Effects of rutin and buckwheat seeds on energy metabolism and methane production in dairy cows

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ABSTRACT

Flavonoids are secondary plant metabolites with several health promoting effects. As dairy cows often suffer from metabolic imbalance and health problems, interest is growing in health improvements by plant substances such as flavonoids. Our group has recently shown that the flavonoids quercetin and rutin (a glucorhamnoside of quercetin) are bioavailable in cows when given via a duodenal fistula or orally, respectively, affect glucose metabolism, and have beneficial effects on liver health. Furthermore, flavonoids may reduce rumen methane production in vitro through their antibacterial properties. To test the hypothesis that rutin has effects on energy metabolism, methane production, and production performance in dairy cows, we fed rutin trihydrate at a dose of 100 mg/kg of body weight to a group of 7 lactating dairy cows for 2 wk in a crossover design. In a second experiment, 2 cows were fed the same ration but were supplemented with buckwheat seeds (*Fagopyrum tartaricum*), providing rutin at a dose comparable to the first experiment. Two other cows receiving barley supplements were used as controls in a change-over mode. Blood samples were taken weekly and respiration measurements were performed at the end of each treatment. Supplementation of pure rutin, but not of rutin contained in buckwheat seeds, increased the plasma quercetin content. Methane production and milk yield and composition were not affected by rutin treatment in either form. Plasma glucose, β -hydroxybutyrate, and albumin were increased by pure rutin treatment, indicating a possible metabolic effect of rutin on energy metabolism of dairy cows. In addition, we did not show that in vivo ruminal methane production was reduced by rutin. In conclusion, we could not confirm earlier reports on in vitro methane reduction by rutin supplementation in dairy cows in established lactation.

Key words: dairy cow, rutin, quercetin, methane, metabolism

INTRODUCTION

Flavonoids belong to the large group of secondary plant metabolites with phenolic structures (Formica and Regelson, 1995; Nijveldt et al., 2001). Flavonoids have been reported to have various health-promoting effects due to their antiviral, antibacterial, anti-inflammatory, and antihepatotoxic properties. Quercetin, as one of the best described flavonoids in plants, is mainly bound to sugars forming glycosides, such as rutin, whereas quercetin aglycone is bound to rutinose (Nijveldt et al., 2001). Rutin is one of the most consumed flavonoids in human nutrition and is found, for example, in tea and buckwheat (Formica and Regelson, 1995; Materska, 2008). Interest is growing in the use of plant-derived substances as a potential natural alternative to using synthetic agents and pharmaceuticals to improve animal health and productivity (Materska, 2008; Rochfort et al., 2008).

In view of the beneficial effects flavonoids have in humans and rodents (Panchal et al., 2011; Galleano et al., 2012), increasing interest has been noted in possible health benefits among dairy cows by plant bioactive compounds and, in particular, flavonoids. Associated with the onset of high milk production, dairy cows experience negative energy balance often followed by metabolic imbalances and health problems. Our group has recently shown that quercetin contained in rutin is much more bioavailable in cows compared with quercetin aglycone when administered intraruminally (Berger et al., 2012), whereas quercetin aglycone requires administration via a duodenal fistula to be systemically available (Gohlke et al., 2013a). Some beneficial effects on performance (Guo et al., 2010), glucose metabolism, as well as milk composition (Gohlke et al., 2013b) and liver health (Stoldt et al., 2015) were reported in dairy cows. Furthermore, it is known from other species that flavonoids affect energy, in particular glucose and lipid

Received July 20, 2015.

Accepted December 1, 2015.

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metabolism (Hoek-van den Hil et al., 2013; Wein and Wolfram, 2014).

Some in vitro studies using pure flavonoids (Oskoueian et al., 2013; Seradj et al., 2014) or plants rich in flavonoids (Wang et al., 2013; Niderkorn and Macheboeuf, 2014) revealed the potential to reduce methane production without negative effects on fermentation processes (Oskoueian et al., 2013; Berger et al., 2015). Flavonoids have been shown to alter the rumen microbiome and to reduce the population of protozoa and methanogenic bacteria (Santra et al., 2012; Oskoueian et al., 2013), which is presumably the underlying mechanism of reduced methane production. Methane is a potent greenhouse gas with enormous climate-warming potential (Etim et al., 2013), and ruminant livestock is an important contributor to anthropogenic methane emission (Pitesky et al., 2009). Furthermore, methane production by rumen microbes implies loss of gross energy for the cow. Thus, a potential reduction in methane production by flavonoids would be environmentally favorable.

