Review: Enhancing gut health in dairy cows.

*Animal*

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**Supplementary Material S1: Herd diagnosis of acidosis**

*For acute acidosis:* Ruminal distension, diarrhea (often with grain in the feces and a sickly, sweet smell), abdominal pain, tachycardia, tachypnea, staggering, recumbency, coma, a marked decline in milk yield, and death may occur. The key considerations in the diagnosis of acute cases of acidosis are the history of:

1. *Access to sugars*: Sources of sugars include forage beets, turnips, cereal hays that are frost affected (e.g. oats, wheats), sugar, fruit and molasses.
2. *Access to rapidly fermentable starch:* Grains have the following order of risk of acidosis: wheat, triticale, barley, oats, maize, and sorghum. Concentrates differ in rates of fermentation due to the following:

* Chemical composition (higher rates of fermentation for sugars and starches – see above)
* Level of processing (finer flake, smaller particle size – more rapid)
* The physical structure of starch is also important – maize starch is less available for example than wheat starch
* Non-starch polysaccharides influence the availability of starches – grain sorghum or milo is less rapidly available
* The extent of ruminal fermentation. This is also influenced by rumen outflow rates – the higher the rate of outflow, the less that is fermented.

1. *Adaptation:* The less adapted to the substrate, the greater the risk to the cows. Most acute cases result from unlimited access to rapidly fermentable substrate and are usually obvious, e.g. beef cattle breaking into grain storage facilities.

*For Subacute Acidosis:* While access to fermentable feeds is important to the diagnosis of subacute cases, the focus must be on the herd examination, as clinical signs of acidosis can be relatively subtle in the individual animal.

Steps to evaluate the risk include chemical analysis of individual feed components and residual total mixed rations (TMR) after feeding to obtain the percentage of dry matter, NDF, acid detergent fibre (ADF), crude protein (CP), starch, sugar, and non-structural carbohydrates (NSC) content. This will allow estimation of the overall chemical composition of diet and for comparison with recommended requirements. This information should be combined with an evaluation of the physical characteristics of the feed and production system to identify sub-optimal rumen function and ruminal acidosis. Herd and cow examinations need to include:

1. *Feeding behaviour:* Feeding behaviour of the herd including the following should be observed: percentage of cows cud chewing at rest should exceed 50%, sorting behaviour of a TMR, and DMI and whether cows are allowed to go straight to pasture after milking or are held to provide even access. Cows that have a low rumination time, are sorting their feed, have a cyclic feeding pattern, or low DMI may be at risk of ruminal acidosis. Cows that are low in the social order, which are frequently first lactation cows, often eat last and therefore can be exposed to feed with a different effective fibre content or chemical composition resulting from sorting from the previous cows and may increase their risk of ruminal acidosis. The animal’s increased risk of ruminal acidosis will be dictated by what they sort for: concentrate (increased risk) or forage (typically decreased risk). All feed sources should be assessed for forage or chop length or particle size if applicable, and quality using relevant characteristics, i.e. stage of maturity of pasture, type of pasture or forage.
2. *Physical examination:* Cattle should be checked for acidosis between 2 to 4 hours after feeding in the milk parlour or after receiving a partial or total mixed ration. These can be checked for ruminal pH – stomach tubing cattle is a quick and easy method for checking rumen contents. If more than 4 out of 10 cows have a low pH <6.5 on stomach tube or <6.0 on rumenocentesis, then, because these findings are present with other signs of acidosis, it is worth undertaking preventive steps to control acidosis. Within a herd, groups of cattle may be diagnosed with different ruminal conditions. The sensitivity and specificity of using rumen pH values as a predictor of acidosis from rumen fluid collected using a stomach tube is 0.68 and 0.84, respectively, and from rumenocentesis is 0.74 and 0.79, respectively.

