Dietary supplementation of essential oils in dairy cows: evidence for stimulatory effects on nutrient absorption

H.-S. Braun†, K. T. Schrapers†, K. Mahlkow-Nerge2, F. Stumpff1 and J. Rosendahl†

1Institute of Veterinary Physiology, Faculty of Veterinary Medicine, Freie Universität Berlin, Oertzenweg 19b, 14163 Berlin, Germany; 2Agrarwirtschaft, FH Kiel University of Applied Sciences, Grüner Kamp 11, 24783 Osterrönfeld, Germany

(Received 17 October 2017; Accepted 14 May 2018; First published online 20 July 2018)

Results of recent in vitro experiments suggest that essential oils (EO) may not only influence ruminal fermentation but also modulate the absorption of cations like Na+, Ca2+ and NH4+ across ruminal epithelia of cattle and sheep through direct interaction with epithelial transport proteins, such as those of the transient receptor potential family. The aim of the current study was to examine this hypothesis by testing the effect of a blend of essential oils (BEO) on cation status and feed efficiency in lactating dairy cows. In the experiment, 72 dairy cows in mid-to-end lactation were divided into two groups of 36 animals each and fed the same mixed ration with or without addition of BEO in a 2×2 cross-over design. Feed intake, milk yield and composition, plasma and urine samples were monitored. Feeding BEO elevated milk yield, milk fat and protein yield as well as feed efficiency, whereas urea levels in plasma and milk decreased. In addition, plasma calcium levels increased significantly upon BEO supplementation, supporting the hypothesis that enhanced cation absorption might contribute to the beneficial effects of these EO.

Keywords: calcium, feed efficiency, rumen, feed additive, hypocalcemia

Implications

Dairy cows were offered a blend of essential oils (BEO) and its effect on cation status and performance was investigated. The study provides evidence that recently described effects of essential oils (EO) on calcium transport across the ruminal epithelium in vitro might be applicable in vivo. Supplementing certain EO may improve calcium metabolism, which could impact future hypocalcemia prevention strategies in dairy farming.

Introduction

In recent decades, the use of EO in ruminant nutrition has received increasing attention. So far, the main focus has been on modulating ruminal microbiota. The potential of EO as antimicrobial agents was realized as early as 1965 when Borchers (1965) demonstrated that thymol arrested deamination of amino acids in vitro in rumen samples. Follow-up studies have since established that, at least in vitro and at appropriately high concentrations, EO modify fermentation patterns with effects on volatile fatty acid production, protein metabolism or both (Calsamiglia et al., 2007).

At least, as interesting as the antimicrobial potential of EO is their powerful ability to modulate cellular signaling pathways in sensory perception, inflammation and pain through receptors from the transient receptor potential (TRP) family of ion channels (Holzer, 2011; Nilius and Szallasi, 2014). Interestingly, many such EO receptors (e.g. TRPA1 and TRPV3) are found in the epithelia of the intestine (Holzer, 2011), including the rumen of cattle and sheep (Rosendahl et al., 2016). Recently, the impact of EO or so-called phytonutrients on ruminant immune status, insulin regulation or oxidative stress was reviewed with special notice to the involvement of TRP channels (Oh et al., 2017).

Essential oils may also influence the expression patterns of other transport proteins expressed by the rumen (Mirzaei-Alamouti et al., 2016). The effects of EO may thus well exceed their interaction with microbial populations in the rumen.

