130 Genetic Analyzer (Thermo Fisher Scientific Inc.). Real-time PCR was performed with the use of a LightCycler (F. Hoffman-La Roche AG); SYBR Green I was used as the fluorescent dye. Melting curve analysis and agarose gel electrophoresis were used to confirm the specificity of the PCR products. Quantification cycle values and amplification efficiencies obtained with the use of LinRegPCR version 2013.0 (29) were imported into qBASE+ version 2.6.1 (Biogazelle) for all subsequent calculations and quality controls. The geometric mean of the reference gene abundances was used for normalization. The data are presented as the ratio of the copy numbers of the genes of interest to the geometric mean of the reference gene abundances.

Statistical analyses. All statistical analyses were conducted with the use of SAS software, version 9.4 for Windows (SAS Institute Inc., Chicago, IL). Descriptive statistics and tests for normality were calculated with the use of the UNIVARIATE procedure included in Base SAS software. With respect to interindividual absorption differences, plasma flavonols were tested with the use of the Grubbs test for outliers; animals whose plasma flavonol concentrations were identified as outliers were excluded from further absorption comparisons. Data regarding the plasma concentrations of flavonols, metabolites, hormones, xylose, and tracer enrichment were analyzed by repeated measurement ANOVA with the use of the MIXED procedure included in the SAS/STAT software. The model included the fixed-factors diet (levels: C, F), quercetin. Least squares means (LSMs) and their SEs were calculated with the use of the Tukey-Kramer procedure. The SLICE procedure of the MIXED procedure was used to conduct partitioned analyses of the LSM for 2-way interactions. Effects and differences were considered statistically significant if \(p \leq 0.05\). Results are presented as LSM ± SE.

Results

Feed intake and growth performance. Feed intake differed between groups neither on day 1 and day 2 nor before blood sampling on day 3, day 7, and day 8. Mean ± SE BW was 45.3 ± 2.1 kg in CQ−, 47.0 ± 1.6 kg in CQ+, 45.1 ± 1.7 kg in FQ−, 4

Quercetin and bovine neonatal glucose metabolism
Plasma flavonol concentrations on day 7 of life were detectable only in quercetin-supplemented groups. Plasma flavonol concentrations in calf 19 (CQ+) were considered outliers according to the Grubbs test and were excluded from further analyses (Supplemental Figure 1). Mean ± SE plasma concentrations of total flavonols during 24 h after ingestion were 161 ± 20.5 nmol/L in CQ+ and 197 ± 18.7 nmol/L in FQ+, respectively, and the individual flavonols quercetin, isorhamnetin, tamarixetin, and kaempferol accounted for 69.4%, 15.1%, 11.3%, and 4.2% of total flavonols in plasma. No statistically significant differences were observed between the dietary groups. Plasma concentrations of total flavonols increased with time more markedly in FQ+, resulting in a significant diet × time interaction (P < 0.01; Figure 2). The AUC for plasma total flavonols over a 10-h period after feeding was higher in FQ+ (2.27 ± 0.20 μmol/L · h) than in CQ+ (1.64 ± 0.15 μmol/L · h).

Plasma glucose concentrations increased in all groups (P < 0.01) after feed intake on day 7 but were higher in C-fed than in F-fed calves (P < 0.01; Figure 3A). The increase of plasma glucose concentrations lasted for 4 h in CQ+ and FQ+ and for 5 h in the corresponding control groups. Plasma lactate concentrations were higher (P ≤ 0.05) in C-fed than in F-fed calves and increased after feeding only in CQ− (Figure 3B). Plasma glucose concentrations decreased within the first 2 h after feeding (P < 0.01) and increased to basal levels between 8 and 10 h after feeding (P ≤ 0.05) in all groups. Quercetin treatment did not affect lactate, urea, or NEFA plasma concentrations, but increased plasma glucose 2 h after feeding (P ≤ 0.05 for Q+ vs. Q−).