To investigate whether selected flavonoids affect health, energy metabolism, and methane emission in dairy cows, we conducted a feeding trial with rutin including indirect calorimetry gas exchange measurements (Derno et al., 2009). Because quercetin is largely degraded by rumen microbes when given orally (Berger et al., 2012), we chose rutin as a likely rumen-protected source of quercetin (Berger et al., 2012) for supplementation. Given that buckwheat has a high rutin content (Fabjan et al., 2003) and is suitable as feed for ruminants (Amelchanka et al., 2010; Kälber et al., 2012), we also tested whether supplementation of tartary buckwheat (*Fagopyrum tartaricum*) seeds have properties comparable to pure rutin isolate. We hypothesized that oral supplementation of rutin as a pure substance or via buckwheat seeds would have beneficial effects on energy metabolism, methane production, and milk yield and composition in dairy cows in established lactation.

MATERIALS AND METHODS

Animals and Diet

The study was conducted in compliance with the German animal protection regulations with approval of the authorities of the land Mecklenburg-Vorpommern, Germany (Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischereiwesen Mecklenburg-Vorpommern, Germany; LALLF M-V/TSD/7221.3-2.1-019/12).

In the study, 7 German Holstein cows with >7,000 kg of milk in their first lactation and an average BW of 608 ± 14 kg (mean $\pm$ SE) were monitored over a

Table 1. Ingredients and chemical composition (g/kg of DM, unless otherwise noted) of TMR diet (means)

Item	Measurement
Ingredient	
Grass silage	173
Corn silage	332
Straw	34.9
Grass hay	38.9
Corn (ground)	79.5
Soybean meal	19.9
Rapeseed meal	20.1
Sugar beet pulp ¹	37.7
Concentrate ²	256
Minerals ³	8.9
Feed lime	4.5
Chemical composition	
DM (g/kg of FM ⁴)	508
Crude ash	67
CP	161
NDF	343
Crude fat	30
ME ⁵ (MJ/kg of DM)	11.2
NE _L ⁵ (MJ/kg of DM)	7

¹Arp, (Thordsen, Rautenberg GmbH & Co. KG, Sollerupmühle, Germany): 7.3 MJ of NE_L/kg of DM and, 153 g of utilizable protein/kg of DM.

²Concentrate 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.

³Rinderstolz 9522 lactation (Salvana Tiernahrung GmbH, Sparrieshoop, Germany): 92% crude ash, 20% calcium, 5% phosphorus, 6% magnesium, 8% sodium, and 0.03% retinol, 0.06% β -carotene, 0.0005% 25-OH-vitamin D₃, and 0.40% DL- α -tocopherolacetate.

⁴FM = fresh matter.

⁵Calculated as recommended by the German Society of Nutrition Physiology (GfE, 2004).

period of 4 wk starting on d 100 of the second lactation. The cows were kept in tiestalls, had free access to water, and were offered a TMR for ad libitum intake. The diet composition was calculated in accordance to recommendations of the German Society of Nutritional Physiology (GfE, 2004). Chemical composition of the TMR was determined according to Weender standard procedure (Naumann and Bassler, 1993) and is shown together with the feed components in Table 1. The DMI and milk yield were recorded daily, milking time was daily at 0630 and 1630 h.

Cows were fed a TMR only (control) or with daily supplementation of 100 mg of rutin trihydrate (Carl Roth GmbH, Karlsruhe, Germany) per kilogram of BW for a period of 2 wk in a crossover design, resulting in a 4-wk experimental period. The rutin dose used was based on the results of a previous study of our group (Berger et al., 2012). Subsequently, in a second experiment, 2 of the cows received the same TMR plus

6.5 g/kg of BW tartary buckwheat seeds (*Fagopyrum tartaricum*; Deutsche Saatveredelung AG, Lippstadt, Germany) daily for 2 wk with a rutin content of 1.54% DM, which is equivalent to 100 mg of rutin/kg of BW. Seeds were ground to a particle size of 3 mm. Two control cows received an equal amount of barley for the same period of time in a change-over mode. For both supplements, daily amounts were divided into 2 equal portions, given at 0730 and 1630 h, and mixed into a small amount of TMR before regular feed submission.

