Assessment of rumen acid base metabolism in high yielding dairy cows

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Rumen acid base status:

• Most important for rumen function and therefore for health and yield of the animal
• Mainly influenced by feeding; other factors also of importance
Introduction

Two general approaches to assess rumen acid base status:

1. Direct measurement using rumen fluid

2. Indirect measurement using other parameters
Rumen fluid sampling

Direct measurement

Rumenocentesis

Stomach tubes
Rumen fluid

Direct measurement: pH in rumen fluid

• Stomach tubes (several types available)

  – Advantages
    • Quick and easy
    • Larger volumes are possible

  – Disadvantages
    • Saliva contamination
    • pH tends to be higher
    • Under-diagnoses SARA?
Rumen fluid

Direct measurement: pH in rumen fluid

• Rumenocentesis (ventral rumen sack punctured)

  – Advantages
    • No saliva contamination
    • Quick and easy

  – Disadvantages
    • Small volume
    • Milk withdrawal time in some cows
    • Invasive (peritonitis, abscesses, haematomas)
1. Study

Aim of the study part 1

- Evaluation of different stomach tubes
  - One tube type (Hamburger) only suitable for small volumes, therefore only 4 presented here)
  - Success rate in obtaining rumen fluid
  - Accuracy of the obtained data
  - Suitability for daily use
  - Technical reliability

- Two trials in this part of the study
  - 4 rumen fistulated cows
  - 80 cows on a Hungarian dairy farm
Material and methods

• Dirksen probe

• Geishauser probe

• water hose with metal gag

• Select collector
Animals

Trial 1
- 4 rumen fistulated cows
  - Not lactating
  - Fed hay and concentrates
  - kept at the University ruminant clinic

Trial 2
- 80 Holstein cows
  - Average 42 kg milk/day
  - Fed TMR
  - Hungarian farm (1000 cows)
Sampling

Trial 1  (4 weeks)
- latin square
- sampling 3 times a day
  - sample via stomach tube
  - sample directly from fistula
- sampling time
  - 7:00 am, 12:00, 5:00 p.m.

Trial 2  (4 days)
- each tube applied in 20 cows
- sampling once a day
  - sample via stomach tube
- sampling time
  - morning, after milking
Parameters

- Success rate of sampling
- Time for sampling of 200 ml and 2 L rumen fluid
- Adverse reactions of cows

- Rumen fluid analysis
  - odour, colour, viscosity
  - pH
  - methylene blue reduction time
  - number and viability of protozoa

In trial 1, rumen fluid collected by tubes were compared to samples simultaneously taken via fistula from the same sampling site.
Results

- collection of rumen fluid was possible in all cases
  - each tube was tested
    - 48 times in rumen fistulated cows
    - 80 times in the field trial
Results

- time for sampling of 200 ml rumen fluid
  - time for insertion of the tube included
Results

• Trial 1 time for sampling of 2 L of rumen fluid

- sampling time using water hose significantly longer

\[ \ast = p < 0.05 \]
Results

- Transfaunation volume (2 liters) could not be sampled (%)
  - in most cases sampling of volume large enough for transfaunation was possible
  - Best results using select-collector (sampling possible in 90%)
Results

- reactions of cows

- significantly more reactions when water hose was used

* = $p < 0.05$
Results

Mean pH-values of samples taken by tubes and via fistula

Trial 1

- no significant differences between pH-values
  - Smallest numerical differences when select-collector was used
Results

Mean pH-values of samples taken by tubes in the field

• no significant differences between the probes
  – pH indicating SARA were measured in samples taken via stomach tubes
Aim of the study part 2

