Influence of different morphological parts of buckwheat (*Fagopyrum esculentum*) and its major secondary metabolite rutin on rumen fermentation *in vitro*

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ABSTRACT: It was hypothesized that buckwheat, especially its flowers, influences foregut fermentation in ruminant animals because it is rich in phenolic compounds. The entire fresh aerial buckwheat herb, or its parts (leaves, stems, flowers and grain), were incubated for 24 h together with pure ryegrass (1:1, dry matter basis) in an *in vitro* ruminal fermentation system (Hohenheim Gas Test). Additionally ryegrass, supplemented with 0, 0.5, 5, or 50 mg rutin trihydrate/g dry matter, was incubated. Contents of extractable phenols (g/kg dry matter) were the highest in buckwheat flowers (88), followed by leaves (63), and the lowest in ryegrass (8). The levels of production of total gas and volatile fatty acids demonstrated that the nutritional value of buckwheat was slightly lower than that of ryegrass. Compared to ryegrass alone, ruminal transformation of dietary protein-N into ammonia was lower with 50 mg rutin, buckwheat flowers and buckwheat leaves. Thus, these treatments appeared to have partly protected dietary protein from ruminal degradation. Rutin, at the highest level, buckwheat flowers and the total aerial fraction of the buckwheat plant suppressed methane per unit of total gas by > 10%, either at elevated (rutin) or reduced total gas volume. This indicates that the ways of the influence on the ruminal fermentation pattern differed between pure rutin and buckwheat. *In vivo* studies have to confirm these potentially beneficial effects of buckwheat if used as forage for ruminants and clarify the role of further phenolic compounds present in buckwheat.

Keywords: *Fagopyrum esculentum*; ruminant feed; rumen fermentation; methane; ammonia; plant secondary compounds

Abbreviations: DM = dry matter, HGT = Hohenheim Gas Test, NDF = neutral detergent fibre, TEP = total extractable phenols, VFA = volatile fatty acids

Phenolic compounds present in forage plants may have anti-nutritive or even toxic effects on ruminants but they are also considered as nutrients and moreover as modulators of the ruminal fermentation (Lowry et al., 1996; Busquet et al., 2006; Szumacher-Strabel and Cieślak, 2010). The scientific interest in the latter function is growing because this knowledge could be the basis of tools to influence the ruminal lipid metabolism towards producing more healthy fatty acids (Lourenço et al., 2008; Cabiddu et al., 2010) and to mitigate ruminal methane production (Carulla et al., 2005; Patra and Saxena, 2010; Jayanegara et al., 2011). However, research is still mainly aimed at tannins and saponins (Patra and Saxena, 2010; Szumacher-Strabel and Cieślak, 2010) and to a lesser extent at non-tannic phenols which, however, also appear to affect rumen fermentation (Jayanegara et al., 2011).

It has been shown in tropical forage plants that the reliability of the occurrence of phenol-related effects on rumen fermentation increases with concentration (Jayanegara et al., 2011). However, compared to the above-mentioned plants, plants grown in temperate climatic regions are often poor

