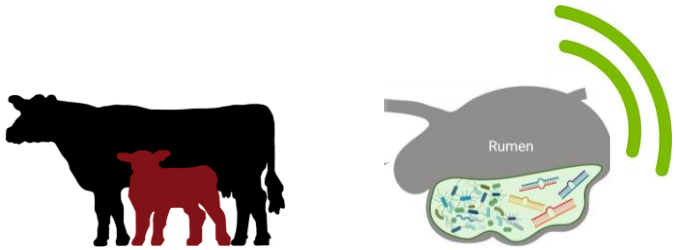
Biomarkers of rumen health:
what do they tell us in terms of diagnosis and prevention?



Qendrim Zebeli

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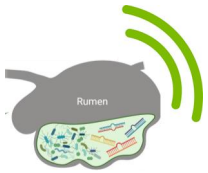
vetmeduni vienna

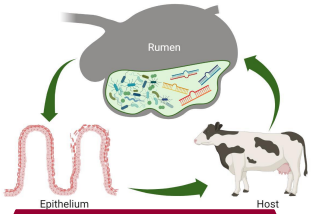
Dairy conference „How to limit ruminal acidosis in ruminants?“

20-21st March 2024, Krakow, Poland

Main aspects of this talk

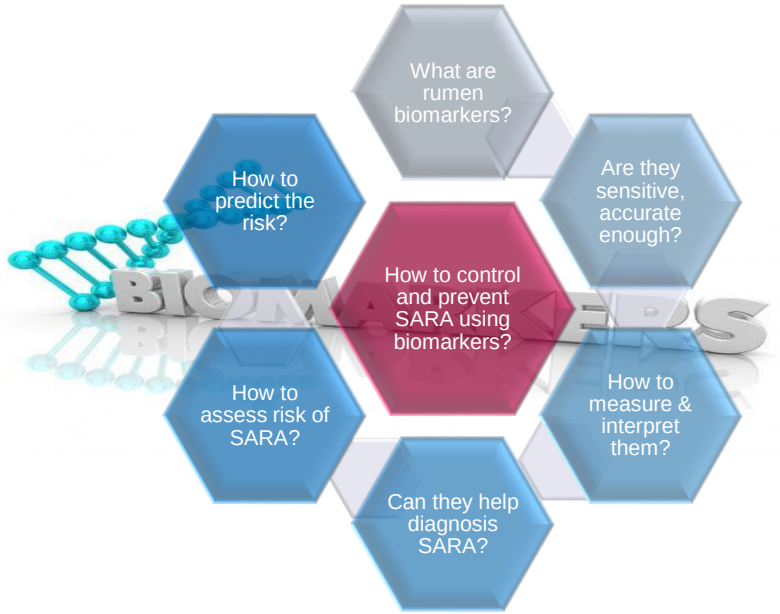


Direct biomarkers of rumen health



Indirect biomarkers of rumen health

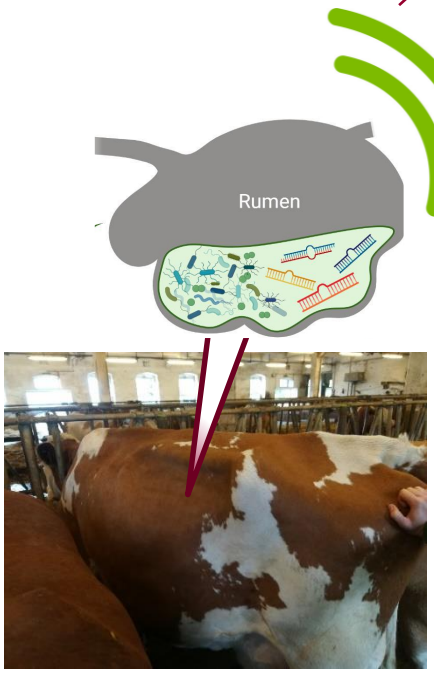
Prediction, control & prevention



Rumen biomarkers = rumen signals

- (easy) Measureable
- Specific
- Sensitive
- Accurate/reproducible

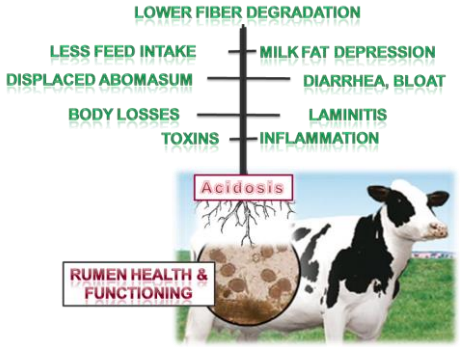
1. Direct biomarkers
2. Indirect biomarkers



Recognize/diagnose rumen health disorders

Understand/predict/control

Prevent the disorder/its sequelae



pH - a direct biomarker of rumen health

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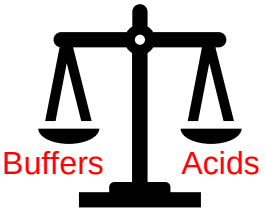
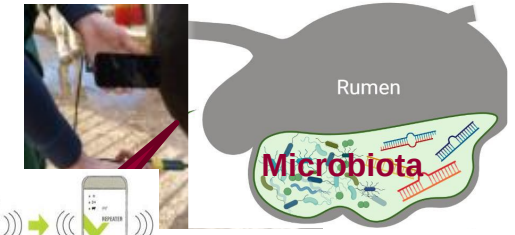




Rumen alkalosis

8,0
7,8
7,6
7,4
7,2
7,0
6,8
6,6
6,4
6,2
6,0
5,8
5,6
5,4
5,2
5,0
4,8
4,6
4,4
4,2
4,0

diseased

Healthy

SubAcute Rumen Acidosis (SARA)

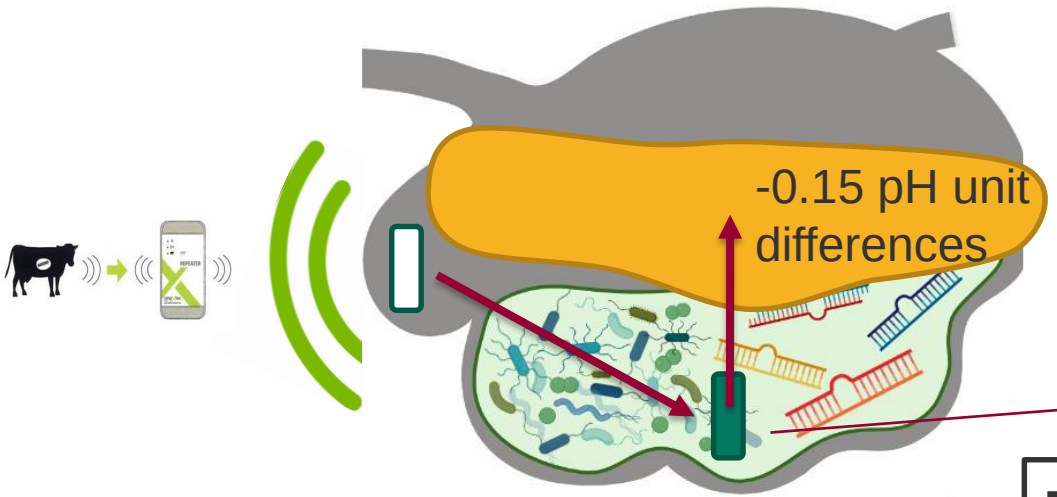
Suboptimal rumen health status

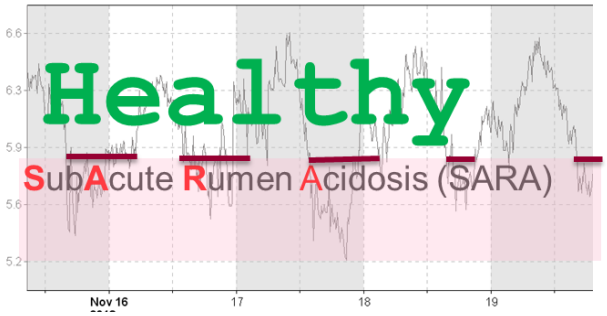
5-6 h/d < 5.8

Acute rumen acidosis

diseased

Is the pH accurate enough?





- Depending on location, different pH values
- ☞ Adjustment is needed
- ☛ Diurnal variation is also to be considered (**length** better than single points)

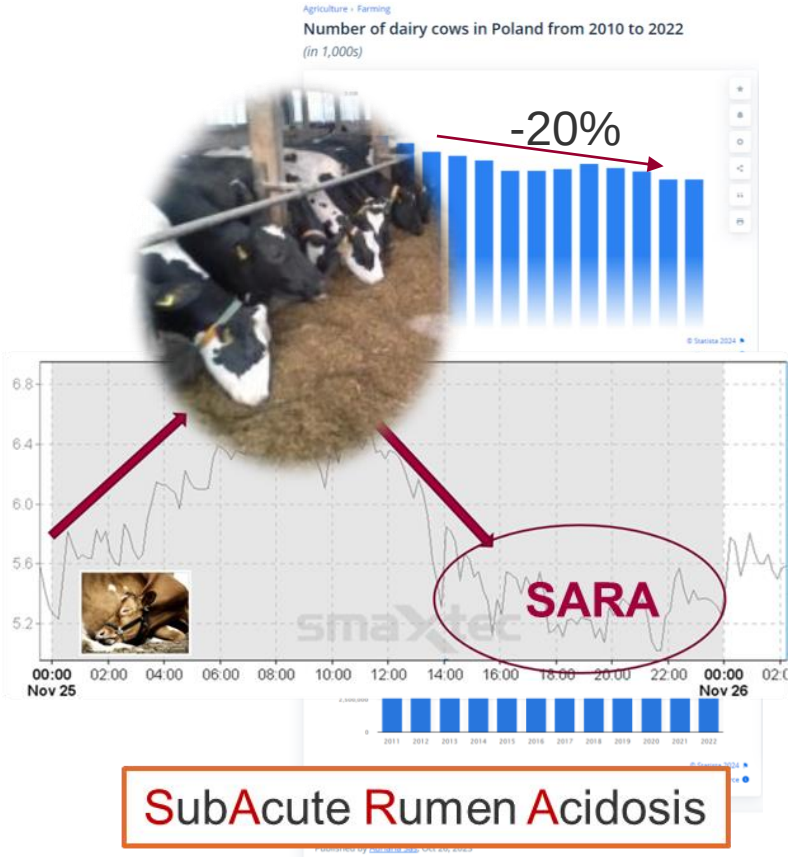
How common is SARA?

- Limited information surveys with **rumenocentesis** based on rumen pH (**5.5 or 5.6** threshold)
  - 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)
  - **Poland** → 14% (30/213) (Stefanska et al., 2017)

With spot measurements, SARA remains underdiagnosed!