Plasma insulin concentrations increased after feed intake (P < 0.01) only in C-fed calves and were highest 2 and 3 h after feeding in CQ+ and CQ−, respectively (Figure 3C). Plasma glucagon concentrations decreased within the first 2 h after feed intake in CQ+ and CQ− but did not change with time in FQ+ or FQ− (Table 1). Plasma insulin and glucagon concentrations were higher in C-fed than in F-fed groups (P < 0.01). The insulin

**Figure 1** Plasma concentrations of glucose (A) and xylose (B) before and after feeding with milk replacer and 0.5 g/kg BW xylose in 3-d-old calves that were fed either C or F on days 1 and 2 and that were supplemented with quercetin (CQ+, FQ+) or were not supplemented with quercetin (CQ−, FQ−) from day 2 onward (50 mg/kg BW daily). The data represent LSMs ± SEs; n = 7 except FQ+, n = 6. Labeled LSMs at a time without a common letter differ, P ≤ 0.05. BW, body weight; C, colostrum; D, diet; F, formula; LSM, least squares mean; Q, quercetin; T, time.

**Figure 2** Plasma concentrations of total flavonols in 7-d-old calves before and after feeding with milk replacer supplemented with quercetin (50 mg/kg BW). Calves received colostrum or formula on days 1 and 2 of life and milk replacer from day 3 onward. The data represent LSMs ± SEs; CQ+, n = 5; FQ+, n = 6. BW, body weight; CQ+, colostrum with quercetin; D, diet; FQ+, formula with quercetin; LSM, least squares mean; T, time.

**XYLOSE ABSORPTION.** Plasma glucose and xylose concentrations on day 3 of life increased until 3 h after feeding and decreased to baseline concentrations 24 h after xylose intake (P < 0.01; Figure 1). Plasma concentrations of glucose and xylose were higher in C-fed than in F-fed calves (P ≤ 0.01). Both monosaccharides showed a quercetin × time interaction (P ≤ 0.05); xylose concentrations, in particular, were lower at 7 and 8 h after feeding in quercetin-fed calves. In addition, quercetin × diet × time interaction (P < 0.06 for glucose and P < 0.01 for xylose) indicated a faster increase of glucose and xylose (especially in FQ+) and for xylose, an earlier decrease in CQ+. The AUC (0–24 h) for xylose was greater (P ≤ 0.05) for C-fed than for F-fed calves and tended to be greater (P < 0.1) in calves receiving no quercetin (LSM ± SE were 38.3, 35.3, 34.2, 31.5 ± 1.4 nmol/L · h for CQ−, CQ+, FQ−, and FQ+, respectively).
to glucagon ratio in plasma increased \((P < 0.001)\) after feed intake in all groups but indicated no differences in response to quercetin supplementation in C-fed and F-fed calves (diet \(\times\) time interaction: \(P \leq 0.05\); Table 1).

Plasma concentrations of catecholamines decreased after feeding \((P < 0.01)\) and recovered to baseline concentrations 10 h after feeding (Figure 3D, E). Recovery of adrenaline was faster when quercetin was fed (quercetin \(\times\) time interaction; \(P \leq 0.05\)) than in C-fed calves, and plasma concentrations of adrenaline were higher 5 h after feeding in C-fed than in F-fed calves. Mean noradrenaline but not adrenaline concentrations were higher after quercetin supplementation \((P \leq 0.05)\).

**Tracer studies.** On day 7, glucose FPU and Ra\(_{oral}\) were higher \((P \leq 0.05)\), and Ra\(_{i.v.}\) tended to be lower \((P < 0.1)\) in F-fed than in C-fed calves (Table 2). When corrected for differences in xylose absorption, glucose FPU still tended to be higher \((P < 0.1)\) in F- than C-fed calves. Enrichment of \([^{13}\text{C}]_6\)-glucose increased to maximal values 2 h after ingestion (Table 2), and recovered to baseline values within 24 h \((P < 0.01)\). Maximal enrichments of \([^{13}\text{C}]_6\)-glucose and enrichment during first 4 h and during 24 h were higher in C-fed than in F-fed calves \((P \leq 0.05)\); Figure 4A). Significant diet \(\times\) time and quercetin \(\times\) time interactions were present \((P < 0.01)\); Figure 4A): From 0.75 to 3 h, enrichment of \([^{13}\text{C}]_6\)-glucose was lower in FQ\(_2\) than in C-fed groups \((P \leq 0.05)\); Figure 4A). This effect was confirmed by the AUC (0–4 h) result for \([^{13}\text{C}]_6\]-glucose enrichment, which was lower in F-fed than in C-fed calves \((P < 0.01);\) Table 2). When \([^{13}\text{C}]_6\)-glucose enrichment was corrected for xylose absorption, AUC (0–4 h) was higher \((P \leq 0.05)\) in quercetin-fed calves and tended to be higher \((P < 0.1)\) in C- than in F-fed calves (Table 2).