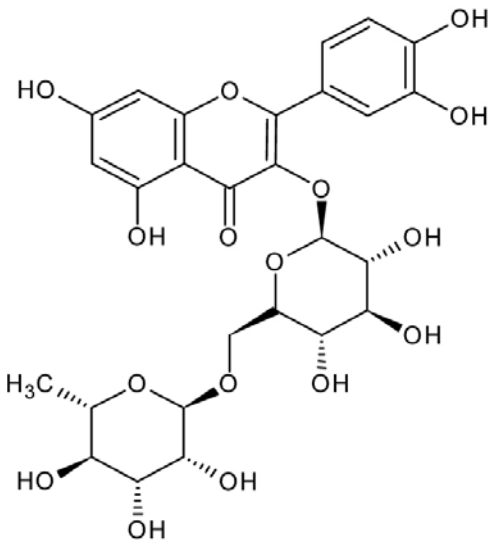

Figure 1. Chemical structure of the flavonoid rutin

in total phenols (Broudiscou et al., 2000; Fraisse et al., 2007; Kälber et al., 2011). Common buckwheat (*Fagopyrum esculentum*) is a dicotyledonous crop plant growing in temperate regions which contains comparably high concentrations of phenolic compounds (Hinneburg and Neubert, 2005). The flavonoid rutin is its main phenolic constituent (Figure 1), and also hyperoside and chlorogenic acid occur in buckwheat in substantial amounts (Hinneburg and Neubert, 2005; Kalinova et al., 2006). These compounds cause, i.a., antioxidative effects (Azuma et al., 1999; Hinneburg et al., 2006). Buckwheat is therefore considered to have the potential to serve as a functional food (Li and Zhang, 2001). Buckwheat is less common in livestock nutrition. However, it has recently been shown that different forms of buckwheat (grain, fresh herb or ensiled herb) are suitable as parts of the diet for poultry (Leiber et al., 2009) and for ruminants (Amelchanka et al., 2010; Kälber et al., 2011). The fresh buckwheat herb was found to promote the transfer of α-linolenic acid from feed to milk when fed to dairy cows (Kälber et al., 2011, 2012). This has been assumed to result from a lower level of ruminal biohydrogenation of this fatty acid. Concerning its methane mitigating potential, the use of buckwheat has been less clear so far (Broudiscou et al., 2000; Amelchanka et al., 2010). Concentrations of phenols largely differ in different fractions of the buckwheat herb (Kalinova et al., 2006), leading to the question whether effects might depend on the morphological part of the plant. Especially the flowers are expected to be effective (Kälber et al., 2011), but experimental data allowing to differentiate between different buckwheat-related effects on ruminal fermentation are still required.

Therefore the objective of the present study was to test *in vitro* whether different morphological parts of the buckwheat plant differently affect ruminal fermentation and methane formation. As rutin is the major secondary compound of buckwheat, its individual effect was investigated in parallel to the buckwheat preparations by adding it in pure form to ryegrass, a common forage containing low concentrations of phenols.

MATERIAL AND METHODS

Plant materials and rutin

Common buckwheat (*Fagopyrum esculentum* var. Lileija; seeds obtained from Fenaco Sämereienzentrum Niderfeld, Winterthur, Switzerland) was sown on 1 ha of intensively fertilized sandy soil in central Switzerland in July. At the end of August, when the buckwheat was fully flowering, plants were sampled from 7 plots randomly distributed across the whole area. From this pool, a part of the entire aerial material was left intact. The rest was separated into stems, leaves and flowers immediately after harvest. Samples were then oven dried at 60°C for 24 h. At the end of September, mature buckwheat grains were collected by hand from the same field, by taking samples across the whole area again. Additionally, pure ryegrass hay (*Lolium multiflorum*, 1st cut, at ear-emerging stage) was obtained from a local plant breeder. All plant materials were ground to pass through a 1.0 mm sieve. Pulverized (+)-rutin trihydrate with a purity of 97% was purchased from Alfa Aeser GmbH & Co KG (Karlsruhe, Germany).

Plant analyses

Plant materials were analysed in triplicate for dry matter (DM), organic matter, nitrogen and ether extract by standard methods of AOAC (1997), as described in Kälber et al. (2011). Crude protein was calculated as N × 6.25. Neutral detergent fibre (NDF) was determined according to Van Soest et al. (1991) using α-amylase but no sodium sulphite. Total extractable phenols (TEP) were ana-

lysed in triplicate by the modified Folin-Ciocalteu method using gallic acid as equivalence standard (Jayanegara et al., 2011). The non-NDF carbohydrates were calculated as the remaining organic fraction.

In vitro incubation

Rumen fluid was obtained from a rumen-cannulated lactating Brown Swiss cow before the morning feeding on four different days. The donor cow was fed white-clover ryegrass hay *ad libitum* and 0.5 kg/day of a concentrate for dairy cows (UFA 149; UFA AG, Herzogenbuchsee, Switzerland). The cow was housed and cared for according to the Swiss legislation for animal welfare. The rumen fluid was transported for immediate use to a laboratory in airtight containers at an ambient temperature of 39°C. The rumen fluid was strained through four layers of gauze to remove the particles. Then it was immediately mixed with preheated Menke buffer (Menke and Steingass, 1988) at a ratio of 1:2. This mixture is hereinafter called incubation fluid.