In cattle, the absorption of Ca2+ is of particular interest as low plasma Ca2+ levels are the cause of the metabolic disorder milk fever (Martin-Teresa and Martens, 2014). In many epithelia, the transport of divalent cations is known to be mediated by certain members of the TRP channel family (Wilkens et al., 2009; Martens et al., 2018). Intriguingly, EO have been shown to enhance the transport of Ca2+ and other cations across numerous preparations (Nilius and Szallasi, 2014), with studies of ruminal epithelia in vitro...
suggesting stimulatory effects on the absorption of Na\(^+\), NH\(_4\)\(^+\) and Ca\(^{2+}\), possibly related to the expression of TRPV3 by ruminal tissue (Rosendahl et al., 2016). In support of this hypothesis, it was recently demonstrated that the bovine TRPV3 mediates the transport of all three ions and that transport is stimulated by the same EO that stimulate transport across the intact ruminal epithelium (Schrapers et al., 2018). This leads to the hypothesis that offering EO to cows might enhance cation absorption through TRP channels, thus facilitating the absorption of minerals and nutrients with positive effects such as improved production and health status. Accordingly, the present study focuses on the effect of a BEO on feed efficiency, nitrogen metabolism and calcium status in dairy cows.

**Material and methods**

**Experimental procedures, animals and diet**

The study was performed using a patented, commercial BEO (BTX12; PerformaNat GmbH, Berlin, Germany, patent US 9693971). A premix of 25 g was added to the concentrate feed, which was added to the total mixed ration (TMR). In total, animals were fed a daily dose corresponding to 1.2 g EO, with menthol as the major active compound (>80%) in addition to smaller amounts of eugenol and anethol.

The feeding trial followed a 2 × 2 cross-over design and was carried out at the agricultural teaching and testing institute of the state of Schleswig Holstein at Futterkamp approved by the responsible authority, the Ministry for Energy Transition, Agriculture, Environment and Rural Areas, State of Schleswig Holstein (V242-7224.121-25). A herd of 72 primi- and multiparous Holstein–Friesian cows in mid-lactation were randomly assigned to two groups which were fed either BEO or the control diet. The first BEO feeding period lasted 20 days followed by a period of 40 days in which cows of both groups were fed a standard diet without BEO to prevent carry-over effects. The second BEO feeding period of 20 days followed in which the cows that had previously been fed the control diet were fed BEO and vice versa.

Each group consisted of nine or 11 heifers and 25 or 27 multiparous cows, with the total mean for both groups at 2.3 ± 0.1 lactations, 170 ± 8 days in milk and milk yield at 37 ± 1 kg/day at the start of the trial. Cows were milked twice daily in a milking parlour at 0600 and 1500 h. A TMR was presented directly after milking before the start and at the end of each feeding period. The results of both samples were averaged directly after morning milking before the start and at the end of each experimental period were used to calculate individual milk yield and feed intake.

Milk fat, protein and urea were analysed twice weekly and the corresponding values for fat corrected and energy corrected milk yield (FCM and ECM) were calculated. Feed efficiency was calculated by dividing milk yield by the dry matter intake (DMI). To investigate the effect of BEO on metabolites and minerals, blood samples were collected both on the day before and on the last day of each feeding period directly after morning milking. Blood samples were taken from the coccygeal vessel using vacutainers (Greiner Bio-One International GmbH, Kremsmünster, Austria). After centrifugation, plasma was separated and stored at −20°C until analysis of metabolites and minerals. In serum samples, calcium, magnesium, phosphorus, total protein, creatinine, urea, β-hydroxybutyrate (BHB) and non-esterified fatty acids (NEFA) were analysed using an automatic biochemistry analyser (COBAS C-311; Roche, Mannheim, Germany). Unfortunately, due to technical problems, NEFA values were unreliable and had to be discarded.

Spontaneous urine was collected on 2 consecutive days directly after morning milking before the start and at the end of feeding period. The results of both samples were averaged to minimize daily fluctuations. In urine samples, calcium, magnesium, urea and creatinine were analysed. Urinary pH

| Table 1 Diet ingredients and chemical composition of the total mixed ration for dairy cows |
|---------------------------------|------------------|
| Feed items (% of dry matter)    |                  |
| Com silage                      | 35.2             |
| Grass silage                    | 24.1             |
| Wheat straw                     | 2.1              |
| Rapeseed meal                   | 13.3             |
| Soybean meal                    | 3.4              |
| Grain of maize                  | 5.9              |
| Dried sugar beet pulp           | 7.0              |
| Rye grains                      | 8.0              |
| Urea                            | 0.1              |
| Vitamin premix                  | 0.2              |
| Feed lime                       | 0.6              |
| Cattle salt                     | 0.2              |
| Chemical composition (g/kg dry matter)\(^1\) |                   |
| Dry matter                      | 411              |
| CP                              | 153              |
| Crude fat                       | 33               |
| Starch                          | 179              |
| NDF                             | 389              |
| ADF                             | 217              |
| ADL                             | 25               |
| Calcium                         | 6.1              |
| Magnesium                       | 2.1              |
| Phosphorus                      | 3.8              |

