



Impaired gut health: the root of systemic disorders in cattle

Qendrim Zebeli



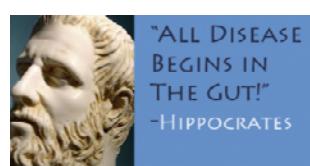
An inside-outside approach to dairy cow health



University of Veterinary Medicine, Vienna (Vetmeduni Vienna)

Gut and disease

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"ALL DISEASE
BEGINS IN
THE GUT!"
-HIPPOCRATES

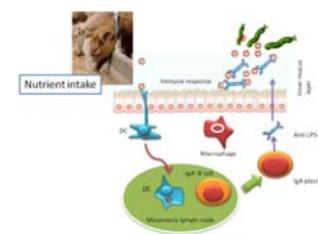


Genotype & environmental stressors
(Antimicrobials, pathogen, diet, disease)
Hormones & Nutrition

Gut homeostasis ↔ Gut microbiome

Bacteria, mucusols, and
metabolic signatures
Health/gut

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Zebeli et al. 2011 Innate Immunity

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Diet for health and performance

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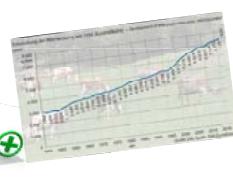
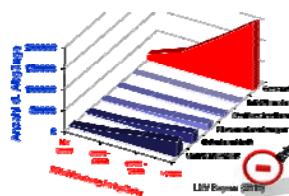
- Low productivity
- Welfare, ethical issues



1. Supporting high milk yields
(feed efficiency)



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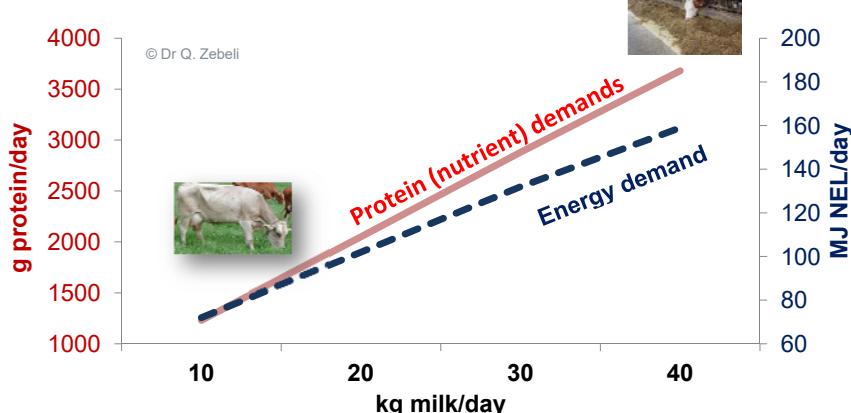
1. Avoid nutrient deficiencies/health risks related to diet (support health/welfare)



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High the performance, higher the demands!

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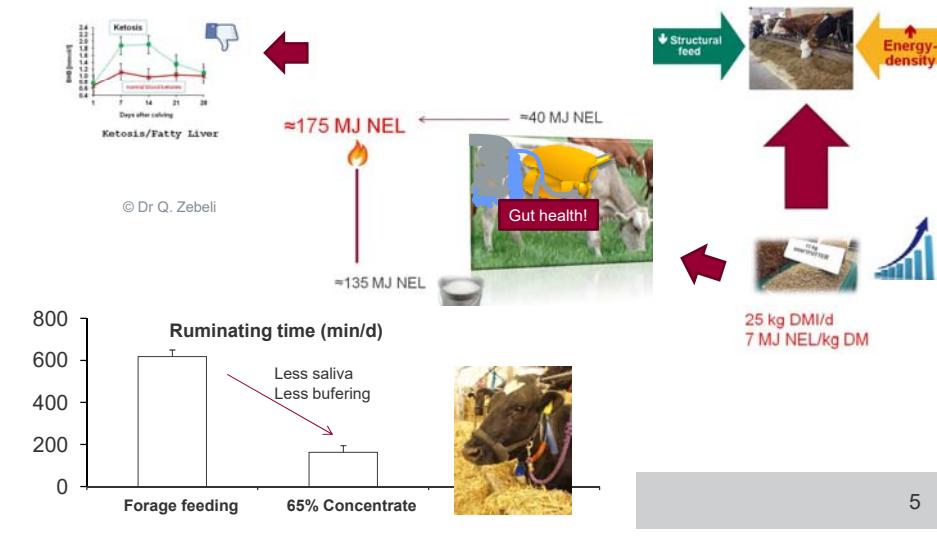
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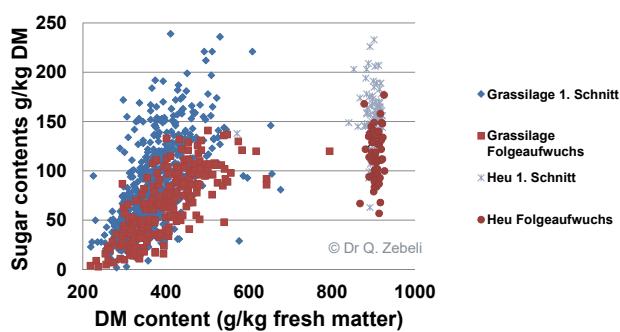
Diet balancing becomes increasingly difficult

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Sugars in silages and hays in AUT

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Futtermittellabor Rosenau (2017)

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Rumen fermentation load

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Lactate → SCFA

High grain diets

≈120 mol short-chain fatty acids (SCFA) (6-7 kg/daily):
70-75% of energy requirements

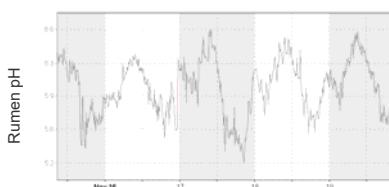
High fermentation load acidifies the rumen

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Physical fiber
Dietary fiber Particle size
Bicarbonate
Risk of SARA
Starch amount Starch fermentability
Lactate VFA

Rumen pH drop

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PhD work P Pourazad, Vetmeduni Vienna

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SARA thresholds

If longer than 5-6 h/day (Zebeli et al. 2008 J Dairy Sci)
If longer than 3 h/day (Plaizier et al. 2008 Vet J)

How common is SARA?

