Effects of a 6-wk intraduodenal supplementation with quercetin on energy metabolism and indicators of liver damage in periparturient dairy cows

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ABSTRACT

Periparturient dairy cows experience metabolic challenges that result in a negative energy balance (EB) and a range of postpartum health problems. To compensate for the negative EB, cows mobilize fatty acids from adipose tissues, which can lead to fatty liver disease, a periparturient metabolic disorder. Flavonoids, such as quercetin (Q), are polyphenolic substances found in all higher plants and have hepatoprotective potential and the ability to prevent or reduce lipid accumulation in the liver. In ruminants, few studies on the metabolic effects of Q are available, and thus this study was conducted to determine whether Q has beneficial effects on EB, lipid metabolism, and hepatoprotective effects in periparturient dairy cows. Quercetin was supplemented intraduodenally to circumvent Q degradation in the rumen. Cows ($n = 10$) with duodenal fistulas were monitored for 7 wk. Beginning 3 wk before expected calving, 5 cows were treated with 100 mg of quercetin dihydrate per kilogram of body weight daily in a 0.9% sodium chloride solution for a total period of 6 wk, whereas the control cows received only the sodium chloride solution. The plasma flavonoid levels were higher in the Q-treated cows than in the control cows. A tendency for higher postpartum (pp) than antepartum (ap) plasma flavonoid levels was observed in the Q-treated cows than in the controls, which was potentially caused by a reduced capacity to metabolize Q. However, the metabolic status of the Q-treated cows did not differ from that of the control cows. The pp increases in plasma aspartate aminotransferase and glutamate dehydrogenase activities were less in the Q-treated cows than in the control cows. The Q had no effect on energy expenditures, but from ap to pp

the cows had a slight decline in respiratory quotients. Irrespective of the treatment group, the oxidation of fat peaked after calving, suggesting that the increase occurred because of an increased supply of fatty acids from lipomobilization. In conclusion, supplementation with Q resulted in lower pp plasma aminotransferase and glutamate dehydrogenase, which indicated reduced liver damage. However, the direct effects of Q on the liver and the implications for animal performance remain to be investigated.

Key words: transition dairy cow, quercetin, metabolism, liver health

INTRODUCTION

The periparturient period is defined as the 3 wk before calving and the 3 wk after calving (Drackley, 1999) and is a period with enormous metabolic challenges for dairy cows that results in a range of postpartum health problems. After the onset of and during the first 40 to 80 d of lactation, the nutrient and energy requirements for milk production exceed energy intake, which leads to a negative energy balance (EB; Bell 1995; Coffey et al., 2002).

To compensate for the negative EB, cows mobilize FA from adipose tissue. Hallmarks of typical periparturient metabolic disorders (Goff and Horst, 1997; Gross et al., 2013), fatty liver disease and ketone body formation, occur if the uptake of NEFA in the liver exceeds the oxidation capabilities or the secretion of re-esterified triacylglycerol (TG) via very low-density lipoproteins, which are generally very low in ruminants (Bremmer et al., 2000). Fatty liver disease and negative EB are associated with a greater incidence of health problems such as mastitis, metritis, milk fever, ketosis, decreased reproductive performance caused by delayed uterine involution and ovarian activity, and impaired immune function (Staples and Thatcher, 1990; Bobe et al., 2004; Esposito et al., 2014).

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In the periparturient period, 40 to 60% of high-yielding dairy cows develop subclinical ketosis, whereas 2 to 15% and up to 40% develop clinical ketosis (McArt et al., 2012) and fatty liver disease (Bobe et al., 2004), respectively. These conditions reduce animal welfare and milk production and can lead to stock and economic losses, all of which negatively affect the profitability of farms (Suthar et al., 2013; McArt et al., 2015).

Phytochemicals that have potential benefits for the health of animals are commercially available, but studies that demonstrate their effectiveness are rare. Additionally, the increase in public concern with the use of pharmaceuticals in animal production requires a closer examination of the use of phytochemicals as alternatives to pharmaceuticals (Rochfort et al., 2008).

Flavonoids are polyphenolic substances found in all higher plants, including vegetables and grains (Nijveldt et al., 2001). Some flavonoids have hepatoprotective potential (Harborne and Williams, 2000), and quercetin (**Q**) is one of the most potent flavonoids (Ader et al., 2000). Several studies, primarily with rodents and pigs, found decreased plasma lipid levels (Hoek-van den Hil et al., 2013; Jung et al., 2013; Wein and Wolfram, 2014) and prevention or reduction of lipid accumulation in the liver after **Q** treatment (Aguirre et al., 2011; Beltrán-Debón et al., 2011; Joven et al., 2012). The underlying mechanisms of these effects included an improvement in the Ω - and β -oxidation of FA in the liver and an upregulation of the genes involved in lipid metabolism (Kobayashi et al., 2010; Jung et al., 2013; Hoek-van den Hil et al., 2013). Additionally, **Q** had an effect on glucose metabolism and antidiabetic properties (Aguirre et al., 2011). For ruminants, information on the metabolic effects of **Q** is scarce. Although **Q** is largely degraded in the rumen, we recently reported that **Q** is bioavailable in cows after administration directly into the duodenum (Berger et al., 2012; Gohlke et al., 2013a). Therefore, in the present study, we investigated the potential metabolic effects of **Q** administered via a duodenal cannula, thereby circumventing degradation in the rumen. Our aim was to determine whether **Q** had metabolic effects during the periparturient phase in dairy cows by investigating the effects of **Q** on energy and lipid metabolism and milk production. We hypothesized that supplementation with duodenal **Q** in periparturient dairy cows would have beneficial effects on the EB and parameters of lipid metabolism and hepatoprotective function.