Many studies have used rumenocentesis to assess the pH in the ventral ruminal sack. The procedure itself is valued differently among researchers. Most studies render the technique both safe for the animal and suitable for field diagnosis (Kleen *et al.*, 2004; Tajik *et al.*, 2011; Duffield *et al.*, 2004; Atkinson, 2014). While these papers report the clinical outcome of tested animals, Gianesella et al., (2010b) controlled several pathophysiological parameters and found no significant alterations in cows that received rumenocentesis. Strabel *et al.*, (2007) performed necropsies on animals one week after rumenocentesis and found some pathological alterations. The authors therefore disapproved of the method on animal welfare grounds. The other way to directly assess ruminal pH is to extract ruminal fluid by ways of probes, a technique which is also valued differently: While it appears to be less accurate than rumenocentesis as shown by Duffield *et al.*, (2004), Steiner *et al.*, (2015) showed the reliability of stomach tubes in a very elaborate study. It has to be concluded that both techniques, rumenocentesis and the use of stomach tubes, will deliver sufficient information on ruminal pH. The choice will largely depend on animal welfare legislation and personal preference.

Indwelling rumen sensors have also become available allowing dynamic measurements of pH, being a feasible tool to monitor acidification of the rumen in real time. Yet, it has become evident that indwelling sensors stay in the reticulum. This technique has been developed over the last 10 years into commercially available systems (Gasteiner *et al*., 2012; Sato *et al.*, 2012; AlZahal *et al.*, 2011; Mottram, 2015a). It was soon noted that the systems create a huge amount of data that is difficult to read, that drifts in electrode stability occur, and that battery life may be an issue. Even if the data delivered by these systems may need careful interpretation as they are not exactly mirroring the ruminal pH (Neubauer *et al.*, 2017), two trends to achieve analysis of this data are discernible: First, the course of ruminal pH data can be linked to recorded events in the life cycle of the cow or farm management, as presented by Mottram (2015b). The results clearly show the need to take external events into consideration when interpreting ruminal pH and health of the GIT in general. Another option is presented by Denwood *et al.* (2017). Here, ruminal pH data is aggregated into sine-curves. The results indicate that the course of ruminal pH is not only affected by events, but largely follows a daily, individual pattern. Any alteration from this natural pattern has therefore to be valued as abnormal and can be shown to result in change of production.

Cattle with rumen perturbations consistent with subacute acidosis may present with a range of clinical and subclinical signs that include; diarrhea, poor body condition, a dull and lethargic demeanor, dehydration, a lack of rumen fill, lameness, weak rumen contractions, depression in milk fat, and inappetence.

The herd should also be examined for the following:

1. *Dung check:* If a high percentage of cattle are scouring, especially if the dung bubbles and contains grain – the risk of acidosis is high. The dung can contain undigested fiber, particles greater than 1.5 cm. Differential diagnoses include very lush grass and parasites.
2. *Lameness check:* Only swelling of the coronary band occurs at the same time as ruminal acidosis, but herds that have had acidosis causing other typical foot problems associated with acidosis often have active acidosis, especially if there has been no effort to control it. Changes observed in hooves such as ‘poverty lines’ and paint brush haemorrhage indicate acidosis, but the acidotic episode occurred perhaps months before examination.
3. *Check the bulk vat/tank:* Milk fat to protein ratios less 1.02 to 1 for cows in the first 100 days in milk provide a weak, but useful, indication of acidosis. The test is not sensitive, that is not all cows with a low test are likely to have acidosis, but cows with acidosis are very likely to have low fat test. The sensitivity and specificity for using a fat:protein ratio as a predictor of acidosis is 0.54 and 0.81, respectively. Unsaturated fatty acids have also been implicated in milk fat depression without relationship to ruminal acidosis.
4. *History:* If cattle have bled from the mouth (or nose) or a high prevalence of liver abscesses are reported, these indicate that it is likely the herd has had acidosis. Some acidotic herds have a history of increased respiratory disease, but this finding is not specific, as there are many other causes of respiratory disease.