Does sampling site influences measured rumen pH?
in comparison to central rumen compartment:
- caudoventral lower pH
- cranial higher pH
(among others Höltershinken et al. 1992, Duffield et al. 2004)

low correlation coefficients (0 - 0.6) between probe and rumenocentesis, Enemark et al. 2004)

\[ R^2 = 0.11 \] (Enemark et al. 2004)
Aims of the study part 2

to compare pH

1. Between rumen fluid taken by stomach tubes and fluid taken manually from the same site at the same time
2. Between rumen fluid taken by stomach tubes and an indwelling submersible rumen pH bolus

in fistulated cows.
Rumen pH comparisons

Dirksen $r = 0.44$, $P < 0.01$

Geishauer $r = 0.50$, $P < 0.01$
Rumen pH comparisons

water hose $r = 0.66$, $P<0.01$

Select collector $r = 0.59$, $P<0.01$
Aims of the study

Aims of the study to compare pH

1. Between rumen fluid taken by stomach tubes and fluid taken manually from the same site at the same time
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in fistulated cows.
Rumen Acid Base Status

pH-Sensor

Radiotransmission of Data
(pH and temperature)

Receiver

Internet

Software for Analysis
Rumen pH comparisons

Association between the pH values measured by indwelling sensor and stomach tube was low and not significant ($r = 0.15$, $P=0.52$)
Discussion/Conclusions

- Collection of rumen fluid is possible using tubes.
- Time for sampling is short, method suitable in practice.
- Samples taken by probe can be used:
  - Not different from fistula samples.
  - Very low pH-values measured.
- Effect of vacuum applied by pumps (CO$_2$-evacuation) in contrast to rumenocentesis and water hose?
Discussion/Conclusions

- Saliva influences pH only if added in large percentage (> 20% Höltershinken et al. 1992)
- Measured pH depended on time and location within the rumen
- There is no such a thing like „the rumen pH“,
- Diagnostic value of a single measurement?
- Do we measure in the correct substrate: fluid versus rumen mattress?
- Automatic submersible systems will very likely improve the situation, interpretation needs further research?
2. Study

- Wittek T, Kricziokat J, Fürll M. Buffer capacity and pH of rumen fluid in cattle during dry period and different stages of the lactation. Tierärztl Prax 2010; 38(G): 141-146.
Rumen Acid Base Status

Measurement methods:

- **pH:**
  - Measurement of the $\text{H}^+$ ion concentration and application of the Henderson-Hasselbalch-equation
  - Often used, easy to perform

- **Titration:**
  - Also includes weak acid and bases (buffer substances)
Aims of the study

- To compare assessment of rumen acid-bases status by pH-measurement or titration of rumen fluid
- Do additional and/or more detailed results provided by titration justify the increased laboratory work effort required for titration?
- Are methylene blue test and sedimentation-flotation-test suitable to assess rumen acid-base status
Material and Methods

- 293 samples of rumen fluid (209 cows and 84 heifers) in 10 farms of one region

- Sampling period:
  - 1. Dry period, 30 d a.p. to calving, (n=86);
  - 2. Post partum period, 0 to 7 d p.p., (n=79)
  - 3. Early lactation, 8 to 30 d p.p., (n=67)
  - 4. Mid-lactation, 80 to 150 d p.p., (n=61)

- 3 to 5 h after morning feeding taken by Geishauser probe (500 mL rumen fluid discarded)
Material and Methods

- Thermos flask, pre-warmed, filled to the brim and closed immediately
- Methylene blue test and sedimentation-flotation-test (Kraft und Dürr 2004)
- pH-measurement (electronic pH-Meter TM 39/Set/S, Sensortechnik Meinsberg GmbH)
- Reference range for rumen fluid pH: 5.8 - 7.2 (Kraft und Dürr 2004)
- Titration (Lachmann und Schäfer 1985)
Material and Methods - Titration

- **Titration with 1n HCl**
  - Titration result (mmol/L) = 25 x volume HCL
  - Positive result = Base excess

- **Titration with 0.1n NaOH**
  - Titration result (mmol/L) = 2.5 x volume NaOH
  - Negative result = Base deficit
Material and Methods

Buffer capacity of rumen fluid = Titration result (base excess or base deficit)

pH measured in sample – 6.8

(how resistant is the rumen fluid to changes of pH value)

pH 6.8 was the titration end point
Results

- Samples with pH values outside of the reference range:
  - 20 of 293 samples pH < 5.8
  - 21 of 293 samples pH > 7.2
- No differences between cows and heifers
- No differences between farms feeding TMR (3) and farms with component feeding (7)
Results