MATERIALS AND METHODS

Animals and Diet

The current study was conducted in compliance with German animal protection regulations and with

approval of the authorities of the state of Mecklenburg-Vorpommern, Germany (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischereiwesen Mecklenburg-Vorpommern, Germany; LALLF M-V/TSD/7221.3–2.1–019/12).

German-Holstein cows ($n = 10$; $>9,000$ kg of milk in first lactation) with an average BW of 759.9 ± 25.7 kg (mean $\pm$ SE) were equipped with duodenal fistulas as previously described (Gohlke et al., 2013a). The cows were monitored for a total of 7 wk, beginning 4 wk before the estimated date of their third calving. The animals had straw bedding in tiestalls, free access to water, and were offered a TMR for ad libitum intake. The composition of the diet was calculated in accordance with the recommendations of the German Society of Nutritional Physiology (GfE, 2001) with respect to gestation and lactation. The feed components and chemical composition of the TMR were determined according to the Weender standard procedure (Naumann and Bassler, 1993; Table 1). The DMI and milk yield were recorded daily. The milking frequency was twice daily at 0400 and 1430 h, and the composition of the milk was determined weekly at the Landeskontrollverband für Leistungs- und Qualitätsprüfung Mecklenburg Vorpommern e.V. The EB was calculated according to Reist et al. (2002).

Beginning 3 wk before expected calving, 5 cows were supplied daily with 100 mg of quercetin dihydrate (Carl Roth GmbH, Karlsruhe, Germany) per kilogram of BW for a total of 6 wk. The daily amount of **Q** was divided into 3 identical portions, mixed into a volume of 20 mL of 0.9% NaCl solution (Serumwerk Bernburg AG, Bernburg, Germany) and was administered at 0630, 1200, and 1800 h via the duodenal fistula. The control (**CTR**) cows ($n = 5$) received the same volume of 0.9% NaCl solution without the **Q**. After calving, a cow of the **CTR** group developed clinical ketosis and 3 cows, 1 of the **Q** treated and 2 of the **CTR** group, retained placentas. The cows were treated appropriately.

Respiration Measurements

The cows were transferred to open-circuit respiration chambers (Derno et al., 2009) for measurements of energy expenditure at 4 wk antepartum (**ap**) before **Q** treatment and after 1 (2 wk **ap**) and 6 wk [3 wk postpartum (**pp**)] of the **Q**/NaCl treatment. A cow of the **CTR** group was too tall to fit in the chamber, and thus measurements were determined for only 9 cows.

The cows were halter-trained and acclimated to the chambers before the experiment. The cows were adapted once they began to eat, to lie down, and to ruminate in the chamber. On the afternoon before the respiration measurements, the cows were placed in the chambers

Table 1. Ingredients and chemical composition of the diet (means $\pm$ SD)

Item	Amount		
	Far-off ¹	Close-up ²	Early lactation ³
Ingredient (g/kg of DM)			
Grass silage	1,000	182.5 $\pm$ 32.6	211.8 $\pm$ 14.7
Corn silage		429.9 $\pm$ 33.1	356.1 $\pm$ 25.5
Grass hay		63.1 $\pm$ 3.0	43.5 $\pm$ 23.7
Straw		63.0 $\pm$ 3.0	
Soy extract meal		64.8 $\pm$ 3.7	58.9 $\pm$ 39.7
Corn, ground		31.6 $\pm$ 1.1	87.9 $\pm$ 16.9
Rape extract meal		68.1 $\pm$ 22.0	36.6 $\pm$ 27.1
Sugar beet pulp ⁴			40.6 $\pm$ 20.8
Concentrate ⁵		92.9 $\pm$ 24.4	143.6 $\pm$ 32.4
Minerals ⁶		11.4 $\pm$ 1.3	8.7 $\pm$ 0.5
Feed lime			5.0 $\pm$ 0
Chemical composition			
DM (%)	36.8 $\pm$ 7.7	43.1 $\pm$ 0.9	44.6 $\pm$ 0.6
CP (% DM)	18.3 $\pm$ 1.3	14.9 $\pm$ 1.2	16.2 $\pm$ 0.6
Crude fat (% DM)	3.7 $\pm$ 0.6	2.9 $\pm$ 0.2	2.8 $\pm$ 0.1
Crude fiber (% DM)	25.4 $\pm$ 2.7	20.8 $\pm$ 0.4	17.5 $\pm$ 0.4
ME (MJ/kg of DM)	10.7 $\pm$ 0.6	10.6 $\pm$ 0.1	11.1 $\pm$ 0.1
NE _L (MJ/kg of DM)	6.4 $\pm$ 0.4	6.5 $\pm$ 0.1	7.0 $\pm$ 0.1

