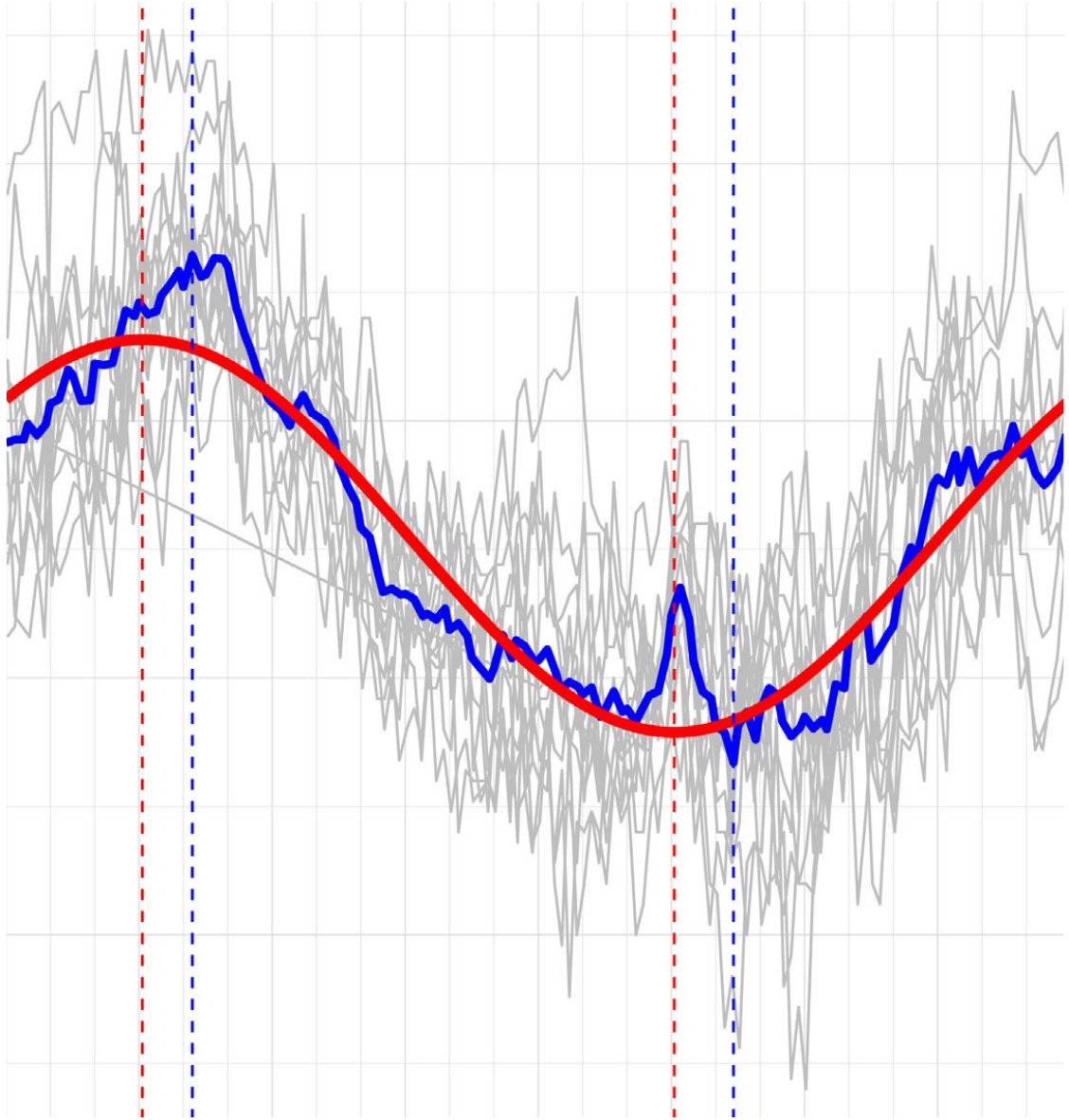

Reticuloruminal boluses for SARA diagnosis – how can we best use them on dairy farms?

Nicholas Jonsson
University of Glasgow
Glasgow, Scotland, UK

University of Glasgow
School of Biodiversity, One Health & Veterinary Medicine

Topics

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- Acids and the rumen
- pH boluses
- Using pH bolus information
- Motility boluses
- Using motility bolus information
- Temperature
- Integration
- Future

Note on SARA

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Sub-acute rumen acidosis is a term that I think should be replaced with something that better describes the syndrome of chronic, fluctuating low reticuloruminal pH with intermittent inappetence, diarrhoea, milk compositional changes, elevated inflammatory markers, and increased susceptibility to left-displaced abomasum and lameness.

However, in this presentation I will use the term as it is.

Acids: p*H* and pK_a

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$$\mathbf{pH = -\log_{10}([H_3O^+])}$$

$$HA_{(aq)} + H_2O_{(l)} \rightleftharpoons H_3O^+_{(aq)} + A^-_{(aq)}$$

$$K = \frac{[H_3O^+][A^-]}{[H_2O][HA]}$$

$$pK_a = -\log_{10} K_a$$

When the protons are given freely (dissociation is high), the numerator is high, the negative logarithm is low, and the acid is strong.

(H_2O is considered a constant, so not in the equation for the acidity constant K_a).

Acids: p*H* and pK_a

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Example – **hydrochloric acid**

$$HCl\ (aq) + H_2O\ (l) \rightarrow Cl^-\ (aq) + H_3O^+\ (aq)$$

$$K_a = \frac{[H_3O^+\ (aq)]\cdot[Cl^-\ (aq)]}{[HCl\ (aq)]}$$

$$K_a = 10^7$$

$$pK_a = -7.0$$

Almost all of the protons are dissociated from the anions.

Ruminal acids (VFAs/SCFAs)

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In rumen, in usually recorded decreasing order of abundance,

acetic acid: $CH_3COOH + H_2O \rightleftharpoons CH_3COO^- + H_3O^+$ (**pK_a = 4.76**)

propionic acid: $CH_3CH_2COOH + H_2O \rightleftharpoons CH_3CH_2COO^- + H_3O^+$ (**pK_a = 4.88**)

butyric acid: $CH_3CH_2CH_2COOH + H_2O \rightleftharpoons CH_3CH_2CH_2COO^- + H_3O^+$ (**pK_a = 4.82**)

lactic acid: $CH_3CHOHCOOH + H_2O \rightleftharpoons CH_3CHOHCOO^- + H_3O^+$ (**pK_a = 3.86**)

In all cases, pK_a is positive, so in an aqueous solution, most VFAs are in the associated (bound) state.

Fate of ruminal acids

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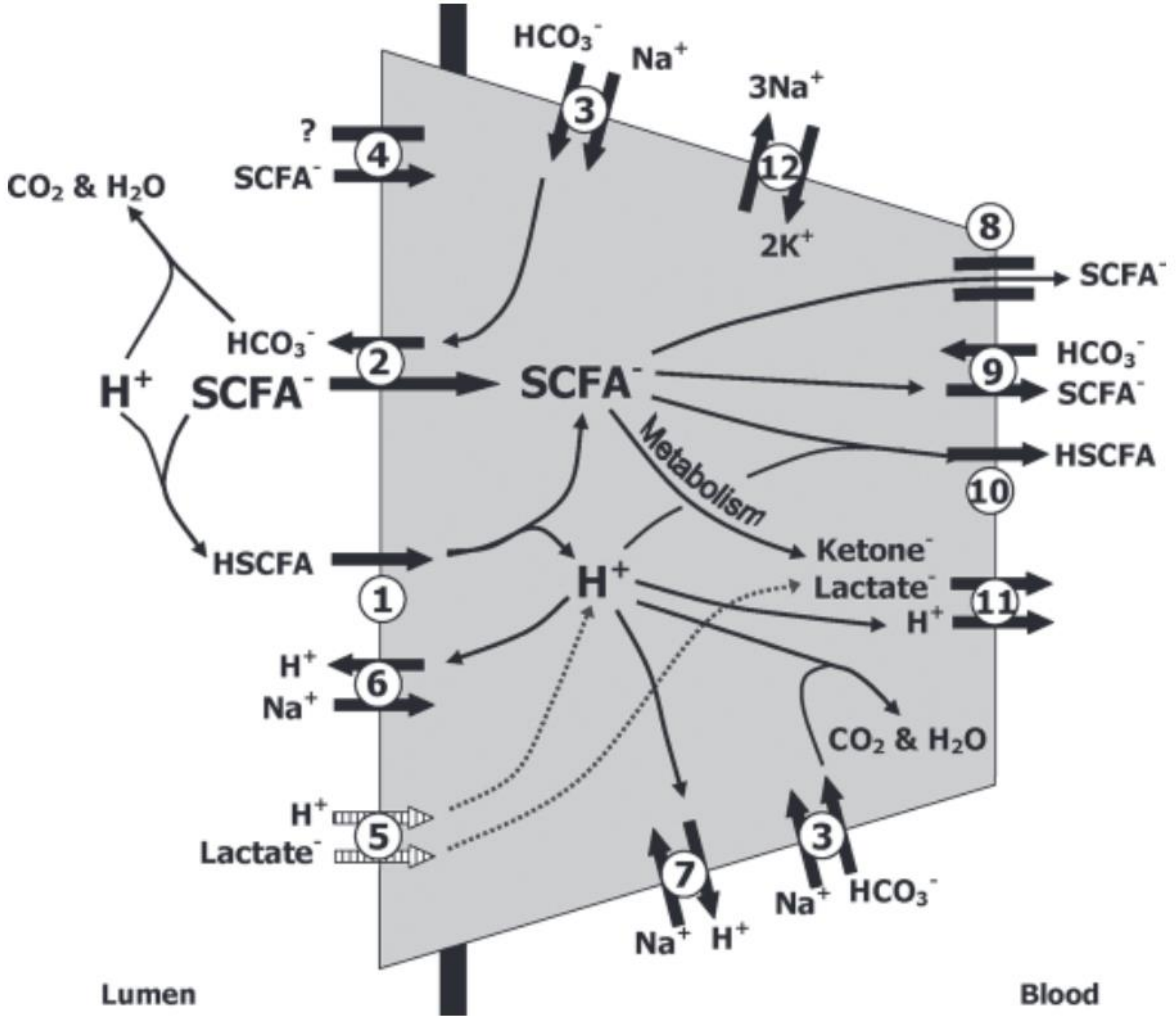
RUMINANT NUTRITION SYMPOSIUM: Role of fermentation acid absorption in the regulation of ruminal pH[1,2]

J. R. Aschenbach,*[3] G. B. Penner,† F. Stumpff,* and G. Gäbel‡

*Institute of Veterinary Physiology, Free University of Berlin, D-14163 Berlin, Germany;
†Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 5A8;
and ‡Institute of Veterinary Physiology, University of Leipzig, D-04103 Leipzig, Germany

Most SCFAs/VFAs are in undissociated form, which is lipophilic and can passively diffuse across epithelial cell membranes.

Free protons (hydronium ions) require active transport or neutralization.



J. Anim. Sci. 2011. 89:1092–1107
doi:10.2527/jas.2010-3301

Potentiometric glass pH sensors

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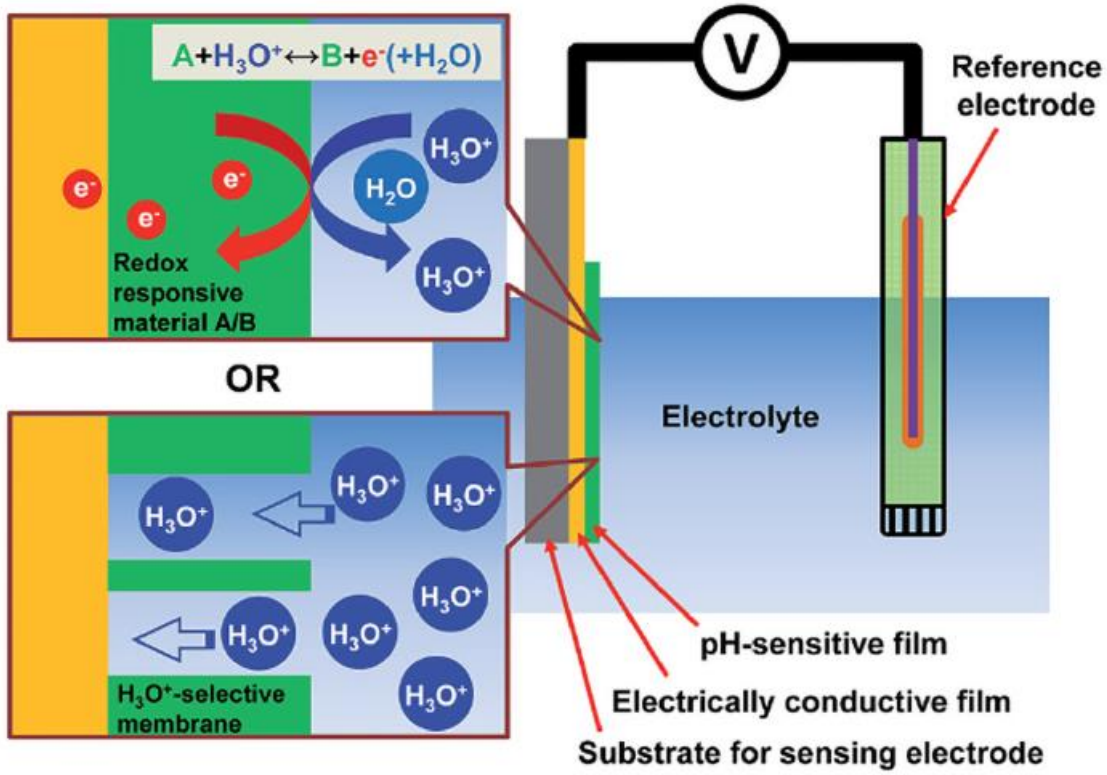
pH meters

- Commonly glass ion-selective electrodes – often solid-state.
- Measure the potential difference between a reference solution and the test solution.
- Nernst equation used to estimate the pH of the test solution, given the pH of the reference solution and the potential difference (mV) between the solutions.



Han, Chan Su et al. 2022. Invited review: Sensor technologies for real-time monitoring of the rumen environment. Journal of Dairy Science, Volume 105, Issue 8, 6379 – 6404 https://doi.org/10.3168/jds.2021-20576

Potentiometric glass pH sensors

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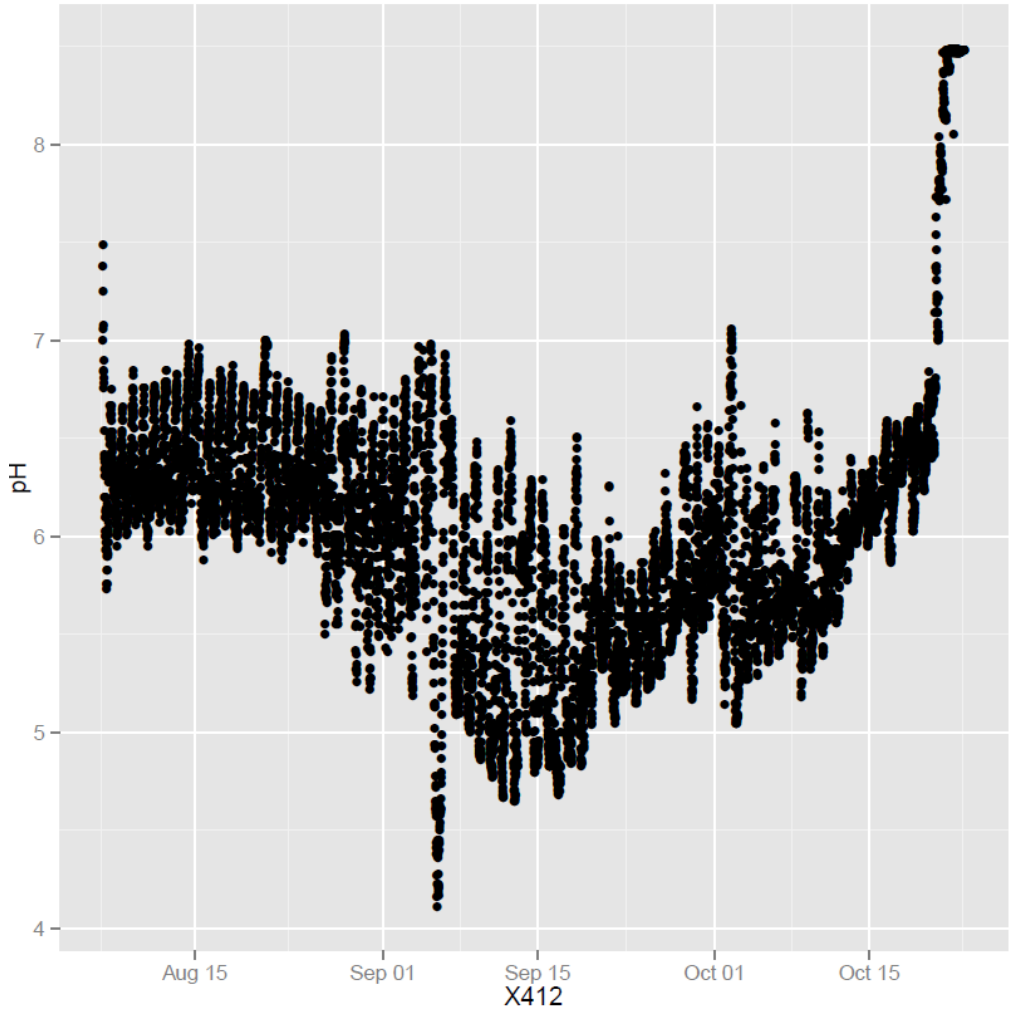
Electrode drift

- Most potentiometric measures of pH are subject to electrode drift, where there is a progressive divergence of readings from true values.
- Caused by degradation or contamination of the electrode, or progressive changes in the pH of the reference solution within the device.
- Historically has been an issue with ruminal devices from about 90 d. Currently expect 150 d with accuracy pH ± 0.4.