The Hohenheim Gas Test (HGT) apparatus with modified pistons was used for incubation (Menke and Steingass, 1988; Soliva and Hess, 2007). 30 ml of incubation fluid was filled in each piston together with the experimental diets. The first set of experimental diets consisted of 200 mg DM of pure ryegrass hay with additions of 0, 0.1. 1 and 10 mg rutin trihydrate, equivalent to 0, 0.5, 5 and 50 mg/g ryegrass hay. The highest dosage of rutin was similar to the content of the entire buckwheat plant described in the literature (Broudiscou et al., 2000; Kalinova et al., 2006). The second set of experimental diets always consisted of (DM based) 100 mg of pure ryegrass and 100 mg of different morphological parts of the buckwheat plant: entire aerial part of the plant, flowers, leaves, stems and grain. After being filled and air-tightly closed, each piston was immediately placed into the preheated HGT incubator and incubated at 39°C for 24 h. Incubations were carried out four times on four different days; each experimental diet was incubated in duplicate in each run (n = 8 per dietary treatment). After incubation, the gas volume was determined on a calibrated scale printed onto the pistons. Subsequently, the incubation fluid was decanted through the main outlet for further analysis. A gas sample was drawn with a sampling injector syringe through the airtight septum of a second outlet of the pistons (Soliva and Hess, 2007) and analysed on a gas chromatograph (5890, series II, Hewlett Packard, Avondale, USA) for the concentration of methane.

In the incubation fluid, pH and ammonia concentration were determined with a potentiometer (model 632, Metrohm, Herisau, Switzerland) equipped with the respective electrodes. As the system was intensively buffered, the pH remained unaffected by treatments and ranged between 7.0 and 7.3. The concentrations of volatile fatty acids (VFA) were analysed by HPLC (Ehrlich et al., 1981; LaChrom, L-7000 series; Hitachi Ltd., Tokyo, Japan). Bacteria and protozoa were counted in Bürker counting chambers (Blau Brand, Wertheim, Germany) with depths of 0.02 mm (bacteria) and 0.1 mm (protozoa), respectively. Prior to counting, the bacteria were fixed with Hayem's solution (mmol/l: $HgCl_2$, 9; Na_2SO_4, 176; NaCl, 86). Protozoa were differentiated into holotrich and entodiniomorphic forms.

Statistical analysis

Data from the incubation experiment were analysed by the general linear model procedure of SAS (SAS Institute Inc., Cary, USA), with diet as fixed effect and incubation run as random effect. Multiple comparisons among means were carried out by Tukey's method. Tables and figures show arithmetic means of the eight incubations per treatment, standard error of the mean (SEM) and P-values for the diet effect.

RESULTS AND DISCUSSION

Composition of morphological parts of the buckwheat plant

The entire aerial part of the buckwheat herb contained less crude protein, ether extract and fibre, but more non-fibre carbohydrates (like starch, oligosaccharides, sugars) than the ryegrass (Table 1). Within the buckwheat plant, crude protein was particularly concentrated in leaves, followed by flowers, whereas stems were characteristically high in fibre. The grains contained 620 g of non-NDF carbohydrates/kg DM (whereof > 800 g/kg are supposed to be starch, according to Wijngaard and Arendt, 2006). Also buckwheat leaves were rich in

Table 1. Chemical composition of the experimental dietary components (g/kg dry matter; analyzed in triplicate)

	Ryegrass total aerial	Buckwheat				
		total aerial	flowers	leaves	stems	grain
Organic matter	910	917	956	866	923	981
Crude protein	166	110	170	242	61	126
Ether extract	38	15	20	32	7.5	19
Neutral detergent fiber (NDF)	526	460	394	184	621	207
Non-NDF carbohydrates[a]	172	284	284	345	218	620
Total extractable phenols (TEP)	8.2	48	88	63	15	9.0

[a]calculated as organic matter – crude protein – ether extract – NDF – TEP

non-NDF carbohydrates. Except for a higher crude protein content, the proximate composition of the grain matched well the values published in other studies (Farrell, 1978; Pomeranz, 1983; Leiber et al., 2009). The composition of the total buckwheat herb was also similar to that analysed in material harvested in another year from the same cultivation site (Amelchanka et al., 2010). The buckwheat herb contained almost six times more total extractable phenols than the ryegrass, and this level was congruent with that found in other studies (Broudiscou et al., 2000; Kalinova et al., 2006). The observation that the highest TEP concentration occurred in the flowers and not in the leaves, however, differs from the findings of Kalinova et al. (2006).