¹6–3 wk antepartum (ap); grass silage was fed ad libitum.²3–1 wk ap.³1–3 wk postpartum (pp).⁴Arp (Thorsen, Rautenberg GmbH & Co. KG, Sollerupmühle, Germany): 7.3 MJ of NE_L/kg of DM and 153 g of utilizable protein/kg of DM.⁵Concentrate MF 2000 (Vollkraft Mischfutterwerke GmbH, Güstrow, Germany): 33% extracted soy meal, 20% corn, 17% wheat gluten, 8% extracted rapeseed meal, 5% sugar beet pulp, 2% sodium hydrogen carbonate, 1.3% calcium carbonate, 0.2% sodium chloride, 8.0 MJ of NE_L/kg of DM, and 204 g of utilizable protein/kg of DM.⁶Rinderstolz 9522 lactation (Salvana Tiernahrung GmbH, Sparrieshoop, Germany): 92% crude ash, 20% calcium, 5% phosphorus, 6% magnesium, 8% sodium, and retinol, β -carotene, 25-OH-vitamin D₃, and DL- α -tocopherolacetate.

and were provided with rations. Drinking water was freely available throughout the study.

The gas exchange measurements began at 0630 h and the concentrations of CO₂, O₂, and CH₄ were measured at 6-min intervals throughout 23.9 h; the data were normalized to 24 h. The feed intake and physical activity were measured continuously for each individual cow, and the production of CO₂ and CH₄ and the consumption of O₂ were calculated as previously described (Derno et al., 2013).

Within the respiration chamber, technicians wearing face masks connected to the outside chamber air via flexible tubes to prevent adulteration of the gas exchange measurements performed the administration of Q or NaCl, daily milking, and measurements of rectal body temperature. The cows were milked at 0630 and 1630 h; the milk yield and body temperature were recorded daily. The measurements of body mass were recorded before and after stays in the respiration chambers. The heat production, fat oxidation (**FOX**), carbohydrate oxidation (**COX**), and respiratory quotient (**RQ**) were calculated as previously described (Derno et al., 2013).

Blood Sampling

Blood samples were drawn from the left jugular vein by venipuncture every week of the study on Monday, Wednesday, and Friday at 1200 h using the vacutainer system (Becton, Dickinson and Company, Plymouth, UK). Similarly, the blood samples for flavonoid analysis were collected weekly on Mondays (0800 and 1200 h) and on Fridays at 1200 h. Lithium-Heparin vacutainers (10 mL) were used to prepare plasma for the analysis of flavonoid concentrations; potassium-EDTA vacutainers (10 mL) were used for all other plasma parameters.

When in the respiration chambers, blood samples were collected from the cows through a catheter using potassium-EDTA monovettes (9 mL; Sarstedt AG & Co., Nümbrecht, Germany). The day before respiration measurements, all cows were equipped with a jugular vein catheter (Certofix Mono; B. Braun Melsungen AG, Melsungen, Germany), which was fixed with 2 stitches and a bandage (Optiplaste C; BSN medical S.A.S., Vibraye, France) and was connected to 2 m of tubing (original perfusor tubing; B. Braun Melsungen AG) to collect blood samples from outside the chamber.

Sample Processing and Analyses

The blood samples were immediately centrifuged at $1,500 \times g$ at 4°C for 20 min, and the plasma aliquots were stored at -80°C . The concentrations of glucose, cholesterol, TG, NEFA, BHBA, albumin, urea, aspartate aminotransferase (**AST**), glutamate dehydrogenase (**GLDH**), and creatine kinase (**CK**) were analyzed at the clinic for cattle of the Stiftung Tierärztliche Hochschule, Hannover, Germany, and at the Institute of Nutritional Physiology at the Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, with an automatic enzymatic analyzer (ABX Pentra 400; Horiba Medical, Montpellier, France) using commercial kits [glucose: Kit No. 553–230, cholesterol: Kit No. 553–127, and AST: Kit No. 553–256 G (MTI Diagnostics, Idstein, Germany); TG: Kit No. A11A01640 (ABX-Horiba Medical); NEFA: Kit No. 600–215S or Kit No. 434–91795/436–91995 (Wako Chemicals GmbH, Neuss, Germany); BHBA: Kit No. RB1007, urea: Kit No. LT-UR 0050 or 0010 (Labor & Technik Eberhart Lehmann GmbH, Berlin, Germany); GLDH: Kit No. 11929992216 (Roche Diagnostics GmbH, Mannheim, Germany); albumin: Kit No. A11A01664, CK: Kit No. A11A01632 (Axon Lab AG, Reichenbach, Germany)]. The albumin and CK were measured only once a week on Wednesdays. The glucagon was analyzed at the Institute of Reproductive Physiology at the Leibniz Institute for Farm Animal Biology using a commercial RIA (Kit Ref RIA-1258; DRG Instruments GmbH, Marburg, Germany).