Respiration Measurements

During the last 2 d of each 2-wk treatment (rutin or TMR), cows were transferred to open-circuit respiration chambers (Derno et al., 2009) for 48-h measurements of components of energy expenditure. On the first day of measurement, cows were fed ad libitum. On the second day, residual feed was taken away at 0630 h and cows were not fed again until 1630 h (restrictive feeding) to expose cows to a metabolic challenge, as we suspected that flavonoid effects could be more pronounced under challenging conditions. Cows were fed again with TMR ad libitum.

Cows were acclimated to the chambers several times before the experiment until they started eating, laying down, and ruminating in the chamber. In the afternoon before the first day of respiration measurement, cows were placed in the chambers and were provided with their respective rations. Drinking water was freely available the entire time.

Gas exchange measurements were started at 0700 h on each respiration day. Concentrations of CO₂, O₂, and CH₄ were measured at 6-min intervals throughout 23.9 h and data were normalized to 24 h. Feed intake and physical activity were measured continuously for each cow individually, and production of CO₂ and CH₄ as well as consumption of O₂ was calculated as described previously (Derno et al., 2013). Within the respiration chamber, daily milking and cleaning were performed wearing a facemask connected to ambient air via flexible tubes to prevent adulteration of gas exchange measurement. Cows were milked at 0630 and 1630 h. The BW was determined before and after the stay in the respiration chamber.

Heat production, fat oxidation, carbohydrate oxidation, and respiratory quotient were calculated as described previously (Chwalibog et al., 1996; Derno et al., 2013). For calculation of the respiratory quotient, which reflects the metabolism of absorbed nutrients, metabolic CO₂ production (i.e., corrected for fermentative CO₂ production) was used. Heat production was calculated according to Brouwer (1965) with total CO₂

production as Brouwer's equation includes fermentative heat production (term for methane production).

Blood Sampling

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 connected to 4 m of tubing (Original Perfusor Line, B. Braun Melsungen AG, Melsungen, Germany), to allow blood sampling from outside the chamber. After 1 wk of each treatment and during the stay in the respiration chamber, blood samples were taken at 0630, 0830, 1030, 1530, 1730, and 1930 h through the catheter using potassium-EDTA and lithium-heparin monovettes (9 mL, Sarstedt AG & Co., Nümbrecht, Germany).

Sample Processing and Analyses

Blood samples were immediately centrifuged at 1,500 × *g* at 4°C for 20 min and plasma aliquots were stored at −80°C. Cholesterol, glucose, triglycerides (TG), fatty acids, BHB, albumin, urea, and lactate concentrations were analyzed with an automatic enzymatic analyzer (ABX Pentra 400, Horiba Medical, Montpellier, France) using commercial kits (Glucose: Kit 553–230, cholesterol: Kit 553–127, all MTI Diagnostics, Idstein, Germany; TG: Kit A11A01640, ABX-Horiba Medical, Montpellier, France; fatty acids: Kit 600–215S or Kit 434–91795/436–91995, all WAKO Chemicals GmbH, Neuss, Germany; BHB: Kit RB1007, urea: Kit LT-UR 0050 or 0010, all Labor & Technik Eberhart Lehmann GmbH, Berlin, Germany; albumin: Kit A11A01664, lactate: Kit A11A01721, all Axon Lab AG, Reichenbach, Germany). Plasma TG, lactate, fatty acids, and glucose concentrations were analyzed in all samples, whereas cholesterol, albumin, BHB, and urea were only analyzed at 1030 h and not on the restrictive day of respiration measurements.

Glucagon and insulin in samples taken at 0630, 0830, and 1030 h were analyzed using commercial RIA (Kit RIA-1258 and 1257, DRG Instruments GmbH, Marburg, Germany). Both variables were only measured during the ad libitum day of respiration measurements.

Plasma flavonoid content in 0830-h samples was analyzed as described by Gohlke et al. (2013a). Plasma quercetin and quercetin metabolites kaempferol, tamarixetin, and isorhamnetin were measured; concentration values presented in this paper are total plasma flavonoids, calculated as the sum of concentrations of quercetin and its metabolites.

Statistical Analysis

Data obtained from the measurements in respiration chambers were analyzed using SAS Institute Inc. (2014) with a mixed model including the fixed effects of diet (with or without rutin), feeding regimen (ad libitum or restrictive during the stay in respiration chamber only), the interaction between diet and feeding regimen as well as effect of order of treatment assignment (rutin or control TMR given first), and effect of period in which cows received different diets (rutin or control; Kaps and Lamberson, 2004). Individual effect of cow (subject) was included in the model as random.