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| **Supplementary material S2.** Summary of studies on induction of subacute ruminal acidosis (SARA) and excessive grain feeding on rumen LPS, inflammation and milk fat | | | | | | | |
| Study | Intervention | Digesta pH | Rumen LPS | Blood plasma LPS | Cytokines | Acute phase proteins | Milk fat |
| Gozho et al., 2007. 4 lactating dairy cows | Control: TMR, 31.1 % DM NDF, 37.2 % DM NFC). SARA: 75 % control TMR and 25% wheat barley pellets (17.6 % DM NDF, 62.2 % DM NFC). fed separately for 5 d | Average dairy pH reduced from 6.24 to 6.01 (P < 0.001). Duration < pH 5.6 increased from 187 to 309 min/d (P < 0.001) | Increased from 24,547 to 128,824 EU/mL (P < 0.001) | ND | ND | SAA from 287 to 498 mg/L (P = 0.03). HP no effect. LBP not determined. | No treatment effect |
| Emmanuel et al., 2008. 8 lactating primiparous Holstein cows | Diets with: 0, 15%, 30%, and 45% barley grain (35.4, 38.8, 42.1 and 45.5 % DM NFC). Fed for 21 d, with 11 d of adaptation and 10 d of measurements | Rumen pH lower in 45% barley treatments than in 0% barley treatment. Rumen pH values higher than in other studies | 30 and 45% barley treatments higher (P < 0.01) rumen LPS (50,021 and 80,870 EU/mL) than groups fed 0 or 15% barley  (6540 and 79,000 EU/mL) | ND | ND | 30 and 45% treatments higher (P < 0.001) SAA (21, 825 and 32,782 ng/mL) than 0 and 15% treatments  (9,255 and 6,886 ng/mL).  No treatment effect on HP. LBP higher in 45% treatment (10,056 ng/mL) than in other treatments (5,627 ng/mL average | ND |
| Khafipour et al., 2009a. 8 lactating dairy cows | Control: TMR, 35.7 % DM NDF, 32.7 % DM NFC, 26.1 % DM starch). SARA: 75 % control TMR and 21% wheat barley pellets (30.4 % DM NDF, 40.4 % DM NFC, 33.4 % DM starch) fed together for 7 d | Average dairy pH reduced from 6.17 to 5.97. Duration < pH 5.6 increased from 118 to 279 min/d | Increased from 28,184 to 107,152 EU/mL (P < 0.005) | From <0.05 to 0.52 EU/mL (P < 0.001) | ND | SAA from 167 to 439 mg/L (P =0.01)  HP from 0 to 476 mg/L (P < 0.001)  LPB from 18.2 to 53.1 mg/L (P < 0.05) | Reduced (P < 0.01) from 3.30 to 2.93% |
| Khafipour et al., 2009b. 8 lactating dairy cows | Control: TMR, 36.3 % DM NDF, 33.5 % DM NFC, 21.7 % DM starch). SARA: 76 % control TMR and 24% pellets of ground alfalfa 35.2 % DM NDF, 33.4 % DM NFC, 21.7 % DM starch). for 7 d, fed together for 7 d. Gradual step-up pellet content | Average dairy pH reduced P<0.01) from 6.35 to 5.78. Duration < pH 5.6 (P<0.01) increased from 112 to 558 min/d | Increased from 42,122 to 145,593 EU/mL (P < 0.001) | Both undetectable | ND | SAA from 23.1 to 6.9 mg/L (P < 0.05)  HP from 56 to 12 mg/L (P<0.001)  LPB from 7.2 to 2.6 mg/L (P = 0.09) | Reduced (P < 0.05) from 3.22 to 2.32% |
| Dionissopoulos et al. (2012). 2 groups of 8 rumen- lactating Holstein dairy cattle | Adapted to high forage diet for 5 wk. Afterwards high forage diet (HF, 46.9% DM NDF, 27.9% DM NFC, 7.4% DM starch) or high concentrate diet (HC 32.3% DM NDF, 46.0% DM NFC, 27.9% DM starch) for 3 wk. | Time below pH 5.6 (P <0.05)  HF: 3 min/d  HC: 594 min/d | Rumen LPS (P < 0.05, EU/mL)  HF: 5771 EU/mL  HC: 28,851 EU/mL | ND | ND | LBP increased in morning sample only (P<0.05)  HF: 11.1 ug/mL  HC: 15.4 ug/mL | Milk fat (P < 0.05)  HF: 3.94%  HG: 3.41% |