The plasma concentrations of adrenaline and noradrenaline were determined at the Institute of Behavioral Physiology at the Leibniz Institute for Farm Animal Biology with HPLC using electrochemical detection as previously described (Weber et al., 2013). To inhibit the breakdown of catecholamines, semicarbazid was added to the monovettes before sampling the blood. The glucagon and catecholamine concentrations were measured on only the days of the respiration measurements.

The plasma flavonoid content was analyzed at the Institute of Animal Nutrition and Physiology of Christian-Albrechts-University, Kiel, Germany, as previously described (Gohlke et al., 2013a). The plasma Q and Q metabolites with an intact flavonol structure (kaempferol, tamarixetin, and isorhamnetin) were measured individually; the flavonol concentrations presented in the current paper are total flavonoids, calculated as the sum of the concentrations of Q and its metabolites.

Statistical Analyses

The data were analyzed statistically with SAS/STAT 9.3 (SAS Institute Inc., Cary, NC) using repeated-measures ANOVA (PROC MIXED). The factors were Q (yes or no), day relative to calving and the Q $\times$ day interaction. The covariance structure of the repeated factor day was modeled by autoregressive first-order [AR (1)]. The basal, ap, and pp periods were evaluated separately. The effects were significant at $P \leq 0.05$, and the differences between treatments (Q or CTR) were compared with the Tukey-Kramer tests. The data are presented as least squares means $\pm$ standard error. Because the interaction effects in the analysis of respiration measurements were not significant, they were removed from the final model.

RESULTS

Zootechnical Data

The Q treatment had no effect on BW, but BW decreased ($P < 0.001$) in both groups, from 759.9 ± 25.7 kg at 4 wk ap to 601 ± 25.4 kg at 3 wk pp. The DMI was not different between the Q-treated and control groups and between 4 wk ap (10.4 ± 1.2 kg/d) and wk 1 ap (9.6 ± 1.1 kg/d; $P > 0.4$); however, the DMI increased ($P < 0.05$) after calving (13.8 ± 1.1 kg/d) at 2 wk pp. The feed energy intake was higher ($P < 0.05$) after calving in both groups, increasing from 60.6 ± 8.8 MJ of NE_L/d at 4 wk ap to 89.7 ± 8.3 MJ of NE_L/d at 2 wk pp. The composition of the milk was not affected ($P > 0.05$) by the Q supplementation. The milk yield increased ($P < 0.001$), although no difference was found between the Q and CTR cows, from 24.3 ± 3.5 kg/d in the first week of lactation to 32.7 ± 2.6 kg/d on d 19 pp. The latter value for milk yield corresponded to 33.9 ± 3.2 kg/d of ECM. The milk protein levels decreased ($P < 0.001$) in the first 3 wk of lactation from d 3 pp with $4.4 \pm 0.2\%$ to $2.9 \pm 0.2\%$ on d 18 pp, which corresponded to 1.1 ± 0.1 and 1.0 ± 0.1 kg of milk protein/d, respectively ($P = 0.8$). No change ($P = 0.5$) was detected in milk fat concentration with time, which was $5.3 \pm 0.3\%$ in the first 3 wk of lactation. The lactose concentration in milk increased ($P < 0.001$) with $4.2 \pm 0.1\%$ on d 3 pp to $4.7 \pm 0.1\%$ on d 18 pp, which corresponded to 1.0 ± 0.1 and 1.5 ± 0.1 kg of lactose/d, respectively. The EB ap and pp was not affected by the Q treatment but was lower ($P < 0.001$) after calving than before and reached negative values with a nadir of -55.5 ± 17.6 MJ of NE_L/d on d 9 pp.

Plasma Flavonoids

The major flavonoid in plasma was Q with minor levels of kaempferol, tamarixetin, and isorhamnetin (data not shown). The total plasma flavonoid concentrations were higher ($P < 0.05$) in the Q-treated cows than in the CTR cows on Mondays and Fridays at 1200 h throughout the 6-wk period of Q supplementation (166.9 ± 23.4 vs. 5.2 ± 24.1 nmol/L, respectively; Figure 1). The concentrations were higher at 0800 ($P < 0.05$) than at 1200 h (357.3 ± 42.9 vs. 164.0 ± 27.8 nmol/L, respectively). A trend toward a higher 1200-h plasma flavonoid concentration pp than ap was observed (242.1 ± 49.7 vs. 96.9 ± 49.7 nmol/L, respectively; $P = 0.09$), an increase that was equivalent to 150% higher plasma flavonoid concentration after calving. By contrast, the plasma flavonoid levels at 0800 h (1.5 h after the first daily dose at 0630 h) tended to be lower pp than ap (194.46 ± 99.9 vs. 410.3 ± 96.1 nmol/L, respectively; $P = 0.08$).

