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MONITORING OF RUMINAL pH IN PERIPARTAL PERIOD IN DAIRY COW

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INTRODUCTION

The measure of the ruminal pH is used since many decades in cattle research and breeding. It have been used for the investigation on ruminal physiology later for the development of nutrition studies (Monroe and Perkins, 1939). Modernization and diversity of experiments of research technics forged deeper knowledge on the microbial digestion and its adaption to variety of feeds. As a consequence we could improve cattle nutrition while optimizing physiological impact and allow the full genetic performance of an animal to accomplish.

Dairy cattle breeding try to maintain a breeding cycle with good reproductive performance, good transition of the peripartal cow with the least open days, high milking production and everything while keeping the best health status in the herd. All those parameters are interconnected as a good health management of the herd will guarantee the highest production level. Nutrition is the key element of intensive breeding condition. As we have no nutrition without digestion the focus of the digestive feature of cattle and means to monitor it are essential.

Dairy cow's most challenging moment of its carrier is the transition period (2-3 weeks before parturition to 4 weeks postpartum). Drastic metabolism, immune and endocrine changes create a difficult background for the cow and everything have to be done to overcome this challenge. Measurement of ruminal pH have been proven to be a reliable parameter to estimate cow health. No surprise that it have been the studies target of many research team for many years in order to understand its variation and define the range of application.

This work is trying to identify the origin of the variation of acidity in the rumen and to establish the link with rumen health status. We are going to draw a picture of the rumen state while measuring its pH. It is also the occasion to do a review of all technics that have been designed for ruminal pH measurement and to confront them to have an objective vision of their potential.

In a first part an effort will be made to set the landscape of the ruminant digestion and the specificity of transition period in regard of physiology, nutrition and health. The second part will retrace and do an inventory of technical means to monitor ruminal health.

I- THE RUMEN: PHYSIOLOGY AND ACIDITY

1.1. Bovine digestive anatomy and physiology

Well known for its specific type of digestion ruminants are even-toed ungulates animal that have the particularity of chewing the cud regurgitated from its rumen and have a four chambered stomach. Among ruminant cattle is the most studied and known.

1.1.1. Digestive physiology

Food particles in a cow digestive system have a long way out from its ingestion. Beginning by the mouth it is them propelled to the 4 chambered stomach via the oesophagus. Ruminant stomach is a huge cavity having different parts: the rumen, the reticulum, the omasum and the abomasum. A current will then either push back the food to the mouth for rumination or further aborally to the intestines. The exit of the stomach is linked to the small intestine with the duodenum, the ileum and jejunum. The caecum is a small bag-like structure making the link between small and large intestines. The Fig.1 show the general disposition of cow gastro-intestinal tract.

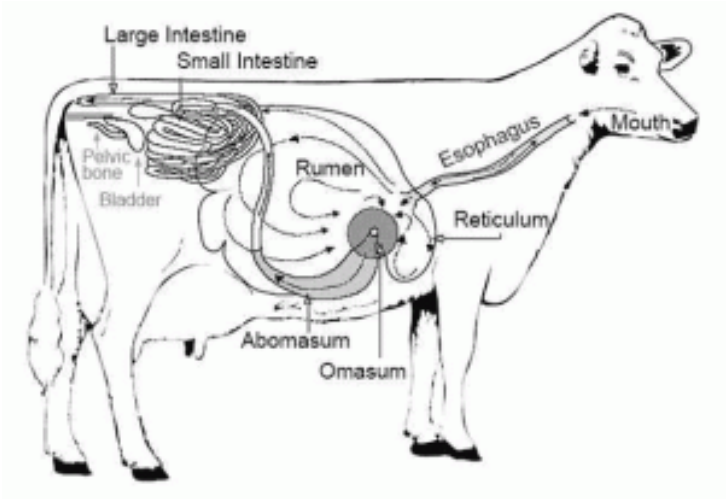


Figure 1: Structure of cattle digestive system (J.E Umphrey and C.R. Staples, 2012)

Rumen, reticulum and omasum are the 3 non glandular parts and the last part is the abomasum which is the glandular part. An overview is presented on the Fig.2 while Fig.3 helps in the topographical orientation.

The reticulum is the first stomach compartment. Its honeycomb structure act as a sieve and retain any hardware from falling down in the digestive tract. Feed that enters the reticulum is later regurgitated and remasticated as part of the cud. The reticulum can contain up to 7.5L of undigested feed and feed being digested (digesta).

The rumen is a large, hollow muscular organ. The rumen develops anatomically in size, structure, and microbial activity as the calf's diet is changed from liquid milk or replacer to dry feed or silages. In the mature ruminant, the rumen nearly fills the entire left side of the abdominal cavity. Ruminal papillae structure and size is intimately linked with the feeding. The "fiber effect" is a phenomenon that describe the influence on the proportion of NDF (Neutral Detergent Fiber) on the rumen wall stratification and absorption. It is a fermentation vat that can hold 150 to 227 L of material and is the site of microbial activity. Its environment is naturally maintained with a temperature range of 37.7 to 42.2^{o}C. If cows are fed a proper balance of forages and grain, the pH should range between 5.8 and 6.4, which allows the growth of many species of bacteria. It have an absorbing capacity through the bottom and side ruminal wall.

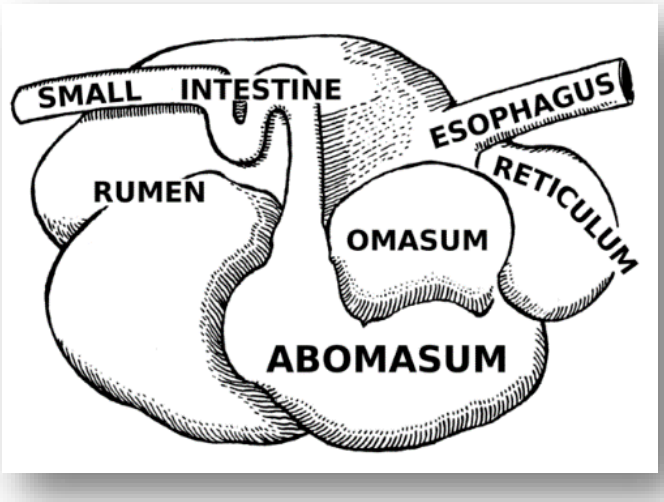


Figure 2: The 4-stomach of cattle / Right aspect (Pearson Scott Foresman, 2008)

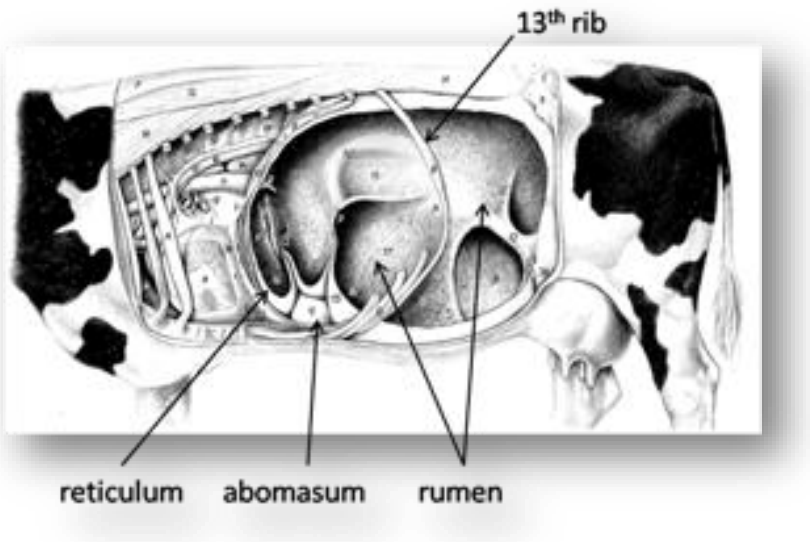


Figure 3: Left aspect of cattle and 4-chamber stomach anatomy (Nickel et al, 1973)

The omasum is sometimes referred to as the "wallet" because of its many layers of muscular flap. In the omasum, the particle size of digesta is reduced, and any excess water is removed before the digesta enters the abomasum. The omasum can contain up to 15L of digesta.