Enrichment of \([^{13}\text{C}]_2\) in blood reached maximal levels about 4 h after tracer ingestion and decreased to basal concentrations within 24 h \((P < 0.01)\) (Figure 4B). Maximal enrichment of \([^{13}\text{C}]_2\) was higher \((P < 0.01)\) in quercetin supplemented calves (Table 2). The AUC for \([^{13}\text{C}]_2\) enrichment from 2–6 h was larger \((P \leq 0.05)\), but that from 6–24 h was smaller \((P < 0.01)\) after quercetin feeding (Table 2). When \([^{13}\text{C}]_2\) enrichment was corrected for xylose absorption, AUC (2–6 h) was highest \((P < 0.01)\) in FQ+.

**Analyses of liver tissue.** The mRNA abundance of G6PC, PC, PYGL, and PCK1 were not significantly altered by diet or quercetin supplementation (Table 3). PCK2 mRNA abundance was lower \((P \leq 0.05)\) in F-fed than in C-fed groups but was not affected by quercetin. Liver glycogen concentration did not differ between the groups.

**Discussion**

Orally administered quercetin aglycone is bioavailable in neonatal calves aged 2 and 29 d, respectively, as we have shown in a previous study (31). Because these previous experiments with the use of a daily oral dosage of 10 mg/kg BW failed to produce significant quercetin effects on plasma metabolites and hormones, we quintupled the dosage to 50 mg/kg BW. Although the dose of quercetin aglycone administered in this study was 5-fold higher, maximum plasma flavonol concentrations measured herein were lower than those measured in 2-d-old calves in the previous study, probably due to altered intestinal permeability. Surprisingly, peak plasma flavonol concentrations measured in...
this study were only slightly higher than those measured in the 29-d-old calves in the previous study (31).

Newborn calves are preruminants; nevertheless, microbial colonization of the rumen starts immediately after birth with Escherichia coli and Lactobacilli spp. as the predominant species (32). Both of these microbes are able to degrade quercetin (33). Berger et al. (20) demonstrated that quercetin aglycone is only poorly bioavailable in adult cows after intraruminal application, and the authors suggested that microbial degradation was responsible. This ruminal degradation, although probably not well developed in 7-d-old calves, might account for some quercetin degradation, but most of the milk passes the rumen because of the esophageal groove reflex in neonatal calves. Nevertheless, the 5-fold larger dose of quercetin aglycone fed to calves in this study did not result in greater flavonol plasma concentrations than the plasma flavonol concentrations observed in the previous study (31).

The GIT develops with age, and permeability and absorption capacity change accordingly (34). The differences between FQ+ and CQ+ are likely explained by differences in GIT maturation between the F- and C-fed calves because colostrum feeding distinctively affects postnatal GIT maturation (9, 11). In addition, microbial activity between F- and C-fed calves might also differ because colostrum contains beneficial bacteria that, when ingested

### Table 1

Mean plasma concentrations of lactate, urea, NEFA, and glucagon in 7-d-old calves fed C or F on days 1–2, supplemented (Q+) or not supplemented (Q−) with quercetin on days 2–8