Plasma Metabolites, Enzymes, and Hormones

The base levels of plasma metabolites, hormone concentrations, and enzyme activities were not different between the cow groups before the start of the Q supplementation, although plasma AST activity had a tendency to be higher in the CTR cows ($P = 0.053$; Table 2). A significant treatment $\times$ day interaction was detected for only glucose in the ap period. With the exception of plasma AST activity, the metabolite and hormone concentrations were not affected by the Q treatment, but some were influenced by day relative to calving (Table 2). The plasma NEFA concentrations increased from basal values to calving and then increased ($P < 0.05$) further pp until 21 d after calving. The plasma BHBA levels showed no difference between the Q-treated cows and the CTR cows, but there was an increase from basal values to calving and then a further increase immediately after calving ($P < 0.05$). The plasma TG and glucose levels decreased ($P < 0.05$) after the onset of lactation. The plasma cholesterol levels decreased from basal values to calving ($P < 0.05$) and then increased pp ($P < 0.05$) from a nadir shortly after calving; lower values were observed in the pp period than in the ap period. The plasma noradrenaline concentrations increased ($P < 0.05$) from wk 2 ap to wk 3 pp (220.5 ± 36.1 vs. 301.9 ± 34.7 ng/L, respectively). For the liver enzymes, the plasma AST activity showed an effect of the intraduodenal Q administration during both ap ($P < 0.05$) and pp ($P < 0.05$) periods, with higher values in CTR cows and an increase ($P < 0.05$) in both groups beginning 1 d after

calving; an increase that was much more pronounced in the CTR cows (Figure 2). By contrast, the plasma GLDH (Figure 3) and CK activities did not differ ($P > 0.05$) between the groups.

Energy Expenditure

The measures of energy expenditure were not affected by Q supplementation but showed time effects relative to calving. The daily heat production increased ($P < 0.001$) from 823.7 ± 32.6 kJ/kg of $BW^{0.75}$ at 4 wk ap (before Q supplementation) to 987.6 ± 32.5 kJ/kg of $BW^{0.75}$ at 3 wk pp, whereas the RQ decreased ($P < 0.05$) from 4 wk ap to 3 wk pp (0.82 ± 0.02 vs. 0.80 ± 0.02 , respectively). The calculated FOX increased ($P < 0.05$) from 4 wk ap (466.8 ± 61.2 kJ/kg of $BW^{0.75}$) to 607.8 ± 60.9 kJ/kg of $BW^{0.75}$ at 3 wk pp. Similarly, the COX increased ($P < 0.05$) from 384.4 ± 85.5 kJ/kg of $BW^{0.75}$ at 4 wk ap to 403.0 ± 85.2 kJ/kg of $BW^{0.75}$ at 3 wk pp, but the highest COX values were found at 2 wk ap with 466.3 ± 87.6 kJ/kg of $BW^{0.75}$.

The production of methane was higher ($P < 0.05$) at 3 wk pp (380.0 ± 36.7 L/d) than at 4 wk ap (313.1 ± 36.7 L/d) and was not affected in the Q-treated cows. By contrast, when related to DMI at 4 wk ap, the production of methane was higher ($P < 0.05$) than at 3 wk pp (37.9 ± 1.0 vs. 30.7 ± 1.5 L/kg of DMI, respectively).

DISCUSSION

In a previous study by our group (Gohlke et al., 2013a) with a single dose of 27 mg of Q/kg of BW per day, the baseline plasma flavonoid values were reached 4 h after intraduodenal administration. In the present study we used 100 mg of Q/kg of BW per day, administered in 3 daily aliquots, to generate a constantly elevated level of plasma flavonoids throughout the day. This amount of Q resulted in higher levels of plasma flavonoids in the Q-treated than in the CTR cows 5.5 h (1200-h samples) after administration of the morning aliquot. Based on the decrease in flavonoid concentration between 0800 and 1200 h, it was assumed that plasma flavonoids were still significantly elevated at least 5.5 h after the last dosing (0600 h; i.e., until approximately midnight, and possibly longer). Thus, the dosing scheme used in the present study likely elevated levels of plasma flavonoids during at least 18 of 24 h.