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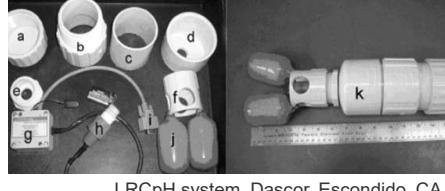
- Limited information surveys with rumenocentesis based on rumen pH (5.5 threshold)
 - Wisconsin, USA → 19% (early) and 26% (mid lactation) (Garrett et al., 1997), 20.1% in early and peak lactation (Oetzel et al., 1999)
 - The Netherlands → 13.8% (0 – 38% on farms) (Kleen et al., 2009)
 - Germany → 20% (Kleen et al., 2013)
 - Italy → 33% (Morgante et al., 2007)



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SARA diagnosis via ruminal pH

- SARA threshold: pH<5.8
 - Spot sampling
 - Stomach tubes, Rumenocentesis, Rumen cannula
 - Location: Rumen
- Diurnal pH- changes!
- Wireless pH-boli
 - In non-fistulated cows
 - Herd level
 - Continuous measurement
- Location: Reticulum!

LRCpH system, Dascor, Escondido, CA



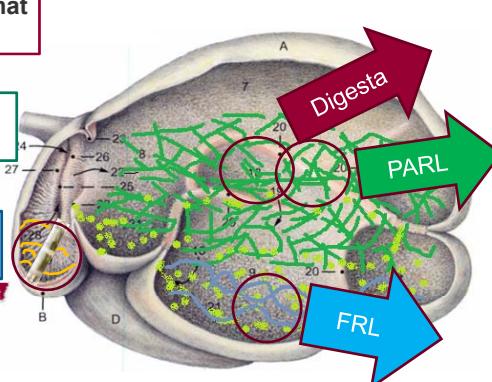
eCowTM

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Reticular vs. ruminal pH

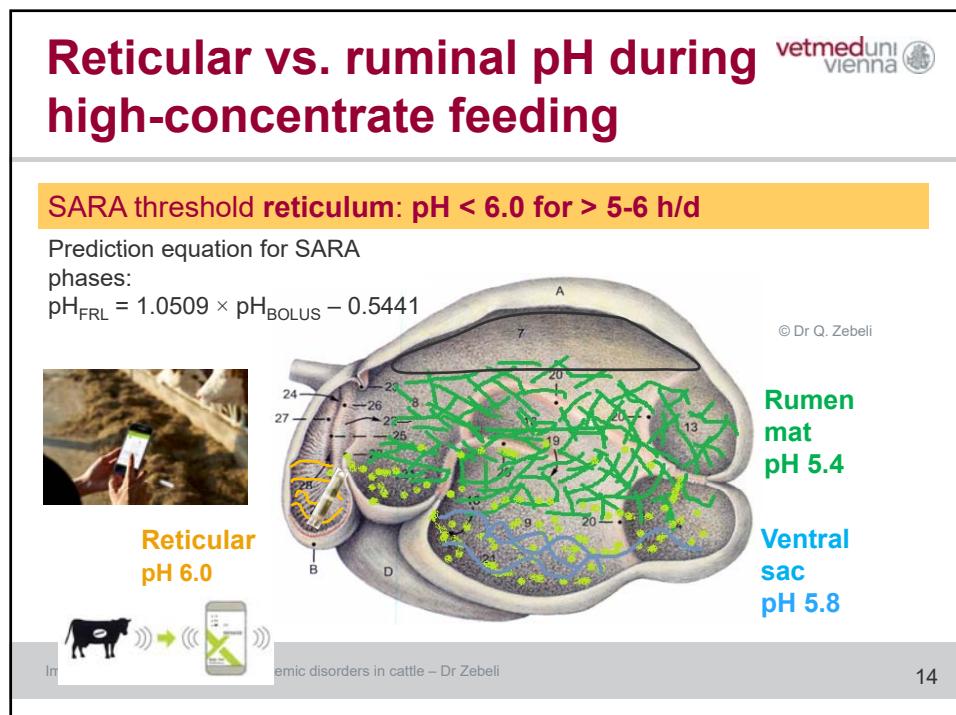
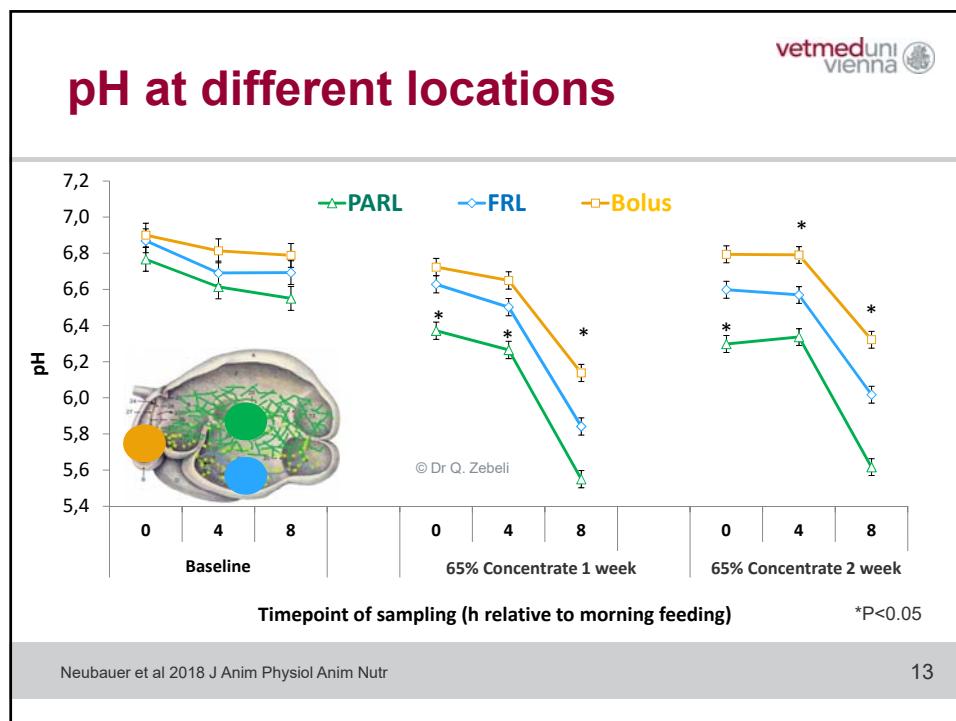
- Particles of rumen mat (Digesta)
- Particle-associated-rumen liquid (PARL)
- Ventral sac: Free rumen liquid (FRL)