Production of fermentation gas and volatile fatty acids

The incubation of ryegrass hay and buckwheat grain led to the highest total gas production (Table 2). By adding 50 mg rutin trihydrate/g to the ryegrass, gas production was further significantly increased. Gas production differed among different morphological parts of the buckwheat plant to some extent. The lowest value was found in the leaves ($P < 0.05$ against the ryegrass treatments and the buckwheat grain treatment). Generally, the diets containing buckwheat forages (entire aerial part, flowers, leaves, stems) resulted in a lower production of gas and total VFA than that found in ryegrass. Gas and VFA production are closely correlated with ruminal nutrient digestibility and energy content of feeds, especially when feeds are low in lipids as they were in the present investigation (Menke and Steingass, 1988). Consequently, these data indicate that the buckwheat-derived forages, compared to ryegrass, have a slightly reduced ruminal efficiency and nutritional value. This is consistent with previous results obtained with a long-term inflow-outflow *in vitro* system (Amelchanka et al., 2010). An expected exception was the buckwheat grain which is rich in non-fibre carbohydrates and therefore concentrate-like. The observation that the rutin applications even increased gas production in the ryegrass incubations, and that gas production was not low especially in flowers, which had the highest total phenol concentrations, suggests that other factors than the polyphenols in general and rutin in particular inhibited rumen fermentation. Rutin may even serve as a substrate for several fibrolytic rumen bacteria (Cheng et al., 1969) and thus may have promoted the overall fermentative activity. In stems, the high fibre content, associated with a low crude protein content, likely prevented a higher gas production. The low gas production from buckwheat leaves, which was not accompanied by a low VFA production, was unexpected, since they contained clearly higher amounts of crude protein and soluble carbohydrates than the ryegrass. These results suggest the presence of an inhibitory factor in the buckwheat herb other than rutin. This could have been fagopyrin, a potentially toxic polyphenol, which is a characteristic compound of buckwheat. Especially the leaves may contain up to 1 g fagopyrin/kg depending on the variety (Eguchi et al., 2009). As fagopyrin was not analysed in the present study, it remains to be investigated whether or not this

Table 2. Influence of rutin and different morphological parts of buckwheat on the production of fermentation gas and volatile fatty acids (VFA) as determined after 24 h of *in vitro* batch fermentation*

	200 mg ryegrass + rutin trihydrate (mg/g)				100 mg ryegrass + 100 mg buckwheat fraction					SEM	*P*-value
	0	0.5	5	50	total aerial	flowers	leaves	stems	grain		
Fermentation gas volume (ml)	52.8^{bc}	57.0^{a}	55.6^{ab}	57.4^{a}	47.7^{de}	49.5^{cd}	45.0^{e}	47.3^{de}	55.3^{ab}	4.11	< 0.001
VFA (mmol)	2.08^{abc}	2.21^{ab}	2.17^{bc}	2.24^{a}	2.00^{bc}	1.98^{c}	2.09^{abc}	1.97^{c}	2.17^{ab}	0.061	< 0.001
Acetate (mmol/mol VFA)	720^{a}	725^{a}	724^{a}	725^{a}	726^{a}	725^{a}	728^{a}	730^{a}	695^{b}	6.1	< 0.01
Propionate (mmol/mol VFA)	187^{b}	186^{b}	186^{b}	185^{b}	187^{b}	186^{b}	186^{b}	183^{b}	204^{a}	4.4	< 0.05
n-Butyrate (mmol/mol VFA)	62.3^{b}	63.1^{b}	63.6^{b}	65.8^{b}	62.2^{b}	66.2^{b}	61.6^{b}	64.0^{b}	76.8^{a}	2.95	< 0.05
iso-Butyrate (mmol/mol VFA)	13.2	8.6	8.2	7.5	9.1	9.5	8.5	8.4	8.9	1.38	0.479
n-Valerate (mmol/mol VFA)	8.7	7.2	7.5	6.5	6.7	6.4	7.4	6.9	7.1	0.95	0.486
iso-Valerate (mmol/mol VFA)	9.4	10.5	10.7	9.7	8.8	6.9	8.7	7.3	8.1	0.67	0.253
Acetate-to-propionate ratio	3.86^{a}	3.92^{a}	3.90^{a}	3.93^{a}	3.90^{a}	3.93^{a}	3.92^{a}	3.99^{a}	3.40^{b}	0.118	< 0.05

*values are the averages of eight replicates obtained from four independent incubations. Values in the same row with different letters are significantly different at $P = 0.05$

compound is a key factor influencing the ruminal fermentation.