Cow numbers and milk yield in Poland

- ↑ Demands (Energy/Nutrients)
  - ↑ Forage quality
  - ↑ **Dry matter intake**
  - ↑ concentrate feeds!
- Balancing the diet difficult!
- SARA, an increasing concern!



pH is an inherent variable of the rumen

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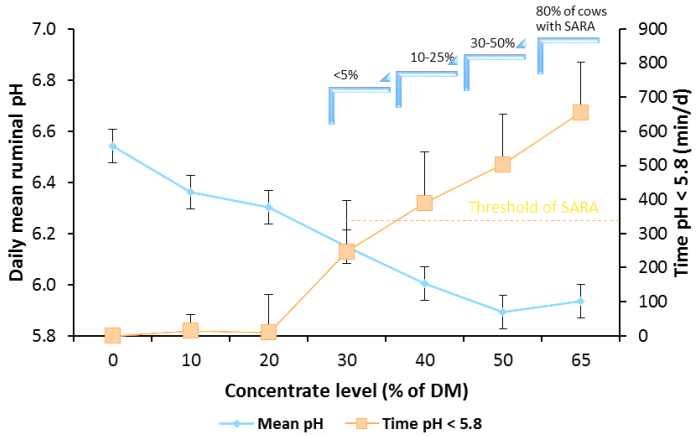
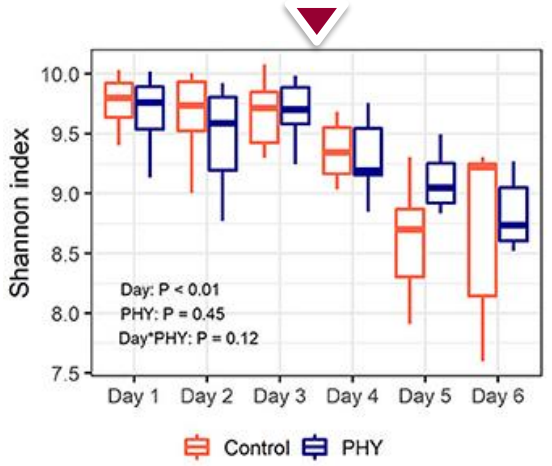
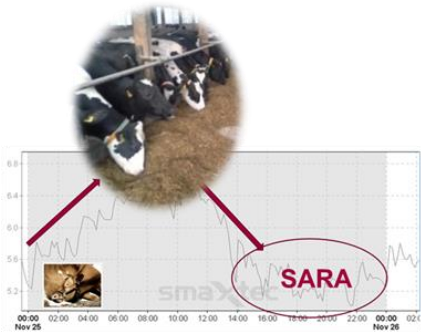
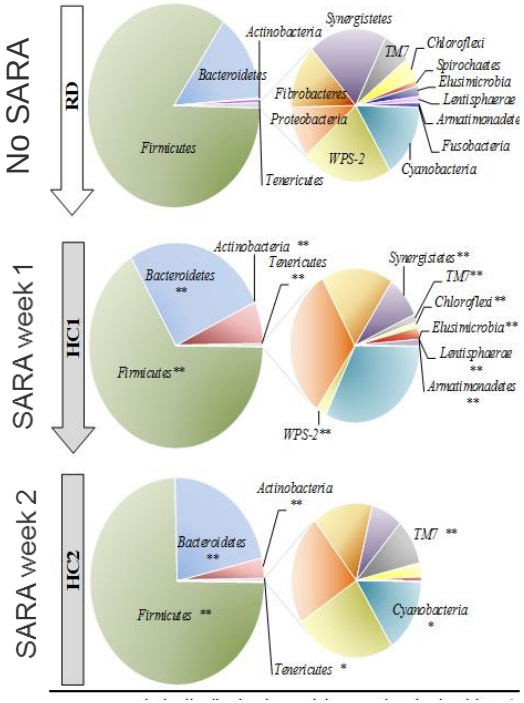
Gradual increase of concentrate level



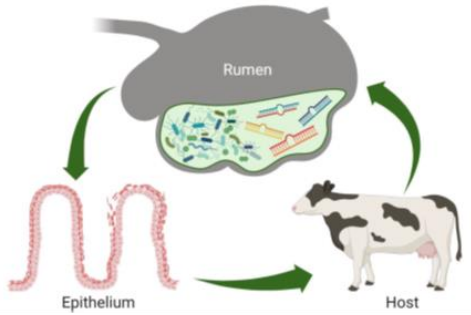


SARA leads to rumen dysbiosis





☑ normobiosis



👎 dysbiosis

- Can dysbiosis be a **direct biomaker of rumen health**?

👍 Theoretically yes

👎 Practically difficult ☛ rumen microbiome too complex - more research needed

Neubauer et al (2019) Anaerobe

Complexity of rumen microbiome

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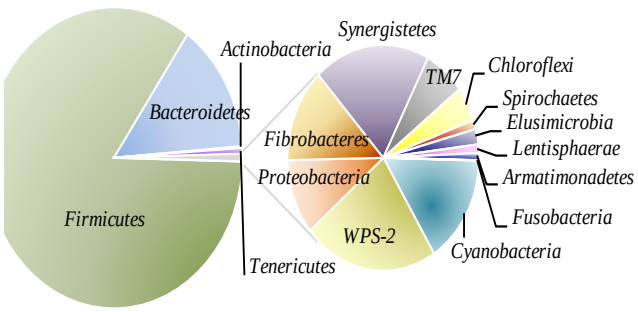
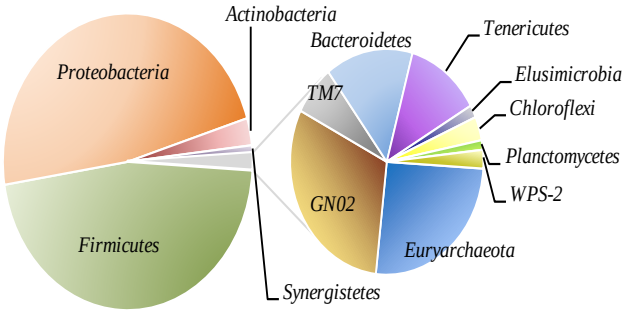
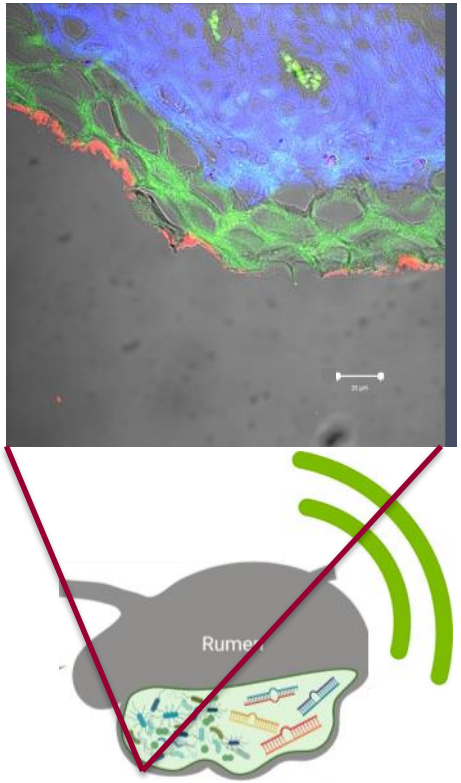
Particle associated Microbiota (PaM)



Epimural Microbiota (EpM)





Microbe-derived biomarkers of rumen

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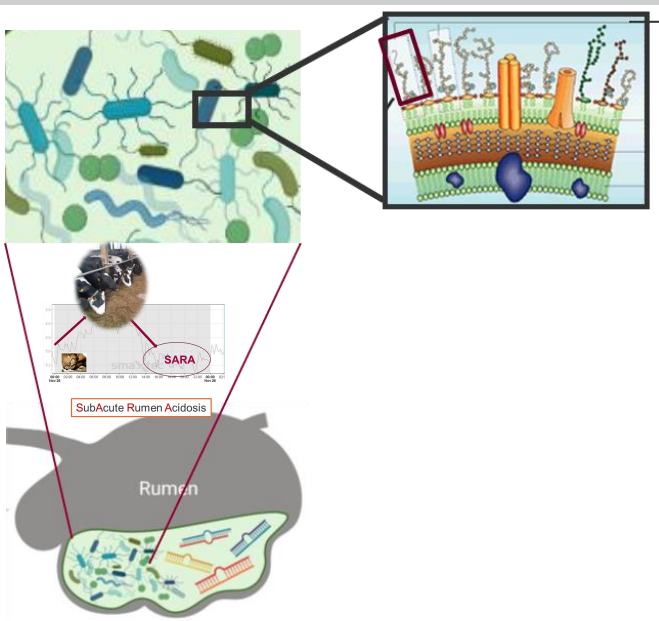
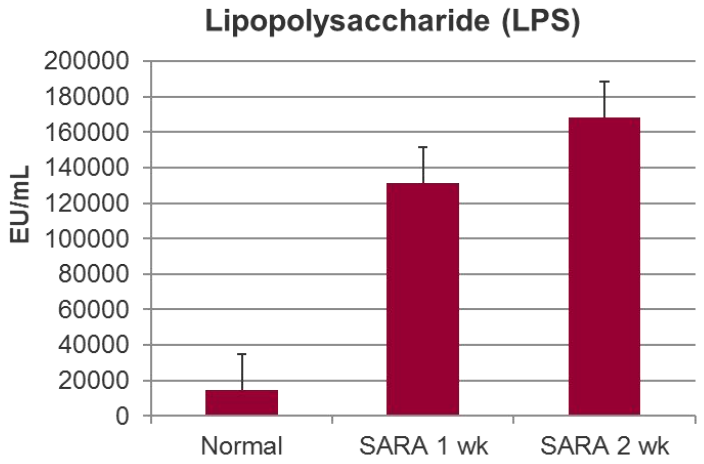
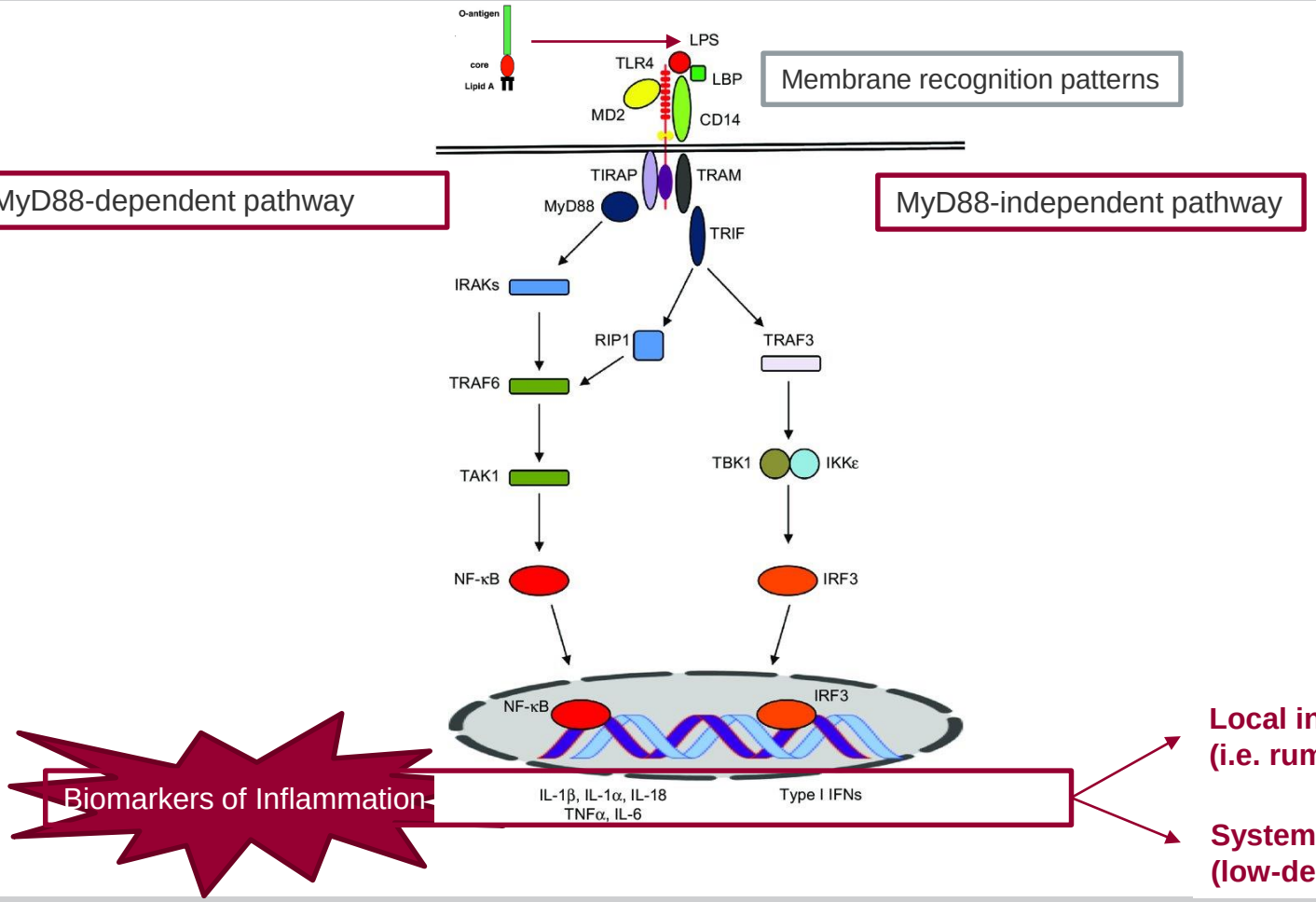