Plasma parameters measured on one time point per day only (cholesterol, albumin, BHB, urea, and flavonoids) were analyzed with a mixed model including the fixed effects of diet, order of treatment assignment, periods, and effect of residence (respiration chamber or not). Individual effect of cow was included in the model as random.

Repeatedly measured plasma metabolites (fatty acids, TG, glucose, and lactate) were analyzed with a repeated measures ANOVA model that included effects of diet, feeding regimen, the interaction term between diet and feeding regimen, effects of orders, and period, residence, time of day (mornings, afternoons), and sampling time relative to supplementation (1–3) as well as interaction between time of day and relative time. Effect of repeatedly sampled cow was considered random.

Data for repeatedly (at 0630, 0830 and 1030 h) measured plasma hormones (insulin and glucagon) were analyzed with another repeated measures ANOVA model that included effects of diet, order, residence, periods

and effects of sampling time relative to supplementation. Effect of repeatedly sampled cow was considered random.

Variance structure for the repeated measures ANOVA models was variance components as it provided better (smaller) model fit parameters. Effects were considered significant at $P \leq 0.05$ and as a trend at $P \leq 0.1$. Data are presented as least squares means $\pm$ standard error.

RESULTS

Performance

Average milk yield (33.2 ± 1.40 kg/d) and DMI (21.6 ± 0.61 kg /d) during the experimental period were not affected by rutin supplementation or feeding regimen. Milk components were not influenced by rutin supplementation and were $3.8 \pm 0.16\%$ fat, $3.2 \pm 0.10\%$ protein, and $4.9 \pm 0.05\%$ lactose. The BW was lower ($P < 0.001$) after respiration measurements than before (606 ± 16 vs. 625 ± 16 kg) and not influenced by rutin supplementation.

Energy Expenditure

Parameters of energy expenditure and gas exchange (Table 2), and in particular methane emission, were not affected by rutin supplementation but by feeding regimen ($P < 0.05$). Dry matter intake in the respiration chamber with ad libitum and restricted feeding, respectively, did not differ (21.7 vs. 21.6 kg/d; pooled SE = 0.40). No significant interaction effect of treatment by feeding regimen was observed on any of the variables.

Table 2. Parameters of gas exchange and energy expenditure during ad libitum and restricted feeding, and with and without (control) rutin supplementation (LSM)

Item	Feeding regimen			Treatment			PSE ²
	Ad libitum	Restricted ¹	P-value	Rutin	Control	P-value	
CH ₄ production (L/d)	612	531	<0.001	570	573	0.753	14.7
CH ₄ /kg DMI (L/kg)	28.3	24.7	<0.001	26.5	26.5	0.954	0.79
CO ₂ production ³ (L/d)	5,915	5,527	0.002	5,689	5,753	0.562	185.6
O ₂ consumption (L/d)	7,079	6,817	0.064	6,902	6,994	0.498	174.1
Heat production/d (kJ/kg of BW ^{0.75})	1,183	1,155	0.136	1,163	1,174	0.550	15.8
RQ ⁴	0.84	0.81	<0.001	0.82	0.82	0.524	0.024
COX ⁵ (kJ/kg of BW ^{0.75})	587	466	<0.001	526	527	0.986	106.5
FOX ⁶ (kJ/kg of BW ^{0.75})	625	709	<0.001	662	673	0.584	96.2

¹Residual feed removed at 0630 h, followed by refeeding at 1630 h.

²PSE = pooled standard error.

³Metabolic CO₂ (corrected for fermentative CO₂ production).

⁴RQ = respiratory quotient.

⁵COX = carbohydrate oxidation.

⁶FOX = fat oxidation.

Table 3. Plasma metabolite, hormone concentrations, and plasma flavonoid levels with and without (control) rutin supplementation (LSM)

Item	Treatment		<i>P</i> -value	PSE ¹
	Rutin	Control		
Fatty acids (μmol/L)	166	148	0.142	8.9
TG ² (mmol/L)	0.14	0.13	0.211	0.003
Lactate (mmol/L)	0.33	0.32	0.420	0.013
Glucose (mmol/L)	3.55	3.47	0.067	0.028
Albumin (g/L)	33.2	31.9	0.013	0.86
BHB (mmol/L)	0.79	0.70	0.068	0.078
Cholesterol (mmol/L)	4.91	5.12	0.654	0.492
Urea (mmol/L)	4.95	4.53	0.204	0.364
Insulin (μU/mL)	11.1	11.1	0.586	0.62
Glucagon (ng/L)	126	131	0.760	3.1
Quercetin (nmol/L)	35.6	5.4	0.003	8.98

¹PSE = pooled standard error.²TG = triglycerides.