The concentrations of plasma flavonoids at 1200 h were higher pp than ap, which suggested an effect of the stage of lactation, the status of the liver, or both on total plasma flavonoid concentrations. The liver is particularly important for the elimination of Q and its metabolites (Lesser and Wolfram, 2006). The

Figure 1. Concentrations of total flavonoids in plasma of cows with (A) or without (B) intraduodenal quercetin (Q) supplementation in the periparturient period. The shaded area indicates intraduodenal Q supplementation from wk -3 to 3 relative to calving. A total amount of 100 mg of quercetin dihydrate per kilogram of BW was given daily in 3 equal doses at 0630, 1200, and 1800 h. Values are LSM $\pm$ SE of $n = 5$ cows per group. The day of calving is the dotted vertical line. Blood samples were collected on Mondays at 0800 (hatched bars) and 1200 h (plain bars) and on Fridays at 1200 h (plain bars). The 1200-h samples were collected before administering the 1200-h dose of Q. Please note the different scaling for treatment (A) and control (B) groups.

Table 2. Plasma levels of metabolites, hormones and enzymes before (basal) and during quercetin (Q) supplementation from 3 wk before to 3 wk after parturition in cows¹

Item	Group		P-value	
	Quercetin	Control	Treatment	Day
NEFA ($\mu\text{mol/L}$)				
Basal ²	264.6 $\pm$ 76.1	272.9 $\pm$ 76.1	0.940	<0.001
ap ³	235.3 $\pm$ 60.8	274.4 $\pm$ 67.2	0.675	0.019
pp ⁴	889.3 $\pm$ 131.6	954.9 $\pm$ 120.4	0.719	0.003
BHBA (mmol/L)				
Basal	0.6 $\pm$ 0.1	0.5 $\pm$ 0.1	0.785	0.788
ap	0.5 $\pm$ 0.0	0.6 $\pm$ 0.0	0.606	0.765
pp	1.4 $\pm$ 0.4	2.3 $\pm$ 0.3	0.136	0.370
Triglycerides (mmol/L)				
Basal	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.674	0.707
ap	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.708	0.172
pp	0.1 $\pm$ 0.0	0.1 $\pm$ 0.0	0.382	0.023
Cholesterol (mmol/L)				
Basal	2.9 $\pm$ 0.3	2.5 $\pm$ 0.3	0.398	0.148
ap	2.2 $\pm$ 0.2	2.1 $\pm$ 0.2	0.821	0.005
pp	2.1 $\pm$ 0.2	2.2 $\pm$ 0.2	0.857	0.002
Glucose (mmol/L)				
Basal	3.6 $\pm$ 0.1	3.7 $\pm$ 0.0	0.258	0.125
ap ⁵	3.5 $\pm$ 0.1	3.7 $\pm$ 0.1	0.246	0.099
pp	2.8 $\pm$ 0.1	2.7 $\pm$ 0.1	0.499	0.006
Albumin (g/L)				
Basal	32.7 $\pm$ 1.0	33.5 $\pm$ 1.0	0.553	— ⁶
ap	31.1 $\pm$ 0.7	30.4 $\pm$ 0.7	0.526	0.679
pp	31.0 $\pm$ 1.2	30.5 $\pm$ 1.1	0.784	0.494
Urea (mmol/L)				
Basal	4.4 $\pm$ 0.4	3.8 $\pm$ 0.5	0.405	0.049
ap	4.3 $\pm$ 0.5	4.4 $\pm$ 0.5	0.869	0.439
pp	3.7 $\pm$ 0.4	3.2 $\pm$ 0.3	0.361	0.116
Aspartate aminotransferase (U/L)				
Basal	61.6 $\pm$ 7.4	85.1 $\pm$ 7.4	0.053	0.178
ap	60.6 $\pm$ 4.5	86.4 $\pm$ 5.0	0.004	0.210
pp	93.5 $\pm$ 14.2	170.4 $\pm$ 13.7	0.003	0.007
Glutamate dehydrogenase (U/L)				
Basal	14.5 $\pm$ 1.8	9.0 $\pm$ 1.0	0.059	0.625
ap	16.0 $\pm$ 3.5	8.7 $\pm$ 3.7	0.187	0.442
pp	23.7 $\pm$ 10.0	37.7 $\pm$ 9.3	0.321	0.344
Creatine kinase (U/L)				
Basal	111.2 $\pm$ 30.6	172.2 $\pm$ 30.6	0.197	— ⁶
ap	78.8 $\pm$ 17.7	115.4 $\pm$ 19.4	0.209	0.705
pp	135.1 $\pm$ 30.9	215.9 $\pm$ 23.9	0.094	0.174
Glucagon ⁷ (ng/L)				
Basal	150.0 $\pm$ 28.7	102.8 $\pm$ 32.1	0.309	— ⁶
ap	134.5 $\pm$ 21.9	112.4 $\pm$ 26.9	0.541	— ⁸
pp	92.7 $\pm$ 23.5	111.8 $\pm$ 24.5	0.591	— ⁸
Adrenaline ⁷ (ng/L)				
Basal	44.6 $\pm$ 9.0	31.2 $\pm$ 10.1	0.354	— ⁶
ap	27.9 $\pm$ 3.2	20.3 $\pm$ 4.1	0.157	— ⁸
pp	27.8 $\pm$ 3.2	21.3 $\pm$ 3.6	0.209	— ⁸
Noradrenaline ⁷ (ng/L)				
Basal	235.2 $\pm$ 42.5	203.3 $\pm$ 47.5	0.633	— ⁶
ap	241.4 $\pm$ 46.3	199.5 $\pm$ 55.3	0.575	— ⁸
pp	286.8 $\pm$ 46.3	316.9 $\pm$ 51.7	0.676	— ⁸