Turnover rate is an important parameter for the 4 chambers of the stomach and the differences are linked with their role. In the reticulorumen the passage time is the longest because the longer micro-organisms and feed particles are in contact the more efficient the fiber digestion is. It represent about 30H for a milking cow fed on hay (Hartnell and Satter, 1979) .In contrast the turnover rate in the abomasum is very short as only small particules transit and the digestion is enzymatic and chemical and quicker than microbial digestion.

1.1.2. The rumen cycle

The cow compartmented stomach could not be an efficient digester if the sacs would not be linked by a series of contraction that create movement of digesta and transfer it from on sac to the other. Here we will focus only on the reticuloruminal cycle.

The reticuloruminal motility is ensured with the rhythmic contraction of ruminal and reticular wall smooth muscle highly innervated with the vagus nerve and the enteric nervous system. We distinguish 3 types of activities:

- **Resting** which is the regular background activity that guarantee a continuous mixing of ingesta within the rumen. Old, recent ingesta and ruminal fluid will then be always mixed together.

- **Ruminating** which is a complex of regurgitation of food toward the mouth via the cardia and the esophagus, the remastication/reinsalivation and the redeglutition.
- **Eructation** which is a sequence of ruminal contraction to expulse fermentative gas out of the rumen through cardia, esophagus and mouth.

Two types of contraction are described: the primary and secondary contraction. The primary contraction that originate in the reticulum and spread around the rumen. Contraction wave followed by relaxation wave in a particular sequence allow some rumen part to empty while contracting and other to fill while relaxing. The secondary contraction occur in circumscribed part of the rumen only and are associated with eructation.

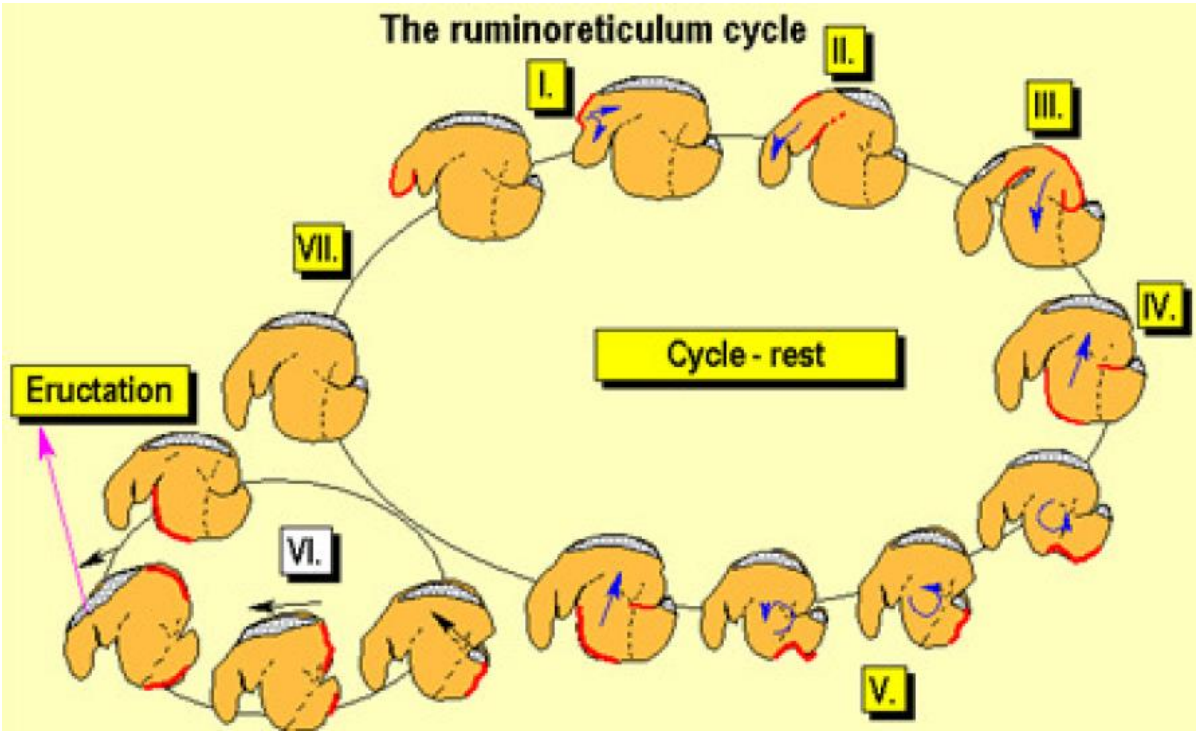


Figure 4: Reticulo-ruminal contraction cycle (Barta Tibor, 2011)

The series of contractions during reticulo-ruminal cycle is as follow: (cf Fig 4).

1. A first weak contraction is mixing reticular content while a second one stronger is evacuating the content out of the reticulum. The role of the reticulum contraction is multiple;
   - It forms a caudal flow of ruminal fluid from the reticulum to cranial ruminal sac,
   - The less dense material (not fermented enough) in the reticulum gets back to the dorsal ruminal sac.
   - It regulates the flow of content from the reticulum to the omasum as well. Its laminated structure helps retaining large particles.
   - It makes regurgitation possible by filling the cardia (dorsal to the reticulum) with reticulum content.
2. Cranial ruminal sac contracts with second reticular contraction. Content flow from cranial sac to caudal sac end in the terminal phase and it gets back toward the relaxed reticulum.
3. The Contraction of caudodorsal sac of the rumen. Pushes content in ventral direction mixing the ruminal content. Gas bubbles are pushed cranially with the help of the contraction of cranial and caudal pillar (fold that separate sacs).
4. The ventral and caudoventral blind sac contract mixing furthermore the ruminal content. A cranio-caudal followed by caudo-cranial contraction complete mixing.

With the last mixing contractions ventral ruminal sac content is frequently sent dorsally close to the reticulum and the cardia where further cycle will determines either this material will goes in the reticulum for further digestion or be regurgitated during rumination.

In case of rumination, the regurgitation is done by the two reticular contraction help by a massive third reticular contraction that propelled ingesta through relaxed cardia for remastication.

This sequential contraction is responsible for the continuous movement of digesta in the rumen. Ruminal cycle occurs 1 to 3 time per minute and the higher frequency is found during feed intake. We will discuss later the impact of this kinetic on intraruminal measurement technics (ruminal fluid composition…) and location and movement of intra-ruminal probes.

1.1.3. Ruminal Digestion and composition of the ruminal microbiota.

Micro-organisms present in the rumen are essential to the ruminant digestion digesting and modifying the ingesta leading to an environement where the cow will either absorb the necessary component according to the mucosa absorbing state or process further aborally in the tract and digest the rest with the abomasal, pancreatic secretion or bile and then absorb it. (Belbis, 2007). Protozoa, fungi and bacteria are the components of ruminal microbiota.

