1.

Post mortem endpoints of ruminal fermentation and anion/proton transporter gene expression as affected by variations in the amounts of physically effective neutral detergent fibre in the diets of growing German Fleckvieh bulls

Zum Einfluss einer variierenden Versorgung von wachsenden Bullen der Rasse Deutsches Fleckvieh auf post mortem Parameter der Pansenfermentation und die Genexpression von Anionen/Protonen Transportern

*Brugger D. , Ettle T., Feser S., Windisch W. M., Bolduan C. – Freising/Poing-Grub

Question: Sufficient alimentary supply with physically effective fibre is crucial for maintaining health and performance of ruminants. Currently, the respective feeding recommendations are re-evaluated with growing German Fleckvieh Bulls (1). In this context, the present study assessed the response of parameters of ruminal fermentation and anion/proton transporter gene expression.

Methods: 70 growing German Fleckvieh Bulls (517 ±32 kg life weight; 314 ±11d of age) were randomly assigned to three dietary treatment groups differing in physically effective neutral detergent fibre (peNDF) (294, 270, 246 g/kg DM) under isoenergetic and isonitrogenous conditions (per kg of DM: 12 MJ ME, 134 g CP) (1). After 191 days of ad libitum feeding, all animals were starved for precisely one day and sacrificed through slaughtering. 20 animals per group were randomly chosen for chemical analyses. Ruminal fluids were analyzed for total short-chain fatty acids (SCFA), ammonia, and pH. Transporter gene expression was quantified by RT-qPCR within total RNA extracts from rumen epithelium. SLC9A3 represents a Na+/H+ antiporter localized in the plasma membrane of rumen epithelial cells. SLC16A1 is a SCFA-/H+ symporter in the basolateral membrane of rumen epithelial cells whereas SLC26A3 is a SCFA-/HCO3- antiporter in the apical membrane. All data was subject to one-way ANOVA.

Results: Reduction of peNDF numerically reduced concentration of SCFAs and, consequently, increased ruminal pH in the lowest supplied group. Ruminal ammonia exhibited a numerically relevant decline within the middle group. The expression of SLC9A3 and SLC26A3 increased with declining dietary peNDF supply. However, these differences were only significant/relevant between the highest and lowest supplied groups. SLC16A1 reacted curvilinear over dietary treatment groups with a significant decline in the middle group compared to the highest and lowest supplied group, respectively.

peNDF/structural value	**g/kg/---**	**294/1.2**	**270/1.1**	**246/0.6**	**SEM**	**ANOVA**
pH	---	7.04	7.06	7.14	0.03	0.06
Total SCFA	mmol/L	55.49	51.30	46.04	3.72	0.19
Ammonia	mmol/L	9.75	8.71	9.25	0.33	0.10
SLC9A3	xfold regulation	1.00^{b}	1.38^{ba}	3.01^{a}	0.12	0.02
SLC16A1	xfold regulation	1.00^{a}	0.70^{b}	1.10^{a}	0.05	0.01
SLC26A3	xfold regulation	1.00^{b}	2.06^{b}	5.68^{a}	0.10	<0.0001

Conclusion: An increased abundance of SCFAs in the rumen is associated with a more efficient absorption of SCFA from the lumen and, conversely, increased transport of HCO_3^- into the lumen in order to buffer the system (2). In the present study, one day of starvation induced a linear decrease in total SCFA with stepwise reduction in peNDF. Consequently, pH in the lowest supplied group was increased. Conversely, the gene expression data points towards a linearly increased expression of SLC26A3 with decreasing peNDF supply. Furthermore, SLC9A3 reacted in a comparable manner to SLC26A3 which indicates a higher necessity to clear protons from the epithelial cytosol in order to stabilize intracellular pH. In summary, we hypothesize reduced peNDF intake induced an increase in SCFA abundance within the ruminal lumen which fostered the necessity for higher $SCFA^-/HCO_3^-$ exchange efficiency at the rumen epithelium in order to stabilize the chemical conditions.

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*Lehrstuhl für Tierernährung, Technische Universität München, Liesel-Beckmann-Straße 2, 85354 Freising (Weihenstephan), E-mail: daniel.brugger@wzw.tum.de

2.

Ruminal absorption of short chain fatty acids as affected by a continuous or interrupted adaptation to a high concentrate diet in dairy cattle

Einfluss einer kontinuierlichen oder unterbrochenen Adaptation an eine kraftfutterreiche Diät auf die ruminale Resorption kurzkettiger Fettsäuren bei Milchkühen

*Qumar M., Khiaosa-ard R., Pourazad P., Wetzels S., Klevenhusen F., Aschenbach J., Zebeli Q. – Vienna/Berlin

Short chain fatty acids (SCFA) are the microbial fermentation end products and are continuously absorbed through the reticulorumen epithelium to provide energy to the animal. Epithelial adaptation plays a key role in SCFA absorption, but these adaptive changes depend on duration and pattern of high concentrate feeding. The current study was undertaken to investigate the absorption of SCFA at different points in response to the pattern and duration of a high concentrate feeding.