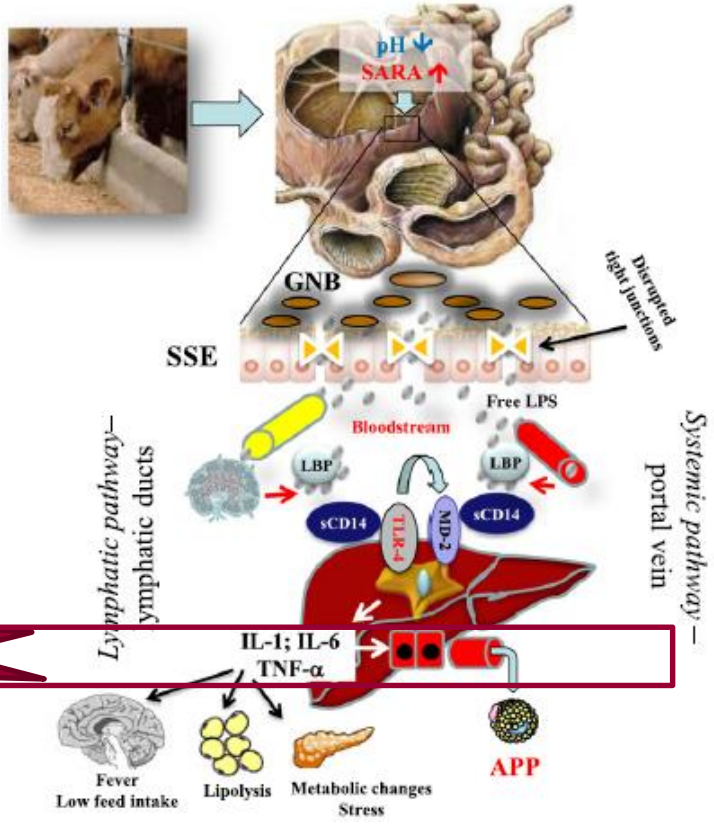
- Can **LPS** be a direct **biomaker of rumen health**?
- 👍 Theoretically yes (**good biomarker**)
- 👎 Practically difficult ☛ still expensive, Analytics not easy

Humer et al 2018 J Dairy Sci

LPS is strong proinflammatory



Systemic inflammation

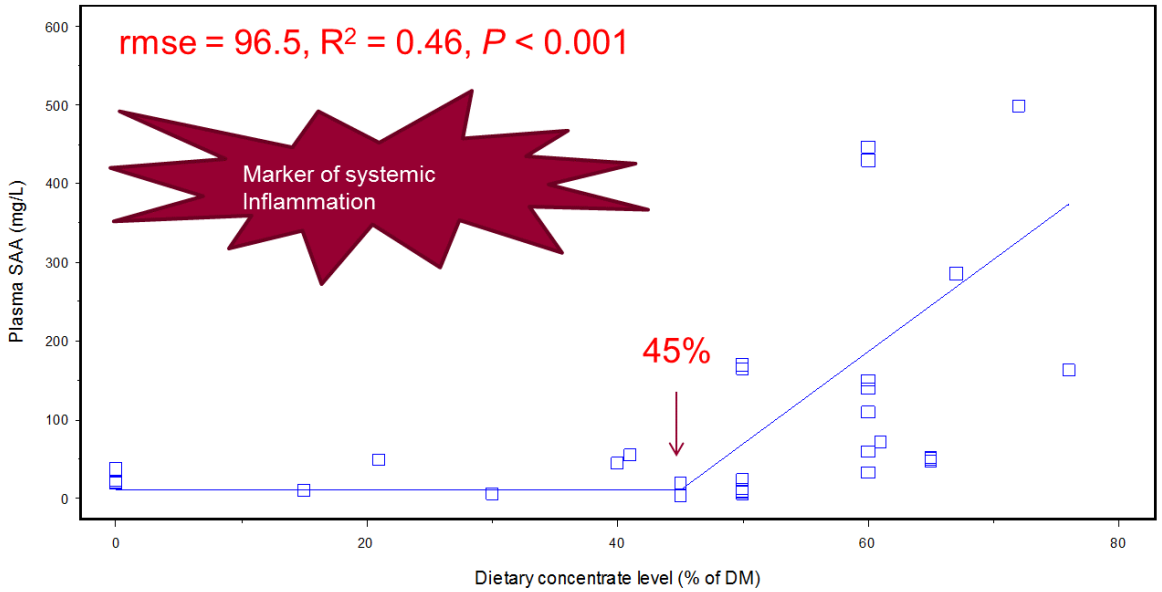


Biomarkers of Inflammation

Systemic inflammation (low-degree inflammation)

Interplay between rumen digestive disorders and diet-induced inflammation
https://www.ncbi.nlm.nih.gov/pubmed/22370295
by Q Zebeli - 2012 - Cited by 114
Res Vet Sci. ... **Zebeli** Q, **Metzler-Zebeli** BU.

Serum amyloid A – Concentrate level in the diet



> 45% easily fermentable concentrates increases the risk of Inflammation

- **SAA, Hp** (APP) are non-specific, indirect biomarkers of rumen health
- Easy to measure (ELISA), yet, still no thresholds established

Rumen biomarkers due to SARA and dysbiosis

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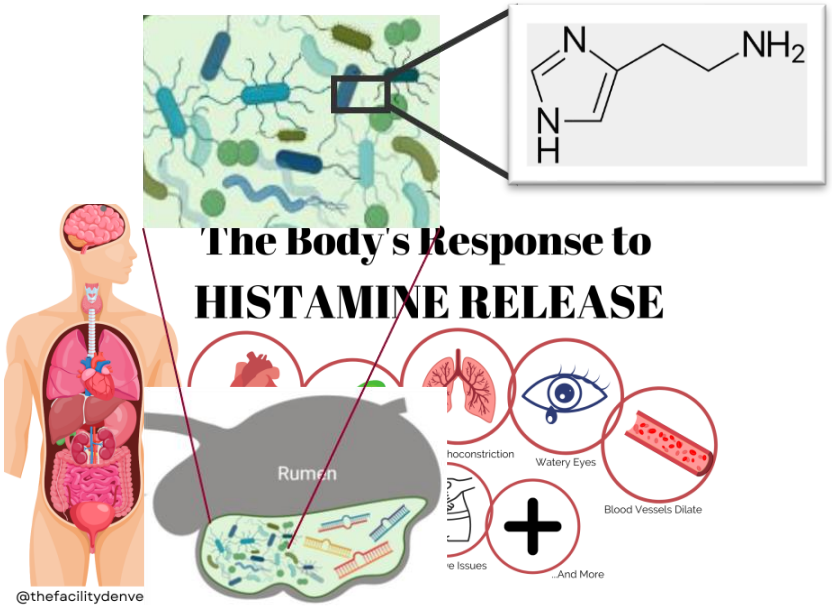
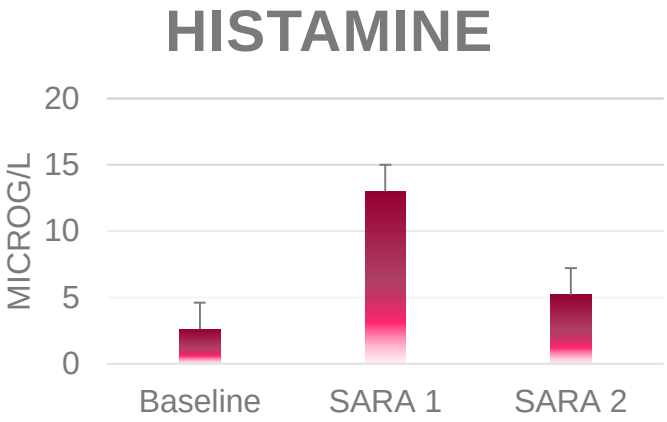
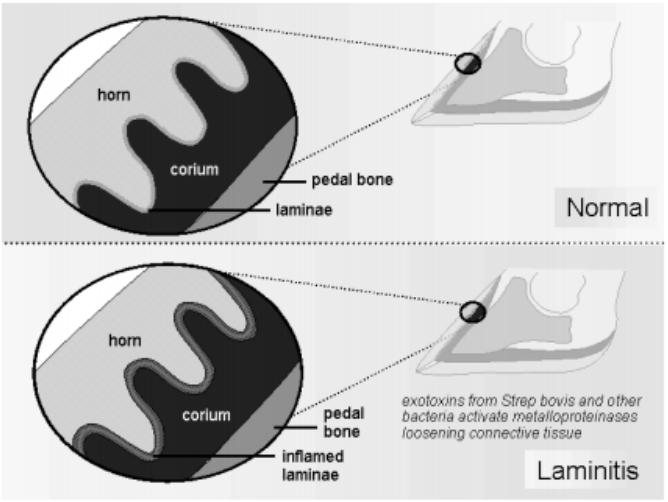
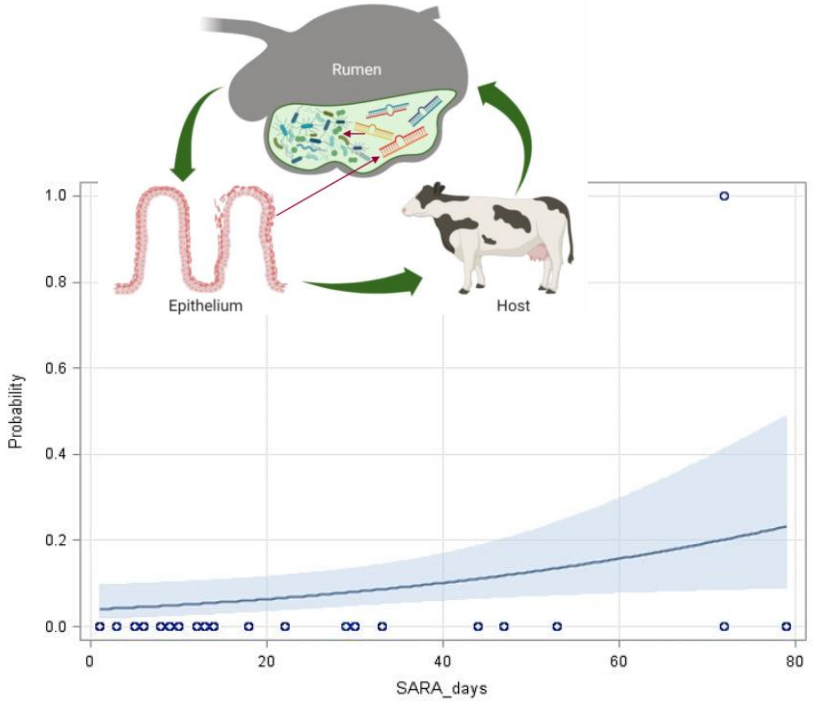