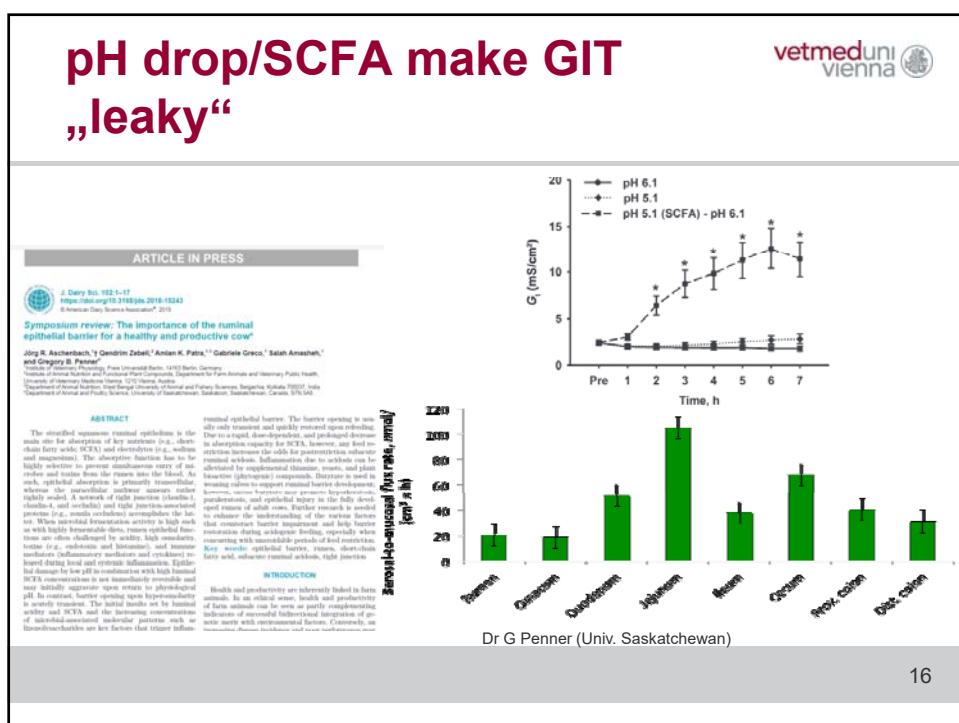
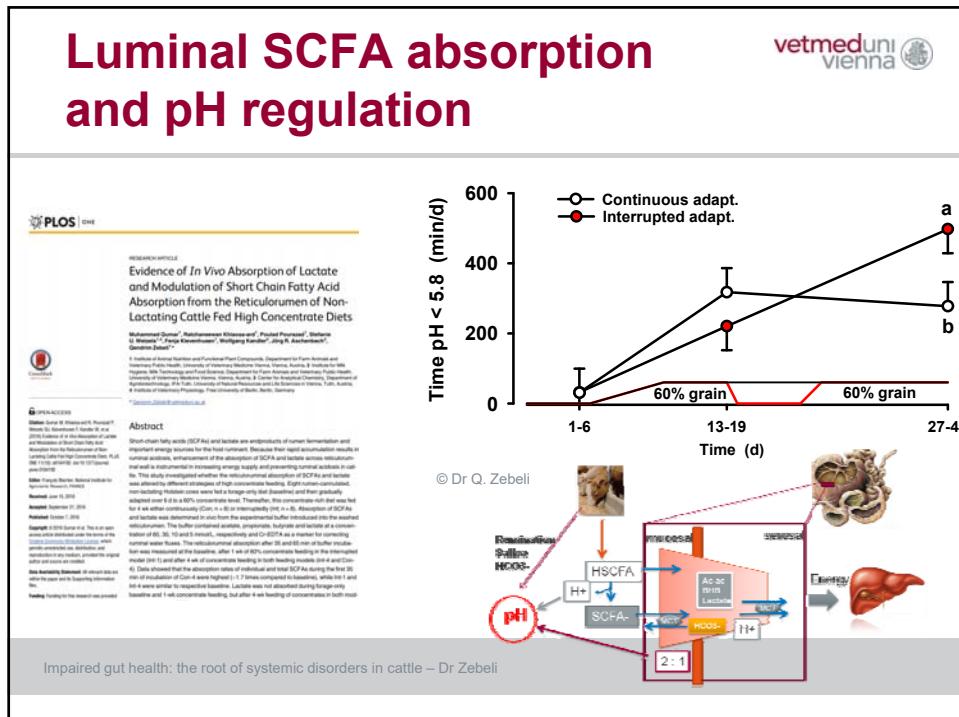