Reticuloruminal boluses

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Currently available

- **smaXtec** – commercial motility, pH and temperature
- **moonsyst** – commercial motility and temperature device; research-only pH device
- **LiveCare Biocapsule** – motility, pH, temperature ----

Defunct

- **Well Cow** – seems to have gone out of production
- **eCow** – one of the early innovators – patented in 2007 – ceased production 2020 – pH only

pH bolus data

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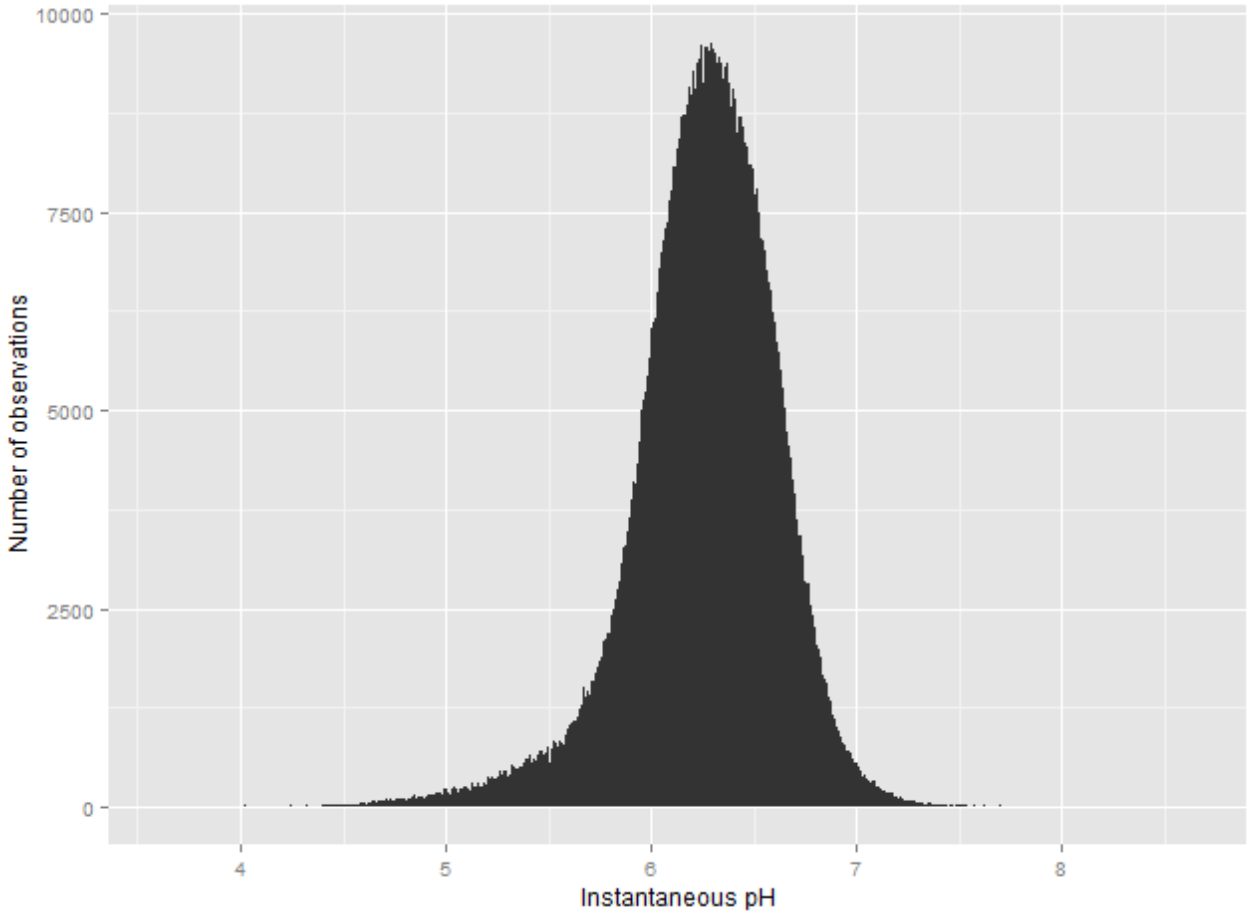
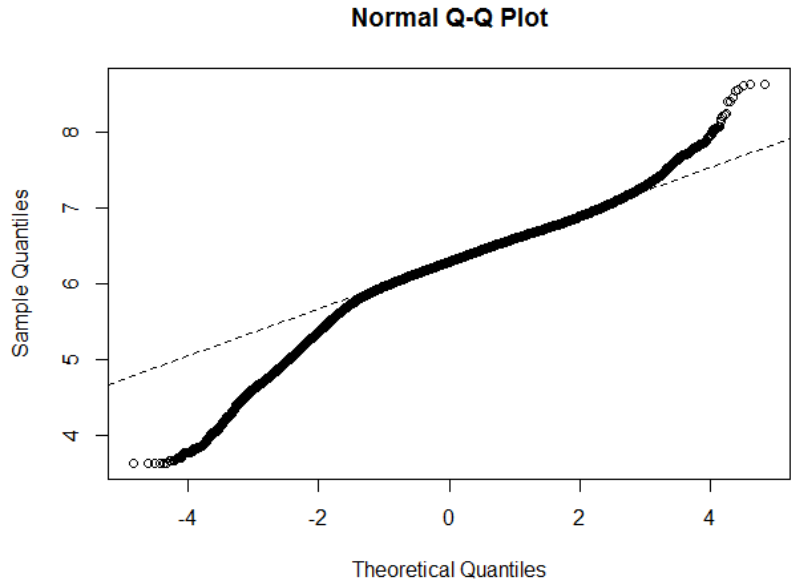
Range: observed pH values are often lower than assumed and not normally distributed.






```
ks.test(cutdata$RuminalpH,pnorm(cutdata$RuminalpH)) Two-sample Kolmogorov-Smirnov test data: cutdata$RuminalpH and pnorm(cutdata$RuminalpH) D = 1, p-value < 2.2e-16 alternative hypothesis: two-sided
```