<table>
<thead>
<tr>
<th>Group</th>
<th>CQ− (n = 7)</th>
<th>CQ+ (n = 6)</th>
<th>QF− (n = 7)</th>
<th>QF+ (n = 6)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea, mmol/L</td>
<td>5.44 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.61 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.65 ± 0.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.16 ± 0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.003</td>
</tr>
<tr>
<td>NEFA, μmol/L</td>
<td>109 ± 17.3</td>
<td>123 ± 18.7</td>
<td>138 ± 17.3</td>
<td>112 ± 18.7</td>
<td>0.62</td>
</tr>
<tr>
<td>Glucagon, ng/L</td>
<td>139 ± 6.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>142 ± 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.8 ± 6.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>71.8 ± 7.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin/glucagon, mol/mol</td>
<td>2.57 ± 0.54</td>
<td>2.23 ± 0.58</td>
<td>2.75 ± 0.54</td>
<td>3.14 ± 0.58</td>
<td>0.34</td>
</tr>
</tbody>
</table>

<sup>1</sup> Data are LSMs ± SEs, analyzed over a 10-h period after feeding. Labeled least squares means in a row without a common letter differ, P ≤ 0.05. Interactions that are not shown (e.g., diet × quercetin and quercetin × time) were not significant (P > 0.05). C, colostrum; F, formula; LSM, least squares mean; NEFA, nonesterified fatty acids; Q−, not supplemented with quercetin; Q+, supplemented with quercetin.

### Table 2

Rates of glucose appearance, first pass glucose uptake, and AUC for 13C6-glucose enrichment and blood 13CO2 enrichment (derived from 13C6-glucose) of 7-d-old calves fed either C or F on days 1 and 2, supplemented (Q+) or not supplemented (Q−) with quercetin on days 2–8

<table>
<thead>
<tr>
<th>Group</th>
<th>CQ− (n = 7)</th>
<th>CQ+ (n = 6)</th>
<th>QF− (n = 7)</th>
<th>QF+ (n = 6)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ra&lt;sub&gt;oral&lt;/sub&gt;, mmol/(kg · h)</td>
<td>1.74 ± 0.26</td>
<td>1.52 ± 0.24</td>
<td>1.12 ± 0.11</td>
<td>1.42 ± 0.14</td>
<td>0.08</td>
</tr>
<tr>
<td>Ra&lt;sub&gt;oral&lt;/sub&gt;, mmol/(kg · h)</td>
<td>3.41 ± 0.31</td>
<td>3.44 ± 0.22</td>
<td>4.29 ± 0.49</td>
<td>4.21 ± 0.29</td>
<td>0.030</td>
</tr>
<tr>
<td>Fractional FPU, %</td>
<td>46.0 ± 9.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.1 ± 6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>72.2 ± 3.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.3 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.013</td>
</tr>
<tr>
<td>Corrected for xylose absorption</td>
<td>3.00 ± 0.27</td>
<td>2.84 ± 0.20</td>
<td>3.36 ± 0.43</td>
<td>2.97 ± 0.23</td>
<td>0.45</td>
</tr>
<tr>
<td>Fractional FPU, %</td>
<td>39.5 ± 10.4</td>
<td>46.4 ± 7.8</td>
<td>63.7 ± 5.5</td>
<td>52.5 ± 5.0</td>
<td>0.07</td>
</tr>
</tbody>
</table>

<sup>1</sup> Data are LSMs ± SEs. Values in a row without a common letter are significantly different, P ≤ 0.05. APE, atom percent excess; C, colostrum; E<sub>max</sub>, maximum plasma enrichment; F, formula; LSM, least squares mean; MPE, mole percent excess; Q−, not supplemented with quercetin; Q+, supplemented with quercetin; Ra, rate of appearance; Tmax, time until E<sub>max</sub> was reached.
by calves, contribute to establishing intestinal microflora such as lactobacillus and bifidobacteria, which are able to degrade quercetin (33, 33). Furthermore, the area of intestinal surface available for mucosa-associated bacteria was likely to be smaller in F-fed calves; hence, we speculate that the greater absorption of quercetin after F feeding was a consequence of intestinal dysbiosis (35) and the reduced microbial degradation of quercetin after colostrum deprivation. However, treatment of F-fed calves with the antibiotic colistin possibly inactivated quercetindegrading bacteria, thereby contributing to elevated plasma flavonol in FQ+ calves.