Neubauer et al 2019

Cow lameness and rumen health





- Per each day with SARA, the likelihood of lameness increases **with 2.5%**
- **Claw disorders are an indirect sign of SARA**

Liver tissue damage due to SARA

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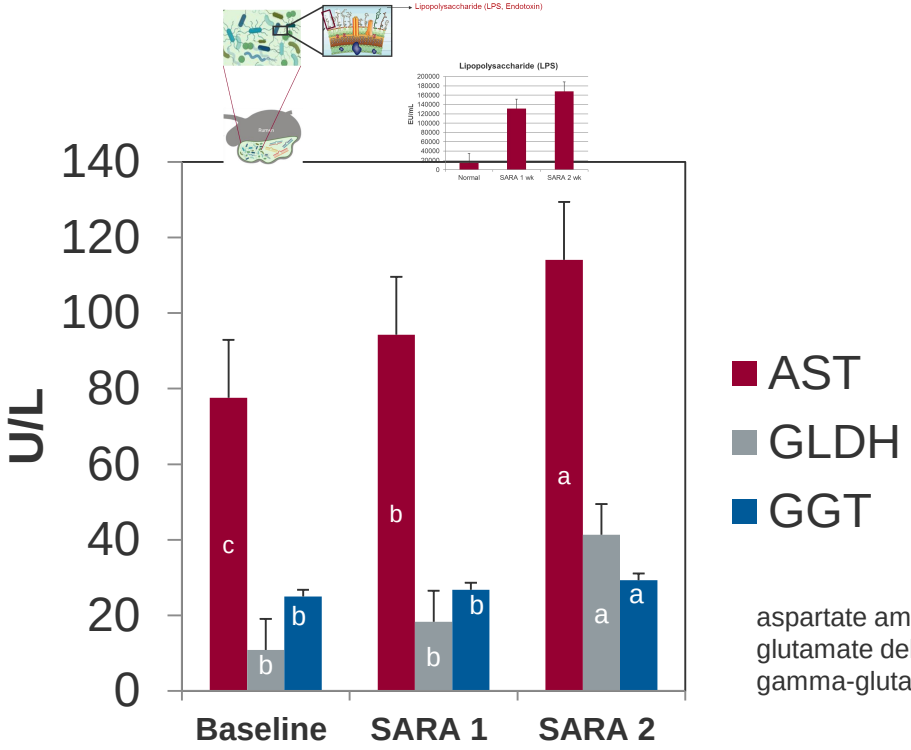

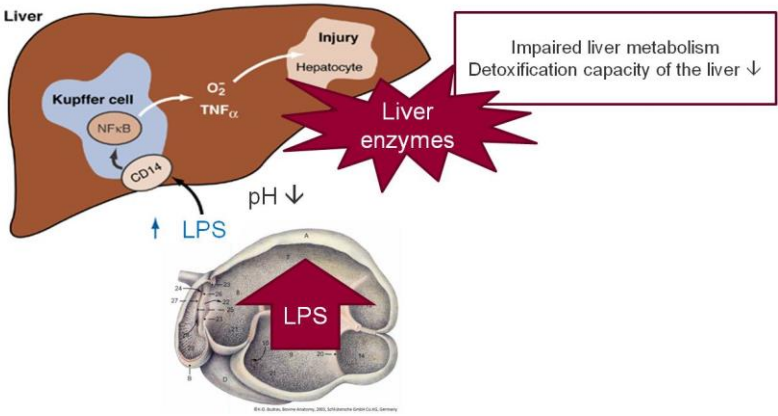

aspartate aminotransferase (AST)
glutamate dehydrogenase (GLDH)
gamma-glutamyltransferase (GGT)

The second SARA bout causes stronger liver damage
Liver enzymes are an unspecific & indirect biomarker of rumen health

Other expectable systemic derailments due to SARA

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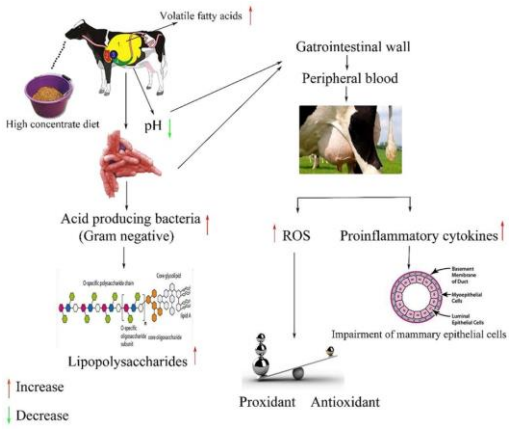
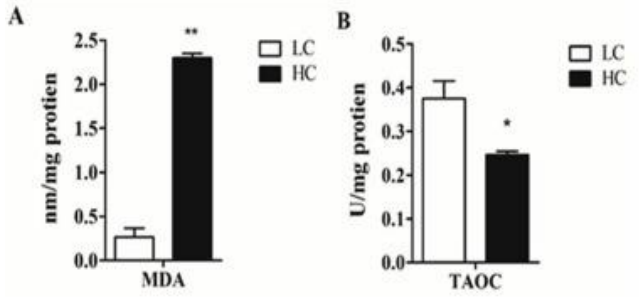
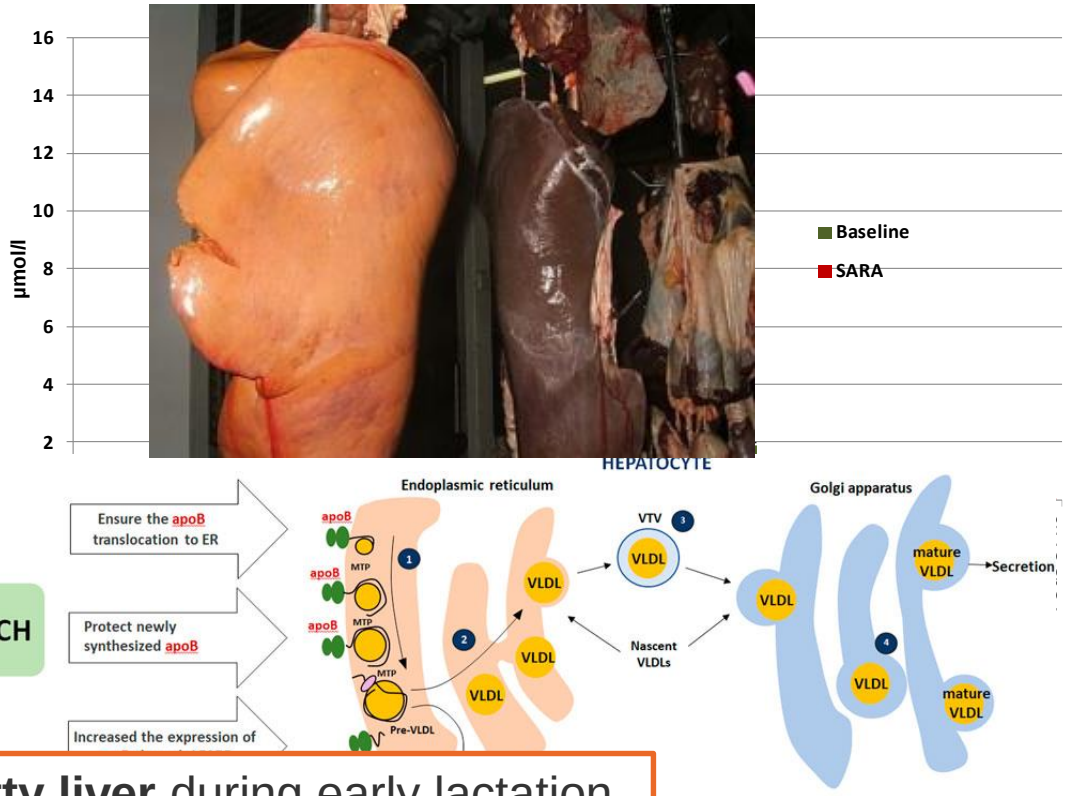




Decreased plasma phosphatidylcholines (PCH)