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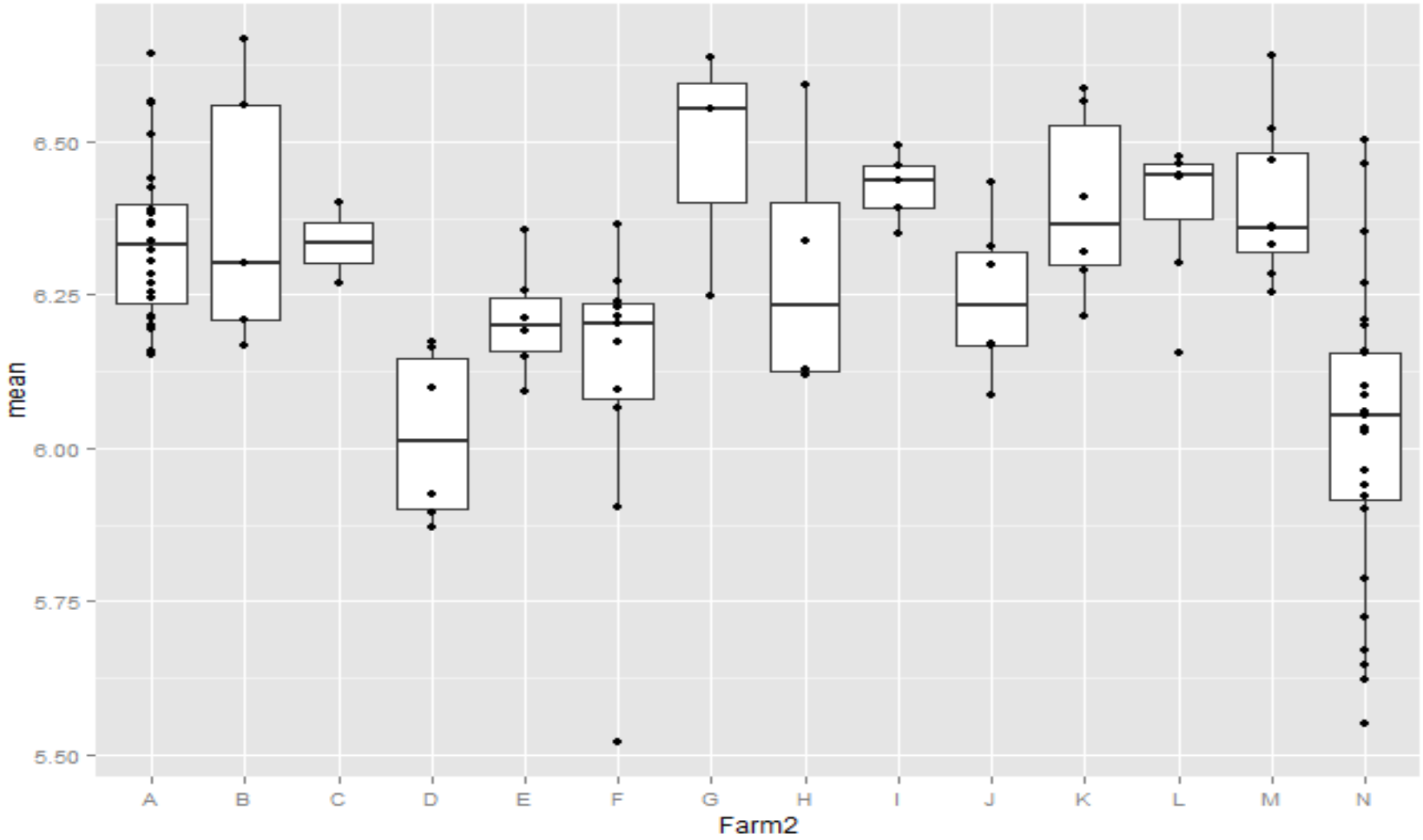
Among farm variation: there is huge variation among farms in mean pH.

```
modelaov <-
aov(cutdata$RuminalpH~cutdata$Fa
rm)
Df  Sum Sq  Mean Sq  F value
Pr(>F) cutdata$Farm
13  16954   1304.2   12119
<2e-16 ***
Residuals 749655 80672 0.1 ---
Signif. codes: 0 ‘***’ 0.001
‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```



pH bolus data

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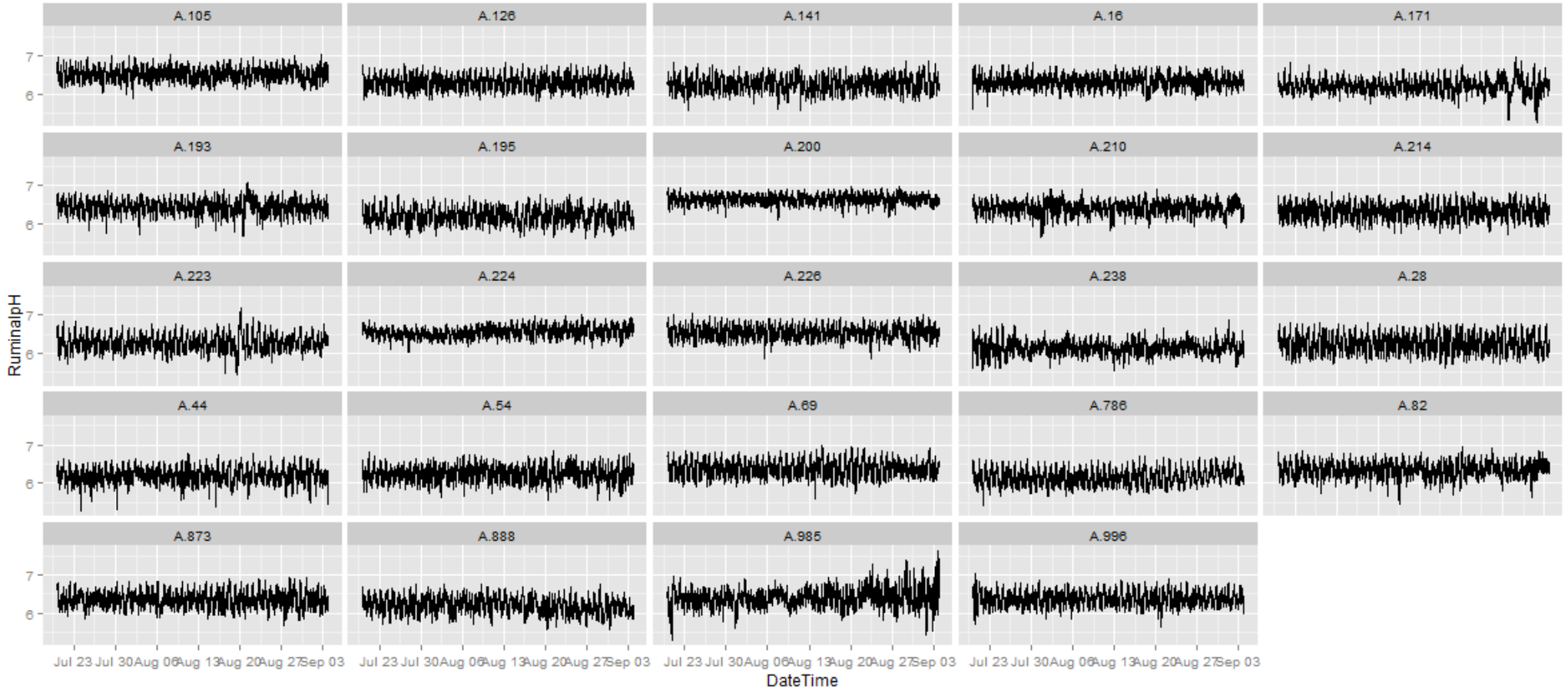
Among animal variation: there is significant and substantial variation among cows on the same rations and on the same farm



pH bolus data

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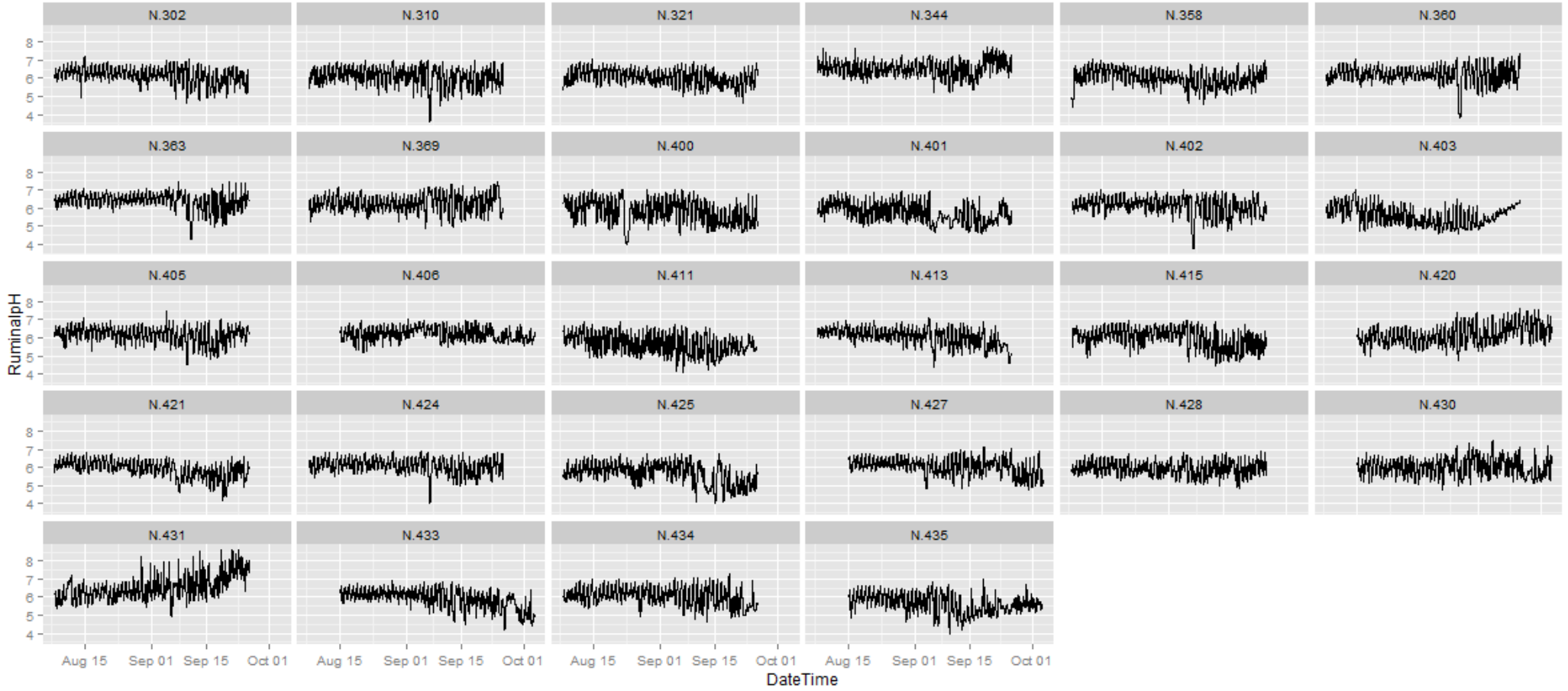
Among animal variation: there is significant and substantial variation among cows on the same rations and on the same farm **– applies also to responses to changes in ration**



pH bolus data

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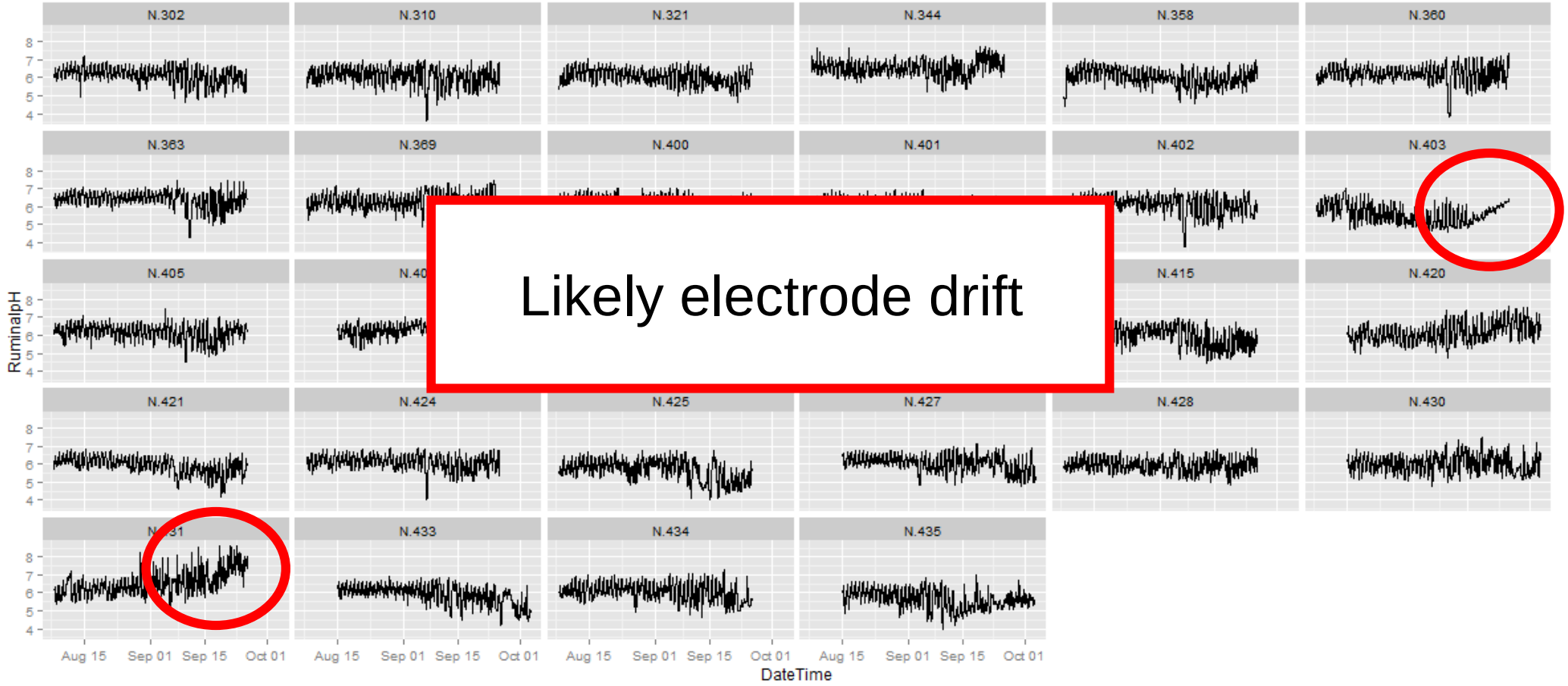
Among animal variation: there is significant and substantial variation among cows on the same rations and on the same farm – applies also to responses to changes in ration



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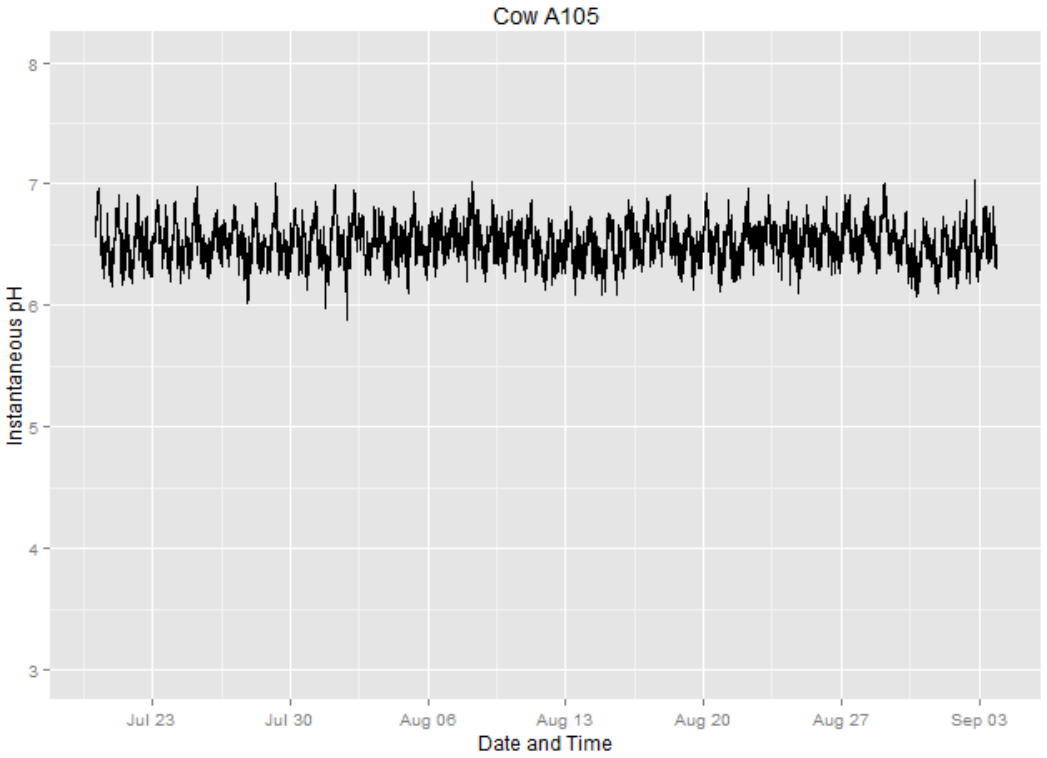
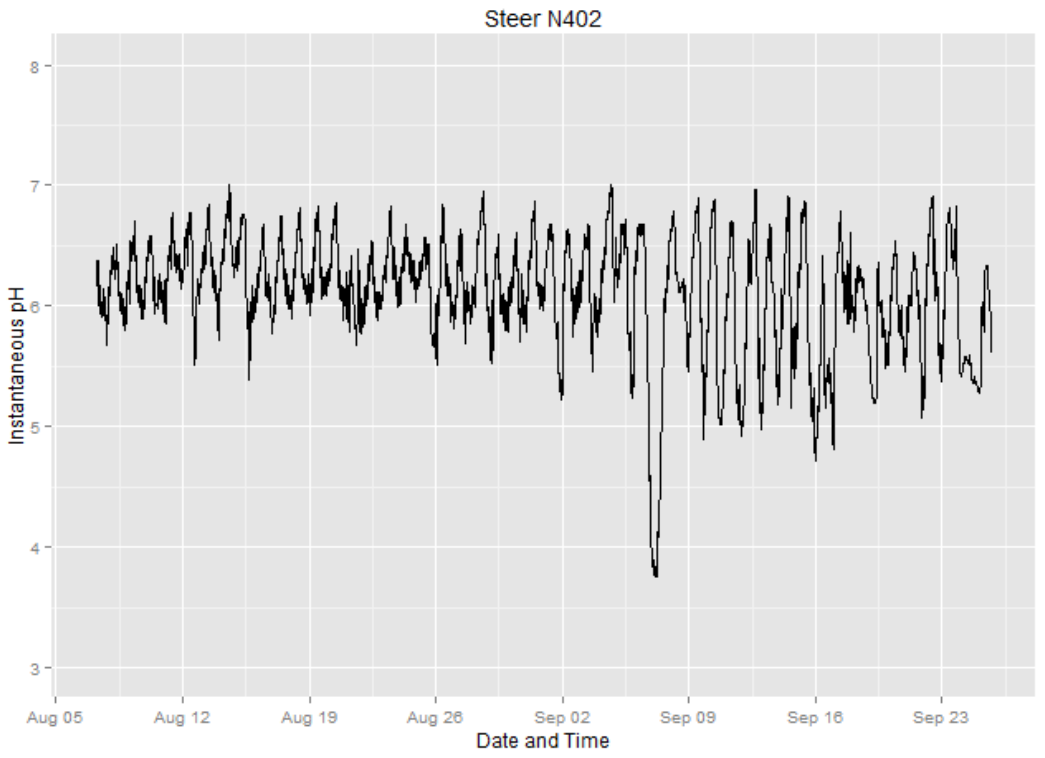
- **Diet effect:** increasing soluble concentrate in the ration results in a higher amplitude and less stable. In this example the dairy on left was 40% concentrate (%DM) and the beef unit on right was increased from 70 to 90% concentrate (%DM).





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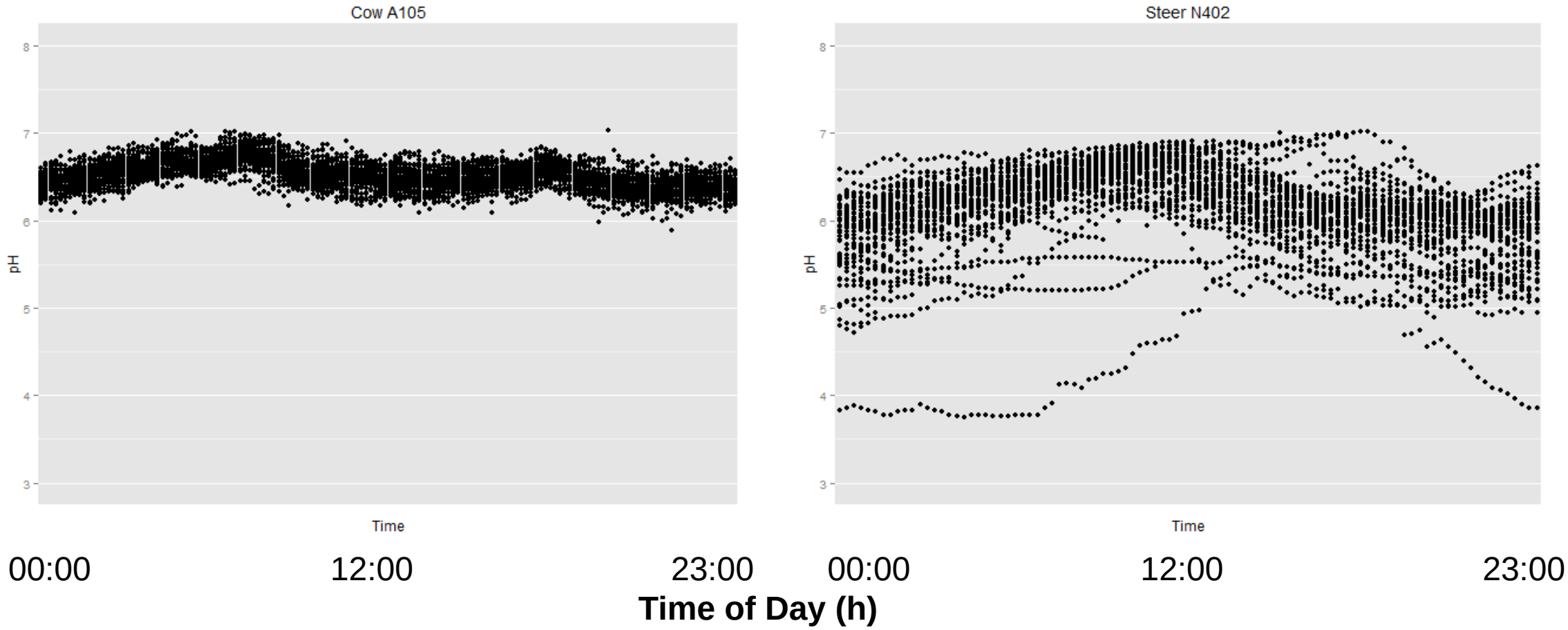
- **Diurnal patterns**: tend to be similar within and among, with variation among animals mainly seen in the amplitude and small shifts in phase.



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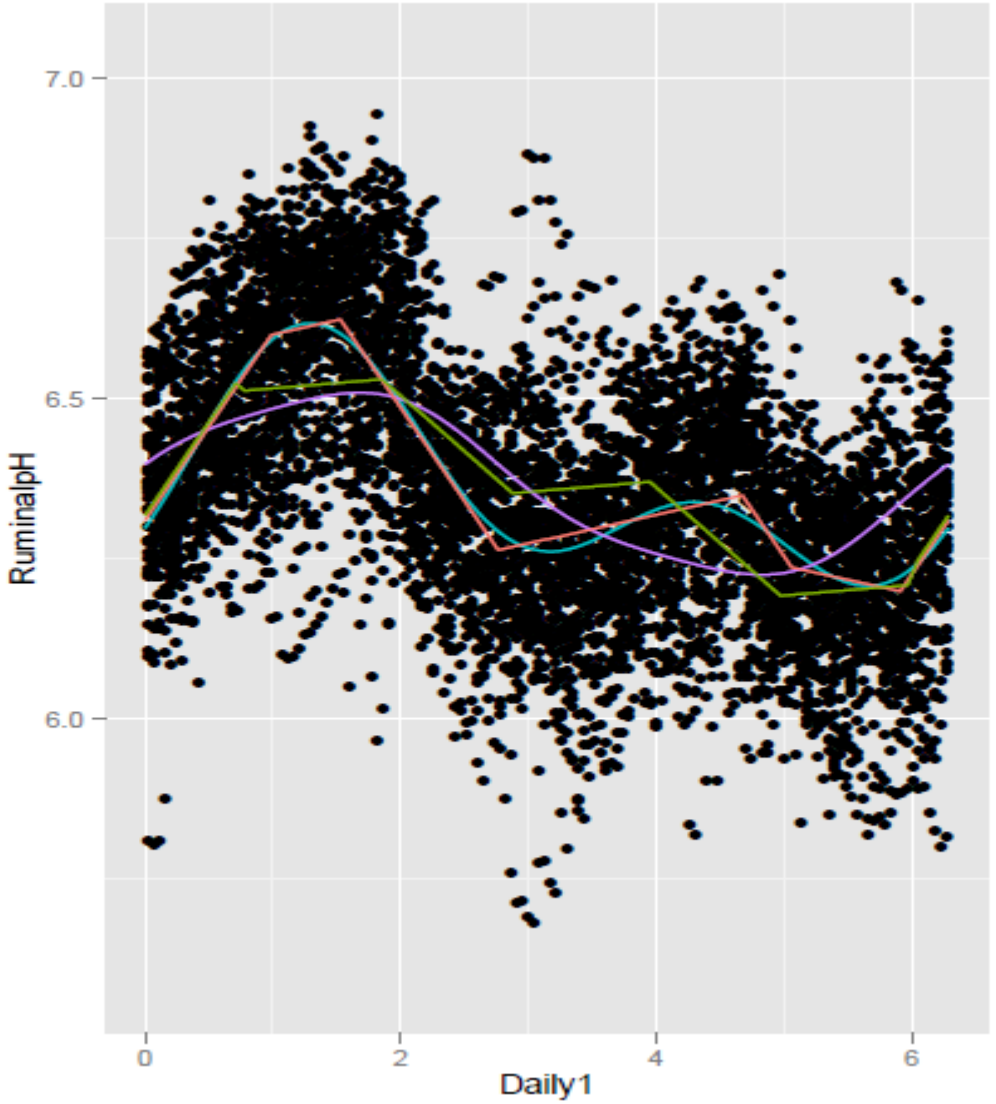

- Every animal on every farm has its own characteristic diurnal rhythm.

pH bolus data analytical approaches

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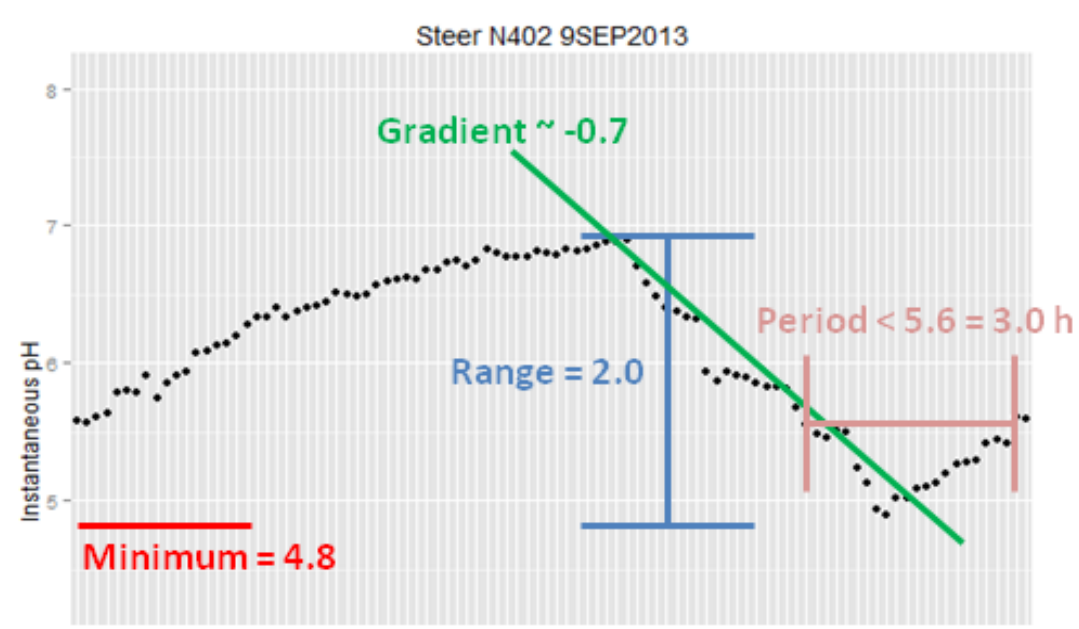
How to convert the data to actionable recommendations?

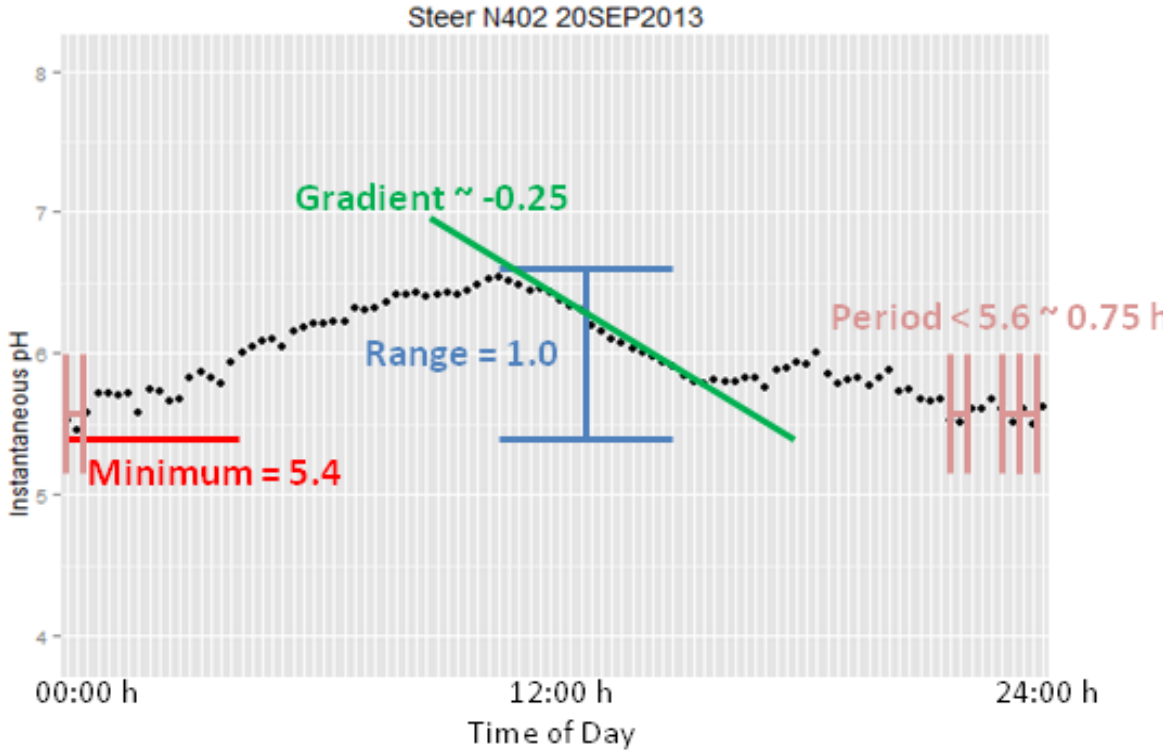
There are two quite different approaches to this:

- **Statistical methods** for classification based on expected range for acceptable or unacceptable states
- **Machine learning** for classification based on patterns of features extracted from pH data

Statistical approaches

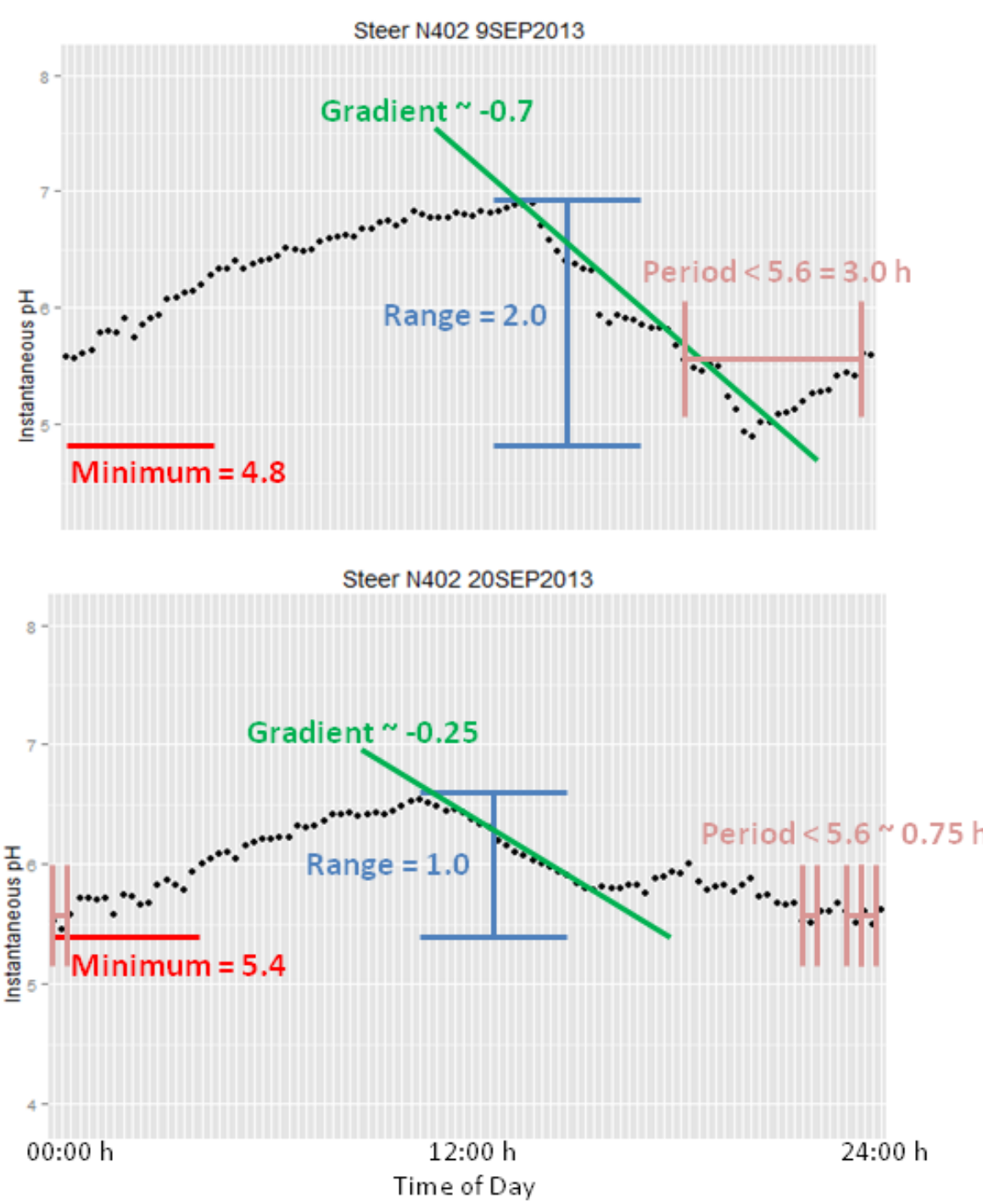
- pH curves can be broken down into distinct components, and each of them can be considered separately or together as an index.
- For real-time application and development of warning systems, each variable requires a threshold value, determined statistically for each animal, herd or population more broadly.





Data statistical approach

- Alternatively, rather than using the various measures to the right, we can analyse each individual cow's pH with respect to its predicted pH given the time of day.