\(^1\)Chemical composition was analysed in three samples of the total mixed ration.

the individual animals left the trough. Feed intake per cow was summed up for each day. Individual milk yield was recorded at morning and afternoon milking using automatic milk counters and was summed up for daily milk yield. The last 3 days before the start and at the end of each experimental period were used to calculate individual milk yield and feed intake.

All data were collected both before the start and at the end of the feeding period. Individual feed intake was measured by feed weighing troughs (Insenter, Marknesse, the Netherlands) as previously described (Pahl et al., 2015). In brief, troughs opened for cows identified by a transponder and closed after

519
Feed efficiency (ECM/DMI and FCM/DMI) significantly increased for cows receiving BEO supplementation, milk yield per DMI tended to increase ($P = 0.066$, Table 2). Fat and protein content of the milk was not influenced by BEO. Fat and protein yield were significantly higher for the BEO group ($P = 0.007$ and $P = 0.003$, respectively). Urea in milk significantly decreased in the BEO group ($P = 0.006$, Table 2).

### Blood parameters
Feeding BEO was associated with a significant rise in plasma calcium levels (control: $2.46 \pm 0.015$ mmol/l, BEO: $2.53 \pm 0.02$ mmol/l, $P < 0.001$) (Table 3). No significant changes were observed in serum levels of magnesium, phosphorus, total protein or creatinine. $\beta$-Hydroxybutyrate significantly decreased in the BEO group (control: $0.78 \pm 0.02$ mmol/l, BEO: $0.65 \pm 0.02$ mmol/l, $P = 0.001$), even though cows in mid-to-late lactation state were not prone to ketosis. Plasma urea levels dropped significantly when BEO was fed ($P < 0.001$, Table 3).

### Urine parameters
In urine, concentrations of calcium and urea were not affected by BEO feeding (Table 4). Urinary excretion of magnesium and creatinine tended to decrease in the BEO feeding group ($P = 0.09$ and 0.08, respectively). Urinary pH significantly increased (control: $8.03 \pm 0.014$ mmol/l, BEO: $8.07 \pm 0.014$ mmol/l, $P = 0.033$). Changes in titratable acid and base were not significant, whereas net acid excretion and acid:base ratio tended to increase ($P = 0.082$ and $P = 0.102$, respectively). Ammonia excretion was significantly lower in the BEO group ($P = 0.013$).

### Discussion
In the current study, cows were fed 1.2 g of a commercial BEO containing menthol as the major active compound (>80%) and smaller amounts of eugenol and anethol. In response to this feed additive, an increase of milk yield, milk fat and protein yield at constant DMI was observed, resulting in a significantly elevated feed efficiency. Plasma calcium concentration increased significantly, whereas urea in plasma and milk decreased. Although this particular BEO has

### Table 2: Response of feed intake, milk parameters and feed efficiency of dairy cows following dietary supplementation with a blend of essential oils (BEO) for 20 days

| Items            | Control | BEO  | SEM  | P-value |
|------------------|---------|------|------|---------|
| DMI (kg/day)     | 19.7    | 19.5 | 0.28 | 0.558   |
| Milk             |         |      |      |         |
| Milk (kg/day)    | 31.8    | 33.0 | 0.32 | 0.014   |
| ECM (kg/day)     | 33.0    | 34.6 | 0.39 | 0.004   |
| FCM (kg/day)     | 32.5    | 34.0 | 0.42 | 0.007   |
| Fat (%)          | 3.25    | 3.79 | 0.05 | 0.352   |
| Fat (kg/day)     | 1.15    | 1.23 | 0.02 | 0.007   |
| Protein (%)      | 3.39    | 3.40 | 0.02 | 0.586   |
| Protein (kg/day) | 1.06    | 1.11 | 0.01 | 0.003   |
| Urea (mg/l)      | 182     | 166  | 3.88 | 0.006   |