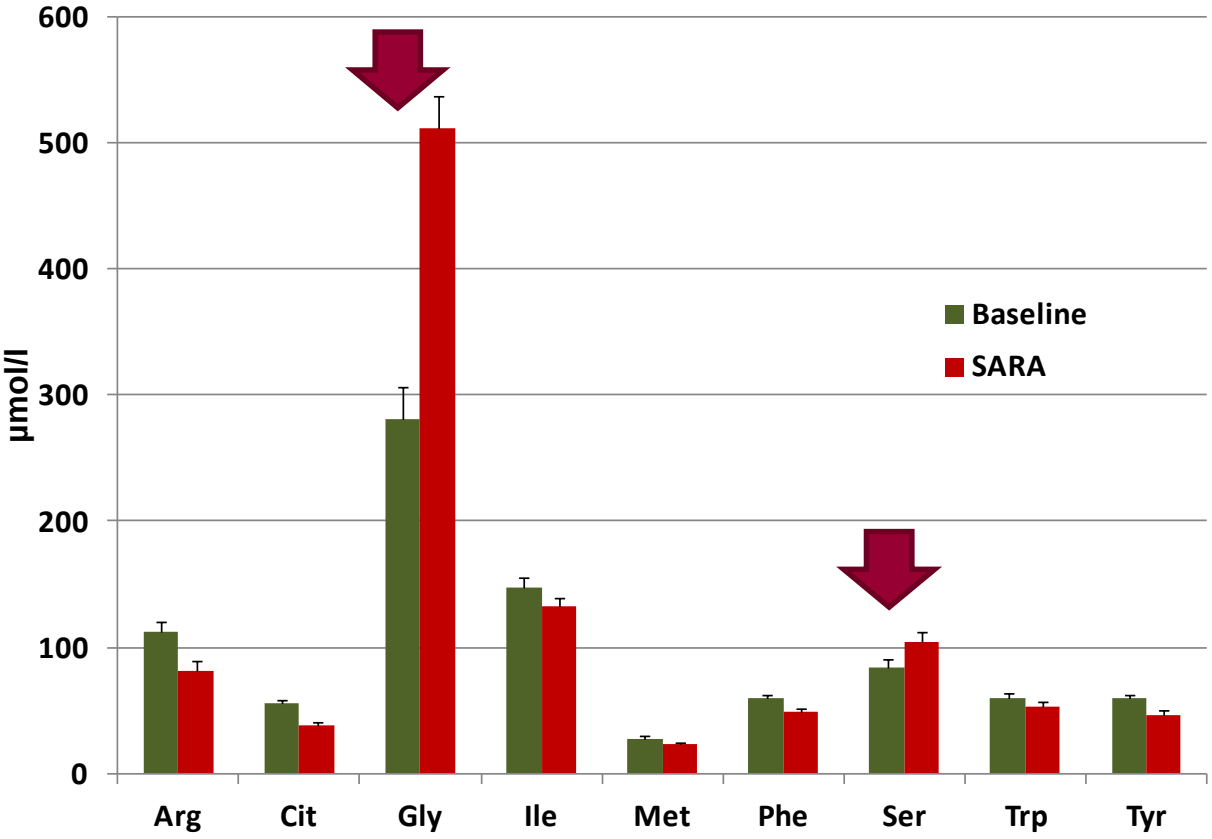


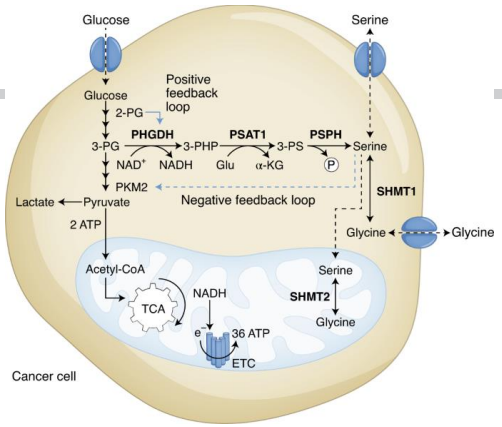
SARA: an additional risk factor of **fatty liver** during early lactation

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

Humer et al. 2018. J. Dairy Sci. 101:9559–9574

Hyperactivation of ser/gly biosynthetic pathway in SARA





https://www.nature.com/articles/s42255-020-00329-9

Warburg Effect

Excess glucose is diverted through the pentose phosphate shunt (PPS)
and **serine/glycine** biosynthesis pathway to create nucleotides

Humer et al. J. Dairy Sci. 101:9559–9574

Low rumen health: feed escapes the rumen

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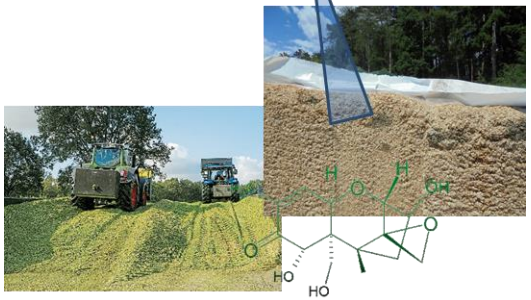
- If rumen is disturbed
- Passage rate increased

- Impaired nutrient degradation/uptake (Lowered feeding value) ⇒ **increased flow to the gut**
- Detoxification capacity is reduced
- Undesirable substances (mycotoxins) may not be fully degraded



Mycotoxins: ruminal dysbiosis & health

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Food and Chemical Toxicology
Volume 162, April 2022, 112900

Short-term exposure to the mycotoxins zearalenone or fumonisins affects rumen fermentation and microbiota, and health variables in cattle

Thomas Hartinger [a], Lena Grabher [a], Cátia Pacífico [a], Barbara Angelmayr [a], Johannes Faas [b], Qendrim Zebeli [a]

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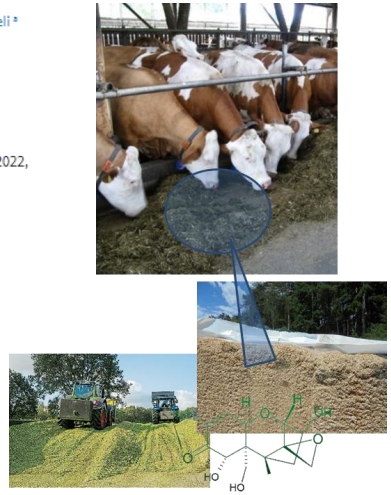
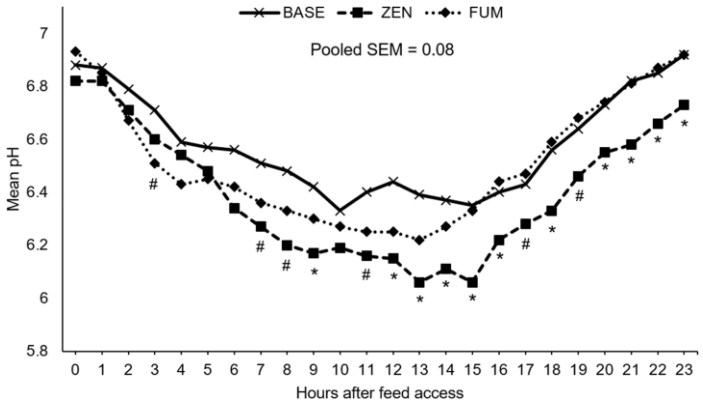
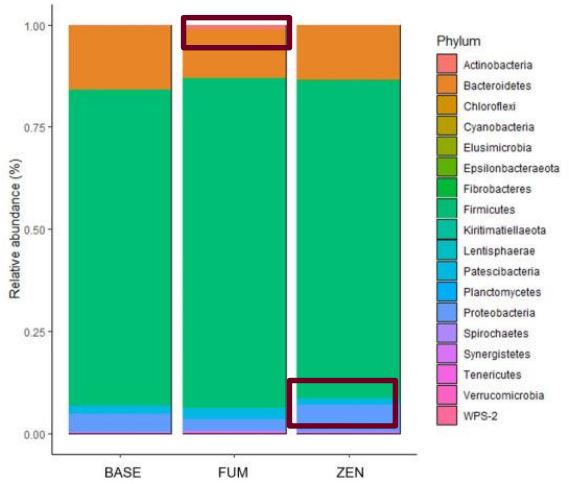
Handling Editor: Dr. Jose Luis Domingo







Fecal consistency – SARA signal?

Score: 1-2

Score: 3

1		**Charakterisierung:** Durchfall, sehr flüssig/Kotpfützen, im bogenförmigen Strahl vom Tier abgesetzt, jauchig, keine Ringbildung **Hinweise auf:** Pansenübersäuerung durch Strukturmangel, hoher Anteil an abbaubarem Protein, Stärkeüberschuss, überschüssige Mineralien, verdorbenes Futter (Pilzgifte), Weide
2		**Charakterisierung:** breiig, der Kot „läuft“ und bildet keine Fladen, spritzt vom Boden beim Absatz, bis zu 2,5 cm hoch **Hinweise auf:** Faserarme Ration, hoher Anteil an abbaubarem Protein, oder bei frischer, saftiger Weide

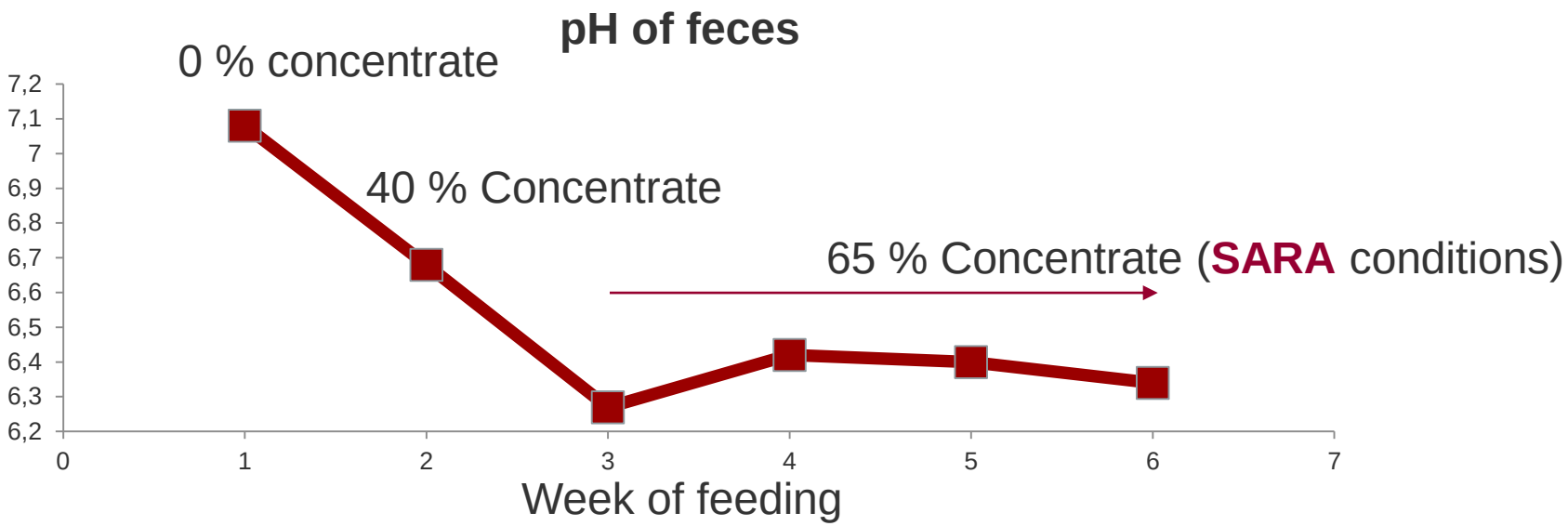
- If feed hygiene, Protein and Mineral supply OK:
  - Increased Passage rate, hindgut fermentation
  - **Rumen or hindgut disorder**

SARA signal – fecal sieving

Screen size	Close up diet (%)	Fresh diet (%)	**Optimal**
> 2 mm	13,0	30,4	< 20 %
1,18 - 2 mm	33,2	42,7	20-30 %
<1,18 mm	53,8	26,9	> 50 %

- if particles suboptimal:
  - Undigested particles, mucous
  - **Interpretation**: rumen dysfunction, increased passage

SARA signal – fecal pH



Fecal pH < 6.6 =

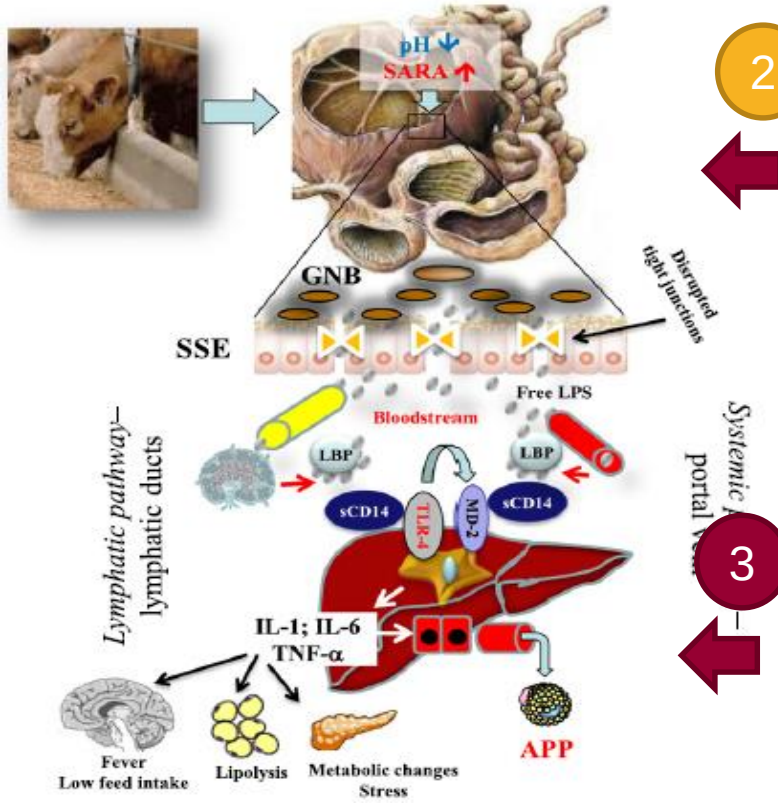
SARA signals

Signals relative to behaviour changes..