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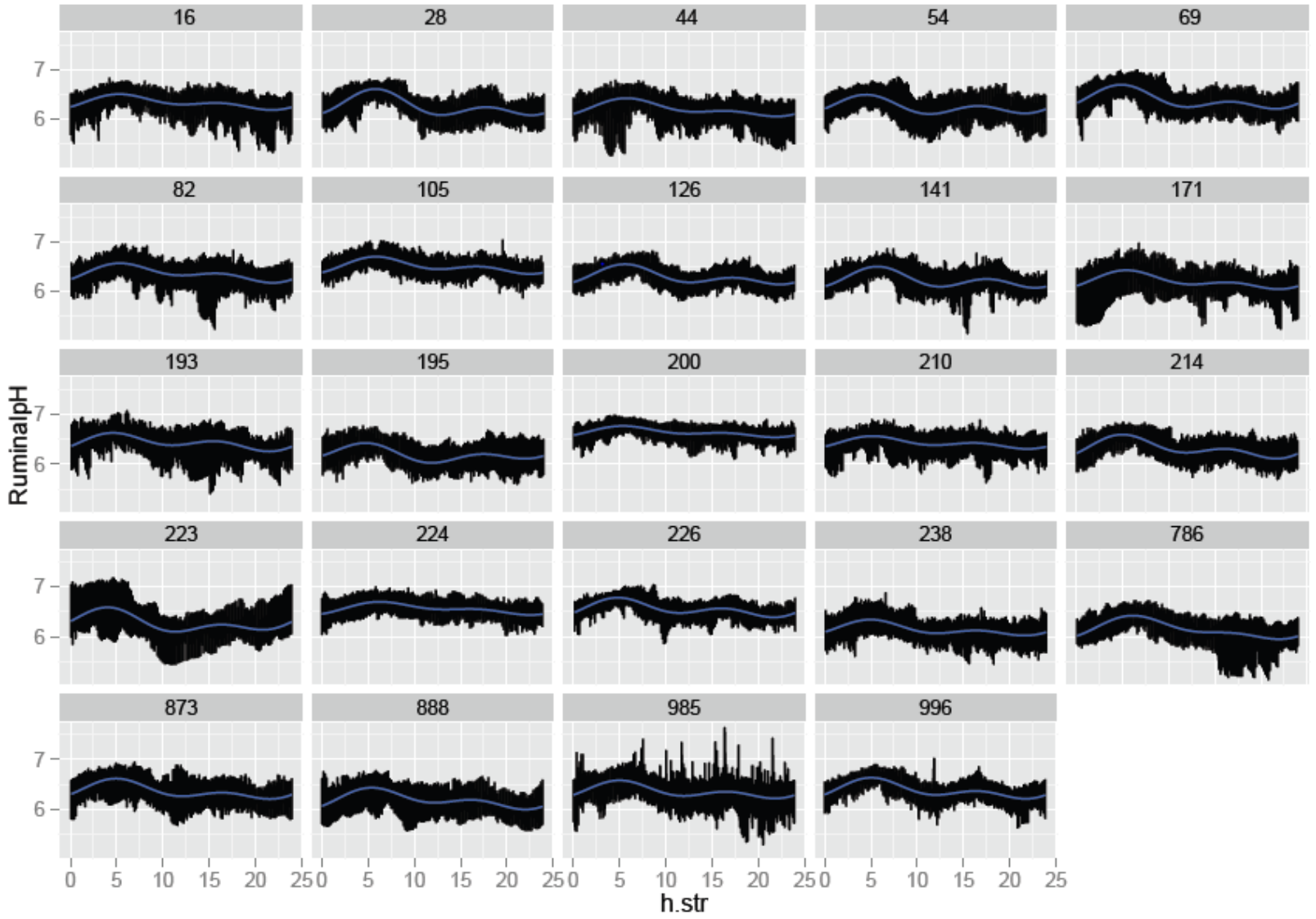
- Can fit a sine curve to capture the diurnal variation for each cow



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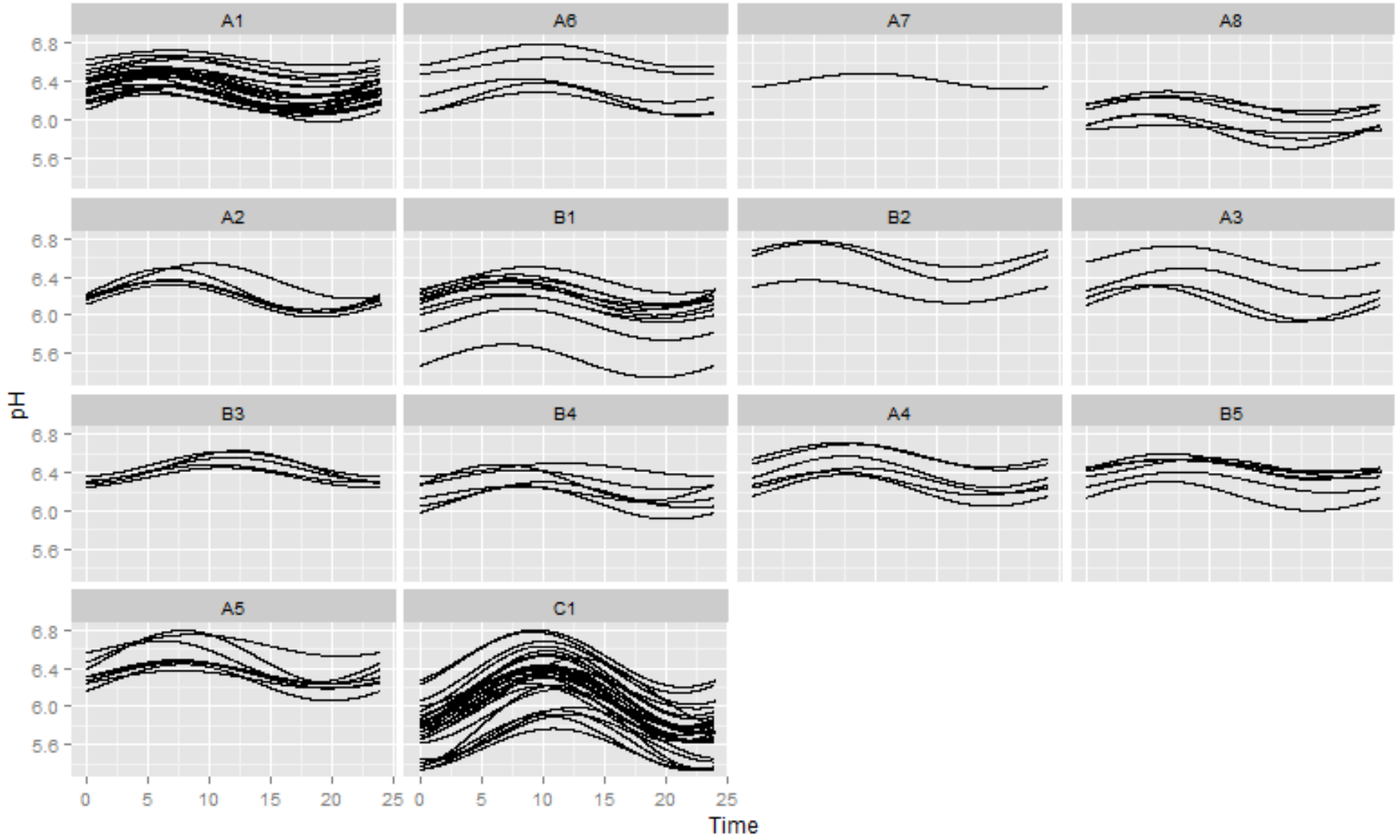
- Sine waves tend to be repeatable among cows within farm as they depend partially on management practices (feeding times, milking times)



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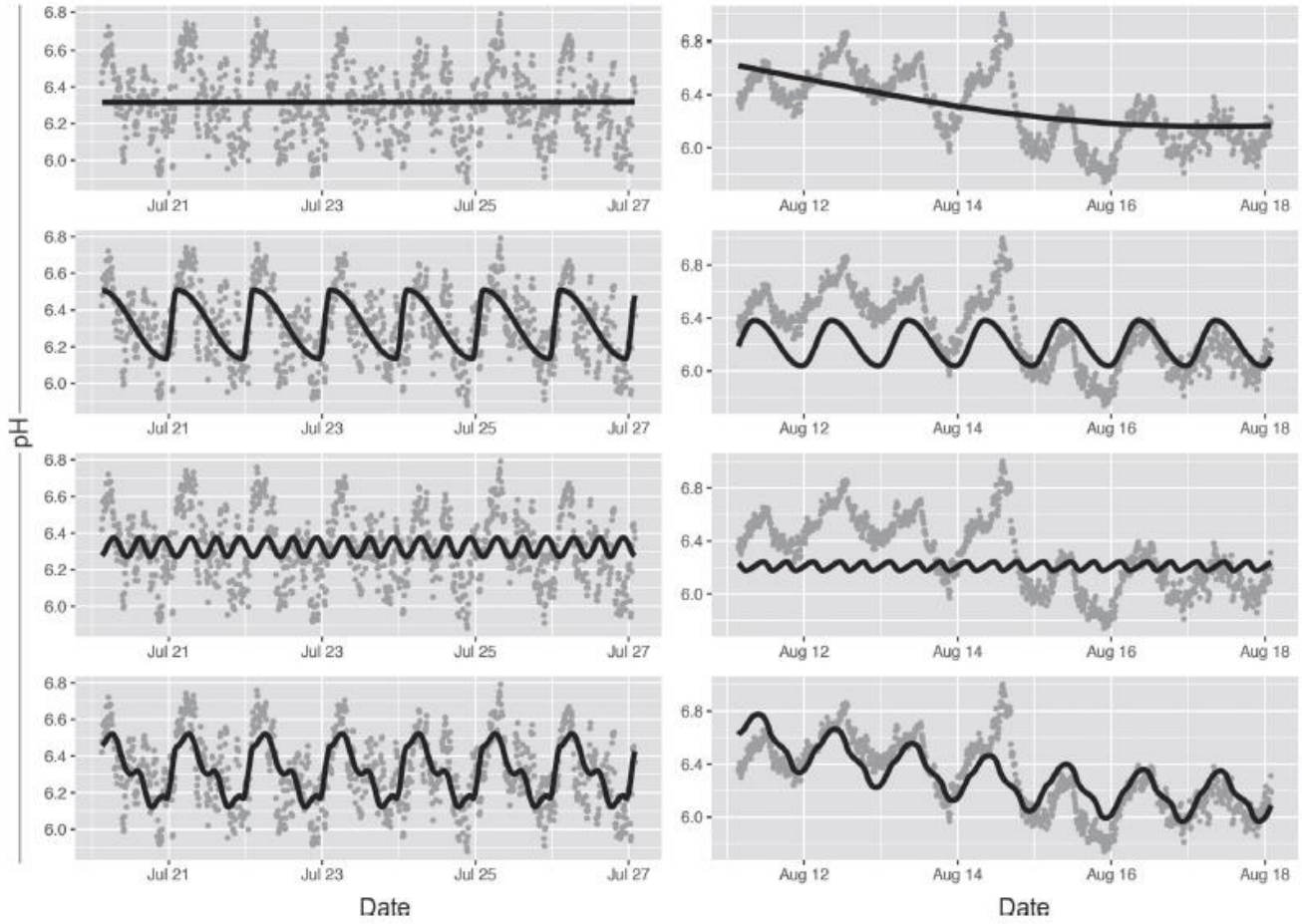

J. Dairy Sci. 101:1–13
https://doi.org/10.3168/jds.2017-12828
© American Dairy Science Association®, 2018.

Describing temporal variation in reticuloruminal pH using continuous monitoring data

M. J. Denwood,*[1] J. L. Kleen,† D. B. Jensen,* and N. N. Jonsson‡§
*Department of Veterinary and Animal Sciences, University of Copenhagen, 1870 Frederiksberg C, Denmark
†CowConsult, Coldinne, 26532, Germany
‡Institute of Biodiversity Animal Health and Comparative Medicine, University of Glasgow, Glasgow, G61 1QH, United Kingdon
§Harbro Ltd., Birkhill Mill, Lanarkshire, ML11 0NJ, United Kingdom

- Including a general additive model for cow and farm (top) plus a sine wave with phase-shift (second row) with a feeding/milking sine wave (third row) gives the complete model for each animal (bottom).

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- High mean absolute residuals were significantly associated with falls in feed intake and milk yield.
- Conventional measures weren't so effective.

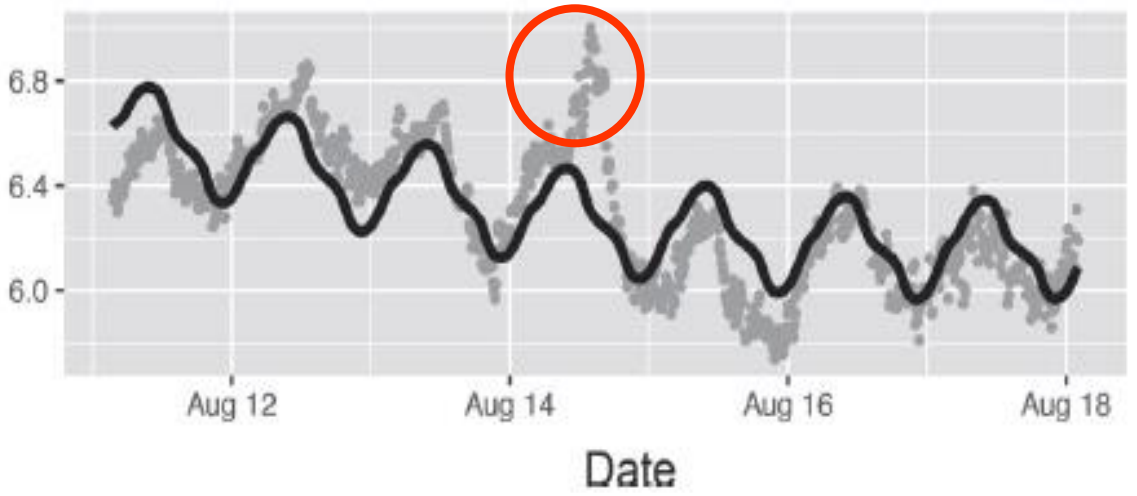

Table 3. Estimates obtained from 2 multivariable linear mixed models relating 4 different daily pH summary statistics to the daily corrected milk yield and daily DMI observed 2 d later[1]

	Daily corrected milk yield		Daily DMI	
Item	Estimate	*P*-value	Estimate	*P*-value
Mean residual	−0.137	0.911	0.019	0.988
Mean absolute residual	−8.633	<0.001	−8.698	<0.001
No. of observations below lowest 1%	0.041	0.056	0.015	0.530
No. of observations above highest 1%	0.003	0.877	0.018	0.396

[1]Summary statistics are mean residual pH, mean absolute residual pH, and number of pH observations below and above the most extreme 1% values for that animal.

pH bolus data - statistical

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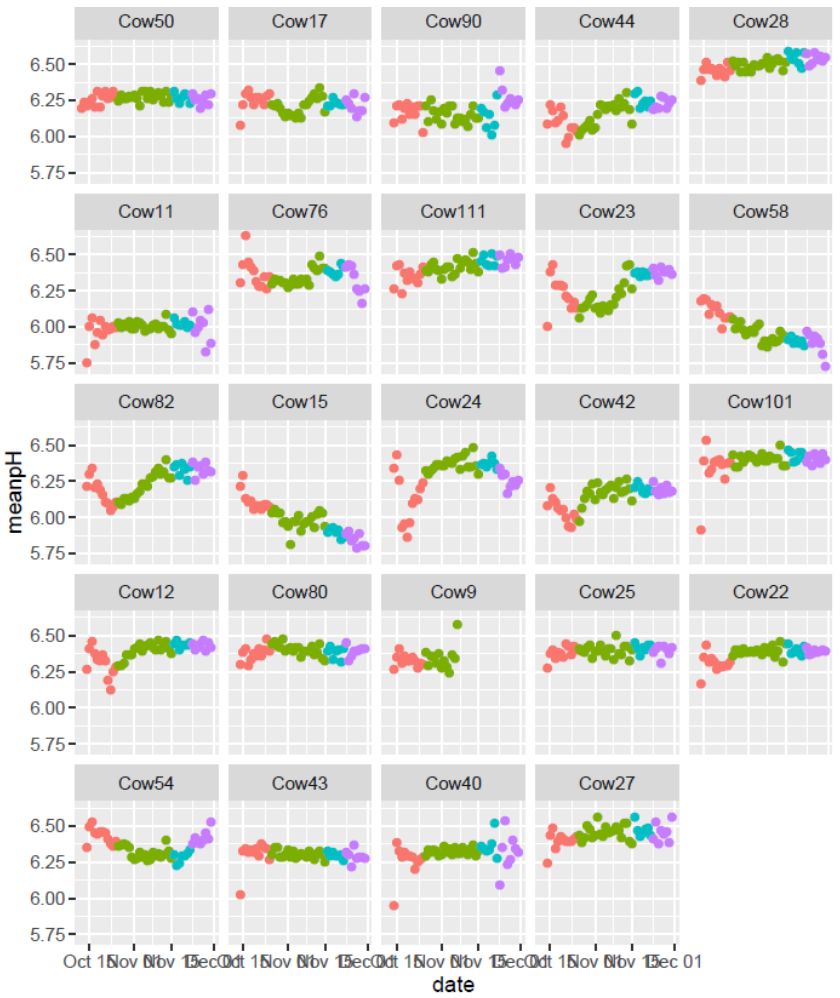
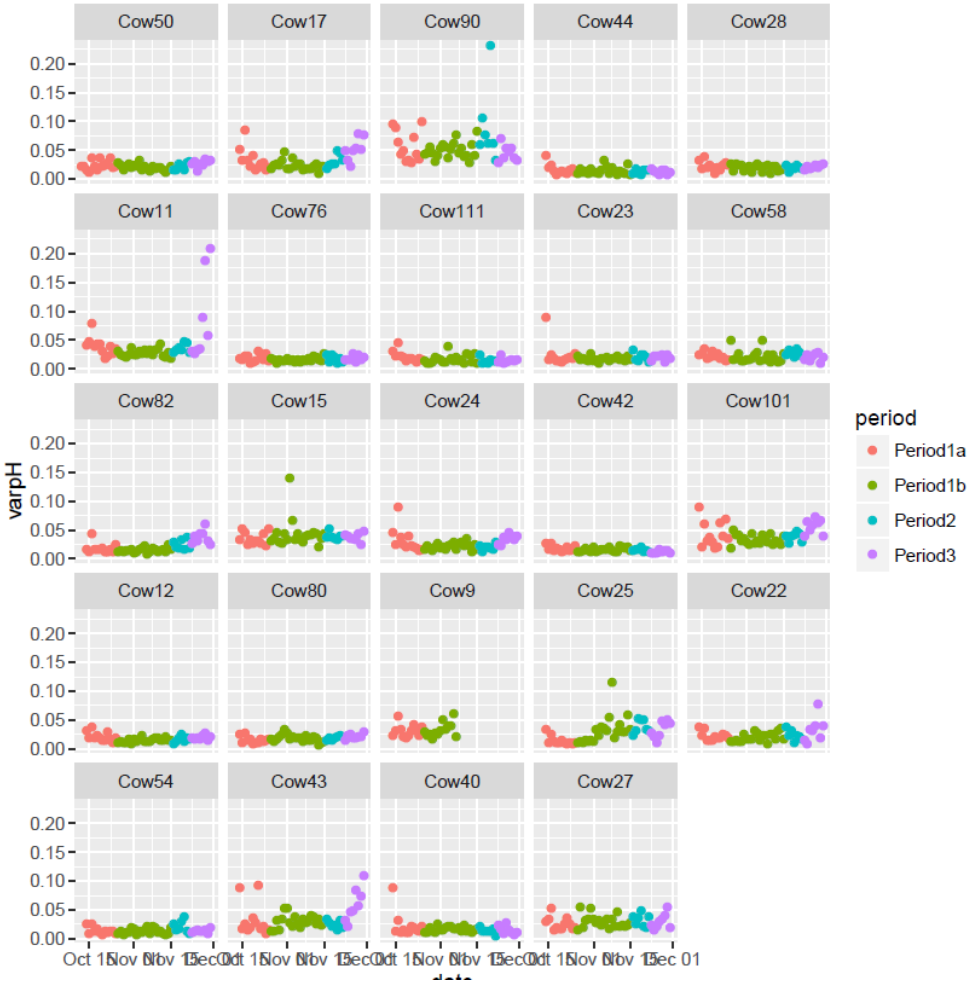
- The Denwood approach requires high density computation and simpler approaches have also worked, including standard deviation and cumulative periods under a given threshold.
- **However, none of the statistical approaches based on pH alone seems to be ideal.**



pH mean over time



pH variance over time

Machine learning

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It seems likely that machine-learning approaches can be applied to pH data but the fundamental problem of potentiometer lifespan and natural variation in a regulated system suggest that pH will not be the most useful tool.

https://cs.stanford.edu/people/karpathy/cnnembed/

pH bolus data