1.1.3.1. Protozoa

Protozoa organism are essentially ciliates from the subclass of the *Trichostomatiae.* Protozoa consume and ferment bacteria to VFA (Volatile Fatty Acid) and ammonia, sequester and ferment starch and recycle nitrogen. Their main products are **Acetate**, **Butyrate** and **dihydrogen** (H2). They hydrolyzes and absorb starch granules and soluble sugars. When feeding a rich carbohydrate diet protozoas will grows fast and store the amylopectine. This will hide nutrient to amylolytic baterias and hinder fast formation of lactic acid by bacterias. They also have numerous interaction with the other rumen inhabitant like the ingestion of endogenous or exogenous bacteria as protein sources for its cellular synthesis. This predation help to regulate the bacterial population leaving more nutrient available. The defaunation will for instance induce an increase of carbohydrate utilizing anaerobe bacteria. Protozoa population is pretty sensitive to pH and varies rapidly with meals. Their disappearance from the ruminal fluid can enhance the acidosis when feeding high amount of concentrate. The protozoal population behavior of protozoa depending on the pH is presented Table 1.

Table 1 : Influence of the pH on the protozoal population

Feeding	Ruminal pH	Protozoal population
Grazing	6.5-7	Many
Concentrate	6.0	Few
High concentrate	<5.5	None

Protozoa represent also a good source of **protein** and **amino acid** for the abomasal digestion.

1.1.3.2. Fungi

Fungi present in the rumen are essentially anaerobic fungi. We distinguish 3 species: *Neocallimastix communis, Pyromonas communis* and *Sphaeromonas communis* ensuring exclusively cellulose (fiber) digestion. They colonize lignified materials, reducing the size of particles via the action of extracellular enzymes. Even highly lignified fibers are degraded by fungi. They attach to particles having rhizoid penetrating through plant tissue and destroys it by proteolysis. Their major products are **VFA, dihydrogen** and **carbon dioxide**. They also produces other minor products as ethanol and lactate. Not always present, they are essential when feeding bad quality roughage. Fungi population is favored by forage rich diet and addition of concentrate can increase it but diet rich in soluble carbohydrate have deleterious effect on its proportion. (Sehgal and Singh, n.d. 2009). Their implication in the determination of ruminal pH is minor but the interaction with the rest of the ruminal flora can have some influences

1.1.3.3. Bacteria

The bacterial population is classified in 4 groups: free living bacteria associated to the ruminal fluid, bacteria associated to feed particles, bacteria associated to ruminal epithelium and bacteria attached to protozoa. We can distinguish them by their metabolism and role in feed digestion within the rumen according to the type of molecule they process.

Fiber digesting bacteria group cellulolytic and hemicellulolytic bacteria: Bacilli (Mostly *Fibrobacter succinogenes Butyrivibrio fibrosolvens, Prevotella ruminicola*-hemicellulose only-) and cocci (Mostly *Ruminococcus flavefaciens* and *Ruminococcus albus)*. Their endproducts and mostly **acetic acid**, **succinate, ethanol, formic acid, carbon dioxide** and **dihydrogen**. They are also digesting complex linear sugar in simpler sugar (Glucose, Cellobiose, Cellotriose) later utilized for their own metabolism. We should mention also pectinolytic bacteria (*Lachnospira multiparus*, *Butyrivibrio fibrosolvens* and *prevotella ruminicola*) that are producing **VFA**, **lactic acid, succinate** and simplifying complex sugar into smaller molecule later metabolized.

Amylolytic bacteria are starch degrading bacteria and is composed of cellulolytic bacteria (F. succinogenes, B. fibisolvens) and non-cellulolytic bacteria (*Streptococcus bovis, Ruminobacter amylophilus, Prevotella ruminicola, Succinimonas amylolytica* and *Selenomonas ruminantium*). Hydrolysis of starch is extracellular. Main products of starch and sugar degradation are **acetic acid, propionic acid, succinate** and **ethanol**. **Lactic acid** is also

produced and its production depend on ruminal pH and is a key feature of ruminal acidosis development.

More simple sugars are utilized by bacteria like *S. ruminatium, S. bovis, B. fibrisolvens* and some *Succinovibrio dextrinisolvens* and *ruminococcus*.

Some bacteria are specializing in acid fermentation. *Megasphaera elsdenii* is a lactic acid fermenter and produce **VFA, carbon dioxid** and **dihydrogen**. Its adaptation to ruminal environment is slow. *Selenomonas ruminantium* is fermenting succinate to produce **propionic acid** and **carbon dioxid**.

Urea is used by ureolytic bacteria that are importante in the metabolism of nitrogen converting urea in ammonia. This activity is see in *Succinivibrio dextrinosolvens, Selenomonas sp., Prevotella ruminicola, Ruminococcus bromii, Butyrivibrio sp.*and *Treponema sp*.

The digestion of proteins is done by various bacteria. Bacteroides, Ruminobacter amylophilus, Prevotella ruminicola and Butyrivibrio are important proteolytic bacteria and presence and activity depend on feeding. Other species like Selenomonas, Succinivibrio, Lachnospira, Eubacterium, Fusobacterium and Clostridium are also seen as protein utilizing bacteria.

Lipid utilizing bacteria are responsible for the lipolysis and the biohydrogenation of lipids of in the ingesta. We can mention amongst them *Butyrivibrio fibrosolvens, Treponema bryantti, Eubactirium sp., Fusicillus sp., Micrococcus sp.* and *Anaerovibrio lipolytica*.

Methanogenic bacteria are mostly utilizing CO2 and H2 produced in fermentation of various sugars and to form methane CH4. *Methanobrevibacter spp*. Is considered to be the major specie of methanogenic bacteria.

Bacteria mostly ferment fiber, starches and sugars to produce **VFA**, H2 and CO2 but also produce most of **microbial protein** and ferment feed proteins to **VFA** and **ammonia**.

A certain category of bacteria that are of particular interest due their role in the pathogenesis of acidosis are *Streptococcus bovis* and the *Lactobacillus ruminis* and *Lactobacillus vitulinus.* When fed easily fermentable carbohydrates they proliferate easily consuming highly fermentable carbohydrate and simple sugar produced by other bacteria. *S.bovis* grow slowly and produce VFA when fed with hay while it grows faster when feeding starch and produce lactic acid. S.bovis resist easily pH of 5.5-5. If pH drop further S.bovis

cannot grow whether Lactobacilli are resistant to highly acidic pH (they represent 90% of the total flora at pH 4-4.5).

The Rumen have a dynamic equilibrium between those inhabitants that create a physicochemical environment that will evolve according to the feeding. The pH is an interesting parameter to follows as it is the direct consequence of microbiota metabolism induce by the feed ingested. Now we have approach the origin of acid produced in the rumen we will see how the rumen tries to get rid of it.

1.1.4. Ruminal mucosa and its variability.

Rumen is specialized in fermentation of structural carbohydrates and have an essential role in the absorption of VFA. To fulfill its tasks the mucosa develop specialized characteristics. Papillae are protruding from the rumen wall and increase greatly the surface area for absorption of VFA. Papillae represent up to 80% of the total ruminal surface Wageningen 2012).