1

- Appetite/Feeding
- Local disorder
- Chewing
- Saliva[illegible]n/Secretions

Systemic disorder



2

GIT **signals**

- pH-drop
- Dysbiosis
- Diarrhea
- Epithel damage (absorption, integrity)
- Toxins

3

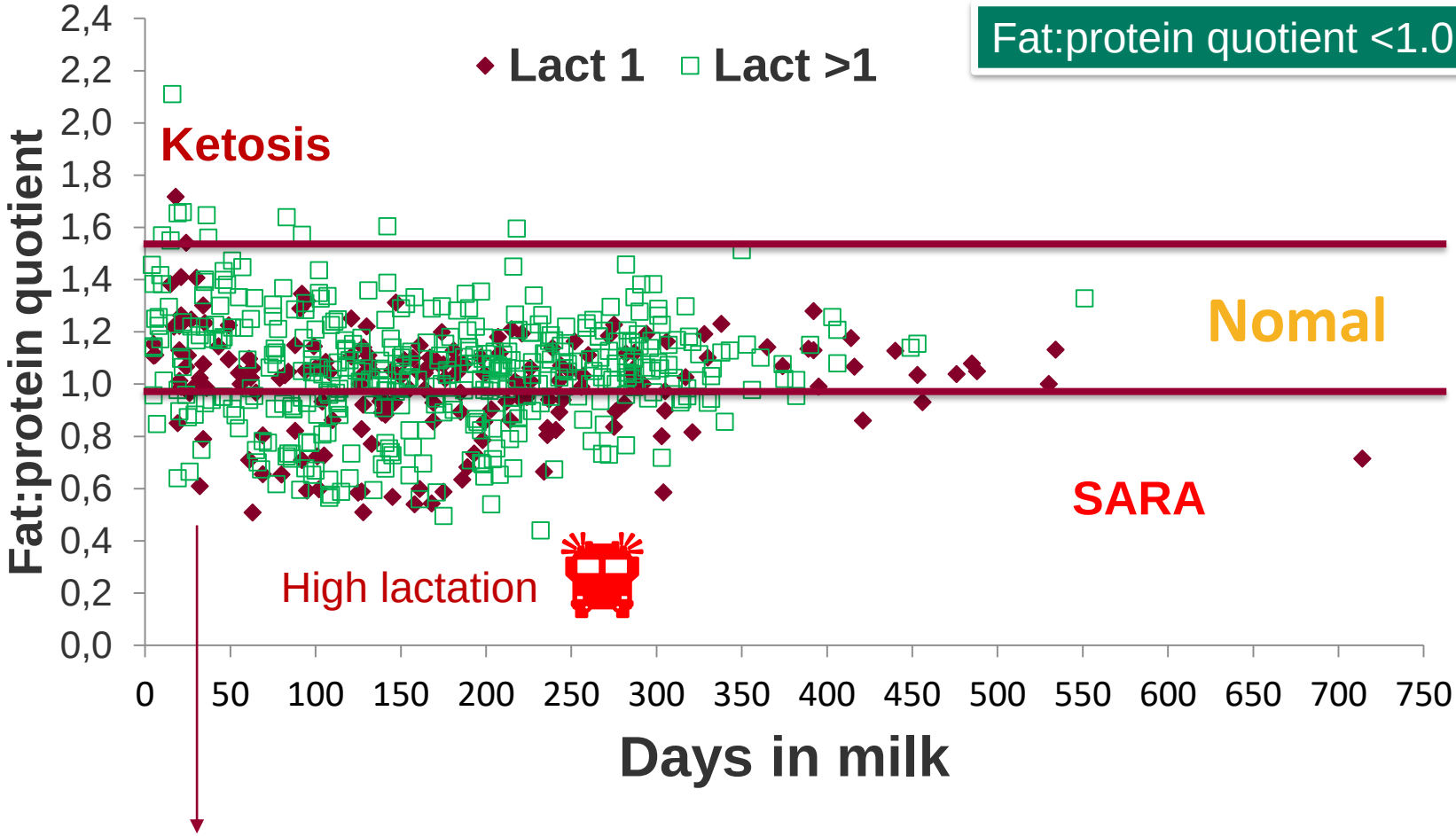
Systemic **signals**

- Liver
- Immune stimulation
- Inflammation
- Oxidatives stress
- Nutrient diversion
- **Milk fat depression**

Interplay between rumen digestive disorders and diet-induced inflammation
https://www.ncbi.nlm.nih.gov/pubmed/22370295
by Q Zebeli - 2012 - Cited by 114
Res Vet Sci. ... **Zebeli** Q, **Metzler-Zebeli** BU.

SARA signal: Milk fat-protein quotient

Fat:protein quotient <1.0 !!



YET: not adequate till day 50-60 of lactation!

SARA signal – Milk fat content

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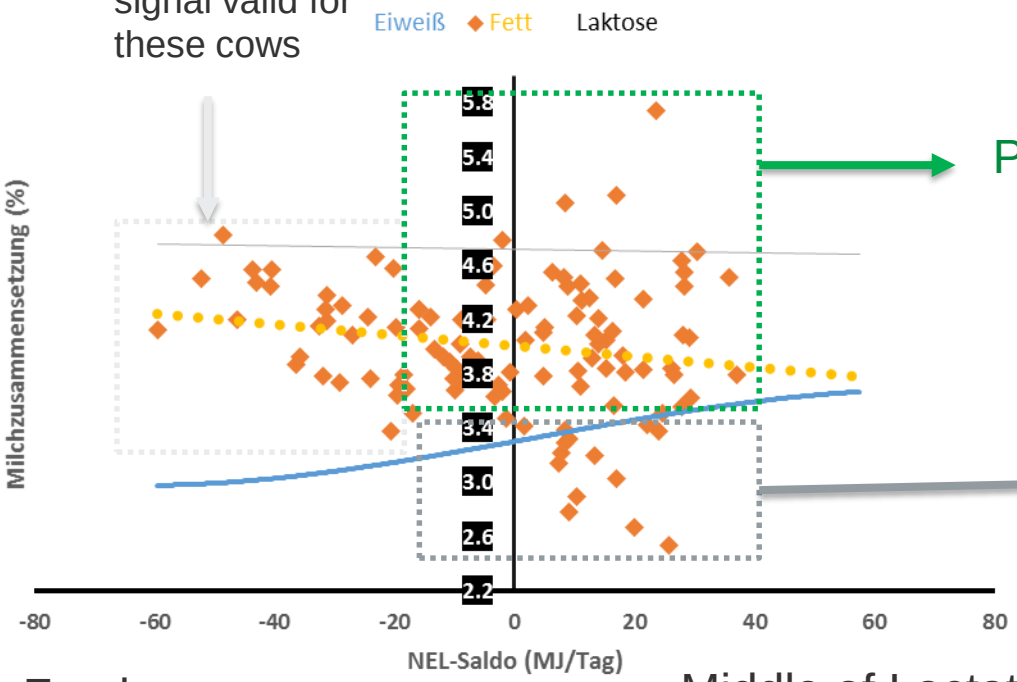
No SARA signal valid for these cows



Potentially no SARA

Pottentially SARA cows

- Fat content ≤3.4%
- FPQ ≤1.4

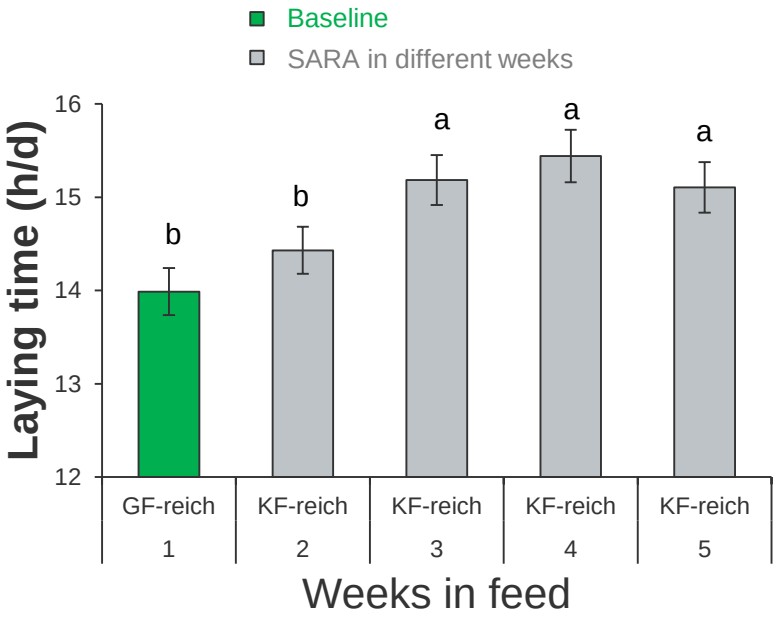
Fresh cows

Middle of Lactation

As long as cows are in NEB, FPQ is not adequate SARA biomarker

(Our own unpublished data)

SARA signal – long laying time, while not chewing?



- Laying longer, chewing less!
- Claw distress?
- Systemic inflammation?
- Depressive cow?