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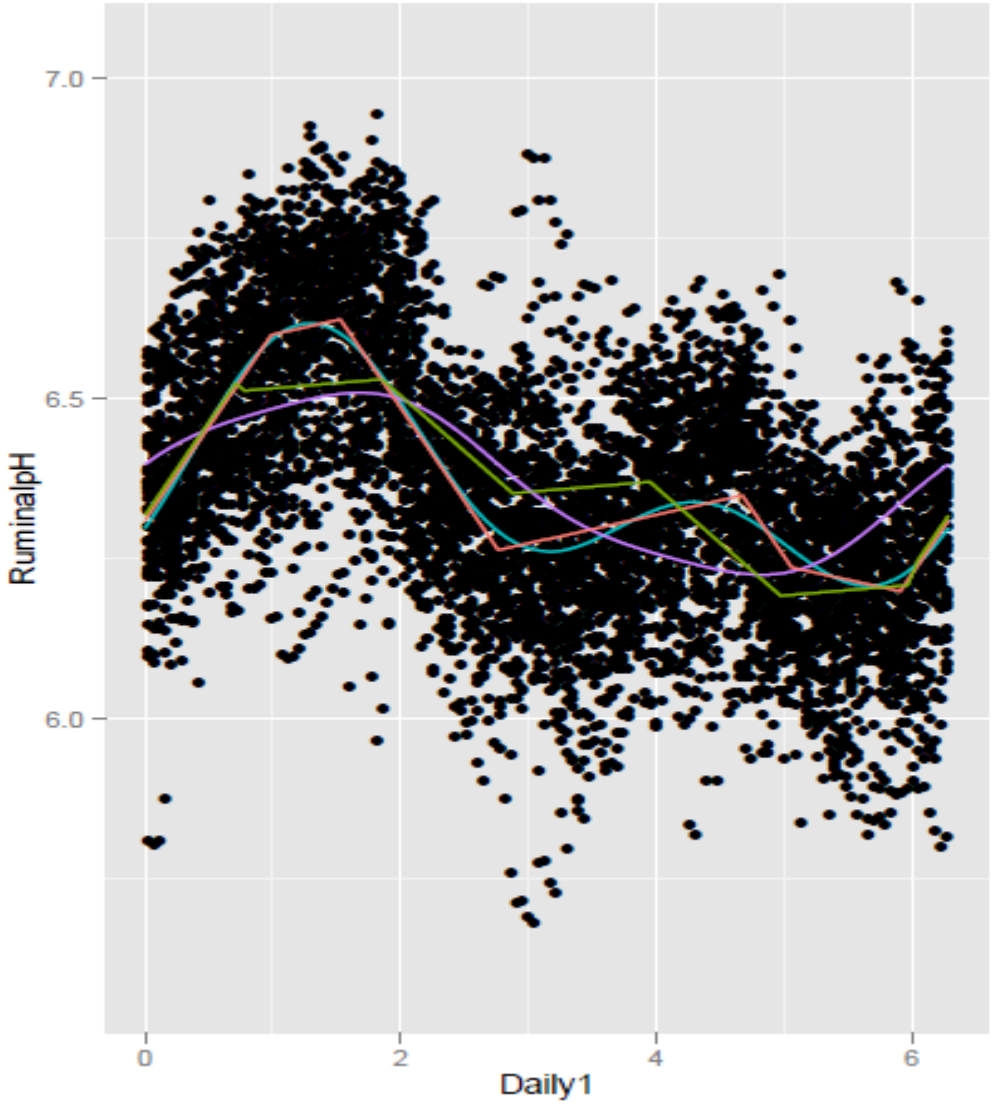

Han et al (2022) concluded, "…**anecdotal evidence suggests that alarm fatigue and limited data interpretability preclude effective use of real-time sensed pH data**. As such, the potential impact of real-time pH monitoring as a tool to improve cattle health management is limited not only by sensor limitations but also by the translation of effectively sensed data into actionable decisions or management recommendations."

https://doi.org/10.3168/jds.2021-20576.

SARA diagnostic criteria

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- Initial Garrett, Nordlund, Oetzel concept was herd-level diagnosis
- Sample ~ 2 × 6 cows by ruminocentesis
- pH thresholds were:
  - ≤ 5.5 = abnormal;
  - 5.6–5.8 = marginal;
  - > 5.8 = normal.
- **A herd was classified as having a problem with SARA if one or more groups had two or more animals with a pH ≤ 5.5**

SARA diagnostic criteria

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More recently Zebeli et al. (2008) proposed

- daily mean ruminal pH < 6.16
- pH < 5.8 for > 5.24 h/d.

These measures are readily achieved using continuous monitoring approaches.

Zebeli, Q. et al. 2008. Modeling the Adequacy of Dietary Fiber in Dairy Cows Based on the Responses of Ruminal pH and Milk Fat Production to Composition of the Diet. Journal of Dairy Science, Volume 91, Issue 5, 2046 – 2066 https://doi.org/10.3168/jds.2007-0572

pH bolus data for SARA research

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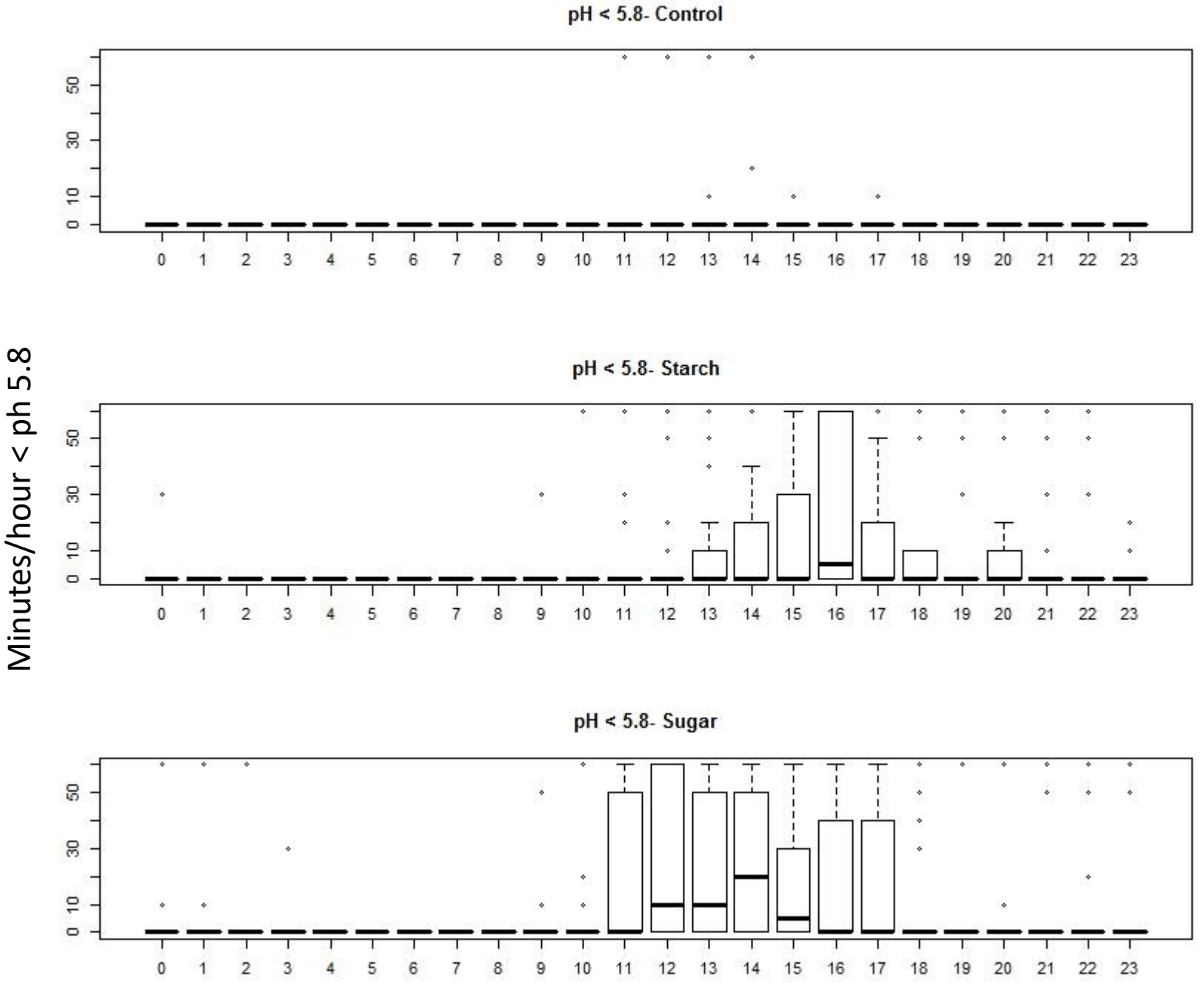
These threshold criteria are widely and successfully used in feeding studies.

Studer et al. 2023. Application note for the use of a wireless device measuring reticular pH under practice conditions in a Swiss dairy herd, Smart Agricultural Technology, 4: 100170
https://doi.org/10.1016/j.atech.2022.100170

Bach et al., 2023. Effects on rumen pH and feed intake of a dietary concentrate challenge in cows fed rations containing pH modulators with different neutralizing capacity, Journal of Dairy Science, 106: 4580-4598
https://doi.org/10.3168/jds.2022-22734

- We adjusted for reticular v ruminal pH (ie + 0.2 pH*) and measured minutes/hour at < pH 5.8 threshold.
- With strong, acute challenge, it is possible to see differences in t < pH 5.8



*Falk et al., 2016: diff = 0.24
https://doi.org/10.3168/jds.2015-9725

pH bolus data for SARA research

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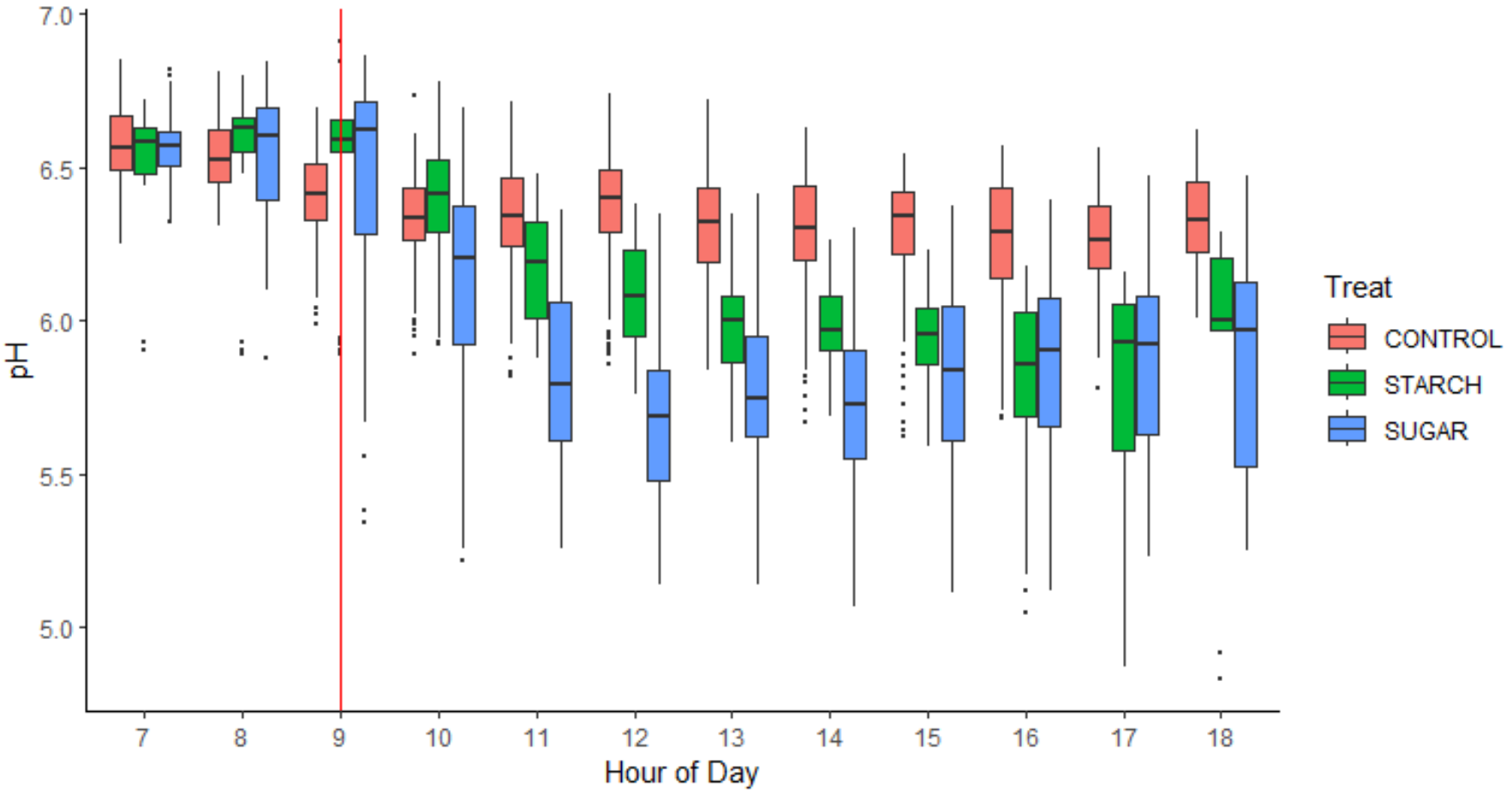
- Similarly, mean pH was significantly different, but only for some timepoints.
- pH bolus data is very useful for comparing the effects of treatments, particularly over short periods and with strong challenges.



pH bolus data for SARA research

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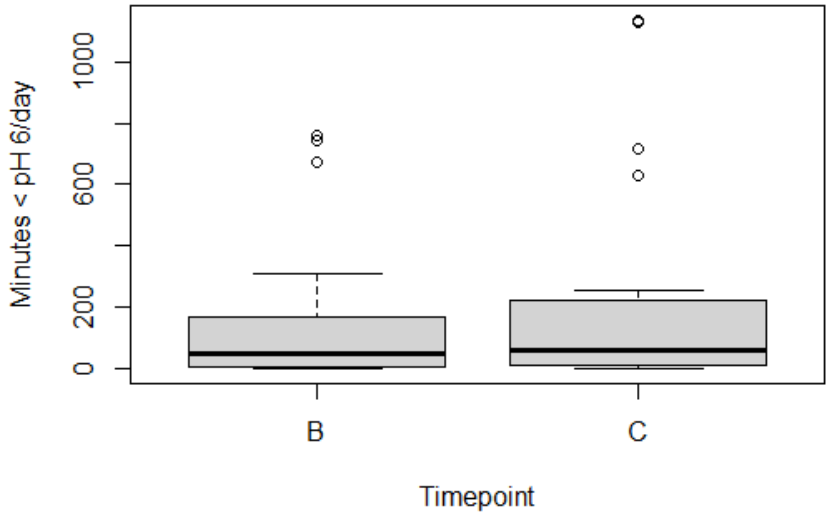
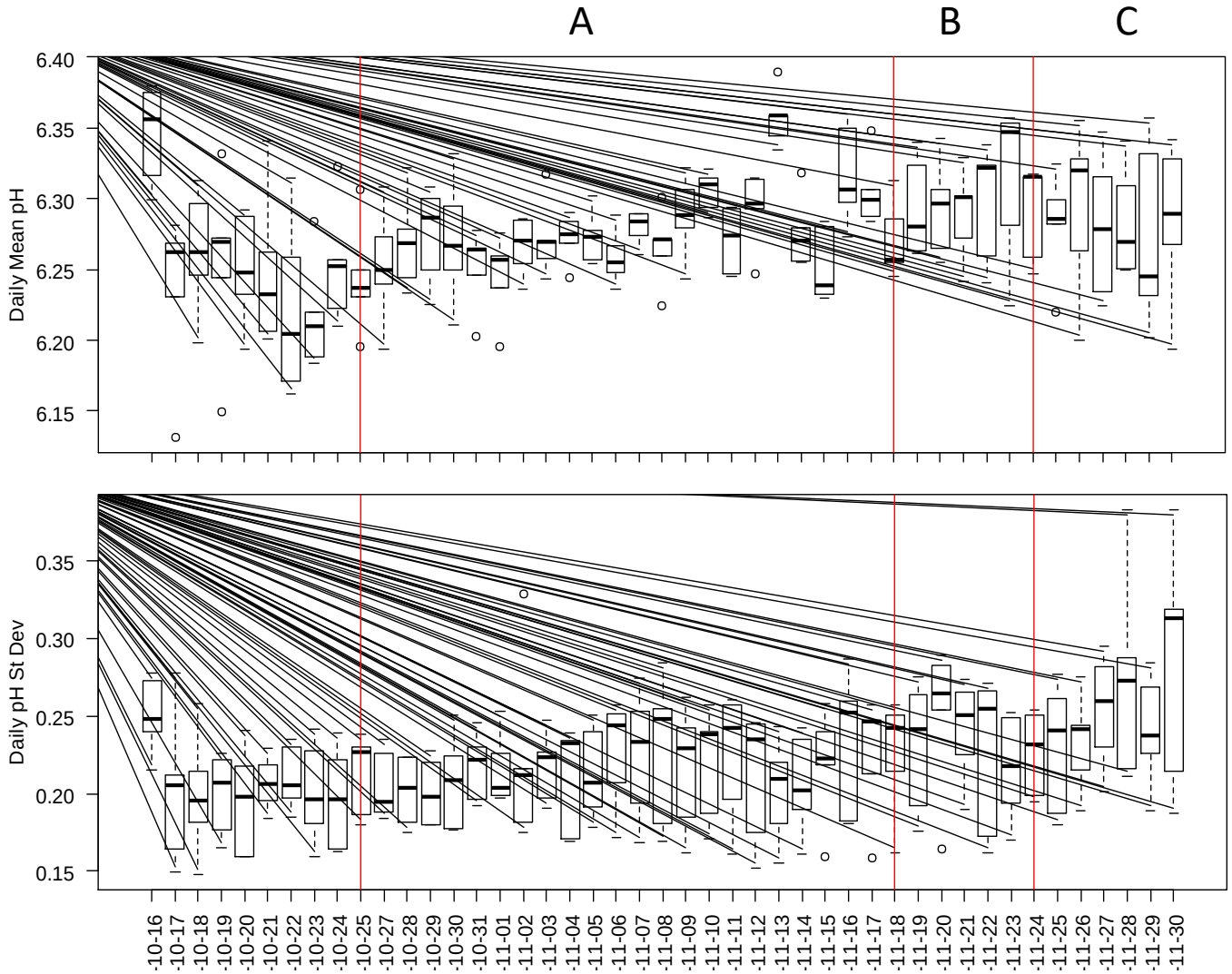
- However, in our hands it has not been very easy to apply when challenges have been more realistic and applied over longer timeframes.