• Significant differences between rumen pH in dry cows (1) and lactating cows (2,3,4)
Results

• Significant differences between titration results of dry cows (1) and lactating cows (2,3,4)
• Widest variability in cow in early lactation (3)
Results

\( r = 0.98 \)

\( P < 0.001 \)
Results

- Rumen pH < 6.0
  - $r = 0.46$
  - $P < 0.05$

- $r = 0.98$
  - $P < 0.001$
Results

- Decreased buffer capacity during post partal period (2) and early lactation (3) in comparison to dry cows (1) and Mid-lactation (4).
Results

- Buffer capacity weak association to rumen pH
  \( (r = 0.26) \)
Rumen Acid Base Status

Sedimentation Flotation Test:

- Freshly collected rumen fluid in a glass cylinder (warm water bath)
- Sedimentation and flotation start immediately and should be completed within 3 - 8 minutes (SAT, sediment activity time)
- Indirect measure of biochemical activity and gas production
Rumen Acid Base Status

Methylene Blue Test:
- Methylene blue (1 mL, 0.03%), add fresh rumen fluid (20 mL) in a glass cylinder
- Biochemically active rumen fluid reduces methylene-blue (decolouration) within 3 minutes
- Indirect measurement of biochemical activity
Results

Not suitable for assessment of rumen acid base status since diagnostic value is low
Discussion/Conclusions

- Titration not necessary in routine diagnostic
- Titration offers possibility to assess buffer capacity of the rumen (robustness of rumen pH)
- Assessment of feeding, diet suitable for ruminants?
- Assessment if rumen buffers are required
- Sedimentation Flotation Test and Methylene Blue test: not suitable to assess rumen acid base status
Rumen Acid Base Status

Indirect diagnostic procedures
Rumen Acid Base Status

Further diagnostic tools:
- Clinical examination
- Milk fat ↓
- Rumination ↓
- Faeces consistency ↓
- Nutrition
- NABE ↓
- Urine pH ↓
- Blood parameter
Rumen Acid Base Status

Further diagnostic tools:
- Clinical examination
- Milk fat ↓
- Rumination ↓
- Faeces consistency ↓
- Nutrition
- NABE ↓
- Urine pH ↓
- Blood parameter
Rumen Acid Base Status

Blood gas analysis
- Compensated metabolic acidosis
- BE decreased
- Blood-pH typically decreased in acute rumen acidosis

- Currently low relevance in practice
- Portable machines becoming available
- Diagnostic value needs to be assessed
Rumen Acid Base Status

Alternative indirect diagnostic approaches:

Urine:
- Central role of the kidney in acid-base-balance
- Studies with contrary results:
  - Urine acid-base status recommended as estimate for rumen acid-base status
  - Diagnostic value low or questionable

Blood:
- Indirect diagnosis using biochemical parameter in blood (Fürll 1994, Seemann and Spohr 2010: e.g. actate, BHB, GLDH, Ca moderate to high diagnostic value)
3. Study

Study on indirect SARA diagnosis

Objectives:

- Measurement and comparison of biochemical parameters in venous blood, parameters of acid-base status in urine and rumen fluid in healthy dairy cows

- Use of biochemical parameters in blood for estimation of rumen acid-base status

- Use of urine acid-base status as estimate for rumen acid-base status
Material & Methods

- Clinically healthy cows
- Milk yield > 8000 kg/a
- Within 100 days after parturition
- Component feeding
- No anionic salts
- No rumen buffers
Material & Methods

Samples:
286 simultaneously taken blood, urine and rumen fluid samples, 2-3 h after feeding

- Venous blood sample (tail vein)
- Rumen fluid sampled by Geishauser stomach tube
  - pH (electronic pH-meter)
- Samples (anaerobical) by catheterization of the bladder
  - pH (electronic pH meter)
  - NABE titration - Jørgensen-Kutas method
Net acid base excretion (NABE)