Methods: Eight rumen-cannulated non-lactating Holstein cows were blocked by BW and randomly assigned to two concentrate feeding models, namely continuous or interrupted feeding of high-grain diet. The experiment consisted of two runs (n=8 per model) with washout period of 8 wk. At the start of each experimental run, all cows were fed a forage-only diet (baseline) and gradually transitioned over 6 d to a 60% concentrate diet. Thereafter, cows with continuous feeding of 60% concentrate were kept on this diet for 4 wk. Interruptedly concentrate-fed animals were kept on the 60% concentrate diet for 1 wk, followed by 1 wk of the forage-only diet and then they returned to the 60% concentrate diet for 2 wk. The temporarily-isolated and washed reticulorumen procedure (WRP) was performed (1) at baseline (Base-I; d-0), after the first wk of the concentrate feeding (INT1; d-14) and 2 wk after the concentrate break (INT2; d-35). With continuous concentrate feeding, WRP was performed at baseline (Base-C; d-0) and at the end of the 4-wk challenge (CONT; d-35). During WRP, digesta was removed from the reticulorumen and stored in an insulated container and then the reticulorumen was cleaned with washing buffer. Afterwards, 20-L experimental buffer containing CaCl2 (2 m*M*), MgCl2 (2 m*M*), NaCl (10 m*M*), NaHCO3 (25 m*M*), K2HPO4 (5 m*M*), sodium acetate (60 m*M*), sodium propionate (30 m*M*), butyrate (10 m*M*), and Cr-EDTA (1.8 m*M*) was infused into the reticulorumen of cows for 65 min. The buffer samples were collected at 0 and 65 min for SCFA concentrations (acetate, propionate, butyrate, and total SCFA) and the absorption rates were calculated. Data were analyzed by the mixed procedure of SAS to test the fixed effect of the concentrate periods balanced for values of the respective baseline (co-variable).

Results: The concentrate feeding periods had a strong effect on absorption rates of all SCFA tested ($P<0.01$). The absorption rate of total SCFA during 0-65 min was lowest in INT1 (489 mmol/h), intermediate in INT2 (638 mmol/h) and highest in CONT (845 mmol/h). These values accounted for 90, 118 and 156% of the averaged baseline. Fractional absorption rate of total SCFA during 0-65 min was similarly affected by the concentrate period and that the rates were 23.2, 29.8 and 37.9 %/h in INT1, INT2 and CONT, respectively. Molar proportions of unabsorbed SCFA changed with the incubation time ($P<0.01$) with less propionate and butyrate but more acetate proportions. This pattern of change, particular of acetate and propionate, was more pronounced with CONT than both INT groups.

Conclusions: Highest SCFA absorption was evident after 4 wk of the continuous high concentrate feeding. Therefore, adaptation of the rumen epithelium, as a mechanism to increase the SCFA absorption capability, appeared to require more than 1-2 wk and the interruption of the concentrate feeding postponed the adaptation.

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* University of Veterinary Medicine, Institute of Animal Nutrition and Functional Plant Compounds, Veterinärplatz 1, 1210 Vienna, Austria, Email: Muhammad.Qumar@vetmeduni.ac.at

3.

Functional and molecular biological evidence for the involvement of TRPV3 and TRPA1 in the absorption of cations by the ruminal epithelium

Funktionelle und molekularbiologische Hinweise auf die Beteiligung von TRPV3 und TRPA1 an der Absorption von Kationen durch das Pansenepithel

*Braun H. S., Hille K. T., Rosendahl J., Aschenbach J. R., Stumpff F. – Berlin

A recent study of the bovine rumen (1) suggests that as previously shown for Na^+ (2), NH_4^+ - induced short-circuit currents are modulated by divalent cations and by menthol and thymol, which are known for their highly selective interaction with three channels of the transient receptor potential (TRP) family: TRPA1, TRPV3 and TRPM8 (3). It was the aim of the present study to identify suitable candidate genes and to study the effect of these agonists on ovine preparations.