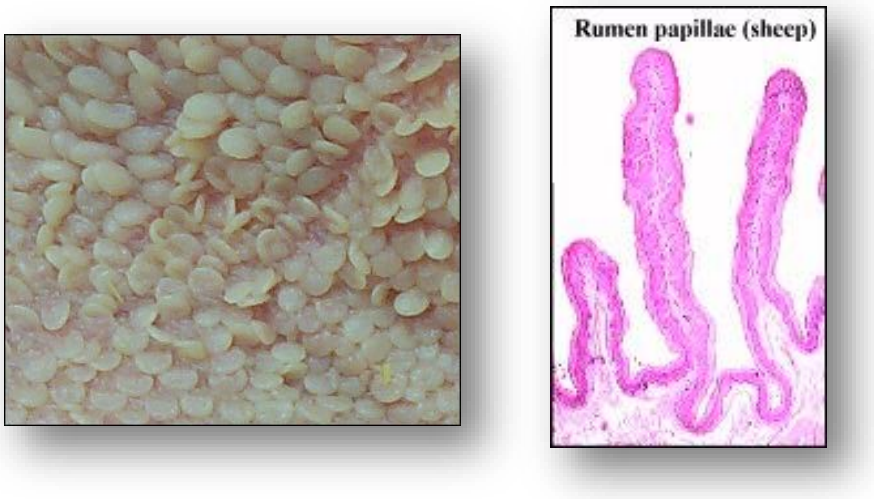


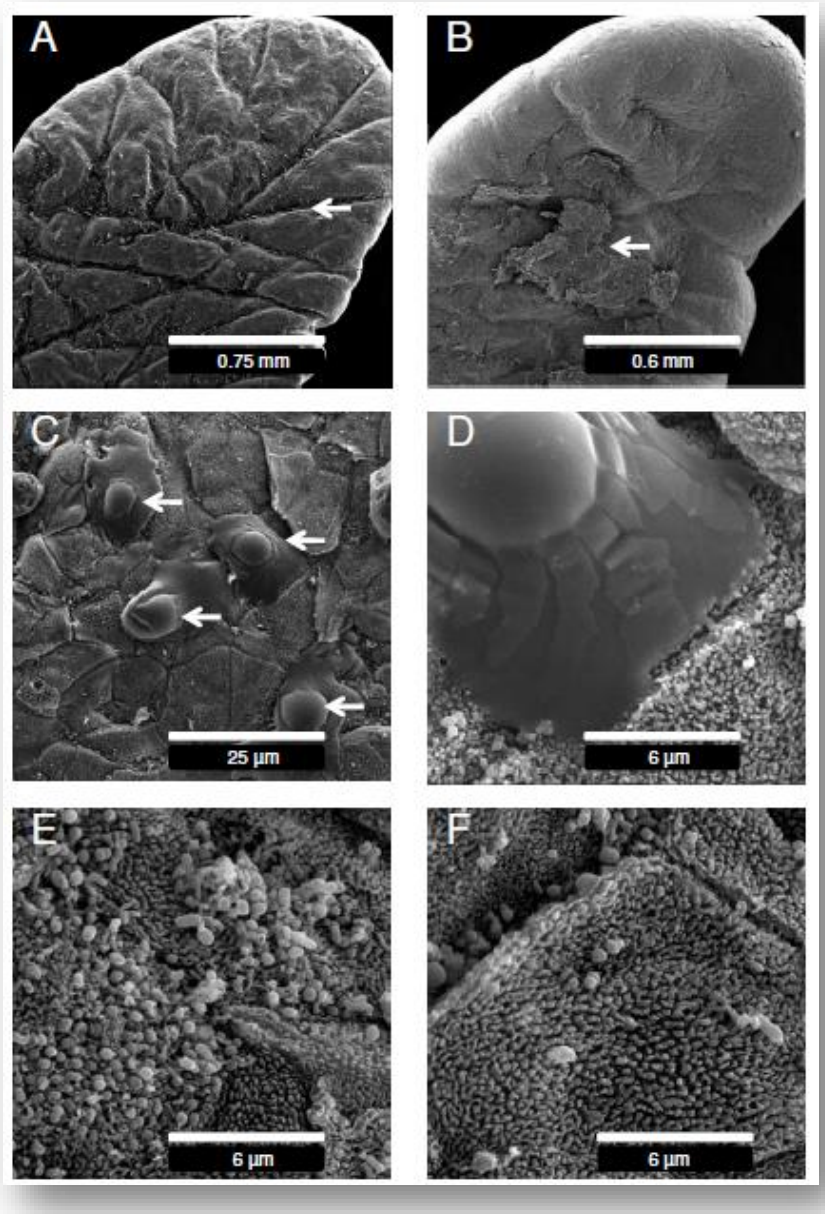
Figure 5: Ruminal mucosa and ruminal papillae (C. Khon, 2003) Macroscopical picture (left) and histological section (right) of Ruminal papillae.

Mucosa is composed of stratified squamous epithelium (SSE). It is composed of 4 distinct strata. The basal lamina with stratum basale and spinosum which is specialized in the metabolic work of ruminal SSE. The stratum granulosum adjacent to stratum spinosum have desmosome junctional complex that guarantee the permeability of the SSE. The most superficial stratum corneum is in direct contact with ruminal milieu with keratinocytes as physical protective barrier (Steele et al., 2011). Papillae play also an essential for microbial

population creating surface of attachment and enhancing the link between microbial fermentation and ruminal absorption.

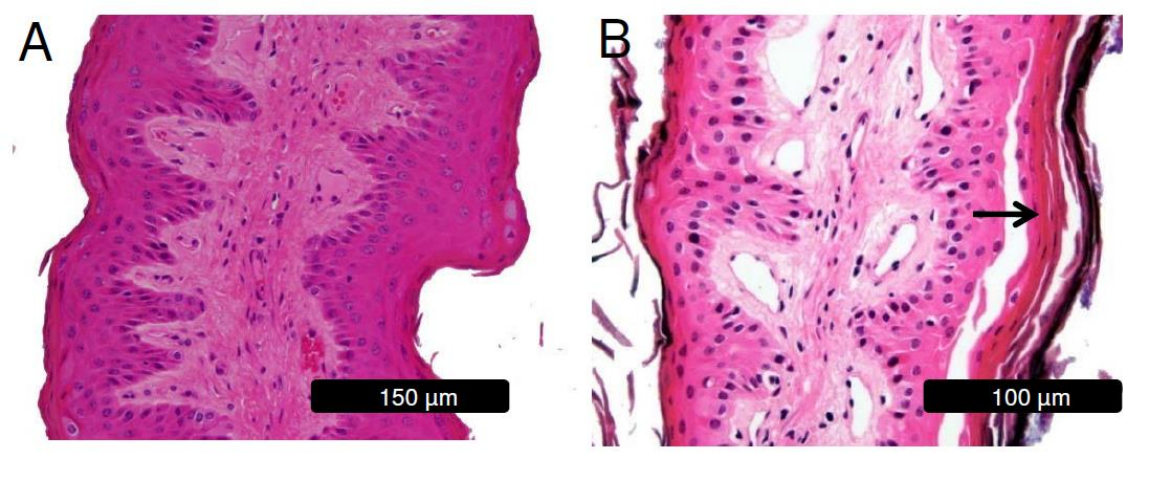
Adaptation of the ruminal mucosa to high level of dietary grain intake is a key physiological event in cattle. Papillae increases in size maximizing the absorption surface. In sheep and cattle higher VFA concentration in the rumen triggers cellular proliferation of SSE and its morphogenesis (Steele et al., 2009).