Feed efficiency:

| kg milk/kg DMI  | 1.62    | 1.70 | 0.032 | 0.066   |
| kg ECM/kg DMI   | 1.68    | 1.79 | 0.032 | 0.023   |
| kg FCM/kg DMI   | 1.65    | 1.76 | 0.033 | 0.023   |

### Table 3: Response of the plasma parameters of dairy cows following dietary supplementation with a blend of essential oils (BEO) for 20 days

| Items (mmol/l) | Control | BEO  | SEM  | P-value |
|---------------|---------|------|------|---------|
| Calcium       | 2.46    | 2.53 | 0.02 | <0.001  |
| Magnesium     | 0.98    | 0.99 | 0.01 | 0.241   |
| Phosphorus    | 1.90    | 1.91 | 0.03 | 0.994   |
| Urea          | 4.28    | 3.92 | 0.07 | <0.001  |
| Total protein | 76.0    | 75.5 | 0.32 | 0.215   |
| BHB           | 0.78    | 0.65 | 0.02 | 0.001   |
| Creatinine (µmol/l) | 65.6 | 68.6 | 0.80 | 0.986 |

**BHB** = $\beta$-hydroxybutyrate.
Table 4  Response of urine parameters of dairy cows following dietary supplementation with a blend of essential oils (BEO) for 20 days

| Items (mmol/l) | Control | BEO | SEM | P-value |
|---------------|---------|-----|-----|---------|
| Calcium       | 1.50    | 1.98| 0.24| 0.163   |
| Magnesium     | 13.6    | 12.6| 0.40| 0.090   |
| Urea          | 214     | 211 | 5.99| 0.772   |
| Creatinine    | 7.78    | 7.29| 0.20| 0.080   |
| pH            | 8.03    | 8.07| 0.01| 0.033   |
| Titratable base| 185 | 191 | 4.12| 0.316   |
| Titratable acid| 70.7| 68.7| 1.80| 0.451   |
| NH₃           | 15.6    | 12.5| 0.88| 0.013   |
| Base:acid     | 2.77    | 2.94| 0.07| 0.102   |
| Net acid excretion | 99.1 | 109.6| 4.19| 0.082   |

not been studied before, a number of other studies have addressed the effects of isolated EO or mix of these agents in feeding trials, with results highly variable and depending on the preparation studied and the study design (Khiaosa-ard and Zebeli, 2013).

In a recent study of cows fed a ketogenic diet, a commercial BEO significantly increased milk fat content, energy-corrected milk and feed efficiency (ECM/DMI) (Drong et al., 2016). However, this occurred with a concomitant rise in BHB and NEFA, both of which rose more strongly when EO were added to the ketogenic diet, suggesting a negative impact of the preparation on energy balance in these challenged animals. In a study of cows fed a more balanced diet, BHB and NEFA were not affected by this blend (Tassoul and Shaver, 2009), which is in contrast to the results of this study showing decreased BHB values in cows offered BEO feeding. However, more work is clearly needed to assess the impact of EO on fat mobilization and energy balance of lactating cows, in particular in the transition period.

An interesting observation in this study was the significant drop in plasma urea levels, which most likely explains the concomitant lower milk urea levels during BEO supplementation. As milk urea nitrogen (MUN) can be used to predict total urinary nitrogen excretion (Nousiainen et al., 2004; Spek et al., 2013), a reduction in total nitrogen excretion can be inferred. A drop in MUN was also observed in a study of ewes fed another commercial BEO (Giannenas et al., 2011), whereas in lactating cows, MUN values that were elevated by monensin supplementation were normalized (Benchaar et al., 2006). As daily milk protein yield increased, nitrogen might have been incorporated more efficiently into protein and amino acids such as glutamine rather than non-protein nitrogen like urea.