Apart from influencing fibre and protein digestion, rutin, and even more quercetin, may influence starch digestion as inhibitors of α-glucosidase (Li et al., 2009). However, propionate, the major end product of starch fermentation (Van Soest, 1994), remained unchanged in its proportion in the present study. Blanch et al. (2010) also demonstrated that the inhibition of α-glucosidase in the rumen of dairy cows does not alter ruminal fermentation patterns even when diets are rich in starch.

Bacteria and protozoal counts

The total bacterial counts did not reveal a clear picture and lacked any significant differences between treatments (Table 3). It can be assumed that any possible effects of the buckwheat plant parts and rutin on distinct bacterial species were compensated by those on the others. By contrast, the protozoa, namely the entodiniomorphids, which accounted for a proportion of about 0.9 of all protozoa, were largely increased in the buckwheat grain incubation compared to all other treatments. This can be explained by the starch provided by the grain because, in the case of a moderate dietary starch level, protozoa are very competitive in ingesting entire starch granules before these can be utilized by bacteria (Jouany and Ushida, 1999). By contrast, the holotrich protozoa did not respond significantly to the dietary treatments (data not shown).

Ruminal ammonia release

Dietary crude protein fulfills two functions in the ruminant: it supplies sufficient degradable protein to rumen microbes as these organisms utilize ammonia for their own protein synthesis, and it provides extra amino acids available for digestion and absorption in the small intestine after bypassing the rumen in an intact form. Compared

Table 3. Influence of rutin and different morphological parts of buckwheat on ruminal microbial counts, ammonia and methane formation as determined after 24 h of *in vitro* batch fermentation*

	200 mg ryegrass + rutin trihydrate (mg/g)				100 mg ryegrass + 100 mg buckwheat fraction					SEM	*P*-value
	0	0.5	5	50	total aerial	flowers	leaves	stems	grain		
Bacteria (10^9/ml incubation fluid)	32.8	36.4	37.3	33.8	30.1	32.0	29.0	30.6	35.6	8.01	0.440
Total protozoa (10^3/ml incubation fluid)	11.4^{b}	9.3^{b}	12.7^{b}	10.8^{b}	11.1^{b}	13.6^{ab}	11.3^{b}	11.1^{b}	19.0^{a}	3.51	< 0.001
Entodiniomorph protozoa (% of total)	88.8	93.5	93.0	93.1	98.9	86.7	96.2	99.1	98.3	3.2	0.113
NH_3 (μmol/ml incubation fluid)	13.0^{ab}	12.5^{bcd}	12.8^{abc}	12.0^{bcd}	11.8^{cd}	12.1^{bcd}	13.9^{a}	10.5^{e}	11.6^{d}	1.28	< 0.001
NH_3-N/dietary N (mmol/mmol)	0.85^{bc}	0.81^{cd}	0.84^{cd}	0.78^{de}	0.93^{b}	0.78^{de}	0.74^{e}	1.00^{a}	0.86^{bc}	0.013	< 0.001
CH_4 volume (ml)	8.15^{a}	8.27^{a}	8.17^{a}	7.86^{a}	6.39^{b}	6.51^{b}	6.69^{b}	6.43^{b}	7.71^{a}	0.821	< 0.001
CH_4/total gas (ml/100 ml)	15.5^{a}	14.5^{bc}	14.7^{abc}	13.6^{d}	13.4^{d}	13.2^{d}	14.9^{ab}	13.6^{d}	13.9^{cd}	0.954	< 0.001