Plasma Metabolites, Hormones, and Flavonoid Content

Plasma fatty acid levels were higher ($P < 0.001$) under restricted (203 ± 11.6 μmol/L) than under ad libitum (109 ± 6.7 μmol/L) feed intake. Plasma fatty acid concentrations were not affected by rutin supplementation (Table 3).

Values of plasma TG were not affected by rutin supplementation (Table 3) or feeding. Lactate values were affected by order of treatment ($P = 0.009$) and were higher if rutin was given first (0.36 ± 0.012 mmol/L) compared with control given first (0.29 ± 0.013 mmol/L).

Plasma glucose concentrations tended to be higher ($P = 0.067$) under rutin supplementation (Table 3). Plasma glucose tended ($P = 0.054$) to be affected by feeding regimen with lower values (3.46 ± 0.037 mmol/L) under restricted feed intake than under ad libitum feeding (3.56 ± 0.021 mmol/L).

Plasma albumin was higher ($P = 0.013$) under rutin supplementation than without (Table 3). The BHB values tended ($P = 0.068$) to be higher under rutin supplementation (Table 3). Plasma values of cholesterol and urea were not affected by rutin supplementation. Because we took blood samples only once on the day of respiration measurements under ad libitum feeding for cholesterol, albumin, BHB, and urea, it was not possible to estimate effects of feeding regimen for these parameters. Plasma insulin was not influenced by rutin supplementation (Table 3). Insulin values were influenced ($P = 0.040$) by order of treatments with higher values if rutin was given first (12.5 ± 0.57 μU/mL) than if control was given first (10.2 ± 0.64 μU/mL). Plasma glucagon was not influenced by rutin supplementation, but by order of treatments ($P = 0.037$), with higher

values if rutin was given first (134 ± 2.9 ng/L vs. 122 ± 3.3 ng/L).

Quercetin was the major flavonoid in plasma samples 1 h after rutin supplementation. Furthermore, minor levels of kaempferol and isorhamnetin were found, yet no tamarixetin could be detected. Total plasma flavonoid concentrations were higher ($P = 0.003$) during the period of rutin supplementation, whereas without rutin supplementation plasma flavonoid content was 7 times lower (Table 3).

Supplementation of ground buckwheat seeds did not increase total plasma flavonoids in comparison with TMR feeding only. Given that plasma flavonoid values did not reach detection level and we focused on flavonoid effects, we decided not to evaluate related results of respiration measurements and blood analyses.

DISCUSSION

The aim of our study was to assess possible beneficial effects of rutin in dairy cows in vivo with emphasis on energy metabolism, methane production, and production performance. Because the systemic availability of quercetin is much better after intraruminal application of rutin compared with quercetin aglycone (Berger et al., 2012), we wanted to investigate effects of oral rutin supplementation. To our knowledge, this is the first study to examine rutin effects when given orally in the pure form and contained in buckwheat seeds in lactating dairy cows.

Rutin trihydrate supplementation of 100 mg/kg of BW elevated total flavonoid levels in plasma by 600% with quercetin as the main metabolite. Indeed, total plasma flavonoid levels were comparably lower than in former studies with quercetin as rutin given intraruminally at 50 mg/kg of BW (Berger et al., 2012) or with

quercetin as aglycone given intraduodenally at 100 mg/kg of BW (Stoldt et al., 2015). The different levels of plasma flavonoids are most likely due to the different ways of flavonoid application and different stages of lactation of the cows examined. As discussed, capability for hepatic detoxification of absorbed flavonoids may differ in regard to the lactation stage (Stoldt et al., 2015). Production performance of cows was not altered by rutin supplementation. Improved milk yield or milk protein content, as reported for rutin (Guo et al., 2010) and intraduodenally administered quercetin (Gohlke et al., 2013b), could not be confirmed. Energy expenditure was not modified by rutin supplementation. Changes found in parameters related to energy metabolism indicate the metabolic shift toward fat oxidation as triggered by the feed restriction period, consistent with former studies (Derno et al., 2013). Because cows were fed *ad libitum* after the restriction period, they were able to catch up to their normal DMI so that total DMI did not differ between days with *ad libitum* or restrictive feeding.