| Li et al., 2012a. 6 non-lactating dairy cows | Control TMR 35.6 % DM NDF, 34.8 % DM NFC, 14.2 % DM starch). Grain-based SARA challenge (GBSC, 22.9 % DM NDF, 50.4 % DM NFC, 33.7 % DM starch): 70 % control TMR, 30% wheat barley pellets fed together for 7 d  Alfalfa-pellet based SARA challenge (APSC, 34.5 % DM NDF, 35.7 % DM NFC, 33.7 % DM starch): 15.9 % control TMR): 63 % control TMR, 37% pellets of ground alfalfa fed together for 7 d | Average daily pH (P< 0.001)  Control: 6.30  GBSC: 5.98  APSC:5.99  Duration < pH 5.6(P<0.001)  Control: 56.4 min/d  GBSC: 298.7 min/d  APSC: 255.3 min/d  Cecal pH (P = 0.05)  Control:7.07  GBSC: 6.79  APSC:6.86 | Rumen LPS (P < 0.01)  Control: 10,405, EU/mL  GBSC:168.391 EU/mL  APSC:30.715 EU/mL  Cecal LPS (P < 0.001):  Control: 16,508 EU/mL  GBSC: 118,522 EU/mL  APSC: 14,458 EU/mL | All treatments undetectable (<0.05 EU/mL) | ND | LBP (P = 0.05)  Control: 8.9 mg/L GBSC: 12.1 mg/L  APSC: 9.5 mg/L | ND |
| Li et al., 2012b, 8 lactating dairy cows | Control: TMR 30.1 % DM NDF, 35.7 % DM NFC). Grain-based SARA challenge (GBSC, 27.2 % DM NDF, 40.6 % DM NFC): 88 % control TMR, 12% wheat barley pellets, fed together for 14 d  Alfalfa-pellet based SARA challenge (APSC, 31.5 % DM NDF, 36.5 % DM NFC): 74 % control TMR, 26% pellets of ground alfalfa fed together for 14 d | Rumen:  Average daily pH (P = 0.02  Control: 6.1  GBSC: 5.8  APSC:5.9  Duration < pH 5.6 (P = 0.02)  Control: 104 min/d  GBSC: 360 min/d  APSC: 278 min/d  Fecal pH no treatment differences. Average 6.63 | Rumen LPS (P < 0.01)  Control: 6,975 EU/mL  GBSC: 19,208 EU/mL  APSC: 15,518 EU/mL  Feces LPS (P < 0.01):  Control: 18,858 EU/mL  GBSC: 50,267 EU/mL  APSC: 21,112 EU/mL | All treatments undetectable (< 0.05 EU/mL) | ND | SAA (P = 0.05):  Control: 16.9 mg/L GBSC: 30.8 mg/L  APSC: 22.2 mg/L  HP:  No treatment differences  Control: 235.1 mg/L GBSC: 264.7 mg/L  APSC: 251.4 mg/L  LBP:  No treatment differences  Control: 20.9 mg/L GBSC: 31.4 mg/L  APSC: 21.8mg/L | Milk fat (P = 0.05)  Control: 3.34% mg/L GBSC: 3.04 %  APSC: 3.22% |
| Danscher et al, 2015. 6 Danish Holstein cows | Control: TMR (43.8 % DM NDF, 26.1 % DM NFC, 19.6 % DM starch). Grain-based SARA challenge (GBSC, (43.8 % DM NDF, 26.1 % DM NFC, 31.8 % DM starch)): 60 % control TMR, 40% wheat barley pellets, fed together for 7 d | Average daily (P = 0.01)  Control: 6.31  GBSC: 6.06  Duration < pH 5.6 (P = 0.02)  Control: 11 min/d  GBSC: 295 min/d  Feces pH (P < 0.001):  Control: 6.49  SARA: 6.04 | ND | ND | IL-6 not affected by treatment, averaged 5.06 ng/mL across treatments | SAA (P < 0.05)  Control: 4.24 mg/L;  SARA11.60 mg/L;  HP (P < 0.05)  Control: 3.57 mg/L;  SARA:22.1 mg/L;  LBP (P = 0.10)  Control 7.54 mg/L  SARA: 10.23 mg/L | Milk fat (P = 0.06)  Control: 5.08%  SARA: 4.14% |
| Li et al., 2016. 8 lactating cows | Control TMR (37.2 % DM NDF, 36.1 % DM NFC, 15.3% DM starch). Grain-based SARA challenge (GBSC, (25.9 % DM NDF, 45.0 % DM NFC, 22.2% DM starch): 79.2 % control TMR, 20.8%wheat barley pellets, fed together for 7 d | Average daily  Control: 6.32  GBSC: 5.93  Duration < pH 5.6  Control: 10.9 min/d  GBSC: 331.6 min/d  Cecal pH: no Cecum treatment differences, average 6.90 | Rumen  Control:17,365 EU/mL  SARA: 146,886 EU/mL  Cecum:  Control: 43,016 EU/mL  SARA: 99,969 EU/mL | Control:0.50 EU/mL  SARA: 1. 58EU/mL | IL-6  Control:4.68 pg/mL EU/mL  SARA: 2.18 pg/mL  TNF-α, no treatment effect, 0.06 ng/mL across treatments | SAA:  Control: 61.5 mg/L  SARA 215.5 mg/L  HP:  Control: 71.1 mg/L  SARA: 200.3 mg/L  LBP:  Control: 8.1 mg/L  SARA: 11.8 mg/L | Milk fat (P < 0.01)  Control: 3.25%  GBSC: 2.82 % |