Methods: RNA of the ruminal epithelium of three lactating cows and two sheep was isolated and transcribed into cDNA. Intron-spanning primer pairs were used to detect the bovine target genes TRPV1, TRPV2 and the bovine and ovine genes TRPV3-6, TRPA1 and TRPM6-8. To ensure binding to the correct target, all amplicons were subsequently sequenced. The Ussing chamber technique was used to measure the impact of different concentrations of menthol or thymol on the short-circuit current (I_{sc}) and conductance (G_t) of ovine ruminal epithelium (N = 7). Stripped tissues were equilibrated in standard, ammonia-free, bicarbonate-containing buffer solutions gassed with 5% CO_2/95% O_2. Mucosal solutions contained short chain fatty acids (pH 6.4) which were replaced serosally by Na-gluconate (pH 7.4). Menthol, thymol or pure solvent (ethanol) were added to the mucosal side of the tissue, yielding end concentrations of 0, 10, 100 and 1000 µM, with n = 18 tissues in each treatment group. Data were compared using Repeated Measures ANOVA on Ranks.

Results: Ovine and bovine ruminal epithelium expressed mRNA for the following channels: TRPA1, TRPV3, TRPV4, TRPM6 and TRPM7. Only a weak band was discovered for TRPV6, while TRPV5 and TRPM8 could not be detected. No reliable band was found for TRPV1 and TRPV2 in bovine ruminal tissue. In the Ussing chamber experiments, 10 µM menthol or thymol showed no effects versus control. At 1000 µM, menthol (N/n = 4/18) and thymol (N/n = 3/18) had a biphasic effect on I_{sc}, which increased sharply after application of the agonists ($p \leq 0.001$) with a subsequent decline to a value below the original level ($p < 0.05$). Tissue conductance G_t also rose abruptly in response to both agonists and continued to rise after I_{sc} values peaked ($p < 0.001$). At 100 µM, effects of both agonists on I_{sc} and G_t were diverse, with some tissues showing a monophasic and other tissues a full biphasic response.

Conclusion: We present evidence for the expression of mRNA encoding for TRPV3, TRPV4 and TRPA1 in addition to the epithelial Mg^{2+} channel TRPM6 by the ovine and bovine rumen. We confirm expression of TRPM7, while TRPM8 was not expressed. Functionally, we demonstrate that menthol and thymol modulate the I_{sc} and G_t across the ovine ruminal epithelium in a dose-dependent manner. In the absence of a chemical gradient, the increase in I_{sc} should reflect effects of the modulators on transcellular transport. In conjunction with work previously done (1,3), TRPA1 or TRPV3 or both emerge as prime candidates mediating the electrogenic transport of Na^+ and NH_4^+ across the ruminal epithelium, with TRPV4 possibly playing an additional role.

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*Freie Universität Berlin, Fb Veterinärmedizin, Oertzenweg 19b, 14163 Berlin, stumpff@zedat.fu-berlin.de

4.

Challenging the porcine intestinal epithelial barrier function by milk

Effekte von Milch auf die porcine intestinale Barrierefunktion

Radloff J., *Amasheh S. – Berlin

Outline: Newborn's only source of nutrition is milk provided by their lactating mothers. It contains proteins, carbohydrates, fatty acids and other essential nutritive substances which help the newborn thrive and establish a competent immune system. However, some milk components in porcine milk have been identified to have an effect on the epithelial barrier function determined by tight junction proteins, including claudin-5 (1), which might increase absorptive capacity of piglet intestinal epithelium. Thus, we hypothesized that milk has an influence on barrier properties, which might be caused by effects on tight junction protein expression and/or localization.

Methods: Piglet intestinal tissue samples were mounted in conventional Ussing chambers and incubated with a half-and-half mixture of either predigested or non-predigested porcine milk with physiological electrolyte buffer solution on the apical side, while only buffer was used on the basolateral side. Transepithelial resistance was reported, and unidirectional paracellular marker flux analyses were performed using sodium fluorescein under voltage-clamp conditions (0 mV). For further analysis, protein preparations of selfsame tissue specimens were performed, and analyzed by PAGE employing specific antibodies raised against tight junction proteins, including members of the tight junction protein family of claudins.

Results: Both, non-digested and pre-digested milk induced an increase of transepithelial resistance from 39.0 ± 4.6 to 62.0 ± 2.2 Ω • cm2, and from 38.7 ± 4.3 to 46.3 ± 3.7 Ω • cm2 within the first 30 min (n= 6 and 7, ***$p<0.001$ and *$p<0.05$, respectively). Fluorescein marker flux measurements revealed no significant effect on respective paracellular permeability and remained stable during the course of the experiment (3 h, n = 6 and 7, respectively). On protein level, no significant change of claudin-5 was detected after incubation with non-digested and pre-digested milk (n=3, respectively).

Conclusion: Although an increased piglet intestinal epithelial permeability might be a benefit regarding the uptake of nutrients, the opposite effect could be observed in our model. Moreover, no significant changes of a major barrier-determining tight junction protein could be observed.

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*Freie Universität Berlin, Fb Veterinärmedizin, Oertzenweg 19b, 14163 Berlin, salah.amasheh@fu-berlin.de