*values are the averages of eight replicates obtained from four independent incubations. Values in the same row with different letters are significantly different at $P = 0.05$

to ryegrass, the crude protein of the entire buckwheat plant, and especially of the stems but not of the leaves and flowers, tended to be better degradable (Table 3). Accordingly, the plant fractions poor in crude protein had a high ammonia release rate resulting in unexpectedly small differences in ammonia concentration in the incubation fluid. A specific feature of phenols, especially of tannins, is their ability to firmly bind to protein at a pH around 6 to 7 and thus to protect the protein from being degraded in the rumen whereas these bonds are cleaved at a low pH in the abomasum (Cortés et al., 2009). Relating the total phenol contents with ammonia-to-dietary-protein ratios across the buckwheat plant parts illustrates that buckwheat phenols also seem to be able to partially protect protein from degradation in the rumen. A significant effect of the treatment with 50 g rutin trihydrate/kg gives reason to assume that in this respect it was namely rutin that exhibited such an activity. It has been shown that the N-efficiency (milk N in relation to excreta N) of buckwheat silage in dairy cows is quite high compared to that of cows receiving ryegrass silage (Kälber et al., 2012). This could have been caused by the apparent protein-protecting property of rutin, deduced from the results of the present study.

Methane formation

The absolute methane production was consistently lower with the buckwheat herb incubations than with ryegrass alone and buckwheat grain (Table 3). Rutin supplementation did not significantly inhibit absolute methane gas production. As methane mitigation measures are of practical relevance only when methane production is decreasing in relation to the animal's productivity (Jayanegara et al., 2011), methane was also expressed as a proportion of total gas volume, which is an indicator of digestibility and energy concentration (Menke and Steingass, 1988). Relative to total gas, methane production was decreased not only by the buckwheat herb but also by the treatments with 5 and 50 mg rutin/kg compared to pure ryegrass (Table 3). The incubation of buckwheat flowers was most effective in decreasing the methane proportion in total gas (–14%). Rutin abated methane per unit of total gas when supplemented at a level of 50 mg/g DM (–12%). However, this reduction was achieved mainly by an increase in total gas production and less so by a numerically lower methane release. This could mean that rutin acted as a substrate for, and promoter of, non-methanogenic bacteria (Cheng et al., 1969) rather than as an inhibitor of methanogens. On the other

hand, rutin is less concentrated in the flowers than in the leaves of buckwheat, and the flowers contain higher amounts of epicatechin instead (Kalinova et al., 2006). This suggests that the effects of buckwheat on methane per unit total gas may be based on inhibitory effects of compounds other than rutin or result from a complex interaction of several plant compounds with different microbial strains. The mitigation of methane emission by up to 13%, as found with replacing half of the ryegrass by the entire aerial part of the buckwheat plant, appears appreciable compared with effects reported for the inhibitory potential of other forages investigated in *in vitro* batch cultures (Garcia-Gonzalez et al., 2008; Soliva et al., 2008; Patra and Saxena, 2010). The lack of treatment-caused changes in acetate-propionate ratio and acetate proportion (Table 2) compared to ryegrass indicates that the methane decline with different parts of the buckwheat herb was not a result of simple depression of fibre digestion and concomitant lack of hydrogen as substrate for the methanogens. It remains unclear why buckwheat did not influence the methane emission variables in a previous study which was carried out with a continuous culture *in vitro* system (Amelchanka et al., 2010).

Incubating the buckwheat grain also resulted in a lower methane emission per unit of total gas (Table 3). Here the mode of action was likely different while it was not based on its phenolic compounds. Replacing half of the ryegrass by the buckwheat grain was equivalent to change the diet from forage only to that with a forage-to-concentrate ratio of 1:1, a measure where a certain reduction in methane emission could be expected (Beauchemin et al., 2008). The acetate-to-propionate ratio (Table 2) was significantly shifted towards propionate when the buckwheat grain was incubated, as was expected from feed components rich in starch (Van Soest, 1994) and is consistent with the decline in methane. As this decrease in methane production was associated with an increase in protozoa count, it was not mediated by a concomitant depression of rumen protozoa which is different from the response to some other effective plant secondary metabolites like saponins (Patra and Saxena, 2010; Szumacher-Strabel and Cieślak, 2010).