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Hindgut acidosis

RUMINANT NUTRITION SYMPOSIUM: Productivity, digestion, and health responses to hindgut acidosis in ruminants^a

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ABSTRACT: Microbial fermentation of volatile fatty acids (VFA) is the primary source of energy for the hindgut, accounting for 5 to 10% of total tract carbohydrate digestion. When dietary amount, or rate, of fermentable carbohydrates increases, without a rise in fiber content, from the small intestine, hindgut acidosis can occur. Hindgut acidosis has been associated with decreased production of short-chain fatty acids (including butyric acid), increased production of volatile fatty acids (VFAs), and decreased pH. The decrease in pH is evidenced by the appearance of acetate casts in feces. The relationship between hindgut acidosis and high-producing animals has data with relatively greater production of VFAs and decreased pH. In cattle fed diets high in starch, ruminal acetate and poor selective intake of fermentable carbohydrates increase. This will increase hindgut acidosis due to the hindgut. As more fermentable hindgut acidosis is demonstrated, the risk of inflammatory hindgut acidosis increases. The balance between ruminal and hindgut may contribute to hindgut acidosis. The balance between ruminal and hindgut acidosis may contribute to hindgut acidosis. The balance between ruminal and hindgut acidosis may contribute to hindgut acidosis. The balance between ruminal and hindgut acidosis may contribute to hindgut acidosis. Continuous small-dose administration of lactobacillus. Continuous small-dose administration of lactobacillus.

Keywords: acetosis, hindgut, rumen

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INTRODUCTION

The role of hindgut acidosis or hindgut fermentation in ruminant nutrition has received little research attention in recent decades. Although the contribution of the hindgut to total tract nutrient digestion is substantially less than the contribution from the rumen, hindgut fermentation is important to animal health and performance. As described subsequently, hindgut fermentation typically provides 5 to 10% of dietary energy but certain conditions can increase its contribution to total tract carbohydrate fermentation in the hindgut. Hindgut fermentation is associated with a decrease in ruminal pH and a subsequent decrease in digesta pH and digesta viscosity. A decrease in digesta pH and viscosity can damage to the animal; it is often indicative of failure of the ruminal fermentation process. As the pH decreases they increase with production level and fermentable carbohydrate intake. But previous review have focused

Too much fermentable substrates escaping the rumen!!

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Hindgut acidosis vs. Rumen acidosis

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Molecular biology, genetics and biochemistry

Grain-rich diets differentially alter ruminal and colonic abundance of microbial populations and lipopolysaccharide in goats

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Table 3
pH and total SCFA concentration in rumen filtrate and colon digesta of goats fed diets containing 0, 30 and 60% barley grain.

Item	% Grain			Pooled SEM	P-value contrast			
	0	30	60		Linear	Quadratic	0 Versus 30 + 60% grain	0 + 30 versus 60% grain
pH								
Rumen	6.4	6.0	5.5	0.13	<0.001	0.726	0.003	0.001
Colon	8.5	8.1	7.0	0.27	0.006	0.248	0.034	0.005
Total SCFA								
Rumen ($\mu\text{mol}/\text{ml}$)	113	120	129	5.48	0.105	0.920	0.162	0.128
Colon ($\mu\text{mol}/\text{g}$)	20	26	78	15.83	0.039	0.338	0.156	0.015

Values are least squares means \pm pooled standard error of the mean (SEM); $n = 5$ for 0% grain diet; $n = 6$ for 30 and 60% barley grain diet.

Quantitatively, the fermentation is less intensive in the hindgut

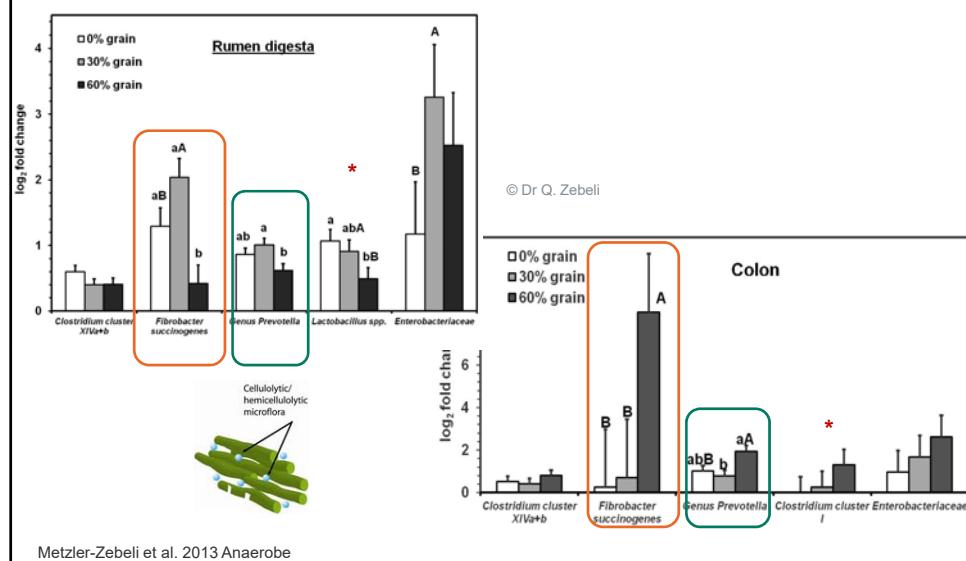
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Microbiota in the rumen and colon due to SARA