Integrity of ruminal wall is ensured by the SSE and guarantee the permeability of ruminal mucosa but when feed change occurs it can upset the mucosa causing erosion of SSE (sloughing of stratum corneum…) (cf fig. 6 and 7) enabling translocation of microbes and LPS into portal bloodstream and subsequently into systemic circulation. Deleterious consequences can be seen on the bacterial population on ruminal mucosa (Steele et al., 2009).



A: rumen papillae biopsied during the high forage diet displaying large crevices (arrow).
B: rumen papillae biopsied during the high grain diet with extensive sloughing of the stratum corneum (arrow).
C&D: surface morphology of epithelium layer below sloughed corneum during high grain diet revealing non-differentiated keratinocytes (arrows).
E: microbial colonization of the rumen epithelium during the high forage diet.
F: microbial colonization of the rumen epithelium during the high grain diet

Figure 6: Scanning electron micrographs of rumen papillae biopsied during the high forage and high grain diets (Steele et al 2009)



A: rumen papillae from the high forage diet with an intact stratum corneum and granulosum.
B: rumen papillae from the high grain diet displaying sloughing of the stratum corneum and demarcation of cells through the epithelial layers (arrow).

Figure 7: Light micrographs of rumen papillae biopsied during high forage and high grain diet (Steele et al 2009)

1.2. The pH and acidity: Origin and meaning

The pH variation in the rumen environment have great importance and its variation define from the mucosal structure to the bacterial composition. The majority of acidity which is produced in the rumen is coming from the microbial metabolism (VFA and lactic acid). Organic acid are produced by the ruminal microflora and the proportion of the different acids depend on feeding and the composition of the flora.

1.2.1. Acid equilibrium in the Rumen.

Acid in the rumen can be either utilized by micro-organism, neutralized by buffers or absorbed. Bacterial metabolism is the major source of acidification with the production of organic acids. Beauchemin et al (2006) did sum up this steady equilibrium with the Fig.8. Among those acids VFA (acetic, propionic and butyric acid) represent 95% of fermentation products (60-150mol/L).The other organic acid of great importance produced in quantity in the rumen is the lactic acid. Altogether they will definite the ruminal production of proton for its majority. An equilibrium is required in order to maintain a constant rumen environment.

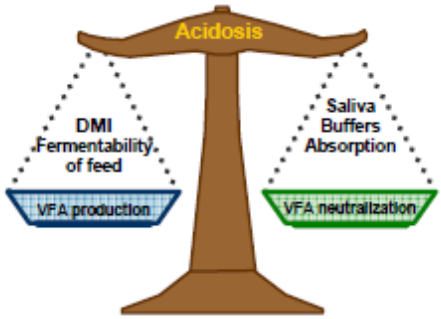


Figure 8: Acid equilibrium in the rumen **(BEAUCHEMIN ET AL., 2006)**

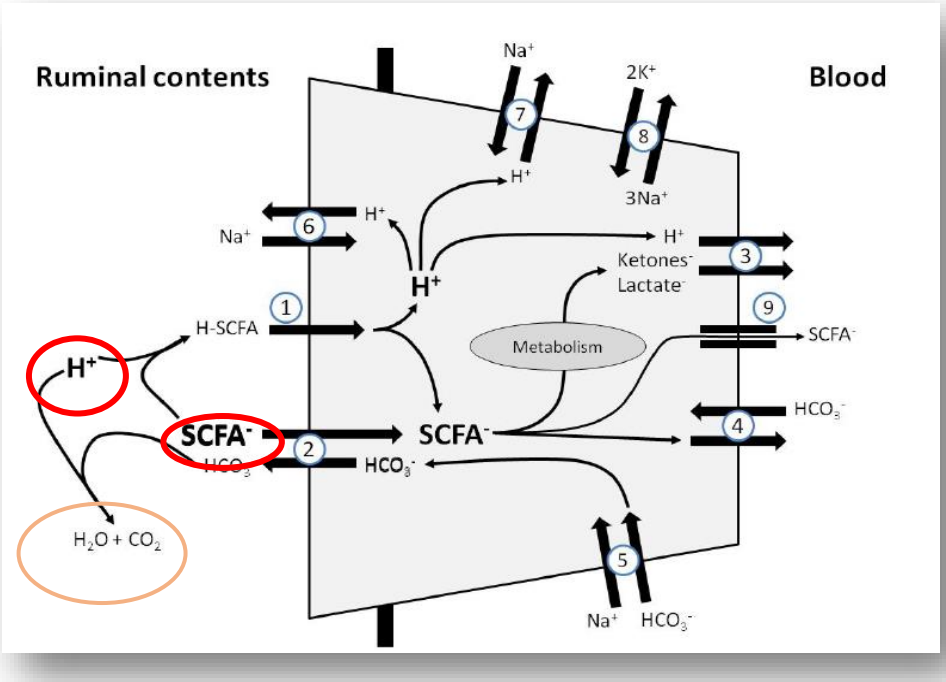
As seen previously acidity in the rumen comes from various bacteria activities (with protozoa and fungi in a lesser extend) and is present under various compound like VFAs, lactic acid, but also succinate and formic acid. This massive acid production needs alkalinity to maintain the pH within the physiological range.

There are two type of acidity removing systems. The absorption of acids by the ruminal mucosa and the neutralization of acidity by buffer system within the rumen. About 30% of acidity is neutralized by buffers and 53% is suppressed with the absorption of acid.(Penner and Aschenbach, 2011).

1.2.1.1. Acid removing system.

Acids and in major proportion VFA are absorbed by the mucosa. 50-80% of SCFA are absorbed through reticuloruminal epithelium. Variation of VFA in the rumen is the signal for the rumen to adapt the absorptive capacity. When the VFA production is increasing the mucosa will adapt to absorb more VFA. This is for instance one of the adaptation of the mucosa during the transition period of the dairy cow (Penner and Aschenbach, 2011). Aschenbach et al (2011) designed a model for the acid movement through the mucosa in the rumen and ruminoreticulum (cf Fig.9).

Combined with H+ or not, VFA absorption is helping to send protons to the circulation. It is worthy to notice that NHE activity is increasing when feeding high concentrate helping the removal of H+ out from the cell to the circulation. Also SCFA-/HCO3- exchanger increase its activity when pH is dropping, helping to maintain the pH in its range.



1) free diffusion of H-SCFA,

2) SCFA-/HCO3- exchanger,

3) basolateral cotransporter Ketones-H+/lactates-H+,

4) HCO3-/SCFA- exchanger,

5) Na+/HCO3- cotransport,

6) and 7) Na+/H+ exchanger (NHE),

8) Na/K pump for electroneutrality.

9) basolateral ion channel for SCFA,

Figure 9 Model of acid absorption through rumino-reticular mucosa. (Aschenbach et al, 2011)

1.2.1.2. Acid neutralizing system

Saliva buffers, mostly HCO3- and H2PO4-, are helping the elimination of acidity. HCO3-. They counter balance the VFA and lactate dissociation equilibrium (pKa of 4,8 and 3,8 respectively) in order to stabilize the pH. One of the key feature of cattle feed is the capacity of feed to buffer the ingesta. For instance long forage particles in diet will promote rumination and saliva production for a better buffering of ingesta. Those are called physically effective non detergent fibers and are favored for a steady pH in the rumen. In a lesser proportion exogenous buffer capacity is found among charged group present in the ingesta like proteins or lignin that can exchange cation (K+, Ca2+, Mg2+) for H+. Among feedstuff the buffering capacity can be classified as follow: Cereal < Grass and low content fiber (corn silage) < legumes and legume proteins.