CONCLUSION

In conclusion, it was demonstrated that buckwheat has a potential to alter ruminal fermentation patterns, including a certain mitigation of methane emission without a concomitant severe decline of rumen microbial productivity. This is particularly true for flowers which also showed the highest concentration of phenolic compounds, indicating that the phenological stage of flowering may be especially effective. The ryegrass-rutin incubations revealed that pure rutin acted differently from the buckwheat incubations and may exhibit its effects rather by triggering than by inhibiting ruminal fermentation. A further important property of rutin appears to be the partial protection of dietary proteins from ruminal degradation. However, buckwheat contains other phenols like fagopyrin, quercetin and epicatechin which may have played an important role as well, an aspect which has to be investigated in future research.

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REFERENCES

Amelchanka S.L., Kreuzer M., Leiber F. (2010): Utility of buckwheat (*Fagopyrum esculentum* Moench) as feed: Effects of forage and grain on *in vitro* ruminal fermentation and performance of dairy cows. Animal Feed Science and Technology, 155, 111–121.

AOAC (1997): Official Methods of Analysis. Association of Official Analytical Chemists, Arlington, USA.

Azuma K., Nakayama M., Koshioka M., Ippoushi K., Yamaguchi Y., Kohata K., Yamauchi Y., Ito H., Higashio H. (1999): Phenolic antioxidants from the leaves of *Corchorus olitorius* L. Journal of Agricultural and Food Chemistry, 47, 3963–3966.

Beauchemin K.A., Kreuzer M., O'Mara F., McAllister T.A. (2008): Nutritional management for enteric methane abatement: a review. Australian Journal of Experimental Agriculture, 48, 21–27.

Blanch M., Calsamiglia S., Devant M., Bach A. (2010): Effects of acarbose on ruminal fermentation, blood metabolites and microbial profile involved in ruminal acidosis in lactating cows fed a high-carbohydrate ration. Journal of Dairy Research, 77, 123–128.

Broudiscou L.P., Papon Y., Broudiscou A.F. (2000): Effects of dry plant extracts on fermentation and methanogenesis in continuous culture of rumen microbes. Animal Feed Science and Technology, 87, 263–277.

Busquet M., Calsamiglia S., Ferret A., Kamel C. (2006): Plant extracts affect *in vitro* rumen microbial fermentation. Journal of Dairy Science, 89, 761–771.

Cabiddu A., Salis L., Tweed J.K.S., Molle G., Decandia M., Lee M.R.F. (2010): The influence of plant polyphenols on lipolysis and biohydrogenation in dried forages at different phonological stages: *in vitro* study. Journal of the Science of Food and Agriculture, 90, 829–835.

Carulla J.E., Kreuzer M., Machmüller A., Hess H.D. (2005): Supplementation of *Acacia mearnsii* tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Australian Journal of Agricultural Research, 56, 961–970.

Cheng K.-J., Jones G.A., Simpson F.J., Bryant M.P. (1969): Isolation and identification of rumen bacteria capable of anaerobic rutin degradation. Canadian Journal of Microbiology, 15, 1365–1371.

Cortés J.E., Moreno B., Pabón M.L., Avila P., Kreuzer M., Hess H. D., Carulla, J.E. (2009): Effects of purified condensed tannins extracted from Calliandra, Flemingia and Leucaena on ruminal and postruminal degradation of soybean meal as estimated *in vitro*. Animal Feed Science and Technology, 151, 194–204.

Eguchi K., Anase T., Osuga H. (2009): Development of a high-performance liquid chromatography method to determine the fagopyrin content of tartary buckwheat (*Fagopyrum tartaricum* Gaertn.) and common buckwheat (*F. esculentum* Moench). Plant Production Science, 12, 475–480.

Ehrlich G.G., Goerlitz D.F., Bourell J.H., Eisen G.V., Godsy E.M. (1981): Liquid chromatographic procedure for fermentation product analysis in the identification of anaerobic bacteria. Applied and Environmental Microbiology, 42, 878–886.

Farrell D.J. (1978): A nutritional evaluation of buckwheat (*Fagopyrum esculentum*). Animal Feed Science and Technology, 3, 95–108.

Fraisse D., Carnat A., Viala D., Pradel P., Besle J.M., Coulon J.P., Felgines C., Lamaison J.L. (2007): Polyphenolic composition of a permanent pasture: Variations related to the period of harvesting. Journal of the Science of Food and Agriculture, 87, 2427–2435.