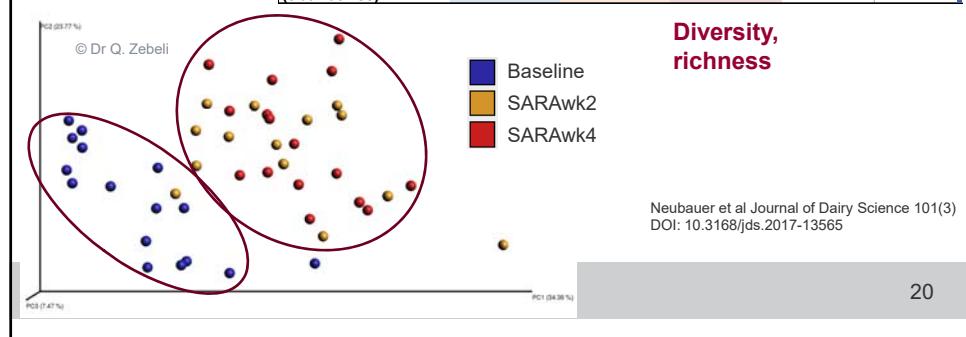
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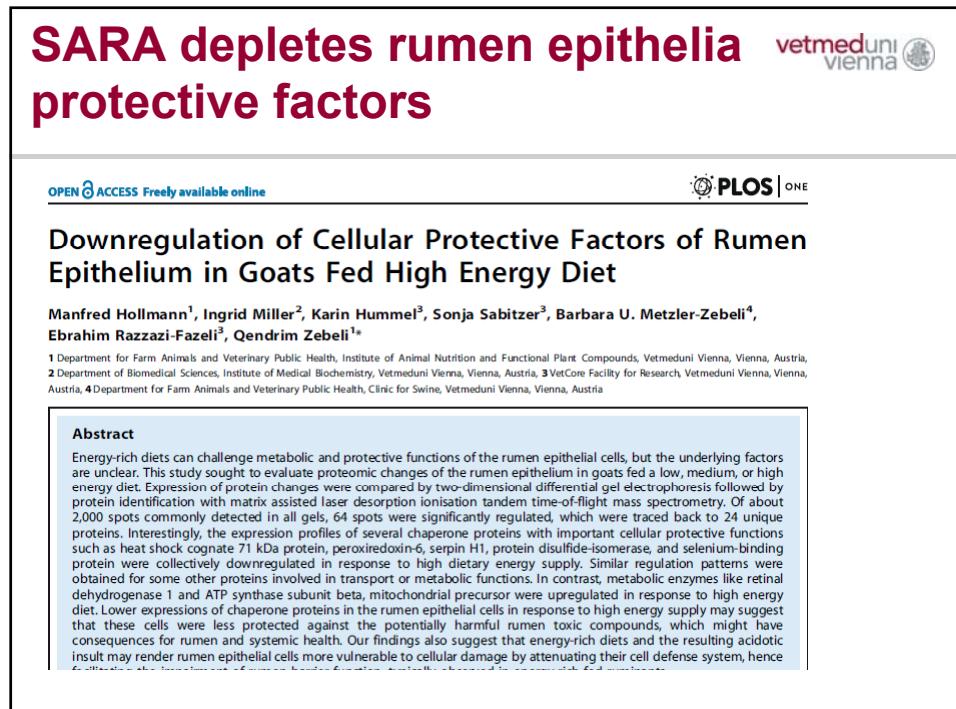
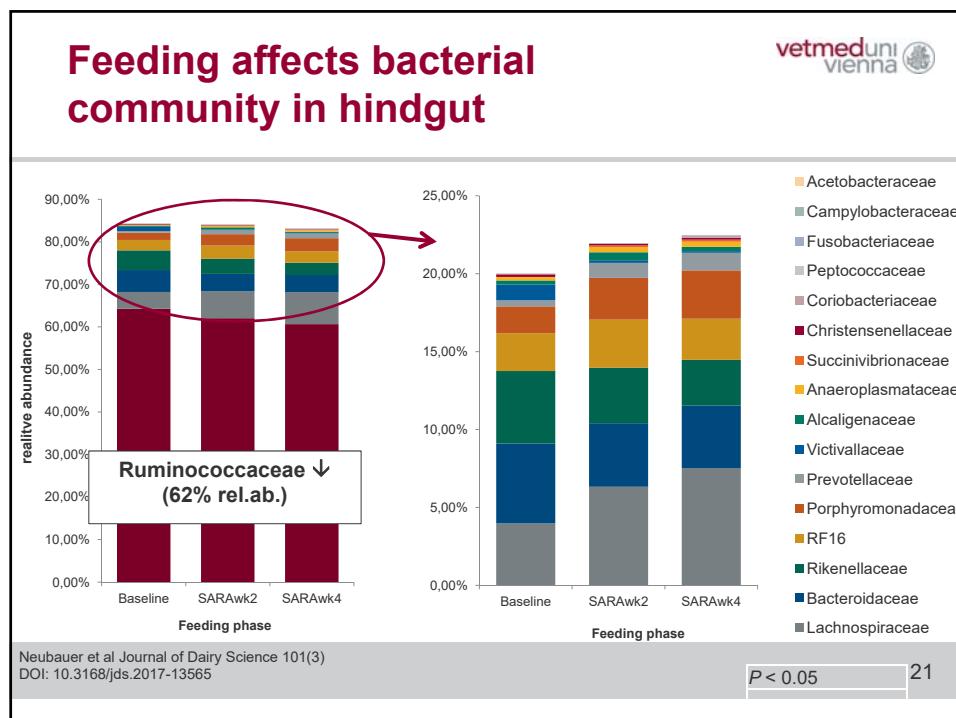
Grain feeding affects bacterial diversity and community structure