¹Values are LSM $\pm$ SE; n = 5 per group.²Values of week before start of quercetin (or NaCl) supplementation.³Ant partum (ap); mean values of treatments 3 wk before calving.⁴Postpartum (pp); mean values of treatments 3 wk after calving.⁵Interaction of treatment $\times$ day; *P*-value <0.05.⁶Only one sample before quercetin supplementation.⁷Plasma glucagon and catecholamine concentrations are from days of respiration measurements only.⁸Only one sample in time period.

Figure 2. Plasma aspartate aminotransferase (AST) activity levels in cows with (Δ ; solid line) or without ($\bullet$; dashed line) intraduodenal quercetin supplementation in the periparturient period. Values are LSM $\pm$ SE of $n = 5$ cows per group. The shaded area indicates the period of quercetin supplementation from wk -3 to 3, relative to calving. The day of calving is the dotted vertical line.

metabolism and elimination of drugs in the liver can be impaired in early-lactating cows (Lake et al., 2011; Merrell and Cherrington, 2011); thus, it is possible that the capacity to metabolize Q in the liver was reduced pp, which could explain the higher plasma Q concentrations pp. However, at 0800 h we observed lower levels of plasma Q pp than ap, which could be attributed to a lower absorption of Q due to faster passage through the digestive tract after calving (Aikman et al., 2008).

Similar to the effects on cows in midlactation (Gohlke et al., 2013b), chronic administration of Q had no effect on DMI, milk yield, or BW. However, in contrast to our previous finding of increased milk protein in midlactation Q-treated cows (Gohlke et al., 2013b), we detected no changes in milk protein in the present study. Whether this result was due to the stage of lactation or another reason is not clear at present. As expected, the EB decreased after calving and reached a nadir 9 d pp, largely caused by the increase in milk yield.

In previous studies, Q lowered concentrations of plasma lipids (Hoek-van den Hil et al., 2013; Jung et al., 2013; Wein and Wolfram, 2014), which was explained by the upregulation of genes involved in β - and Ω -FA oxidation and by the downregulation of ketogenic genes

(Kobayashi et al., 2010). However, in contrast to our hypothesis, the Q treatment did not improve metabolic status compared with that of the control cows. The concentrations of plasma NEFA and BHBA are indicators of energy status and lipomobilization (Cozzi et al., 2011) and are highly associated with hepatic lipidosis (Mostafavi et al., 2013). According to Duffield et al. (2009) and Gonzalez et al. (2011), high lipomobilization is characterized by NEFA >400 $\mu\text{mol/L}$ and subclinical ketosis is characterized by BHBA >1.2 mmol/L. In our study, the cows had greater concentrations of NEFA and BHBA after calving (>850 and >1.3 mmol/L, respectively), which indicated that they suffered from subclinical ketosis. As found in other studies, the levels of plasma glucose were lower pp than ap, which indicated the use of glucose for milk production and thus low availability of systemic glucose (Bell, 1995; Djokovic et al., 2009; Cozzi et al., 2011; Weber et al., 2013). The plasma concentrations of TG, cholesterol, albumin, and urea are indicators of liver function and biosynthetic capacity, which are reduced in cases of fat infiltration and hepatocyte degeneration (Djokovic et al., 2009, 2013). After calving, we found lower levels of plasma TG and cholesterol. Reduced levels of

Figure 3. Plasma glutamate dehydrogenase (GLDH) activity levels in cows with (Δ ; solid line) or without ($\bullet$; dashed line) intraduodenal quercetin supplementation in the periparturient period. Values are LSM $\pm$ SE of $n = 5$ cows per group. The shaded area indicates the period of quercetin supplementation from wk -3 to 3 relative to calving. The day of calving is the dotted vertical line.

plasma albumin and urea may indicate liver cell damage (Grummer, 1993; Djokovic et al., 2009), but we did not find any changes in plasma albumin or urea. The catecholamines provoke lipolysis, particularly during lactation (Vernon, 2005). Increased nutrient intake stimulates the sympathetic nervous system and, thus, the action of noradrenaline, which is accompanied by increases in thermogenesis (Landsberg, 2006). This relationship was consistent with the increase in plasma values of noradrenaline from ap to pp and the higher heat production pp in cows in the present study.