SARA – some reservations

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- Sub-acute is a poorly defined descriptor of the time-course of a disease and is often misinterpreted to refer to either subclinical or mild disease
- The mechanisms by which dietary disturbances lead to pathology in severely affected animals have not been shown to be pH-dependent but have complex pathways, likely including low pH, increased concentrations of endotoxin, biogenic amines, osmotic disturbance and possibly ethanol, among others.
- Finally, although arising from dysfunction of or inability of the rumen to cope with influx, some of the important pathology arises from disturbances in the intestine, caecum and colon.

I therefore conclude that any meaningful diagnostic approach to SARA should include non-pH criteria

SARA diagnostic criteria

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Clothilde Villot and colleagues have been working on combinations of criteria for SARA diagnosis with some early success using relative markers, having found the traditional criteria to be unsatisfactory.

Villot et al. 2018. Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows, Animal,12: 481-490
https://doi.org/10.1017/S1751731117001677

Coppa et al. 2023. On-farm evaluation of multiparametric models to predict subacute ruminal acidosis in dairy cows, Animal, 17: 100826
https://doi.org/10.1016/j.animal.2023.100826

SARA diagnostic criteria

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"**Commonly used pH SARA indicators were not able to discriminate SARA syndrome due to high animal variability and sensor drift and noise**, whereas relative pH indicators developed in this study appeared more relevant for SARA detection as assessed by receiver operating characteristic tests. This work shows that absolute pH kinetics should be corrected for drift, noise and animal variability to produce relative pH indicators that are more robust for SARA detection."

Villot et al. 2018. Relative reticulo-rumen pH indicators for subacute ruminal acidosis detection in dairy cows, Animal,12: 481-490
https://doi.org/10.1017/S1751731117001677

SARA diagnostic criteria

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Helen Golder and Ian Lean are working on integrated diagnostic approaches and improved terminology for SARA – based on Bramley et al. (2008).

The Bramley classification system has three levels: acidotic; suboptimal rumen function; non-acidotic. These levels were identified using cluster analysis.

Differentiation among the categories was based on reticuloruminal pH, VFA, ammonia and lactate concentrations.

Bramley. et al. 2008. The definition of acidosis in dairy herds predominantly fed on pasture and concentrates. Journal of Dairy Science, 91: 308 – 321 https://doi.org/10.3168/jds.2006-601

Monitoring with pH boluses

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- We were asked to estimate how many cows in a herd would need to be equipped with pH boluses to give an accurate estimate of the herd status.