To the extent that they have been validated, the mechanisms behind the in vivo effects of EO are thought to reflect the well-documented antimicrobial effects of EO (Borchers, 1965; Calsamiglia et al., 2007). Various mechanisms for the bactericidal effects of these compounds are discussed, such as an interaction with the lipid membrane itself or proteins embedded therein, ultimately leading to a breakdown in membrane barrier (Jouany and Morgavi, 2007) with a differential impact on Gram-negative and Gram-positive bacteria (Chao et al., 2000).

However, in particular, at the concentrations fed in vivo, the mode of action of EO fed to dairy cows may exceed the antimicrobial effects. We found higher plasma calcium levels – a fact that might relate to the well-documented stimulatory interaction of EO with non-selective cation channels from the TRP channel family (Holzer, 2011; Nilius and Szallasi, 2014; Rosendahl et al., 2016; Schrapers et al. 2018). Effects of EO on the transport of various ions across epithelia of the gastrointestinal tract have been reported not only in human and rat colon (Kaji et al., 2011) but also notably in the ruminal epithelium of cattle and sheep (Rosendahl et al., 2016). Furthermore, we were able to show that menthol and thymol stimulated the transport not just of Na⁺, but also of NH₄⁺ and Ca²⁺ across the isolated ruminal epithelia of sheep and cattle in an ex vivo approach (Rosendahl et al., 2016). In addition to these acute effects, a change in the expression pattern of acid base transporting proteins was recently shown in the ruminal epithelium of sheep fed a blend of extracts from a variety of plants that included peppermint (Mizraei-Alamouti et al., 2016).

Both menthol and thymol stimulate certain non-selective cation channels of the TRP family that are expressed by the tissue (Nilius and Szallasi, 2014; Rosendahl et al., 2016). With due caution, the low-dose BEO fed in this study may have stimulated the transport of calcium from rumen into blood, which might explain the increase of plasma Ca²⁺ observed. An increased ruminal uptake of calcium should enhance calcium homeostasis, with particular relevance for the reduction of hypocalcemia around calving (Goff, 2008).

Based on our in vitro study (Rosendahl et al., 2016), we initially expected to see higher absorption of ammonia as NH₃ from the rumen, and thus higher plasma and milk urea levels in the feeding group receiving EO. However, two factors may have intervened. First, degradation of protein may have been reduced by inhibition of proteolytic bacteria (Mcintosh et al., 2003). Second, given the very high pK value of the strong base ammonia (NH₃) of 9.2, a shift away from the absorption of NH₃ and towards the absorption of the protonated cationic form (NH₄⁺) through a TRP channel can be expected to induce a shift in the acid–base equilibrium of the blood towards less alkaline values. A decrease in pH will cause an activation of glutamine synthetase in the liver (Taylor and Curthoys, 2004; Weiner and Verlander, 2017). This normalizes the blood pH as the protons, which would be released in the course of urea synthesis are incorporated into the glutamine molecule. In the form of the non-toxic glutamine, protons and ammonia can then be transferred to the kidney and excreted, which may explain the higher net acid excretion observed under the BEO in this study.

As the most abundant amino acid in plasma, glutamine plays a central role not only as a shuttle for ammonia and protons but also as a key player in protein metabolism (Taylor and Curthoys, 2004), with the supply limited in stress situations such as calving (Meijer et al., 1993). An increase in glutamine synthesis from ammonium leaving the rumen...
would certainly appear to facilitate protein metabolism in animals challenged by lactation, which might be supported by the reduced NH\textsubscript{3} excretion in cows fed BEO. The possibility that certain BEO have the capacity not only to enhance proton absorption from the rumen but also to shift ammonia detoxification towards glutamine production is speculative. This hypothesis clearly merits further investigation.