To investigate liver damage, we measured the activities of AST and GLDH in plasma. Severe fat infiltration causes hepatocyte destruction with the subsequent release of the cytosolic enzyme AST (Liu et al., 2012). Plasma AST values above 100 U/L indicate hepatic lesions and a fatty liver (Gonzalez et al., 2011; Mostafavi et al., 2013). By contrast, GLDH is specifically expressed in the liver and increased levels in plasma suggest hepatocyte death or sublethal injury (Ok et al., 2013). Both the Q-treated and CTR cows in this experiment showed an increase in levels of plasma AST from ap to pp. Before Q supplementation, the plasma AST tended to be higher in the CTR cows but re-

mained within the normal range ap (Thurmann, 2012). The increase in AST values pp was considerably less in the Q-treated cows than in the CTR cows. This observation was similar to findings for mice and rats with nonalcoholic steatohepatitis and cirrhosis, respectively, in which Q supplementation inhibited the development of steatosis and reduced levels of AST (Peres et al., 2000; Marcolin et al., 2013). Because AST is not liver-specific, we also measured the plasma activity of CK (Kalaitzakis et al., 2010). With CK activity, no difference between the CTR and Q-treated cows was detected, leading us to conclude that the observed effects in regard to AST values were not caused by a release from muscle cells. Thus, our observations were solely because of differences in liver damage between the groups. Plasma GLDH concentrations, by contrast, were not affected by the Q treatment after calving. However, in the CTR cows, the numerical increase in plasma GLDH values from ap to pp was approximately 300%, whereas in the Q-treated cows the increase was only 50% ($P < 0.15$), which indicated that Q supplementation also had a potential hepatoprotective effect with respect to GLDH values. An explanation for why the effect of Q was more apparent with plasma AST than GLDH

could be that GDLH is a mitochondrial enzyme that is only elevated with complete cell destruction, whereas AST is a hepatocellular leakage enzyme released from the cytosol through cell damage (Ok et al., 2013).

The reported effects of Q and other flavonols on energy expenditure in humans (Dulloo et al., 1999; Hursel et al., 2011) and mice (Stewart et al., 2008) are inconsistent (Klaus et al., 2005; Egert et al., 2011). We measured several components of energy metabolism via indirect calorimetry but did not detect effects of Q on energy expenditure. Nevertheless, from the ap to pp period, energy expenditure increased in both groups because of the onset of milk production (Jentsch et al., 2001). The RQ is indicative of changes in oxidative substrate and ranges from 1.0, which represents pure carbohydrate oxidation, to 0.7 for pure fat oxidation (Kim et al., 2013). The cows in our study showed a slight decline in RQ from wk 4 ap to wk 3 pp; furthermore, FOX peaked at 3 wk pp. These results suggest an increase in FA oxidation that reflected the greater supply of FA as an energy source from lipomobilization after calving (Dulloo et al., 1999; Börner et al., 2013).

Together, our results confirmed the typical changes that occur in energy, nutrient, and liver metabolism of periparturient cows associated with diseases in the transition period (Suthar et al., 2013). The primary novel finding of the current study was that Q supplementation was beneficial in periparturient cows because of the reduction in liver damage, which was indicated by increased plasma AST activities and a lower, although not significant, increase of plasma GDLH activities in the Q-treated group. Notably, the pp levels of plasma BHBA were lower, although not significant, in the Q-treated cows than in the CTR cows, an observation that supports our conclusion that Q had a beneficial effect on liver metabolism in periparturient cows because lower levels of BHBA in periparturient cows were associated with a lower risk of ketosis and hepatic lipidosis (Gonzales et al., 2011; Mostafavi et al., 2013). Nevertheless, we recognize that the CTR cows tended to have higher basal levels of plasma AST, but the extent to which the higher level might have affected AST levels pp is not clear. However, possible effects caused by the health of the cows in the CTR group on our findings cannot be excluded, and the observed Q effects must be viewed with caution. To confirm our observations, direct investigations of liver tissue are required to elucidate possible mechanisms of action. Additionally, these results should be verified in a larger number of cows.

We recognize that intraduodenal administration of Q is irrelevant in practical conditions. However, if a rumen-protected Q preparation became available that could be applied orally to pass through the rumen

largely intact, the Q supplementation might provide benefits to alleviate liver disease in transition cows. Such a preparation could be rutin, a glucorhamnoside of Q, which is not degraded in the rumen but leads to absorption of flavonoids, although rutin is not bioavailable when administered in the duodenum of cows (Berger et al., 2012; Gohlke et al., 2013a).

CONCLUSIONS

Our objective was to investigate the effect of Q on energy and lipid metabolism and milk production with a Q dose of 100 mg/kg of BW per day applied intraduodenally for 6 wk, beginning 3 wk before parturition. In conclusion, we did not find any effect of Q on EB, plasma metabolites, or performance in periparturient dairy cows, and the only effect was on the plasma activity of the liver enzyme AST. This lower activity of AST indicated that Q could have hepatoprotective effects. However, further studies are required to substantiate a potential beneficial effect of Q on hepatic health in periparturient dairy cows and to clarify the implications of these effects on animal performance.

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