1.2.2 The pH variation and rumen microbiota.

The link between the composition of the microflora and fauna with the pH is essential as the physicochemical environment of the Rumen determines directly the ability of micro-organism to live and execute its normal metabolism, utilizing nutrient taken from the ingesta and synthetizing chemical component that will either be seen in the free fluid of the rumen (RFL) either present within the cell. The pH scale is considered as a scale where ranges of pH correspond to the optimal condition for micro-organism to develop.

The Rumen Micro-flora and fauna ensure a continuous production of acid in an environment with a continuous standard pH in an anaerobic condition. Physiological pH value varies (Gasteiner et al., 2009) between pH 6.2 and 6.8. It thought that ruminal pH can have 2.5 pH point variation throughout the day for an healthy cow (Gasteiner et al., 2009). Steingass et Zebelli (2008) report that the pH-value in the reticulorumen should be at 6.32 on average in order to maintain physiological conditions and optimal conditions for fermentation. To keep the pH in physiological range the equilibrium have to be kept..

Any variation of pH have incidence on the micro-organism population and in a second time it will influence the ruminal environment. Shift in the equilibrium have consequences on the pH and on the rumen micro-organism composition. This interrelation between pH and micro-organism is the origin of metabolic trouble such as Ruminal Acidosis.

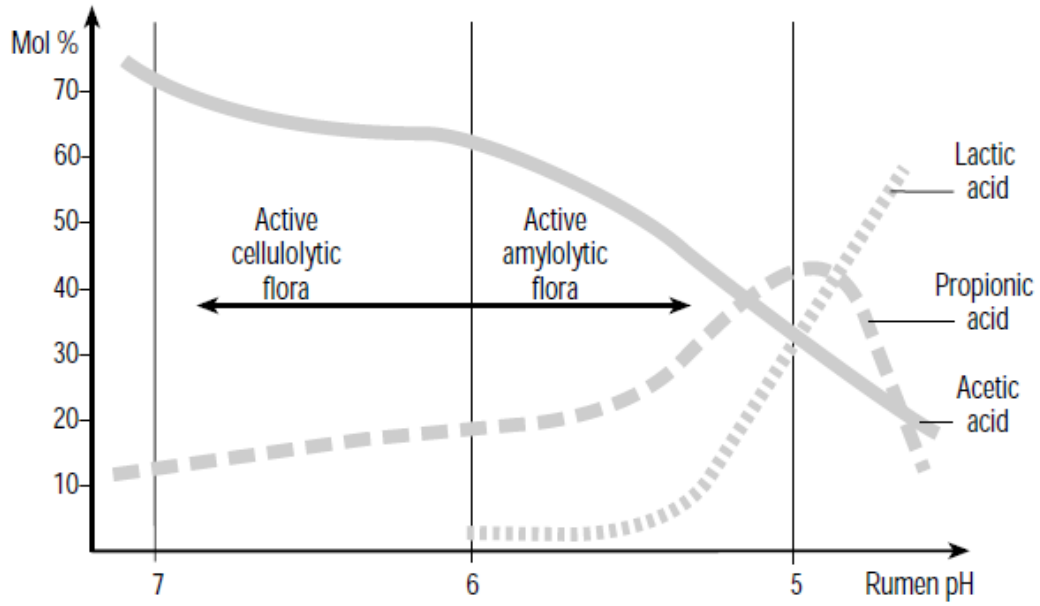


Figure 10: Ruminal fermentation as a consequence of adaptation due to pH regulation. **(KAUFMANN ET AL., 1980)**

In the Fig.10 (Kaufmann et al., 1980) we can follow the fermentation activity in relation to the ruminal pH. This gives also an idea of the composition of the rumen population of micro-organism. Most sensitive to acidity, protozoa are in higher proportion next to the neutrality (pH 7). As seen earlier, they consume bacteria producing VFA and hide nutrient to amylolytic bacteria. When animals are fed high content of soluble carbohydrates (rapidly fermentable carbohydrate) they help regulating bacteria population thus stabilizing the pH. Below pH 6 protozoa population begin to reduce and below 5 it's considered absent from the rumen leaving the place to amylolytic and lactic acid producing bacteria (Lettat et al., 2010).

A pH under 6 (starch based=concentrate feeding) will promote the development of amylolytic bacteria (including lactate producing bacteria) and alter the NDF digestibility and cellulolytic bacteria population (Mackie and Gilchrist, 1979). Cellulolytic bacteria population begin to die leaving the floor to amylolytic bacteria producing more acidity.

From pH below 5.8 lactate utilizing bacteria begin to develop counterbalancing lactate production. This is the first threshold of fermentative pattern with the increase of proportion of propionate/butyrate. and it is usually the beginning of acidotic pathological consequences (early inflammatory response in the rumen appear when ruminal pH drop below pH 5.6 for more than 1 hour, (Gozho et al., 2005)).

Between pH 5.5 and 5 Streptococcus bovis develop in a higher rate as other bacteria are dying leaving nutrient available. Rate of lactic acid production overcomes rate of its removal. When pH<5.1 transport and barrier function of the ruminal epithelium is impaired. At this stage clear signs of acidosis and all signs accompanying acidosis will be seen.

We see here that following the pH of the rumen can have valuable indication of the state of micro-organism population and we will discuss later the need of cut-off value to characterize the level of acidosis.

1.3. The central role of ruminal pH in the peripartal cow

The key and most challenging event in dairy industry is the preparation of the cow for milking onset at parturition. Modern dairy industry being based on high producing cows the need of massive energy intake is required from the very moment of parturition to produce large quantity of milk and ensure a positive energy balance. During dry period the cow is requiring just enough energy to cover expense due to the end of gestation while from day 1 of lactation high producing milk ration have to be fed for sufficient milk production. Needless to say that feeding strategy of dairy cows is essential as a transition have to be done between the end pregnancy low energy requiring period and the milking onset high energy demanding phase. In term of fermentation we need to adapt a ruminal microbial environment designed to digest fibers to an environment capable to digest large quantity of easily fermentable carbohydrate without producing too much acidity to not upset the entire flora. Good feed transition is the secret against energy deficiency and metabolic consequences that are among the most common health issues in dairy industry .The feeding transition can be followed with a change in ruminal digestive physiology and pH profile.

1.3.1. Feeding transition and pH pattern

First of all it is important to have an idea of ruminal pH pattern of normal feeding. The pH pattern depend greatly on the farm strategy and feeding system.