Currently, the assumption that beneficial effects of feeding EO are caused by influencing the gastrointestinal flora represents the most established hypothesis. However, the ability of certain EO to affect epithelial ion transport in vitro might also contribute to their in vivo effects. This approach might provide an explanation for the high variability in the outcome of studies, as the effects of EO on epithelial ion transport are highly dependent on the dose and the substance. Further research is needed to elucidate the complex interaction of EO, gastrointestinal bacteria and the ruminal epithelium, contributing to the multiple observed positive effects on milk yield, feed efficiency and possible impacts on calcium homeostasis.

In conclusion, the tested BEO increased feed efficiency and plasma calcium levels. Possibly, this reflects stimulatory effects of EO on ruminal calcium absorption, which might help to cover the increased calcium demand during lactation.

Conclusion
The blend of plant bioactive EO tested in this study had effects that suggest improved feed efficiency and calcium homeostasis. We propose that in addition to their effects on fermentational patterns; EO activate specific cation-transporting proteins expressed by the rumen, resulting in an increased uptake of cations like calcium and ammonium. Further studies are required to elucidate the fate of the absorbed nutrients with special regard to calcium and nitrogen.

Acknowledgements
The authors gratefully acknowledge the help of M. Grunau, Prof. J. R. Aschenbach (Freie Universität Berlin, Germany) and the staff of the Chamber of Agriculture Schleswig-Holstein in Futterkamp. The project was funded by the European Social Fund (ESF) and the German Ministry of Economics and Technology based on a decision of the German Parliament (03EUABE057).

Declaration of interest
This study was performed primarily for scientific reasons within a conventional academic framework. However, the results of previous studies have led to a patent (US 9693971, involving the authors Friederike Stumpff and Julia Rosendahl, both as employees of the Freie Universität Berlin). At present, Julia Rosendahl, Hannah-Sophie Braun and Katharina T. Schrapers work for PerformaNat, an animal nutrition company with commercial interest in feed additives.

Ethics statement
The feeding trial was carried out at the agricultural teaching and testing institute of the state of Schleswig-Holstein at Futterkamp approved by the responsible authority, the Ministry for Energy Transition, Agriculture, Environment and Rural Areas, State of Schleswig-Holstein (V242-7224.121-25).

Software and data repository resources
All data are available upon request.