| Bilal et al., 2016. 12 groups of 6 Holstein cows | Low concentrate (LC, 37.7 % DM NDF, 33.4 % DM NFC, 25.3 % DM starch) or high concentrate (HC, 31.9% DM NDF, 40.3 % DM NFC, 32.2 % DM starch) for 18 wk | HC significantly higher and more than 180 min/d time < pH 5.6 than  LC | Rumen (P <0.01)  LC: 47,170 EU/mL  HC: 79,040 EU/mL | Jugular blood plasma (P < 0.01)  LC: 470 EU/mL  HC: 860 EU/mL | IL-1β,  TNF-α and IL-6 all moderately, but significantly (P < 0.01) higher in HC than LC | ND | ND |
| Chang et al, 2015. 2 groups of 6 multiparous lactating goats. | Low concentrate (LC, 36.6 %DM NDF, 31.8 % DM NFC) or high concentrate (HC, 34.6% DM NDF, 35.0 % DM NFC) for 8 wk | Duration rumen pH < 5.6  LC: 0 min/d  HC: >240 min/d | Rumen (P <0.01)  LC: 34,700 EU/mL  HC: 60,600 EU/mL | Portal blood plasma (P < 0.01)  LC: 0.65 EU/mL  HC: 1.14 EU/mL | Portal blood plasma  IL-1B (P < 0.01)  LC: 0.27 ng/mL  HC: 1.87 ng/mL  IL-6 (P = 0.05)  LC: 71.23 pg/mL  HC: 224.60 pg/mL  TNF-α (P <0.01)  LC: 16.4 fmol/mL  HC: 147.0 fmol/mL | mRNA expression of SAA, Hp and LBP significantly increased in liver in HC group compared to LC group (P < 0.05) | Milk fat (P = 0.04)  LC: 3.36 %  HC: 2.93 % |
| Chang et al, 2015. 12 goats, 6 per treatment | Low grain diet (LG，36.6% DM NDF) or high grain diet (HG, 27.7% DM NDF) for 6 weeks | ND | ND | Hepatic blood plasma (p = 0.001) LG: 0.40 EU/mL, HG: 1.11 EU/mL  Portal blood plasma (P < 0.001) LG: 0.50 EU/mL, HG: 1.28 EU/mL  Artery blood plasma (P < 0.001) LG: 0.39 EU/mL, HG: 1.07 EU/mL | IL-1β (P < 0.001) LG: 0.06 ng/mL, HG: 0.17 ng/mL  TNF-α (P < 0.001) LG: 15.43 fmol/mL, HG: 59.32 fmol/mL  IL-6 (P = 0.097) LG: 110.38 pg/mL, HG: 139.68 pg/mL | SAA (P = 0.02) LG: 66.18 ug/mL, HG: 353.37 ug/mL  Hp (P = 0.02) LG: 125.20 ug/mL, HG: 341.21 ug/mL  LBP (P < 0.001) LG: 14.78 ug/mL, HG: 40.82 ug/mL | ND |
| Guo et al., 2017, 12 cows, 6 per treatment | Low concentrate diet (LC, 36.5 % DM NDF, 33.8% DM NFC) or high concentrate diet (HC, 31.5 % DM NDF, 39.3 % DM NFC) for 8 wk | Duration rumen pH < 5.6 (P <0.001)  LC: 0 min/d  HC: >180 min/d | ND | Portal blood plasma (P < 0.001): HC > LC  Hepatic blood plasma (P < 0.001):  HC > LC | IL-1B, IL-6, and TNF-α: HC>LC (moderate, but P < 0.05) | mRNA expression of SAA, but not that of Hp and LBP, increased in liver in HC group compared to LC group | ND |
| Qumar et al., 2017. Dairy cows. 8 Cont, 8 INT | Forage-only diet (control, 57% DM NFC) or 60% grain diet for (SARA, 31.8 %DM NDF, 45.2 % DM NFC) 4 wk, continuously (CONT) or with a 1-wk break (INT) | Before SARA:  Average daily pH: 6.38, duration pH<5.5 21.1 min/d  Day 13-19 (SARA)  Average daily pH: CONT: 6.06  INT: 6.06  duration pH<5.5  CONT: 111.7 min/d  INT: 121.3 min/d  Day 27-40 (SARA)  Average daily pH: CONT: 5.93  INT: 6.15  duration pH<5.5  CONT:208.1 min/d  INT: 106.9 min/d | In rumen fluid, grain feeding increase (P < 0,01) from 2369 to 13184 EU/mL  In feces: increase (P < 0,01) from 15677 to 118893 EU/mL.   |  |  | | --- | --- | |  |  | |  |  | |  | ND | SAA and HP not affected by grain feeding, on average 11.1 and 6.1 ug/mL, respectively. | ND |
| 1ND = not determined. APSC = alfalfa pellet SARA challenge, GBSC = grain-based SARA challenge, HC = high concentrate, Hp = haptoglobin, LBP = LPS binding protein, LC = low concentrate, LPS = lipopolysaccharide, NFC = non-fibre carbohydrates, SAA = serum amyloid A, SARA = subacute ruminal acidosis, TMR= total mixed ration | | | | | | | |