1.3.1.1.Physiological pH variation

In the classic system with cows fed twice daily pH pattern present a biphasic curve with a pH drop after feeding classic silage/roughage. The pH reach its lowest from 2 to 4h post feeding and increase continuously until next feeding. (Gasteiner et al., 2015). TMR with concentrate show slightly lower pH but the pattern is identical. The Fig. 11 show this post feeding pattern (Lohölter et al., 2013)

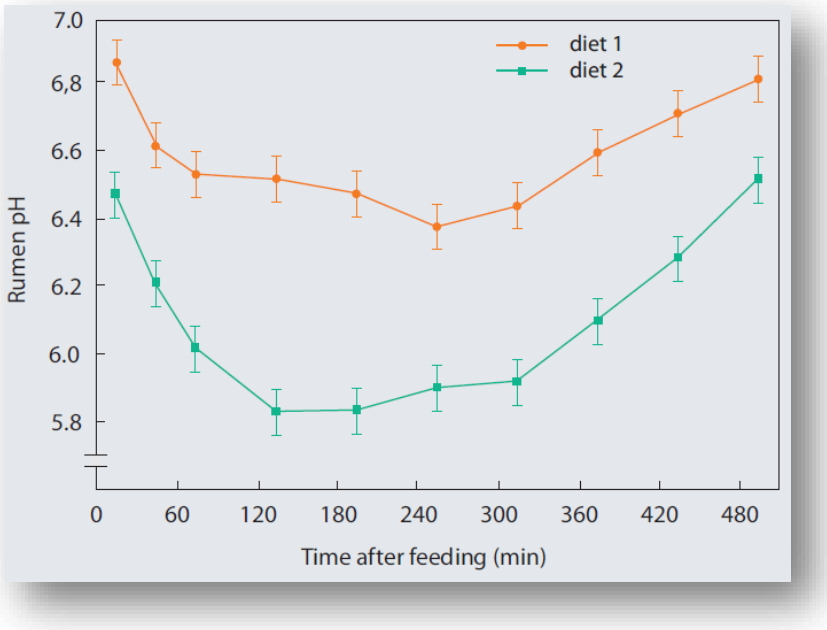


Figure 11 : Post feeding pH pattern (mean pH ventral/dorsal). Diet1=60% corn silage+40%grass silage. Diet2=TMR 36% corn silage+24% grass silage+40% concentrate (DM based) (Loholter et al, 2013)

In the fig.12 we can appreciate this pH pattern over 2 days with the 2 wave pattern dropping after morning and afternoon feeding and reaching the maximum pH before next feeding (Gasteiner and Guggenberger, 2013).

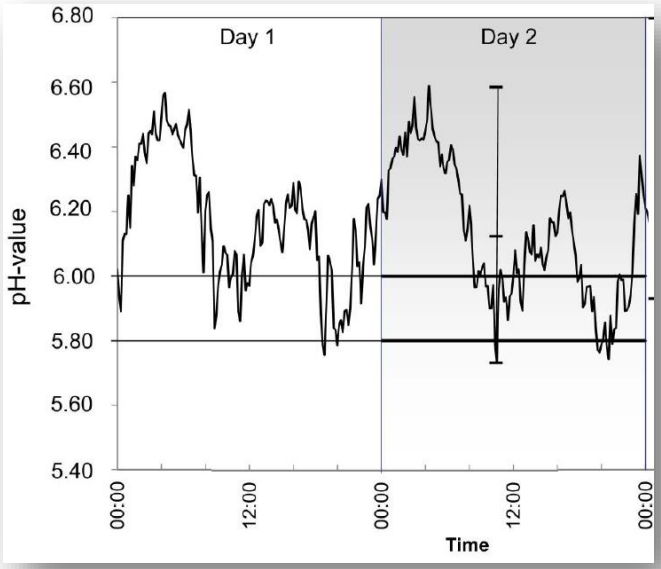


Figure 12: Diurnal pattern of reticulo ruminal pH over 2 days measurment.

Post feeding drop of pH is due to the intake of high amount of nutrient that will be available for flora and fauna digestion that will rapidly begin to ferment the ruminal content producing acidity while saliva isn't produced in adequate proportion to compensate lowering of pH. Acid feeds like silage or roughage is also responsible for this drop. The rumination will balance the pH restoring it in inter-prandial period.

1.3.1.2 Artificial pH patterns

Other feeding types have characteristic pH pattern (see Fig 14). Continuous ad libitum hay feeding is characterized by much more stable and steady pH with higher average pH. More frequent intake of small amount of efficient fibers will promote saliva production and spread more evenly feed intake and rumination giving a more stable pH close to physiological normal range (6.6-6.8) (Gasteiner et al., 2009). Feeding grazing pasture during the day and forage at night cause a distinction of pH pattern. Indeed being richer in energy the feeding pasture will lower the pH and forage will cause a slight elevation at night with the highest pH pic before next morning feeding on pasture. Feeding of concentrate show a lower average pH mean, close to 6.4 with nadir pH of 5.29 with more drastic pH changes. In another experiment AlZahal et al (2009) presented the pH evolution and difference between high forage and high grain feeding in dairy cow (cf fig.13.)