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Index	Baseline	SARA wk2	SARA wk4	SEM	P-value
observed OTU's	1623 ^a	1439 ^b	1389 ^b	30.6	<0.001
Chao1 (richness estimator)	2787 ^a	2402 ^b	2381 ^b	51.8	<0.001
Shannon (count, abundance)	8.88 ^a	8.21 ^b	8.20 ^b	0.070	<0.001
Simpson (abundance)	0.993 ^a	0.982 ^b	0.984 ^b	0.001	<0.001



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SARA depletes rumen epithelia protective factors

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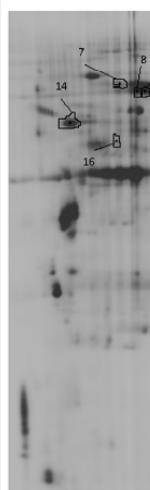


Table 2. Differentially expressed proteins in the rumen epithelium of goats fed diets differing in the energy supply¹.

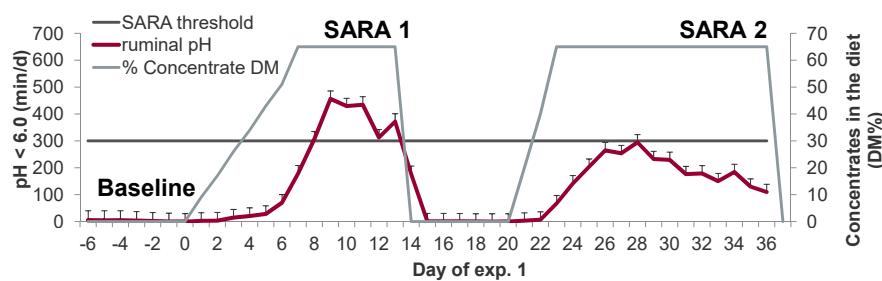
Spot ^a	Theoretical mass (kDa)	pI	Sc ^b (%)	Accession ^c	Annotation	Av. Ratio		
						L vs. M	M vs. H	L vs. H
1	6.6 ^d	9.5	39.3	gi 2318026	Troponin	-1.18	-1.30	-1.33*
2	6.6 ^d	9.5	39.3	gi 2318026	Troponin	-1.22	-1.39	-1.69**
3	6.6 ^d	9.5	39.3	gi 2318026	Troponin	-1.23	-1.46	-1.79**
4	6.6 ^d	9.5	39.3	gi 2318026	Troponin	-1.24	-1.24	-1.54*
5	6.6 ^d	9.5	39.3	gi 2318026	Troponin	-1.28	-1.42	-1.82**
6	6.6 ^d	9.5	39.3	gi 2318026	Troponin	-1.33	-1.54	-2.06**
7	71.2	5.2	9.2	HPV1_BOVIN	Heat shock cognate 71 kDa protein	-1.04	-1.26*	-1.31*
8	66.3	5.5	6.5	gi 193085052	Albumin precursor	-1.15	-1.16	-1.23*
9	66.3	5.5	9.3	gi 193085052	Albumin precursor	-1.41	-1.48	-2.09**
10	66.3	5.5	9.6	gi 193085052	Albumin precursor	-1.47*	-1.31	-1.92**
11	66.3	5.5	6.5	gi 193085052	Albumin precursor	-1.41	-1.36	-1.92**
12	66.3	5.5	7.2	gi 193085052	Albumin precursor	-1.52	-1.38	-2.10**
13	66.3	5.5	9.1	gi 193085052	Albumin precursor	-1.57	-1.22	-1.91*
14	57.2	4.7	6.3	POAT1_BOVIN	Protein disulfide-isomerase	+1.48*	-1.82*	-1.23
15	54.8	6.4	11.4	ALAT1_SHEEP	Retinal dehydrogenase 1	+1.38	+1.13	+1.56*
16	56.2	5.0	11.7	ATPB_BOVIN	ATP synthase subunit beta, mitochondrial precursor	-1.00	+1.73*	+1.72*
17	46.5	9.5	12.0	SEPH1_BOVIN	Sepin H1	+1.35	-1.86**	-1.38
18	52.5	6.0	15.9	SBP1_BOVIN	Selenium-binding protein 1	-1.07	-1.92*	-2.06*
19	58.8	7.4	4.9	HBD_RABT	Histidine-rich glycoprotein	-1.57	+1.70*	+1.12
20	25.1	6.0	14.3	PRDX6_BOVIN	Peroxiredoxin 6	+1.02	-1.59	-1.56*
21	25.1	6.0	27.2	PRDX6_BOVIN	Peroxiredoxin 6	+1.19	-1.50	-1.30*
22	25.1	6.0	18.8	PRDX6_BOVIN	Peroxiredoxin 6	+1.03	-1.54	-1.50**
23	26.7	6.5	18.9	TPS_BOVIN	Tetrosphosphate isomerase	-1.10	-1.27	-1.39**
24	17.3	9.0	25.7	NDX8_BOVIN	Nucleoside diphosphate kinase B	-1.23	-1.55	-1.31*

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Ruminal pH < 6.0 (min/d) during 2 SARA

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Neubauer et al Journal of Dairy Science 101(3)
DOI: 10.3168/jds.2017-13565

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SARA and rumen metabolomic changes