References
Benchchara C, Petit HV, Berthiaume R, Whyte TD and Chouinard PY 2006. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. Journal of Dairy Science 89, 4352–4364.
Borchers R 1965. Proteolytic activity of rumen fluid. Journal of Animal Science 24, 1033–1038.
Calsamiglia S, Busquet M, Cardozo PW, Castillejos L and Ferret A 2007. Invited review: essential oils as modifiers of rumen microbial fermentation. Journal of Dairy Science 90, 2580–2595.
Chan JCM 1972. The rapid determination of urinary titratable acid and ammonium and evaluation of freezing as a method of preservation. Clinical Biochemistry 5, 94–98.
Chao SC, Young DG and Ober C J 2000. Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. Journal of Essential Oil Research 12, 639–649.
Drong C, Meyer U, von Soosten D, Frahm J, Rehage J, Breves G and Dänicke S 2016. Effect of monensin and essential oils on performance and energy metabolism of transition dairy cows. Journal of Animal Physiology and Animal Nutrition 100, 537–551.
Giannenas I, Skoufou J, Giannakopoulos C, Wiemann M, Gortzi O, Lalas S and Kyriazakis I 2011. Effects of essential oils on milk production, milk composition, and rumen microbiota in Chios dairy ewes. Journal of Dairy Science 94, 5569–5577.
Golf JP 2008. The monitoring, prevention, and treatment of milk fever and subclinical hypocalcemia in dairy cows. The Veterinary Journal 176, 50–57.
Holzer P 2011. TRP channels in the digestive system. Current Pharmaceutical Biotechnology 12, 24–34.
Jouany J-P and Morgavi DP 2007. Use of ‘natural’ products as alternatives to antibiotic feed additives in ruminant production. Animal 1, 1443–1466.
Kaji I, Karaki S and Kuvahara A 2011. Effects of luminal thymol on epithelial transport in human and rat colon. American Journal of Physiology-Gastrointestinal and Liver Physiology 300, G1132–G1143.
Khiosa-ard R and Zebeli Q 2013. Meta-analysis of the effects of essential oils and their bioactive compounds on rumen fermentation characteristics and feed efficiency in ruminants. Journal of Animal Science 91, 1819–1830.
Martens H, Leonhard-Marek S, Rügten M and Stumpff F 2018. Magnesium homeostasis in cattle: absorption and excretion. Nutrition Research Reviews (1–7, doi:10.1017/S0954422417000257, Published online by Cambridge University Press 10 January 2018).
Martin-Tereso J and Martens H 2014. Calcium and magnesium physiology and nutrition in relation to the prevention of milk fever and tetany (dietary management of macrominerals in preventing disease). Veterinary Clinics of North America: Food Animal Practice 30, 643–670.
McIntosh FM, Williams P, Losa R, Wallace RJ, Beever DA and Newbold CJ 2003. Effects of essential oils on ruminal microorganisms and their protein metabolism. Applied and Environmental Microbiology 69, 5011–5014.
Meijer GA, van der Meulen J and van Vuuren AM 1993. Glutamine is a potentially limiting amino acid for milk production in dairy cows: a hypothesis. Metabolism 42, 4358–364.
Mirzaei-Alamouti H, Moradi S, Shahalizadeh Z, Razavian M, Amanlou H, Harkinezhad T, Jafari-Anarkooli I, Deiner C and Aschenbach JR 2016. Both monensin and plant extract alter ruminal fermentation in sheep but only monensin affects the expression of genes involved in acid-base transport of the ruminal epithelium. Animal Feed Science and Technology 219, 132–143.
Nilius B and Szalliassi A 2014. Transient receptor potential channels as drug targets: from the science of basic research to the art of medicine. Pharmacological Reviews 66, 676–814.
Cation absorption affected by essential oils

Nousiainen J, Shingfield KJ and Huhtanen P 2004. Evaluation of milk urea nitrogen as a diagnostic of protein feeding. Journal of Dairy Science 87, 386–398.

Oh J, Wall EH, Bravo DM and Hristov AN 2017. Host-mediated effects of phyto-nutrients in ruminants: a review. Journal of Dairy Science 100, 5974–5983.

Pahl C, Hartung E, Mahlkow-Nerge K and Haeussermann A 2015. Feeding characteristics and rumination time of dairy cows around estrus. Journal of Dairy Science 98, 148–154.

Rosendahl J, Braun HS, Schrapers KT, Martens H and Stumpff F 2016. Evidence for the functional involvement of members of the TRP channel family in the uptake of Na\(^+\) and NH\(_3\) by the ruminal epithelium. Pflügers Archiv-European Journal of Physiology 468, 1333–1352.

Schrapers K, Sponder G, Liebe F, Liebe H and Stumpff F 2018. The bovine TRPV3 as a pathway for the uptake of Na\(^+\), Ca\(^{2+}\), and NH\(_3\)\(_2\). PlosOne 13, e0193519.

Spek JW, Dijkstra J, Van Duinkerken G, Hendriks WH and Bannink A 2013. Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: a meta-analysis. Journal of Dairy Science 96, 4310–4322.

Tassoul MD and Shaver RD 2009. Effect of a mixture of supplemental dietary plant essential oils on performance of periparturient and early lactation dairy cows. Journal of Dairy Science 92, 1734–1740.

Taylor L and Curthoys NP 2004. Glutamine metabolism: role in acid-base balance. Biochemistry and Molecular Biology Education 32, 291–304.

Weiner ID and Verlander JW 2017. Ammonia transporters and their role in acid-base balance. Physiological Reviews 97, 465–494.

Wilkens MR, Kunert-Keil C, Brinkmeier H and Schröder B 2009. Expression of calcium channel TRPV6 in ovine epithelial tissue. The Veterinary Journal 182, 294–300.