- A set of pH measurements taken from animals in the same herd at the same time and date was sampled with replacement from the available measurements.
- Absolute difference between the mean of the sample and the true mean of the observed pH from all animals at that date and time in that herd was recorded.
- 1000 iterations – median, 75% quantile and 99% quantile estimates for the mean absolute error associated with each combination of time of day, herd, and number of animals sampled.

Jonsson et al. 2019. Evaluation of reticuloruminal pH measurements from individual cattle: Sampling strategies for the assessment of herd status, The Veterinary Journal, 243: 26-32 https://doi.org/10.1016/j.tvjl.2018.11.006

Monitoring with pH boluses

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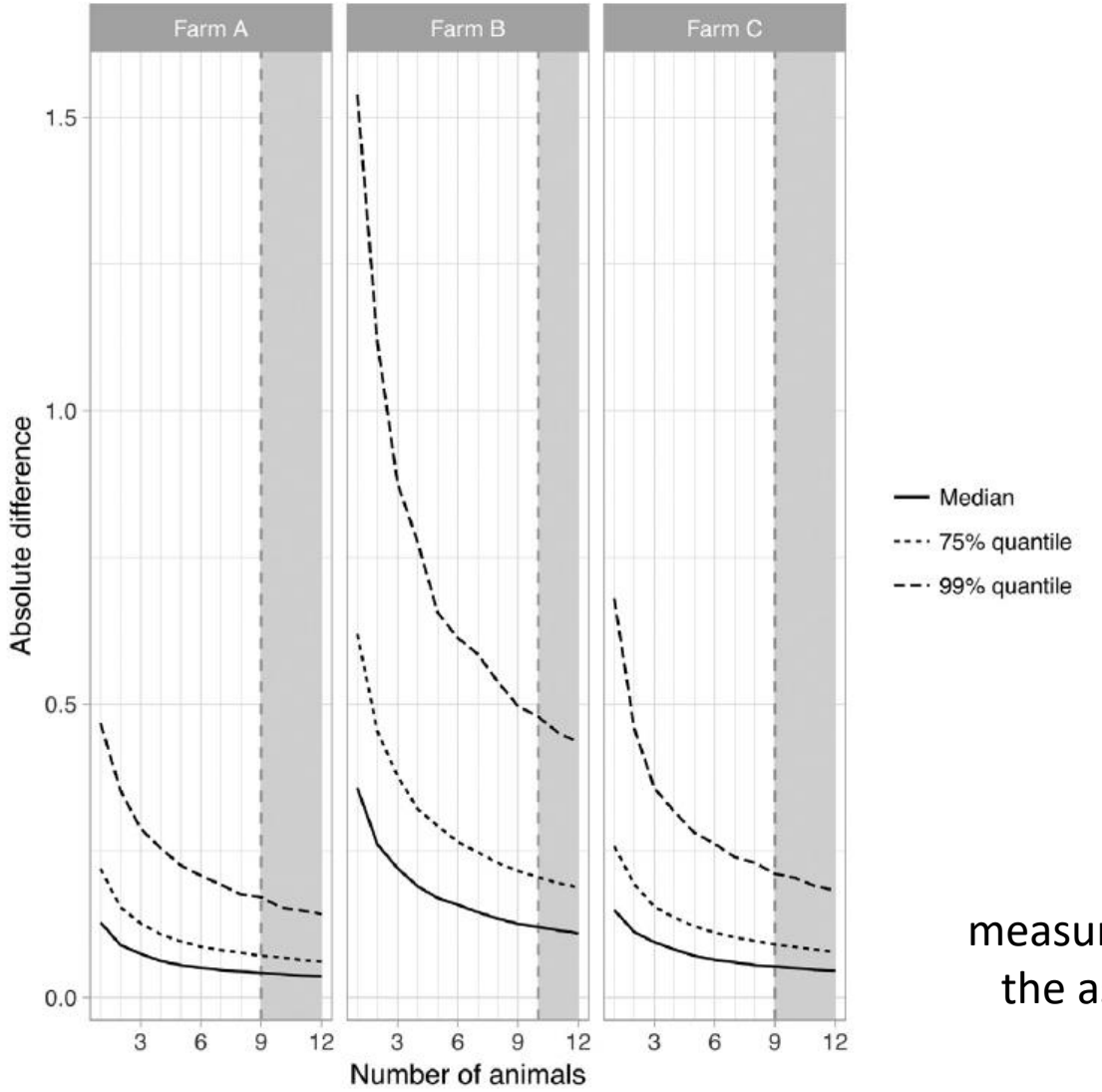

- The more variable the pH within and among animals in a herd, the more boluses required to get an estimate within 0.2 pH units of an overall herd status.
- For most herds, 9 animals equipped with boluses would give a reasonable estimate, but for very uniform herds (such as Farm A in our study) 3 animals with boluses would give a pretty good indication or herd status.

Jonsson et al. 2019. Evaluation of reticuloruminal pH measurements from individual cattle: Sampling strategies for the assessment of herd status, The Veterinary Journal, 243: 26-32 https://doi.org/10.1016/j.tvjl.2018.11.006

Conclusion on pH boluses

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- Continuously monitored pH boluses are excellent for research on the effects of dietary and management changes.
- As a diagnostic, they are challenged by
  - High cost per unit
  - Short life-span per unit
  - Interpretation of data
  - Non-pH factors may be more important

Motility boluses

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- Accelerometers are relatively cheap.
- Accelerometers require relatively little power.
- A known effect of pH decline is reduced reticuloruminal motility.
- **Maybe reticuloruminal motility could be a useful indicator of pH disturbance.**
- We developed prototype motility boluses and trialed them against ultrasound, collars and auscultation.

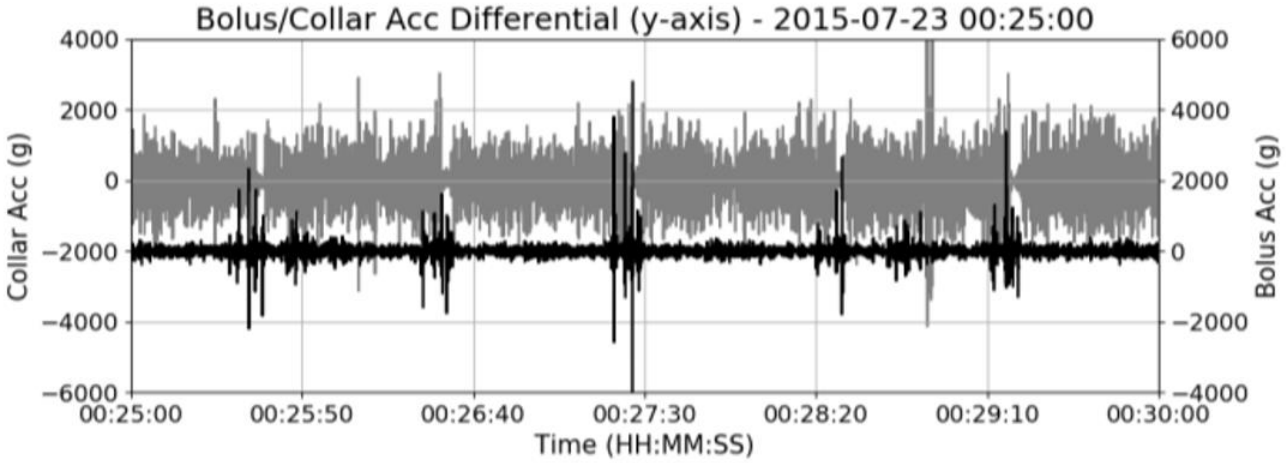

Figure 2. Comparison of accelerometer-derived reticulo-ruminal contraction data and rumination signature using neck-mounted accelerometer. There is close agreement between the putative reticuloruminal contractions and the collar-derived inter-rumination period.

Hamilton et al. 2019. Identification of the Rumination in Cattle Using Support Vector Machines with Motion-Sensitive Bolus Sensors" Sensors 19: 1165. https://doi.org/10.3390/s19051165

Motility boluses

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Accelerometers, hydrophones and gyroscopes all corresponded well with observations on reticuloruminal contractions.

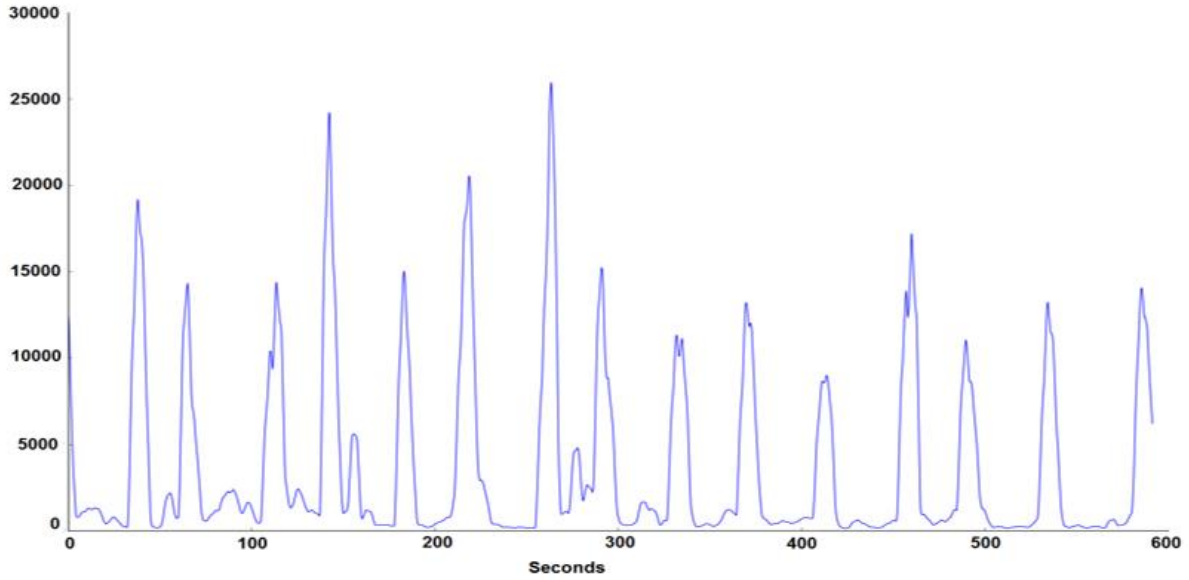

Figure 1. In Study-2, Accelerometers, hydrophones, and gyroscopes were placed in rumen-fistulated Jersey cows. The trace below shows a typical 10-minute recording of variance from a gyroscope in one animal.