**Supplementary material S3.** Dietary or management factors that influence the risk of acidosis

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| Factors that improve rumen stability | Factors that produce rumen instability |
| Fiber | Shorter chop lengths of fiber, sorting of feed that allows avoidance of fiber, lush pastures, poor forages (contaminated or unpalatable) that are avoided by cattle. |
| Constant feed access | Irregular feeding leading to ‘slick bunks’, or allowing differential access to grain in the milking parlor, or accidental additional grain intake |
| Rapidly Fermentable substrate | Sugars cause more instability than starches |
| Protein, amino acids, peptides and nitrogen | Supply of these can provide substrate for microbial protein production. However, the provision of RDP increases supply of VFA and lactate |
| Even feed access | Overstocking (whether feedlots, dairy bunks or pasture), allowing dominant cows more access to forage or concentrates |
| Consistent feed processing | Mixing errors with TMR, over-processing TMR, variable processing of grain |
| Grain species and cultivar | The risk varies between and within grains and other concentrates |
| Grain processing | The greater the degree of processing, the greater the risk, however, this can be moderated by rapid rumen outflow. Type of processing, e.g. steam flaking, dry rolling, tempering will influence the availability of substrate |
| RDP = rumen degradable protein, TMR = total mixed ration, VFA = volatile fatty acids | |

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