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Abstract
 Subacute ruminal acidosis (SARA) causes ruminal dysbiosis, thereby increasing the risk of ruminal metabolic disorders in cattle. We recently showed that supplementation with phytoprobiotic compounds (PHY) or a mixture of AAs (AY) reduced the incidence and severity of SARA by improving ruminal pH and microbiome. The aim of this study was to evaluate the effects of SARA challenge on the ruminal concentrations of bacterial LPS and lipopolysaccharides (LPS), as well as on the blood amino acids (AA). We also evaluated the effects of PHY and AY on the latter variables. Eight ruminal fluid samples were collected from each cow (three samples per cow) at baseline, during SARA challenge, and after treatment. At baseline, the ruminal pH ranged from 6.4 to 7.0, and the total VFA concentration ranged from 1.4 to 1.8 mmol/L. The total VFA concentration increased during SARA challenge, and decreased after treatment. The ruminal pH was significantly lower during SARA compared to baseline. Moreover, a decrease in phosphorus/folates ratio was observed during SARA. Several AA in the blood during SARA were detected. Supplementation with PHY decreased concentrations of LPS (-47%), histamine (-46%), pyroglutamate (-38%), spermidine (-36%) and spermine (-35%) in SARA1 and enhanced in SARA2. Conversely, the concentrations of tryptophane (+26%), several AA, and phosphatidylcholine in SARA1 compared with control group were increased. The concentrations of tryptophane (-21%), histamine (-14%), spermidine (-14%) and spermine (-14%) were increased, whereas in the blood an increase in tryptophane was noticed. In conclusion, the SARA was associated with significant changes in the concentrations of LPS and AA in the rumen fluid and undetectable changes in the blood. We conclude that the administration of PHY and AY counteracted some of these changes and chelate may help in attenuating negative effects of SARA.

Keywords: feed additive; subacute rumen acidosis; ruminal bacteria; blood metabolites; tryptophane; spermidine; spermine; blood metabolites; tryptophane; chelate.

Introduction
 Concentrator-rich diets have become a common practice in the commercial dairy production increasing the risk of SARA. This digestive disorder is characterized by a primary ruminal acidosis, which leads to pH \leq 5.5, causing major microbial imbalances in the rumen (Tsangaris et al., 2000; Khafipour et al., 2009), ammonia release (Kaufmann et al., 2009) and metabolic changes that gastrointestinal dysbiosis during SARA promotes the release of pro-inflammatory agents such as endotoxins, bacterial LPS and long-chain fatty acids (LCFA). Ammonia release (Wang et al., 2013; Mai et al., 2016), which has been implicated in the pathogenesis of ruminal SARA in cattle (Plaizier et al., 2012).

The release of free ammonia by bacterial LPS and

Phase¹

Variable	Baseline	SARA1	SARA2	SEM	P value
LPS	14.581 ^c	131.209 ^b	168.285 ^a	25.722	<0.01
Ethanolamine	3.76 ^c	9.82 ^a	6.42 ^b	0.597	<0.01
Methylamine	37.3 ^{ab}	33.6 ^b	46.0 ^a	5.67	0.07
Isopropylamine	7.50 ^b	17.4 ^a	16.2 ^a	2.65	<0.01
Pyrrolidine	6.68 ^c	12.6 ^a	9.41 ^b	1.257	<0.01
Putrescine	14.0 ^b	32.2 ^a	30.7 ^a	3.65	<0.01
Cadaverine	15.9 ^a	24.2 ^{yz}	24.3 ^y	5.12	0.13
Histamine	2.06 ^b	13.0 ^a	5.19 ^b	3.326	<0.01
Tyramine	1.00 ^a	1.61 ^{yz}	2.14 ^y	0.614	0.18
Spermidine	8.62 ^b	14.9 ^a	17.0 ^a	2.417	<0.01
Spermine	0.70	1.09	1.10	0.431	0.40

^{xyz}Indicate significant ($P \leq 0.05$) changes or a trend (y,z) ($0.05 < P < 0.10$) between the feeding phases.
^aSARA1 = 65% concentrate for 1 wk; SARA2 = 65% concentrate for 2 wk.

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Main pro-inflammatory agents in the rumen

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LPS



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Histamine

Humer et al 2018 J Dairy Sci

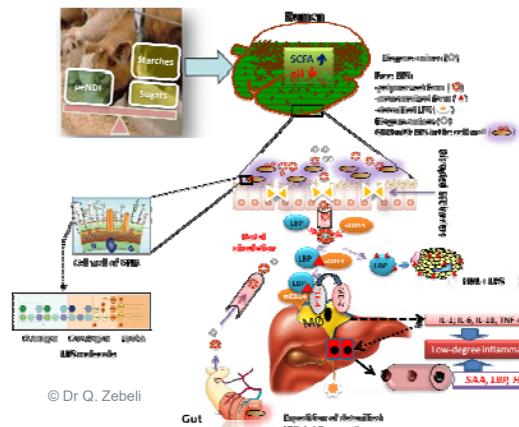
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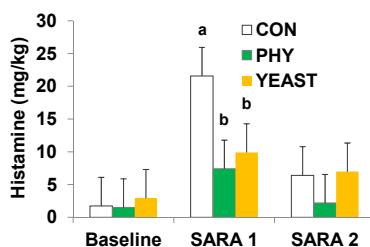
Proposed mechanism for SARA induced inflammation



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AY and PHY affect ruminal Histamine

J. Dairy Sci. 101:9559–9574
<https://doi.org/10.3168/jds.2018-1474>
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Supplementing phytonic compounds or autolyzed yeast modulates ruminal bioactive amines and plasma metabolome in dry cows experiencing subacute ruminal acidosis
 E. Hämmer, L. Kröger, V. Neumann, T. K. Schulz, B. Willeberg, S. Götz, and J. Zebeli*



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Systemic effects

Meena Arif Memon et al. 2019 Microbial Pathogenesis 128, 268-275

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Udder health

Innate immunity and metabolomic responses in dairy cows challenged intramammarily with lipopolysaccharide after subacute ruminal acidosis

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Keywords: acute phase response, dairy cattle, endotoxin, metabolomics, rumen fermentation, SARA