Lack of structure ⇒ **rumen acidification** ⇒ **meal size** ↘ ⇒ **straw intake** ↗

Rivera-Chacon et al. (2022) Animals

Decreased chewing = pH drop

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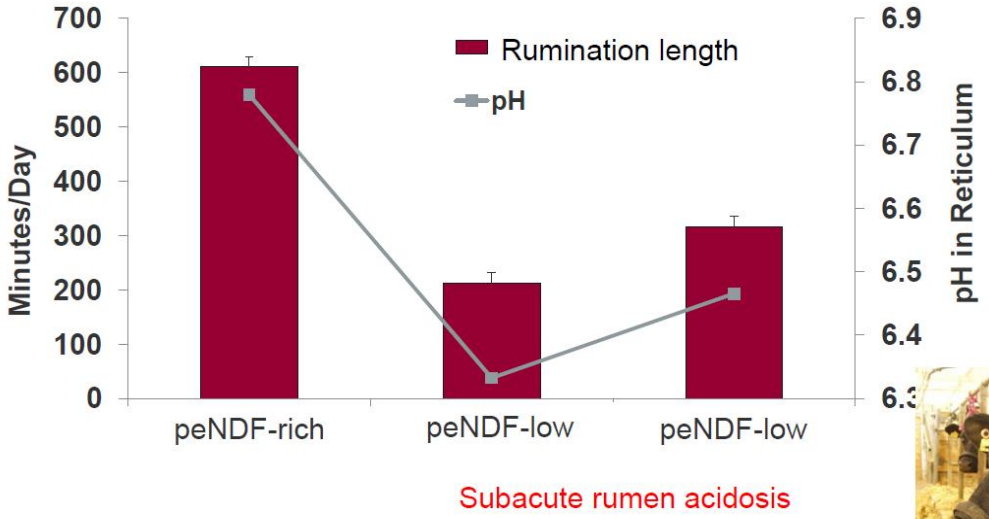

Kröger et al JDS 2017

SARA signal – decreased chewing

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- **Reference**: like >50 chewing/ Bolus

	Chews/Bolus		
	Dry off	Fresh cow	High lactation
Cow 1	74	49	65
Cow 2	71	53	52
Cow 3	67	31	60

4/9 cows (3 fresh cows + 1 high lactation) ⇒ decreased chewing

Decreased chewing = less salivation ⇒ **rumen acidification**

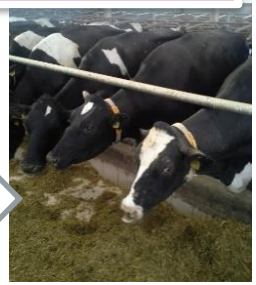
When is the risk highest?

Fresh cows

Dry off

Close up

Calving



High lactation

Late lactation

150-200 d

Risk of SARA

Early lactation – esp. primiparous cows!

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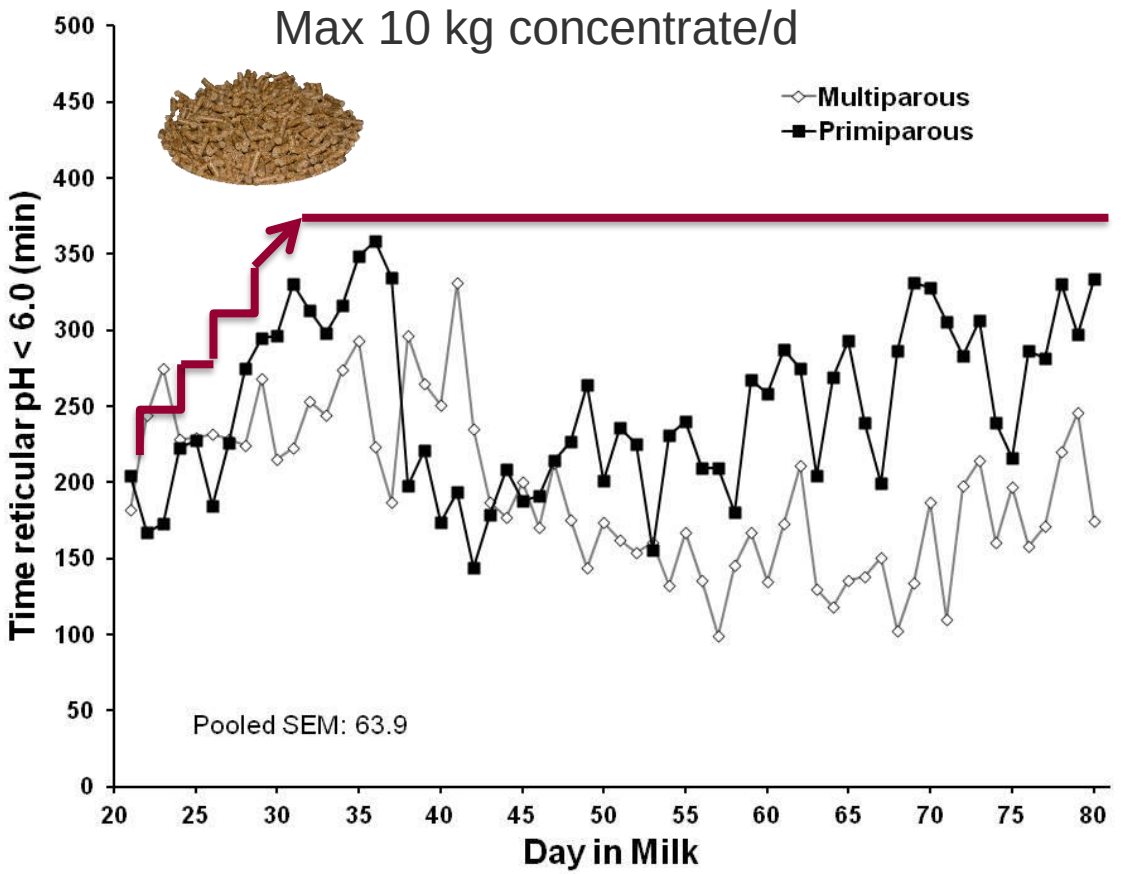
+ concentrate

Partial mixed ration



Check list

- **One or more** SARA biomarkers/signals on dairy herd present?
  - No, everything OK!
  - Yes, please go to the next step
- Next step:
  - To check:
    - Forage of the diet
    - Particle size of the ration
    - NDF-content
    - Starch content
    - Type of concentrate
    - Feed sorting
    - Transition feeding

Check the diet/feeding

- **Particle size**
- **Starch level**
- **Sorting**
- **etc….**

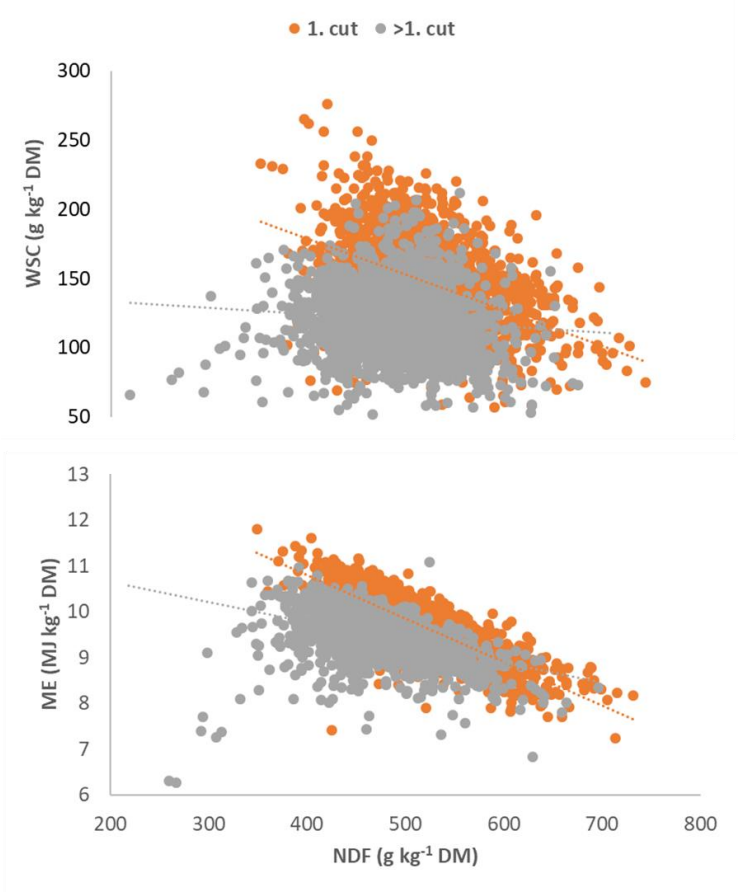
Checking forages

- Grass silage/Hay (Particle size, NDF, sugar content)
- Maize silage (Particle size, Kernel-/Starch proportion)
- Hay/Straw (proportion, appropriately mixed?)
- Others (ie. Brewery spent grain (NDF-rich, no Structure))

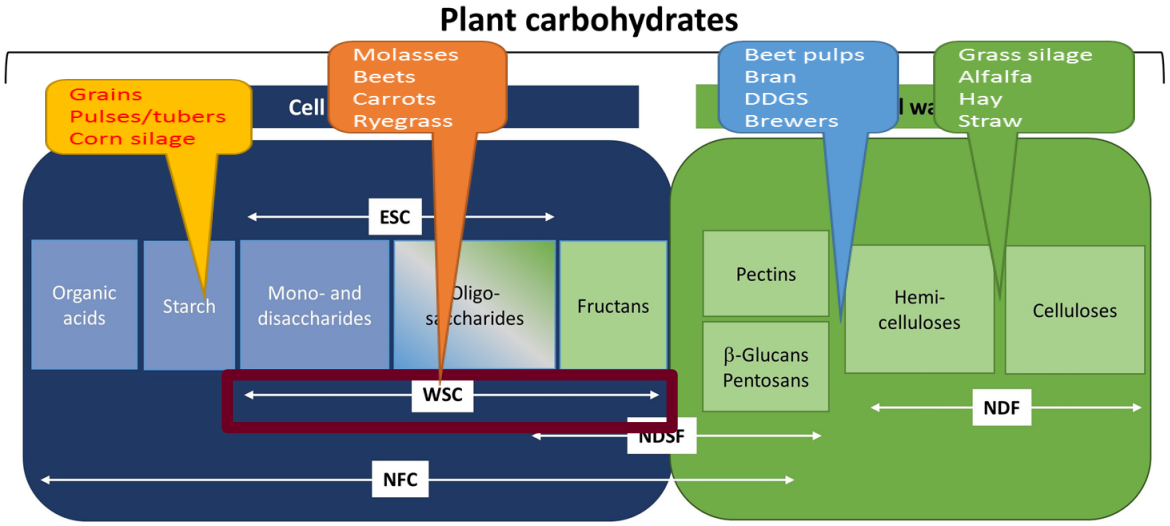
55 % NDF
0 % Starch
15 % Sugars?