Colostrum feeding enhances plasma glucose concentrations. Higher pre- and postprandial plasma glucose concentrations were previously observed in C-fed calves compared with F-fed calves, although lactose intake was similar; these higher concentrations result from slightly greater intestinal lactase activities and enhanced glucose absorption as a consequence of increases in intestinal absorptive capacity that occur after initial colostrum feeding (12, 36, 37). This phenomenon accompanies higher plasma concentrations of insulin, whereas effects on plasma glucagon concentrations are inconsistent in calves and might depend on diet and on the amounts fed to calves (12, 36). The elevated glucose and hormone concentrations observed in this study, together with postprandial plasma insulin and glucagon changes (changes were significant only in C-fed calves) might indicate an advanced maturational stage of the endocrine pancreas after colostrum feeding. The lower insulin to glucagon ratio in C-fed calves may indicate elevated endogenous glucose production and may explain the elevated plasma glucose concentrations in C-fed calves after the immediate postprandial period. In this context, gastrointestinal hormones, such as glucose-dependent insulino tropic polypeptide (GIP), might be involved. Colostrum feeding stimulates GIP secretion in neonatal calves and supports glucose-dependent insulin secretion in the endocrine pancreas (38, 39). Because blood glucose stimulates pancreatic hormone release, glucose concentrations in F-fed calves were probably too low to cause a distinct insulin secretion. On the other hand, higher plasma urea concentrations in C-fed calves might be the consequence of greater protein degradation. Protein intake by colostrum during the first 2 d was slightly higher because colostrum contained little more protein than formula. However, in previous studies, plasma urea concentrations were lower in C- than in F-fed calves (12, 36).

Higher catecholamine concentrations in C-fed calves also indicate a greater development of the endocrine system associated with glucose metabolism. Adrenaline and noradrenaline concentrations were additionally enhanced by quercetin supplementation, probably because quercetin results in the inhibition

![FIGURE 4](https://example.com/figure4.png)

**FIGURE 4** Enrichment of (A) $^{13}$C$_6$-glucose in plasma and (B) $^{13}$CO$_2$ in the blood of 7-d-old calves that were fed either C or F on days 1 and 2 with milk replacer from day 3 onward. In each dietary group, half of the calves received quercetin (50 mg/kg BW daily; CQ+, FQ+; n = 6) and half did not receive quercetin (CQ−, FQ−; n = 7). The data represent LSMs ± SEs. Labeled LSMs at a time without a common letter differ, P = 0.05. APE, atom percent excess; BW, body weight; C, colostrum; D, diet; F, formula; LSM, least squares mean; MPE, mole percent excess; Q, quercetin; T, time.

### TABLE 3 Glycogen content and relative mRNA expression in liver biopsy samples 2 h after feeding in 8-d-old calves fed colostrum (C) or formula (F) on days 1 and 2, supplemented (Q+) or not supplemented (Q−) with quercetin from days 2–8

<table>
<thead>
<tr>
<th>Group</th>
<th>CO−</th>
<th>CO+</th>
<th>FQ−</th>
<th>FQ+</th>
<th>SE</th>
<th>Diet</th>
<th>Quercetin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycogen, % fresh matter</td>
<td>3.71</td>
<td>3.24</td>
<td>3.19</td>
<td>3.55</td>
<td>0.25</td>
<td>0.84</td>
<td>0.91</td>
</tr>
<tr>
<td>Relative mRNA expression of hepatic genes, AU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6PC (BCI140011)</td>
<td>1.38</td>
<td>1.07</td>
<td>1.08</td>
<td>0.82</td>
<td>0.11</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>PC (AY185595)</td>
<td>0.88</td>
<td>1.02</td>
<td>1.12</td>
<td>1.12</td>
<td>0.06</td>
<td>0.19</td>
<td>0.58</td>
</tr>
<tr>
<td>PYGL (NM_001075203)</td>
<td>0.93</td>
<td>0.82</td>
<td>1.17</td>
<td>0.91</td>
<td>0.06</td>
<td>0.17</td>
<td>0.12</td>
</tr>
<tr>
<td>PCK1 (NM_174737)</td>
<td>0.84</td>
<td>1.22</td>
<td>1.26</td>
<td>1.42</td>
<td>0.20</td>
<td>0.48</td>
<td>0.53</td>
</tr>
<tr>
<td>PCK2 (NM_001205594)</td>
<td>1.12</td>
<td>1.37</td>
<td>0.98</td>
<td>0.88</td>
<td>0.07</td>
<td>0.03</td>
<td>0.57</td>
</tr>
</tbody>
</table>