Garcia-Gonzalez R., Lopez S., Fernandez M., Bodas R., Gonzalez J.S. (2008): Screening the activity of plants and spices for decreasing ruminal methane production *in vitro*. Animal Feed Science and Technology, 147, 36–52.

Hinneburg I., Neubert R.H.H. (2005): Influence of extraction parameters on the phytochemical characteristics of extracts from buckwheat (*Fagopyrum esculentum*) herb. Journal of Agricultural and Food Chemistry, 53, 3–7.

Hinneburg I., Kempe S., Rüttinger H.-H., Neubert, R.H.H. (2006): Antioxidant and photoprotective properties of an extract from buckwheat herb (*Fagopyrum esculentum* Moench). Pharmazie, 61, 237–240.

Jayanegara A., Wina E., Soliva C.R., Marquardt S., Kreuzer M., Leiber F. (2011): Dependence of forage quality and methanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis. Animal Feed Science and Technology, 163, 231–243.

Jouany J.P., Ushida K. (1999): The role of protozoa in feed digestion – Review. Asian-Australasian Journal of Animal Science, 12, 113–128.

Kälber T., Meier J.S., Kreuzer M., Leiber F. (2011): Flowering catch crops used as forage plants for dairy cows: Influence on fatty acids and tocopherols in milk. Journal of Dairy Science, 94, 1477–1489.

Kälber T., Kreuzer M., Leiber, F. (2012): Silages containing buckwheat and chicory: quality, digestibility and nitrogen utilisation by lactating cows. Archives of Animal Nutrition, 66, 50–65.

Kalinova J., Triska J., Vrchotova N. (2006): Distribution of vitamin E, squalene, epicatechin, and rutin in common buckwheat plants (*Fagopyrum esculentum* Moench). Journal of Agricultural and Food Chemistry, 54, 5330–5335.

Leiber F., Messikommer R., Wenk C. (2009): Buckwheat: A feed for broiler chicken? Agrarforschung, 16, 448–453.

Li S.Q., Zhang Q.H. (2001): Advances in the development of functional foods from buckwheat. Critical Reviews in Food Science and Nutrition, 41, 451–464.

Li Y.Q., Zhou F.C., Gao F., Bian J.S., Shan F. (2009): Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of α-glucosidase. Journal of Agricultural and Food Chemistry, 57, 11463–11468.

Lourenço M., Cardozo P.W., Calsamiglia S., Fievez V. (2008): Effects of saponins, quercetin, eugenol, and cinnamaldehyde on fatty acid biohydrogenation of forage polyunsaturated fatty acids in dual-flow continuous culture fermenters. Journal of Animal Science, 86, 3045–3053.

Lowry J.B., McSweeney C.S., Palmer B. (1996): Changing perceptions of the effect of plant phenolics on nutrient supply in the ruminant. Australian Journal of Agricultural Research, 47, 829–842.

Menke K.H., Steingass H. (1988): Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Animal Research and Development, 28, 7–55.

Patra A.K., Saxena J. (2010): A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. Phytochemistry, 71, 1198–1222.

Pomeranz Y. (1983): Buckwheat: structure, composition, and utilization. Critical Reviews in Food Science and Nutrition, 19, 213–258.

Soliva C.R., Hess H.D. (2007): Measuring methane emission of ruminants by *in vitro* and *in vivo* techniques. In: Makkar H.P. S., Vercoe P.E. (eds.): Measuring Methane Production from Ruminants. Springer, Dordrecht, the Netherlands, 15–31.

Soliva C.R., Zeleke A.B., Clément C., Hess H.D., Fievez V., Kreuzer M. (2008): *In vitro* screening of various tropical foliages, seeds, fruits and medicinal plants for low methane and high ammonia generating potentials in the rumen. Animal Feed Science and Technology, 147, 53–71.

Szumacher-Strabel M., Cieślak A. (2010): Potential of phytofactors to mitigate rumen ammonia and methane production. Journal of Animal and Feed Sciences, 19, 319–337.

Van Soest P.J. (1994): Nutritional ecology of the ruminant. 2nd ed. Cornell University Press, Ithaka, USA, 476 pp.

Van Soest P.J., Robertson J.B., Lewis B.A. (1991): Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583–3597.

Wijngaard H.H., Arendt E.K. (2006): Buckwheat. Cereal Chemistry, 83, 391–401.

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