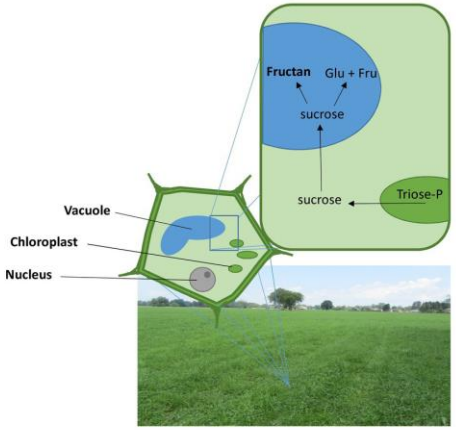
45 % NDF
30 % Starch

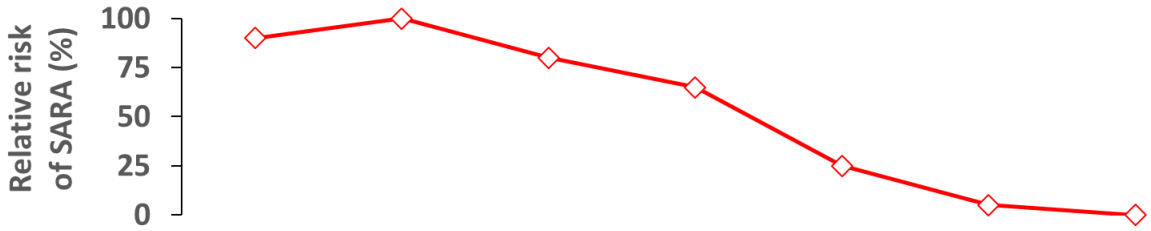
Grass silage in Austria



Water soluble carbohydrates and SARA







Checking particle size and peNDF of the diet

- **300-500g** of fresh diet to sieve
- **40 cycles** of manual sieving
  - **5 times in one direction** (1 movement = 1 x back and forth)
  - Sieve rotation **90°**
  - Each direction 2x
- **Weigh back** the retained feed
- **Calculate** the % of each seive

Results of sieving: calculation of peNDF

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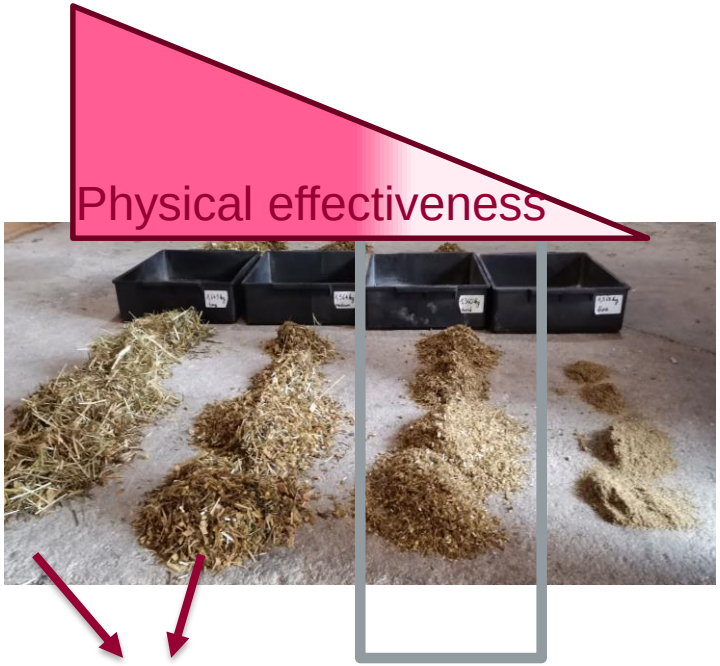
		Actual value	Target value
Partikelgrößen	in Gramm	in %	in %
19 mm	55	17.8%	15-25 %
8 mm	120	38.8%	35-65 %
1.18 mm	111	35.9%	15-25 %
<1.18 mm	23	7.4%	<8 %
Summe	309	100.0%	

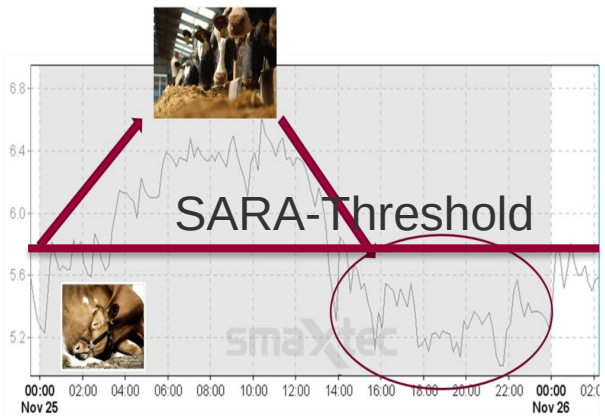
Gehalt an peNDF$_{>8\ mm}$	17.1 %



peNDF>8 mm (d.h. physically effective NDF) = **particles >8 mm** x NDF of Ration

NDF = aNDFom

Predicting SARA risk



Outline

Show full outline

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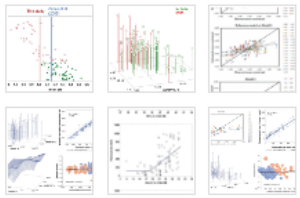

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Journal of Dairy Science
Volume 104, Issue 7, July 2021, Pages 7761-7780

Research

Models to predict the risk of subacute ruminal acidosis in dairy cows based on dietary and cow factors: A meta-analysis

Behzad Khorrami [1 2], Ratchaneewan Khiaosa-ard [1], Qendrim Zebeli [1]

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ABSTRACT

The present research aimed at developing practical and feasible models to optimize feeding adequacy to maintain desired rumen pH conditions and prevent subacute ruminal acidosis (SARA) in dairy cows. We conducted 2 meta-analyses, one using data from recent published literatures (study 1) to investigate the prediction of SARA based on nutrient components and dietary physical and chemical characteristics, and another using internal data of our 5 different published experiments (study 2) to obtain adjustments based on cow status. The results of study 1 revealed that physically effective neutral detergent fiber inclusive of particles >8 mm (peNDF >8) and dietary starch [% of dry matter (DM)] were sufficient for predicting daily mean ruminal pH {y = 5.960 – (0.00781 × starch) + (0.03743 × peNDF >8) – [0.00061 × (peNDF >8 × peNDF >8)]}. The model for time of pH suppression (<5.8 for ruminal pH or <6.0 for reticular pH, min/d) can be predicted with additionally including DMI (kg/d): 124.7 + (1.7007 × DMI) + (20.9270 × starch) + (0.2959 × peNDF >8) – [0.0437 × (DMI × starch × peNDF >8)]. As a rule of thumb, when taken separately, we propose 15 to 18% peNDF >8 as a safe range for diet formulation to prevent SARA, when starch or NFC levels are within 20 to 25% and 35 to 40% ranges, respectively. At dietary starch content below 20% of DM, grain type was

peNDF-requirements met?

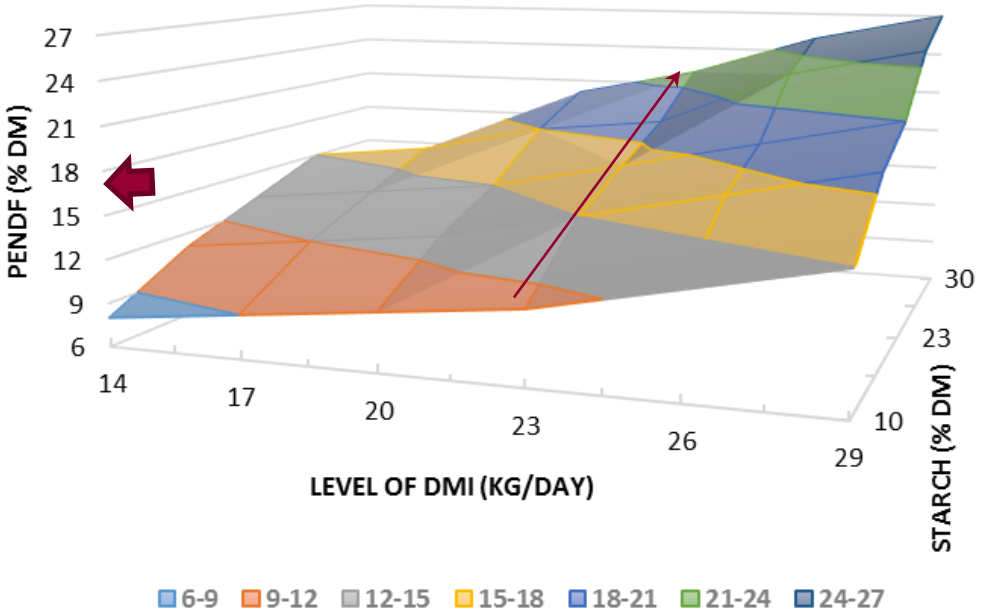
- Yes, for many cows!
- But, for cows in **high starch** diets, probably not

		Actual value	Target value
Partikelgrößen	in Gramm	in %	in %
19 mm	55	17.8%	15-25 %
8 mm	120	38.8%	35-65 %
1.18 mm	111	35.9%	15-25 %
<1.18 mm	23	7.4%	<8 %
Summe	309	100.0%	

Gehalt an peNDF$_{>8\ mm}$	17.1 %

peNDF-requirements depend on:

- Starch concentration of the diet
  - controllable
- The level of DMI of cows
  - Rather incontrollable



GfE 2023

Recommendations for cows

		Lactation phases				
	% of DM	Close-up	Fresh cows	High lactation	Mid-lactation	Late lactation
Maximal amount ↘	Starch	13-15	20-25	24-28	16-20	11-15
Maximal amount ↘	By-pass starch	1-3	5-10	10	2-5	1-2
Minimal amount ↗	peNDF	12-13	17-19	18-23	16-18	12-14
Supply of peNDF	⟶	☑	☑	☒	☑	☑

☛ The more starch + sugar in the diet, more peNDF needed!
☑ by-pass starch reduces the load for the rumen
☛ It should however be fed limited ➲ no hindgut disorders!

Type of concentrates modulate the needs for peNDF

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- Different starch content bzw. buffering capacity
  - Starch- Sugar-rich (grains, molasses etc)
  - Fiber (bran, beet pulp)
  - Protein-/fiber-rich (DDGS, seed meals)

Demand

- Different ruminal fermentability of starch
  - Wheat, triticale
  - barley
  - Corn

Demand

- Different processing (Starch-fermentability)
  - Pelleting (temperature)
  - Grinding (size)
  - Rolling

Demand

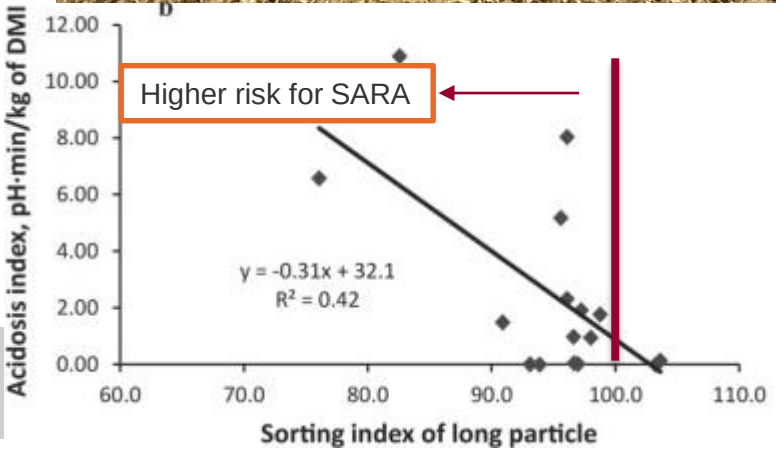
SARA in dairy cows

- Long exposure
- High energy-diet for most part of lactation(s)
- High DMI (>4% of BW)
- Several lactations
- High stress exposure (parturition, grouping, strong diet changes, environmental stress)

Feeding management is highly important

Check feed sorting!

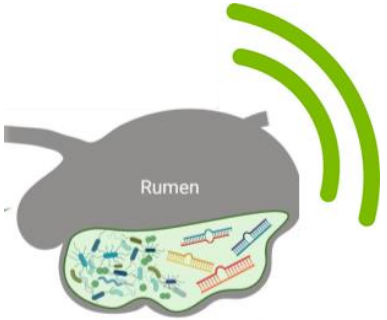
Cows select against forages



Gao and Oba (2014) J Dairy Sci

Conclusions

- Never neglect „suboptimal“ rumen health!
- Direct biomarkers are better, yet in the practice often unavailable
- Systemic biomarkers are promissing, but need more efforts to establish them for practical settings
- ☛ Apply several biomarkers/signals
- ☛ Check the diet and feeding



Thank you for your attention!

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