1 Data are presented as LSMs and pooled standard errors, n = 6. Diet × quercetin interactions were nonsignificant (P > 0.05). AU, arbitrary unit; CO−, colostrum without quercetin; CO+, colostrum with quercetin; FQ−, formula without quercetin; FQ+, formula with quercetin; G6PC, glucose-6-phosphatase; LSM, least squares mean; PC, pyruvate carboxylase; PCK1, phosphoenolpyruvate carboxykinase (cytosolic); PCK2, phosphoenolpyruvate carboxykinase (mitochondrial); PYGL, glycogen phosphorylase.

of catecholamine-inactivating enzymes. Large amounts of dopamine, the precursor to noradrenaline and adrenaline, are formed in the GIT (40) and are immediately inactivated by intestinal sulfotransferases, which are also prominent in the jejunal tissues of C- and F-fed calves (41). Hence, inhibition of sulfotransferases by enteral quercetin (42) might increase plasma concentrations of circulating dopamine and therefore stimulate the synthesis of adrenaline and noradrenaline. Furthermore, quercetin inhibits other catecholamine-deactivating enzymes, such as catechol-O-methyltransferase and monoamine oxidase (43), thereby contributing to elevated catecholamine concentrations in quercetin-supplemented calves.

In the GIT, glucose is absorbed by specific transporters (37). Xylose is more suitable than glucose for use in studying the gastrointestinal absorptive capacity because xylose is absorbed by the same transporters (44) but is barely metabolized (45, 46).

In accordance with previous studies (12, 36, 47), our data reveal diminished sugar absorption in C-deprived calves. The present study revealed that even after a 5 d period of similar feeding (all calves received milk replacer from day 3 onward), C-deprived calves exhibited impaired absorption of labeled glucose; again, this finding indicated that C-feeding per se has a lasting effect on intestinal glucose absorption (48, 49).

In the small intestine, C-feeding improves mucosal growth (thus enhancing the absorptive surface) and stimulates lactose digestion (37). On the other hand, glucose absorption is obviously affected by supplementing quercetin in F-fed calves; this is shown in our study by the slightly larger peak of $^{13}$C$_6$-glucose in FQ+ than in FQ. In addition, plasma xylose increases faster after xylose feeding in FQ+ than in FQ. Quercetin is generally known to inhibit intestinal glucose transport (13, 14).

However, supplementing mice with quercetin aglycone for 28 d enhanced jejunal SGLT-1 abundance, therefore suggesting that quercetin stimulates the protein expression of glucose transporters in the apical membrane (50). Thus, enhanced membrane insertion might explain the observed higher glucose absorption after quercetin feeding in C-deprived calves. Obviously, quercetin effects on intestinal glucose absorption depend on the maturation stage of the postnatal intestine in calves.

A considerable amount of available glucose is already used in the splanchnic bed. According to studies in humans, splanchic glucose utilization decreases with age (51), which is consistent with our data. In 2-d-old calves that were similarly fed either colostrum or formula, FPU was estimated at 80% and 90%, respectively (12); however, in the present study, FPU for glucose in splanchic tissue was ~50% to 70%. When examining corrected FPU for differences in xylose absorption among groups, diet effect on FPU was lessened, indicating that uncorrected FPU is confounded to a small extent by differences in absorptive capacity. However, still, there was a greater FPU for glucose in F-fed calves, and the greater splanchic glucose extraction was probably caused by either an inability to use substrates other than glucose for energy transformation or by an insufficient availability of alternative substrates in the relevant tissues after initial F-feeding. A slightly greater rate of appearance after $[{6,6-^{2}}$H$_{2}$]-glucose ivavenous injection might also be a consequence of higher glucose absorption in C-fed calves. The FPU of glucose in the splanchic tissue was measured at 38% in goat kids and at 6% in 28-d-old piglets under common feeding conditions (23, 52), probably indicating species differences. However, quercetin supplementation did not affect the FPU of glucose in splanchic tissue.