Assessment of acids and bases excreted via urine

• Urine pH

• NABE- net acid base excretion
  – Titration method using HCl and NaOH
  – ↓ NABE indicates excess of anions (acids)
  – NABE (mmol/L):
    • 100 – 200 mmol/l physiological
    • 50 – 99 mmol/l mild acidotic situation
    • 0 – 49 mmol/l moderate acidotic situation
    • < 0 mmol/l severe acidotic situation

• NABE supposed to be more accurate
Material & Methods

Urine-NABE-Titration

**Bases** = $K^+ + Na^+ + Mg^{2+} + Ca^{2+} + HCO_3^-$

**Acids** = $Cl^- + SO_4^{2-} + HPO_4^{2-} + $ organic acids $+ NH_4^+$

**Net Acid Base Excretion (NABE) = bases - acids (mmol/l)**
Material & Methods

Blood

- Biochemical parameters measured in venous blood (automatic analyser Hitachi 912):
  - Metabolites: total protein, albumin, bilirubin, urea, creatinin, glucose, volatile fatty acids, beta-hydroxybutyrate (BHB), lactate, cholesterol,
  - Minerals (ions): Na, K, Cl, Ca, P, Mg,
  - Enzymes: ALP, ASAT, GLDH, CK
Results - rumen fluid

Rumen pH - reference values
physiological 6.2 – 7.2
< 5.8 acidosis
> 7.5 alkalosis

Rumen pH: 6.6 ± 0.47
Results - urine

Urine pH - reference values
physiological 7.0 – 8.4
< 7.0 acidosis
> 8.4 alkalosis

Urine pH: 8.0 ± 0.44
Results – urine pH and rumen pH

Urine pH
< 7.0 acidosis
> 8.4 alkalosis

Rumen pH
< 5.8 acidosis
> 7.5 alkalosis
### Results – urine and rumen pH

<table>
<thead>
<tr>
<th></th>
<th>rumen pH &lt;5.8</th>
<th>rumen pH 6.0 – 7.5</th>
<th>rumen pH &gt;7.5</th>
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<td>urine pH 7.0 - 8.4</td>
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<td>urine pH &gt;8.4</td>
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Urine pH (<7.0) cows having rumen pH <5.8 with a sensitivity of 2.3% and specificity of 97.1%
Results - NABE

NABE - reference values
physiological 80 – 220 mmol/L
< 80 mmol/L acidosis
> 220 mmol/L alkalosis

NABE: 104 ± 70 mmol/L

37.1%  58.6%  4.3%
Results – NABE and rumen pH

NABE
< 80 mmol/L acidosis
> 220 mmol/L alkalosis

Rumen pH
< 5.8 acidosis
> 7.5 alkalosis
Results – NABE and rumen pH

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<td>NABE 80 - 220 mmol/L</td>
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<tr>
<td>NABE &gt;220 mmol/L</td>
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NABE (<80 mmol/L) detects cows having a rumen-pH<5.8 with a sensitivity of 48.8% and specificity of 64.4%
Results – serum biochemical parameter – rumen pH

Lactate (mmol/L)

Glucose (mmol/L)

GLDH (U/L)

BHB (mmol/L)
Results – serum biochemical parameter – rumen pH

Lactate, ROC-curve AUC = 0.43

GLDH, ROC-curve AUC = 0.46

BHB, ROC-curve AUC = 0.61

Glucose, ROC-curve AUC = 0.63
Results - summary

- Rumen-pH 12.3% below pH 6.0, 9.8% below pH 5.8

- Serum biochemical parameter low diagnostic value

- Low to moderate values for sensitivity and specificity for NABE and urine pH

- Low correlation coefficients between rumen pH and NABE: $r = 0.13$
  
  urine pH: $r = 0.19$
Conclusions

- Diagnostic value of biochemical parameters in venous blood and parameters of urine acid-base status to assess rumen acid base status are limited for individual animals
- Measurement of rumen pH currently necessary to assess rumen acid base status
- Introduction of mobile blood gas analysis may improve indirect diagnosis, diagnostic value?
Thank you very much for your kind attention

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