Introduction

Subacute ruminal acidosis (SARA) is a prevalent metabolic disorder in dairy cows known to elicit local and systemic immune responses. We recently showed that cows experiencing SARA and challenged intramammarily with lipopolysaccharide (LPS) exhibited a more pronounced acute phase response compared with healthy cows. In contrast, cows experiencing SARA had a modulated innate immune response and impaired plasma metabolism compared with healthy cows. Subsequently, we conducted an acute metabolic challenge. A total of 18 terminal cows were subjected either to a Control (CON, n = 6) or SARA (n = 12) group. All cows received 100 g of hay and 100 g of straw daily. The CON (CON/LPS) cows were intramammarily challenged with 50 µg LPS from Escherichia coli (E. coli), while the remaining six SARA (SARA/LPS) cows were challenged with 50 µg LPS from *Clostridium perfringens* type C. The challenge was performed using a ED-UC (metabolite-based metabolomics) approach was performed in blood samples 24 h after the LPS challenge. The LPS infusion was performed at 12 h. The results showed that cows experiencing SARA had a higher concentration of acute phase proteins (apoA-I, C-reactive protein, fibrinogen, haptoglobin, serum amyloid A) and endotoxin (LPS) compared with CON/LPS cows. Cows receiving the LPS infusion had a lower plasma concentration of several amino acid and branched-chain amino acids but without differences in SARA cows and healthy cows. In conclusion, our results revealed that an acute metabolic challenge with LPS induced a similar acute phase response in SARA and healthy cows. Further research is required to elucidate the underlying mechanisms and to evaluate its clinical significance for cattle health.

Prolonged udder inflammation due to SARA

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Supplementing a clay-based mineral product improves health

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Supplementation of a clay mineral-based product modulates plasma metabolomic profile and liver enzymes in cattle fed grain-rich diets

E. Hutter¹, T. Kötter², V. Neubauer³, N. Reisinger² and Q. Zebeli^{1,2*}

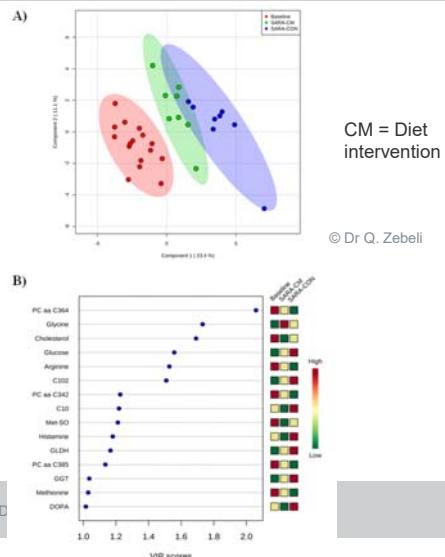
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Grain-rich diets often lead to subacute ruminal acidosis (SARA) impacting animal and systems cattle health. Animal data suggest beneficial effects of a clay mineral (CM) based product on the rumen metabolism of cattle during SARA. This study sought to investigate whether the CM supplement had a similar effect on the plasma metabolome and performance either in addition to or instead of CM as a diet intervention. Fourteen Holstein cattle were assigned to a 4-week SARA challenge (SARA group) and 14 cattle to a control (CM group). All cattle received a diet containing 30% grain and 70% grass silage. In the SARA group, the basal diet was replaced by a forage diet (silaged for 4 weeks, balanced by a 1:1 ratio SARA challenge diet). The CM group received the same diet with eight dry inclusion levels of CM (0–12 g/kg diet). Blood samples were taken and analysed for metabolites related to glucose and lipid metabolism, protein metabolism and energy metabolism. A metabolomic approach was carried out on the plasma samples obtained at the end of the baseline and SARA 1 plasma. Data analysis revealed that the CM supplement reduced the plasma metabolite concentrations of glucose, lactate and glycerol ($P < 0.05$) and reduced the dry matter intake during the recovery phase ($P = 0.02$). Moreover, the SARA-induced decreases in water intake and ruminal pH were partially prevented in cattle receiving CM ($P < 0.05$). The metabolomic approach also had been able to identify effects of CM on plasma metabolite concentrations ($P < 0.05$) in the basal diet. In contrast, the quantification of six metabolites used by the metabolic function was increased in the basal diet over $P < 0.05$. In summary, the data suggest that supplementation of CM has the potential to alleviate the negative effects of high-grain diets on cattle health. The results further support the hypothesis that CM may reduce the risk of ruminal acid-base regulation and diarrhoea.

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Impaired gut health: the root of systemic disorders in cattle – D



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Fix the cause or treat the symptoms?

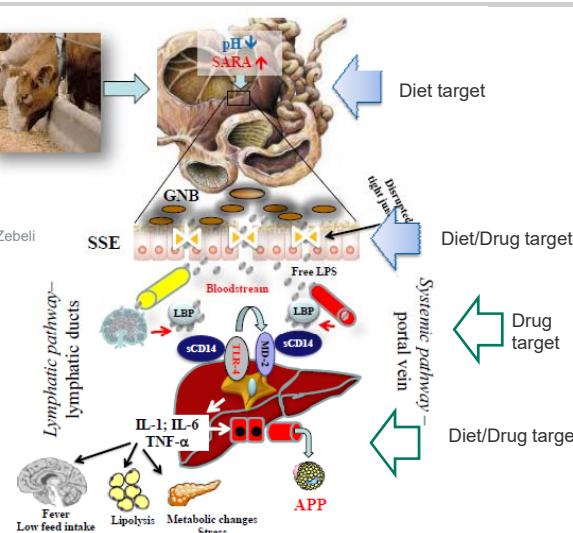
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Dietary target



Diet target

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Zebeli & Metzler-Zebeli 2012, Res. Vet. Sci. doi:10.1016/j.rvsc.2012.02.004



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