As stated before, $^{13}$CO$_{2}$ enrichment in blood after $^{13}$C$_{6}$-glucose administration is only a proxy for true glucose oxidation (27) because we have not measured CO$_{2}$ production in our calves. There are some limitations of the use of $^{13}$C$_{6}$-glucose due to metabolic cycling and labeling of other substrates that contribute to $^{13}$CO$_{2}$ enrichment, such as lactate, pyruvate, and glycerol-3-phosphate. In the present study, $^{13}$CO$_{2}$ enrichment after $^{13}$C$_{6}$-glucose feeding was similar between the dietary groups, but quercetin treatment affected peak enrichment of $^{13}$CO$_{2}$ and its postprandial time course. The lack of feeding effects was unexpected because in 2-d-old calves, $^{13}$CO$_{2}$ enrichment after $^{13}$C$_{6}$-glucose feeding was greater in C-fed calves (12). Interestingly, when corrected for xylose absorption, $^{13}$CO$_{2}$ enrichment (AUC: 2–6 h) was greater in F-fed calves. On the other hand, oral quercetin supplementation might affect glucose oxidation kinetics in splanchic tissue. Studies in perfused rat liver revealed that although quercetin stimulates pyruvate production from glucose, it also reduces glycolysis and therefore leads to the inhibition of glucose breakdown in general (18).

Furthermore, the same authors showed that quercetin inhibits gluconeogenesis and stimulates glycogen catabolism (17). In our study, quercetin supplementation affected neither the mRNA abundances of hepatic gluconeogenic or glycogenolytic enzymes nor glycogen content; this finding is consistent with findings in dairy cows after the intraduodenal application of a comparable dose of quercetin (53).

We found greater mRNA abundances of PCK2 after colostrum feeding, consistent with observations in 5-d-old calves (54). PCK2 codes for mitochondrial phosphoenolpyruvate carboxykinase, which is involved in gluconeogenesis. Because in our previous study we have not found diet-depending differences in gluco-neogenesis (colostrum versus formula; 12), the importance of this finding with respect to effects of diet on endogenous glucose production might be minor.

In summary, our data emphasize the importance of C-feeding during the first stage of life to support gastrointestinal development and the maturation of glucose metabolism in neonatal calves. Furthermore, the feeding of quercetin to newborn calves during the first week of life does not impair glucose metabolism but affects postprandial glucose uptake and splanchic glucose oxidation. These effects partly depend on the diet. To determine whether long-term quercetin supplementation improves neonatal development or enhances productivity, further investigation is warranted.

Acknowledgments

We thank C Fiedler and O Bellmann for assistance with the experimental procedures and veterinary care, as well as P Schulz, U Lüdtke, D Mähling, C Reiko, and U Wiedemuth for laboratory assistance. We also thank Josef Aschenbrenner, Bergphor Futtermittelfabrik, and Lea Hesselmann, EW Nutrition, for assistance. We thank C Fiedler and O Bellmann for assistance with the experimental procedures and veterinary care, as well as P Schulz, U Lüdtke, D Mähling, C Reiko, and U Wiedemuth for laboratory assistance. We also thank Josef Aschenbrenner, Bergphor Futtermittelfabrik, and Lea Hesselmann, EW Nutrition, for assistance. We thank C Fiedler and O Bellmann for assistance with the experimental procedures and veterinary care, as well as P Schulz, U Lüdtke, D Mähling, C Reiko, and U Wiedemuth for laboratory assistance. We also thank Josef Aschenbrenner, Bergphor Futtermittelfabrik, and Lea Hesselmann, EW Nutrition, for assistance.

References

2. Benavides J, Martínez-Valladares M, Tejido ML, Giráldez FJ, Bódas R, Prieto N, Pérez V, Andrés S, Quercetin and flaxseed included in the diet of fattening lambs: Effects on immune response, stress during road transport and ruminal acidi-
Quercetin and bovine neonatal glucose metabolism