Although flavonoids, including rutin and quercetin, were found to reduce rumen methane production *in vitro* by altering rumen microbiota (Oskoueian et al., 2013; Wang et al., 2013), rutin supplementation did not result in a reduced methane emission in our *in vivo* study. This is in accordance with observations of Berger et al. (2015), who found no effects of quercetin and rutin on *in vitro* methane production using the Hohenheimer gas test. Possibly, the residence time of rutin in rumen was too short or the rutin dose used in our experiment was not sufficient to suppress methanogens. It is noteworthy that relative flavonoid doses used in many *in vitro* studies were much higher than in our study and not feasible for animal studies. Although the differences found for plasma glucose concentrations were quite small, they tended to be higher under rutin supplementation. This is in contrast to findings in cows supplemented with quercetin intraduodenally (Gohlke et al., 2013b), where quercetin decreased plasma glucose. It is known that quercetin may affect glucose tolerance and insulin effects (Shao et al., 2013), but plasma insulin was not changed by flavonoid treatment.

Similar to midlactation cows under quercetin supplementation (Gohlke et al., 2013b) plasma BHB values tended to be higher under rutin supplementation. It is noteworthy that quercetin supplementation tended to decrease plasma BHB values in metabolically stressed periparturient dairy cows (Stoldt et al., 2015). Although within physiological range (Alberghina et al., 2011), plasma albumin values were higher under rutin supplementation, which is in contrast to our previous study (Stoldt et al., 2015). As it is established for

carbohydrate metabolism that flavonoids exert their effects differently under different health conditions (Shao et al., 2013; Xu et al., 2014), the influence of cow health and state of lactation on the effects of flavonoid supplementation should not be underestimated and needs to be clarified. Effects of order of treatment found for lactate, insulin, and glucagon are not easy to interpret, because no rutin effects were found for these parameters; thus, a carry-over effect from rutin to control treatment is unlikely.

Oral supplementation of ground buckwheat seeds was not sufficient to increase total plasma flavonoids above the detection limit. We assume low digestibility of the buckwheat seeds was a possible cause, because a recognizable amount of the seed particles was found unmodified in cow feces despite milling. Others (Kälber et al., 2012, 2013) have reported feeding of buckwheat as silage, which seems to be more digestible and suitable as dietary component. Whether buckwheat silage feeding increases plasma flavonoid level remains to be investigated.

Taken together, we found that feeding of rutin increased plasma quercetin content in dairy cows, but not to the same extent as found for intraduodenal quercetin aglycone supplementation (Gohlke et al., 2013a; Stoldt et al., 2015). The present and previous results indicate that the absolute concentrations of quercetin in cow plasma, as well as health conditions and the stage of lactation of the cow, influence the specificity of quercetin effects on energy metabolism and production performance. Effects of quercetin are contradictory under different settings, and health-promoting properties of quercetin seem to be more pronounced in cows under challenged metabolic health conditions (Stoldt et al., 2015), such as in transition cows. However, we could not confirm *in vivo* earlier reports of *in vitro* effects of flavonoids on rumen methane production (Leiber et al., 2012). Feeding rutin or buckwheat silage to metabolically challenged cows is an interesting approach for research, but requires further study.

CONCLUSIONS

In our study, we could not confirm previously shown *in vitro* reductions of rumen methane production by flavonoids using an oral dose of rutin at 100 mg/kg of BW *in vivo*, presumably due to an insufficient local flavonoid concentration in the rumen. Buckwheat seeds do not appear to be a good source for quercetin supplementation in dairy cows. Furthermore, metabolic effects of flavonoids seem to be more distinct in metabolically stressed than in healthy cows.

ACKNOWLEDGMENTS

The authors are grateful to P. Schulz (Institute for Animal Nutrition and Physiology, Christian-Albrechts-University of Kiel, Germany) as well as S. Dwars, K. Grot, C. Arlt, and S. Görs (Leibniz Institute for Farm Animal Biology, Institute of Nutritional Physiology “Oskar Kellner”) for excellent laboratory assistance. B. Stabenow, K.-D. Witt and his team, and D. Oswald, T. Lenke, A. Schulz, and K. Pilz of the “Tiertechnikum” (Leibniz Institute for Farm Animal Biology; Institute of Nutritional Physiology “Oskar Kellner”) are gratefully acknowledged for assistance with animal care and rutin feeding. This work is part of the joint research project AgroClustEr FoCus—Food Chain Plus, and was financially supported by the Federal Ministry of Education and Research, Germany, under the funding initiative “Kompetenznetze der Agrar- und Ernährungsforschung” (BMBF grant number 0315538B).

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