Motility boluses and rumination

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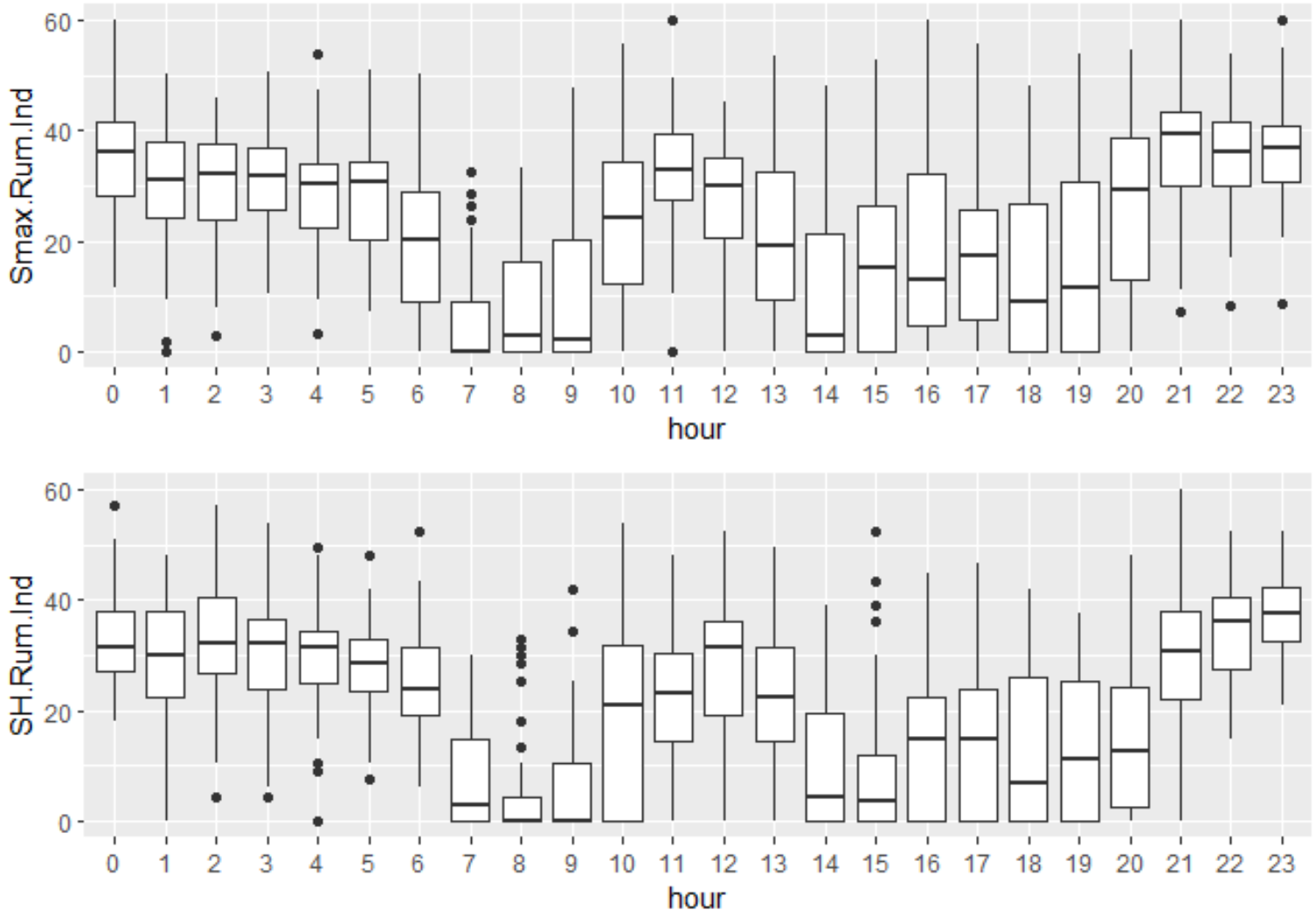
- Discovered smaXtec was ahead of us, so we stopped working with prototypes and had a look at their devices.
- Reticuloruminal boluses are in close agreement with collars for rumination and eating behaviour.



Motility boluses

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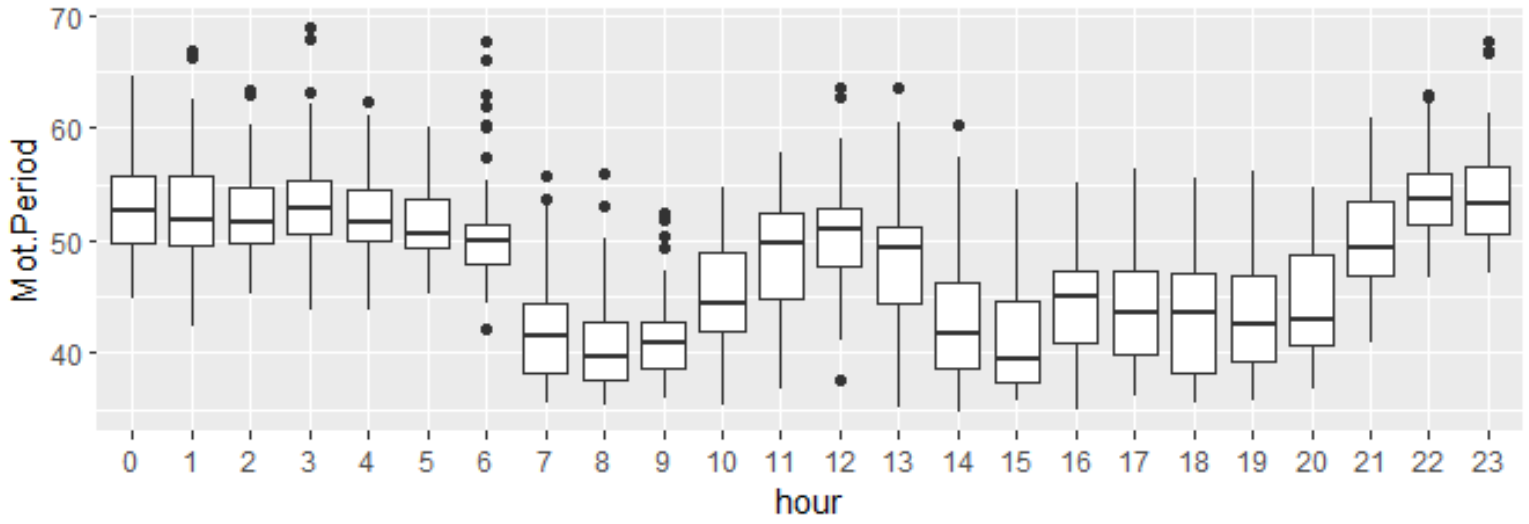
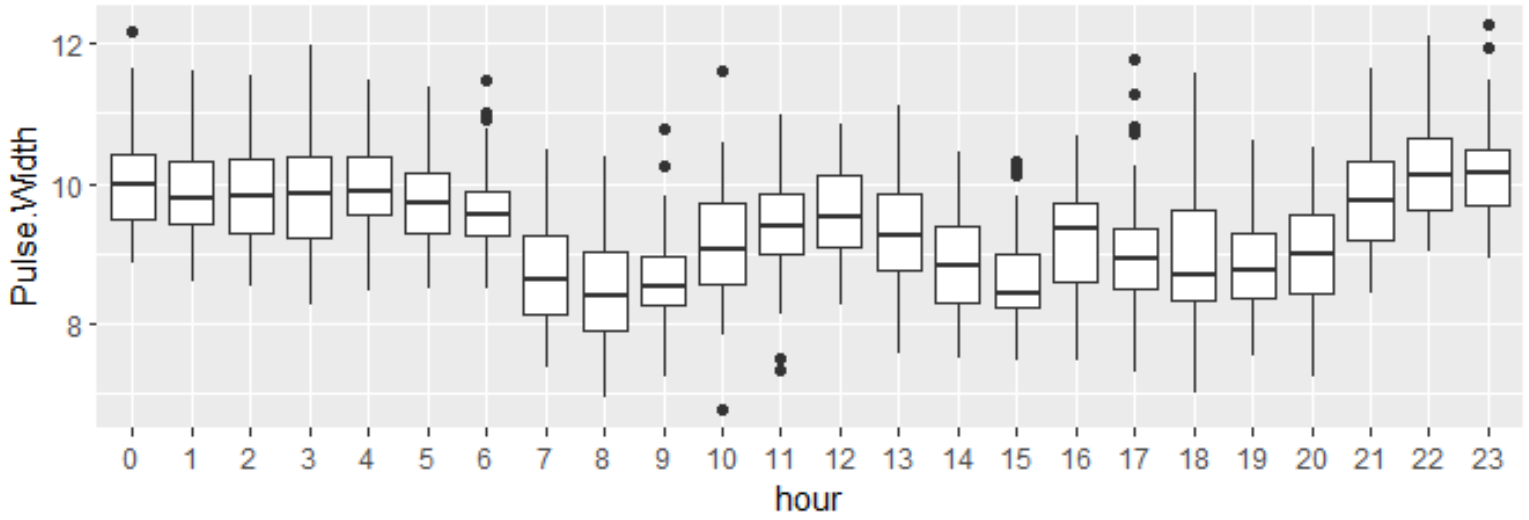
- Data on period (s) and duration (s) of reticuloruminal contractions followed expected diurnal patterns.
- We expect this should be sensitive to reticuloruminal dysfunction associated with acidosis.





Motility boluses and rumination

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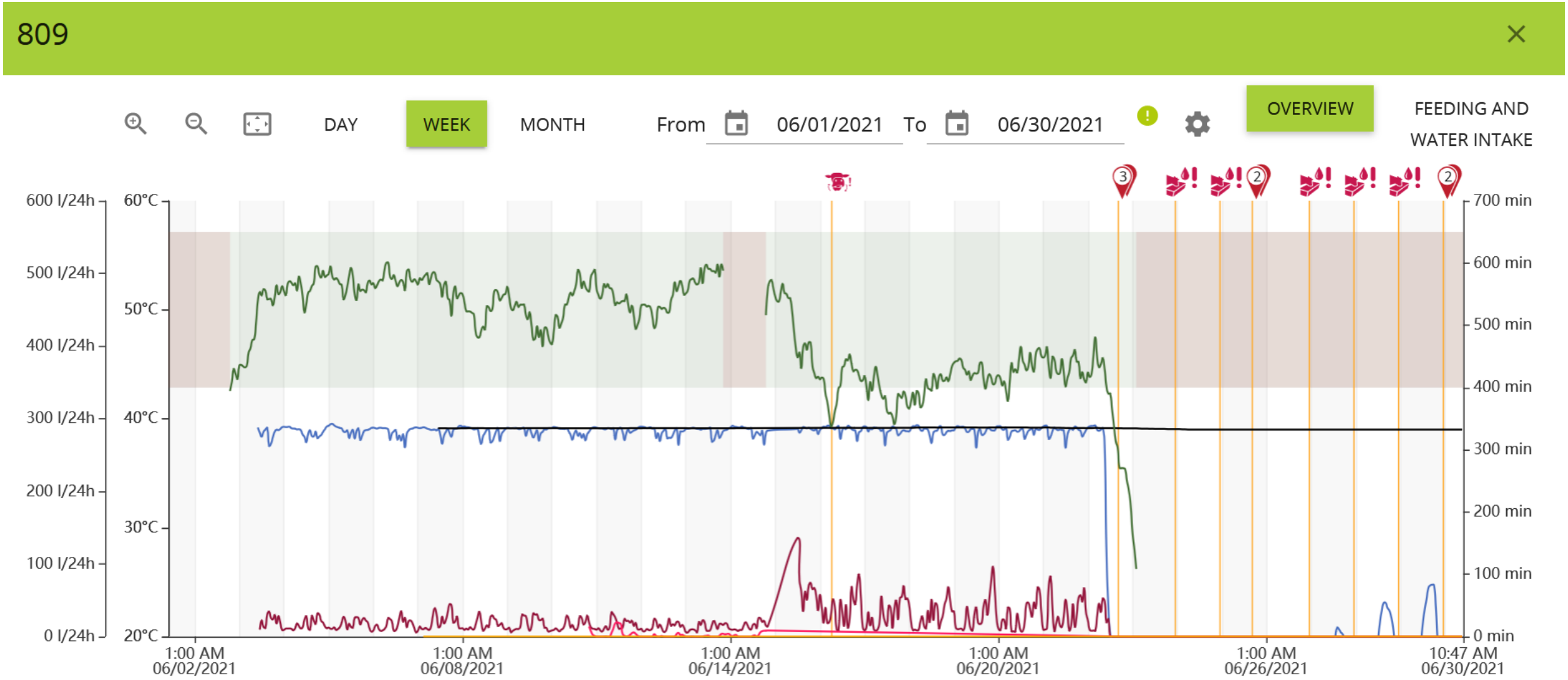

Motility boluses and SARA

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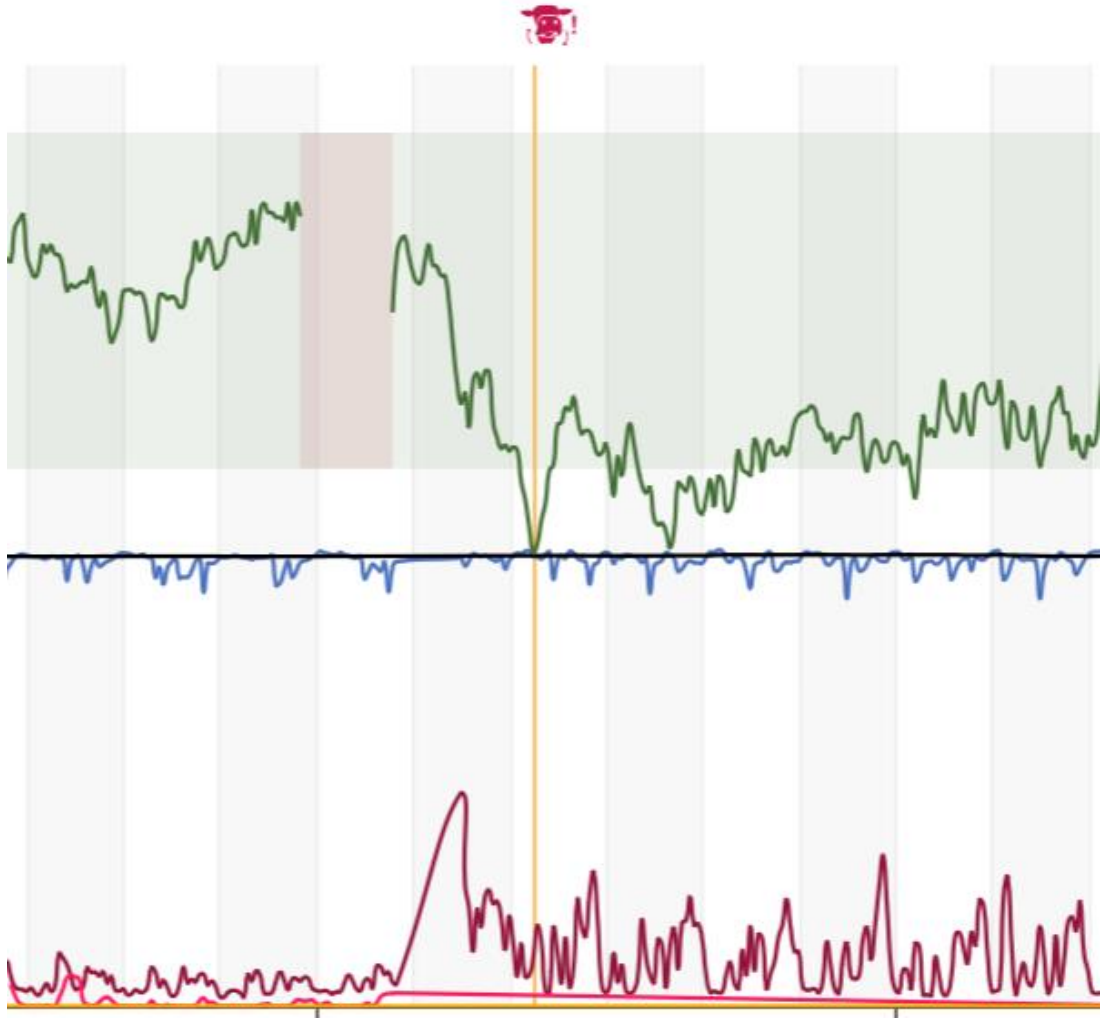
- Reticuloruminal motility provides a good approximation of eating and rumination, and should be a good indicator of SARA.
- The addition of temperature enables AI-based diagnostic procedures that should enable early diagnosis illness and differentiation of some causes.



Temperature measurement

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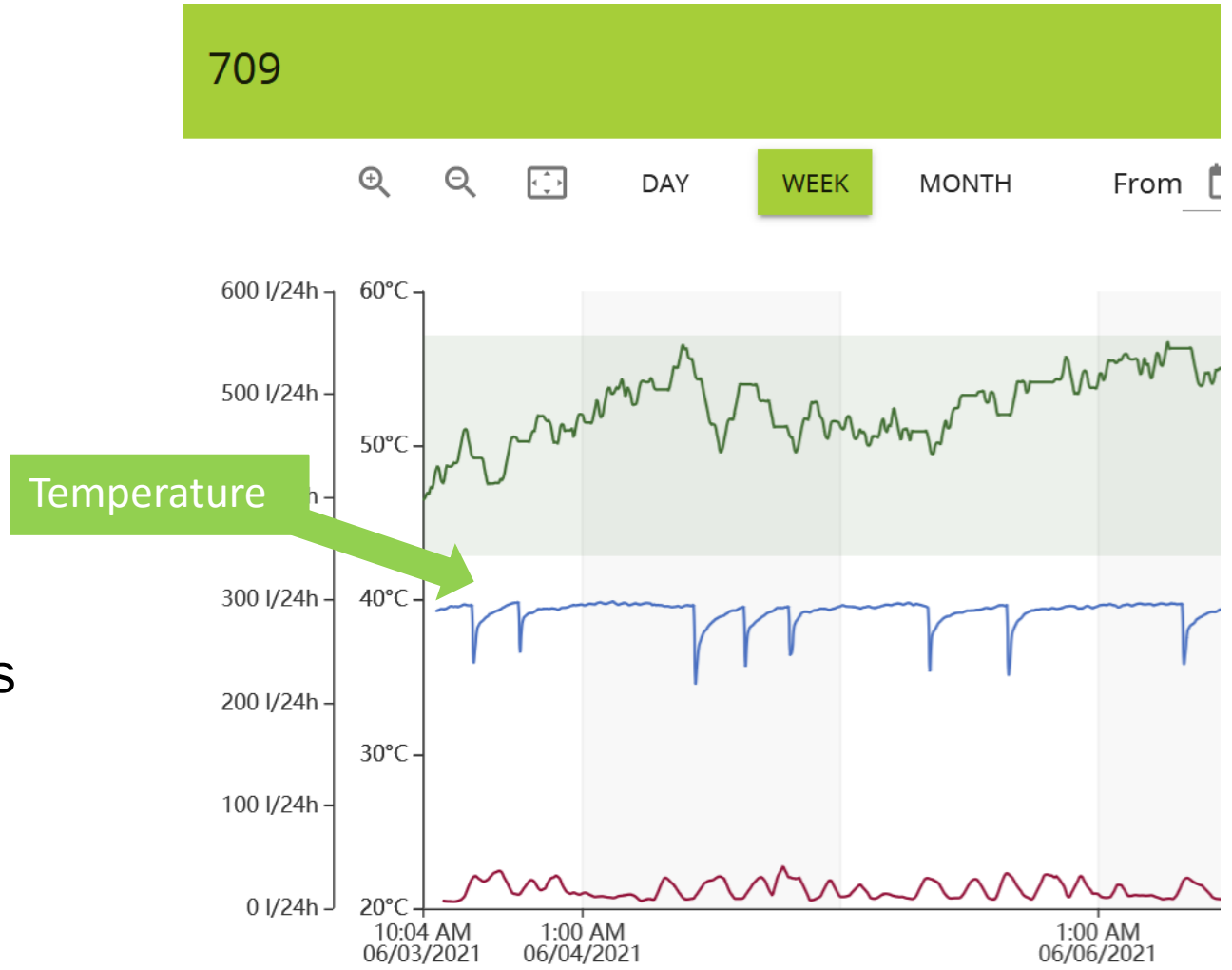
- Most devices have temperature sensors – usually thermistors.
- Temperature variation in the reticulorumen provides good indications of drinking behaviour and reflects core body temperature.
- Although there may be potential for incorporation of temperature data in diagnosis of reticuloruminal acidosis, it is not covered in this presentation.



Alternative technologies

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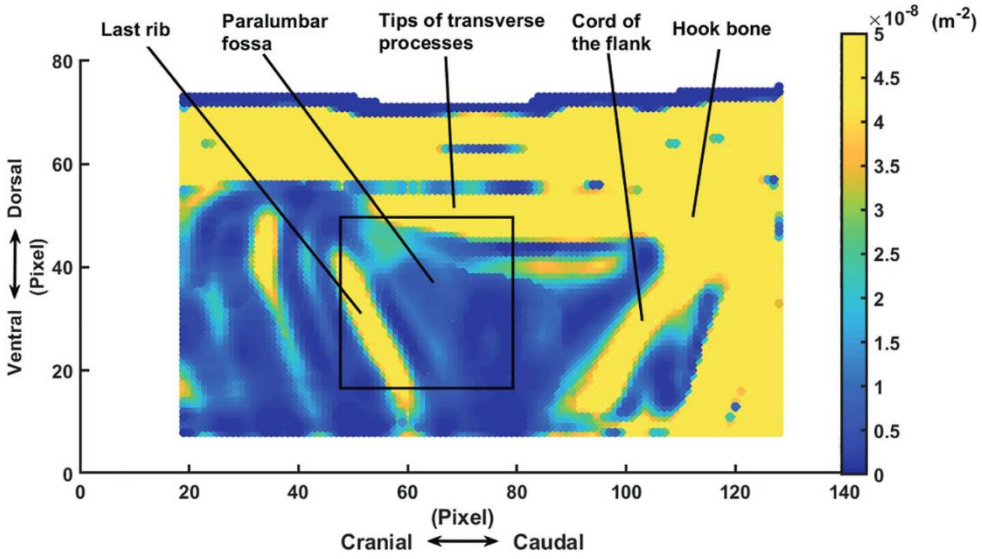
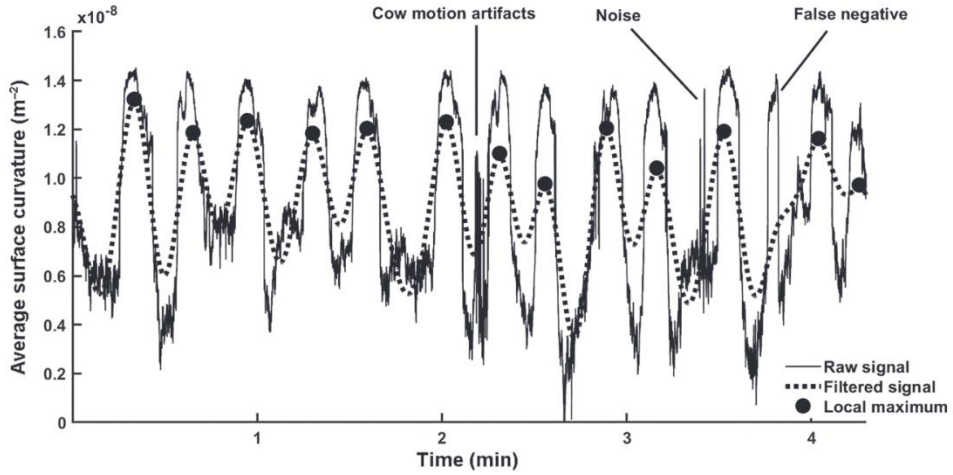
- **Biomarker detection** – reticuloruminal devices that can measure biomarkers associated with acidosis are technically feasible. Han et al (2022) list over 30 electrochemical histamine detectors (quantification of histamine is common with seafoods).
- **Machine vision** – has been demonstrated for detection of ruminal contractions (eg right) – could be incorporated in robotic milking systems. Song et al., Hot topic: Automated assessment of reticulo-ruminal motility in dairy cows using 3-dimensional vision, Journal of Dairy Science,102, 2019, 9076-9081, https://doi.org/10.3168/jds.2019-16550





Integrated early warning/diagnosis

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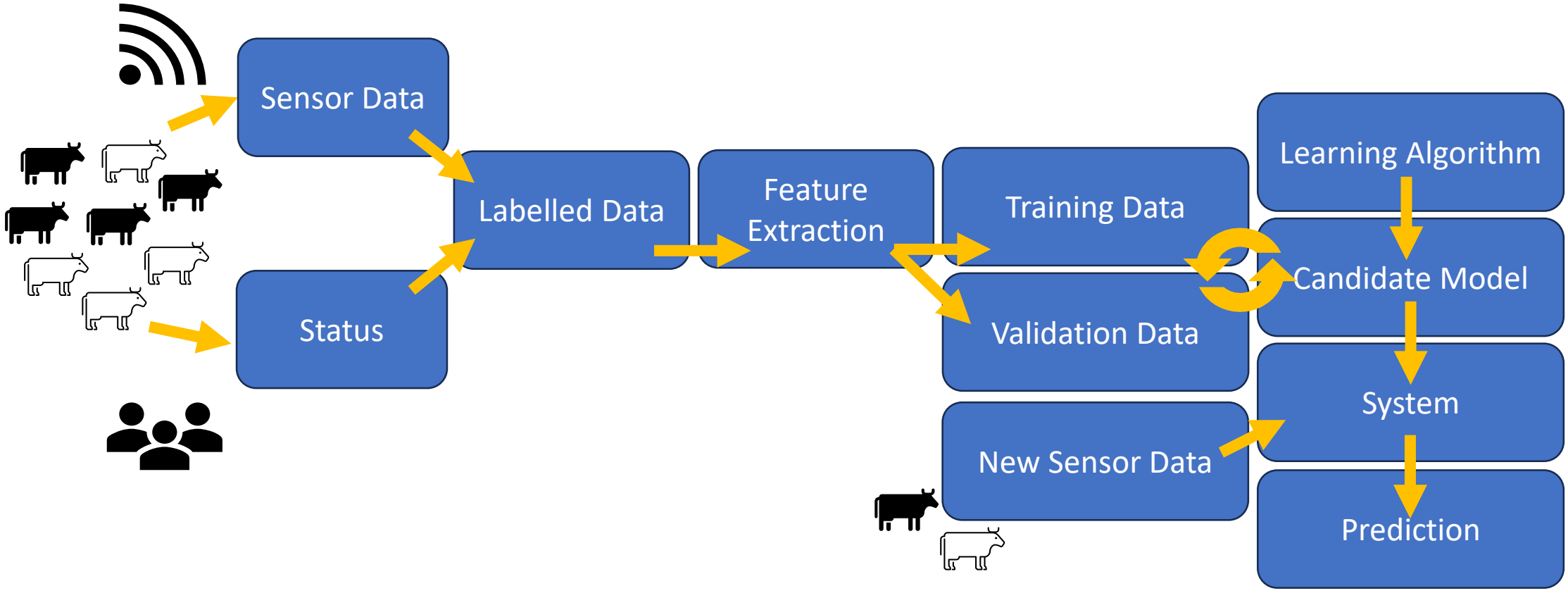
The integration of motility sensors, temperature, with in-line milk measurements holds considerably more promise for disease diagnosis and treatment than pH boluses alone. With any technology in this space, false alarms will be a challenge, particularly early in development.



Thanks to collaborators

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Thanks for your attention

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