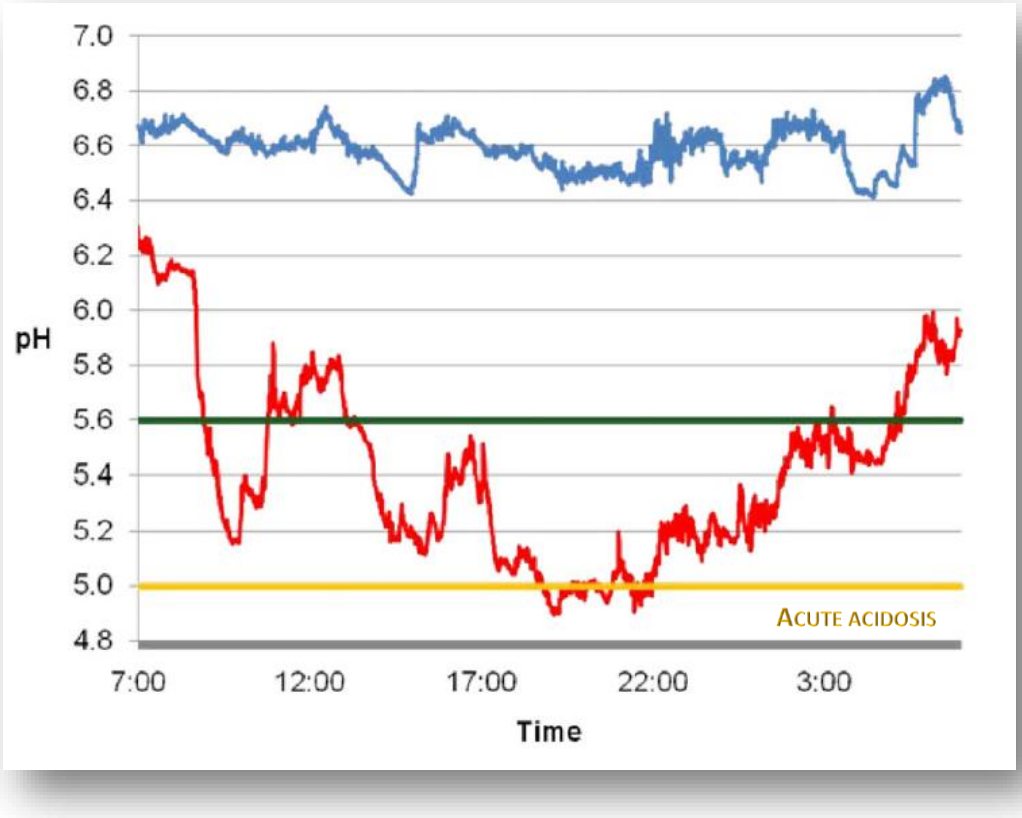
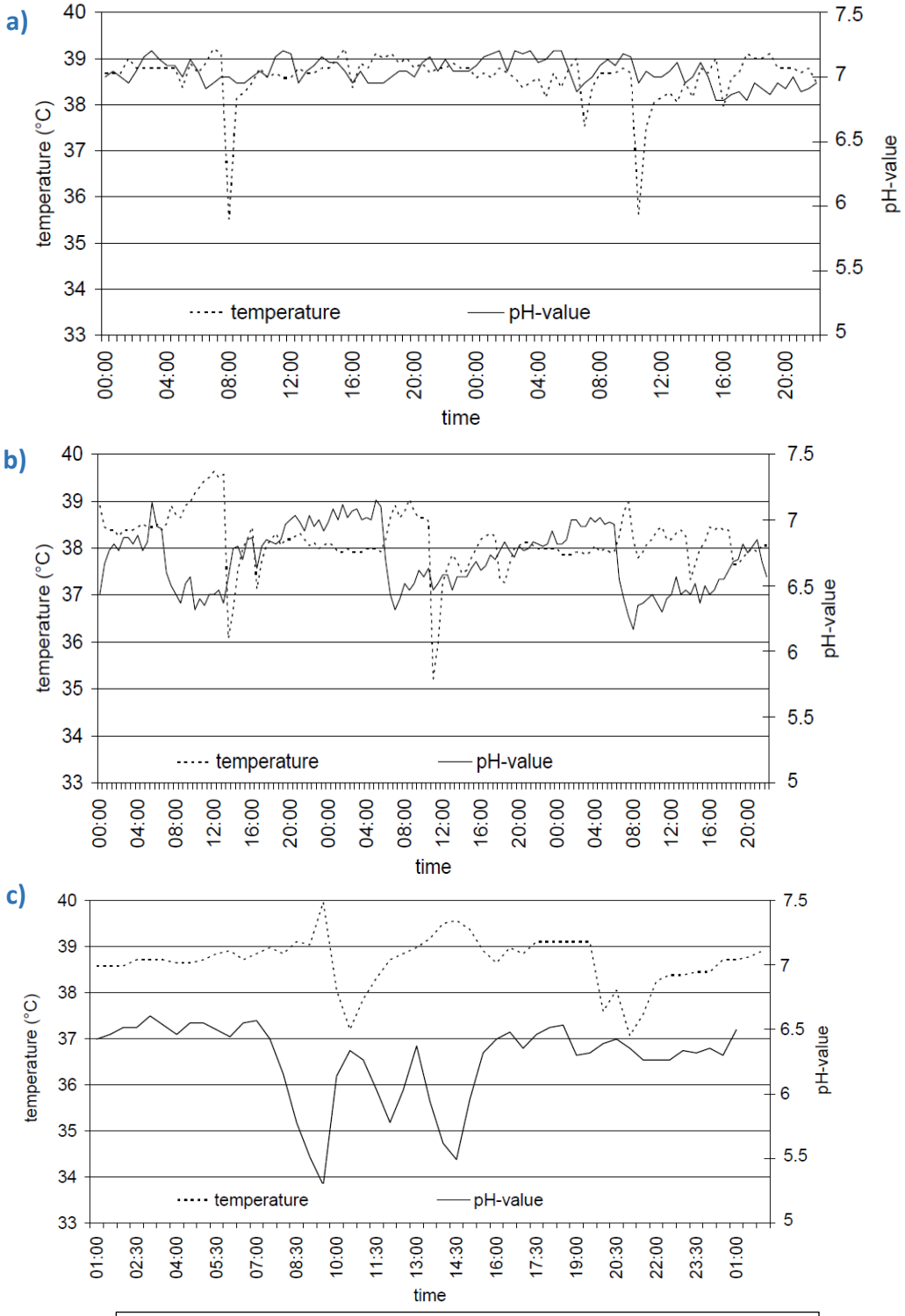


Figure 13 : Continuous rumen pH measurements. (Blue=high forage, red=high grain fedding) (Steele et al 2011)



a) Ad libitum hay feeding; mean pH 6.5 / nadir pH 6.14.
b) Day grass feeding and night forage feeding; mean pH 6.36 / nadir pH 5.34.
c) Roughage to concentrate 50:50 feeding; mean pH 6.37 / nadir pH 5.3

Figure 14: pH pattern under different feeding strategies. (Gasteiner et al 2009)

Farm equipped with Robot milking have a feeding strategy that give a very different pH pattern. Indeed, little and often concentrate feeding ratio and milking allows to reduce pH variation and limit its drop under 6 (Mottram, 2010.) (cf fig.15)

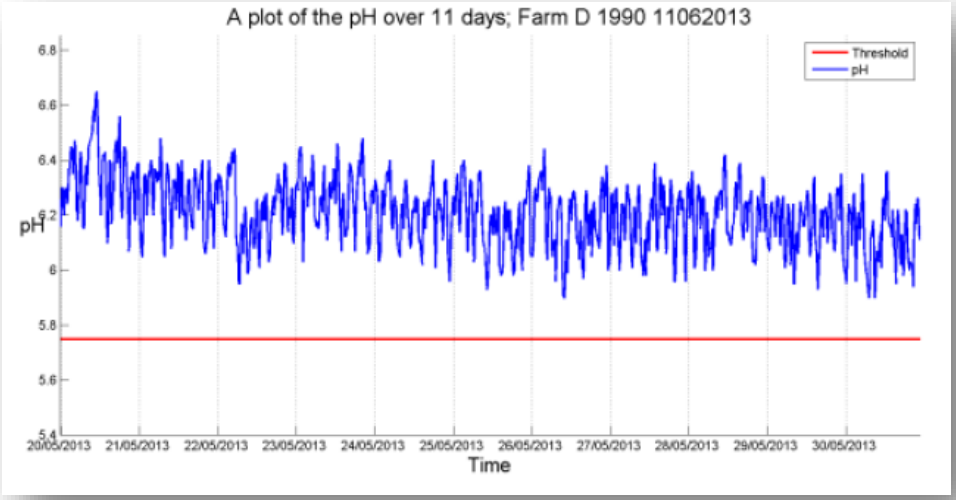
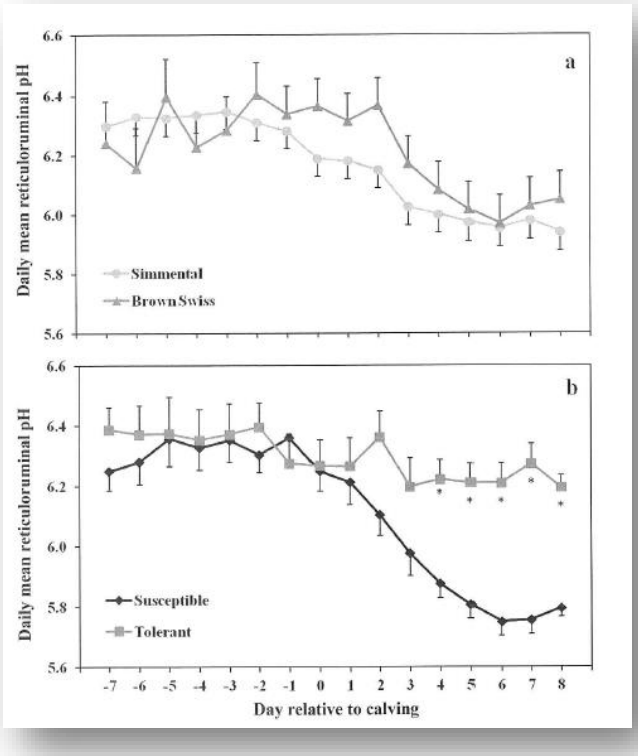


Figure 15: ruminal pH pattern in a milking robot feeding system. **(MOTTTRAM ET AL 2010)**

1.3.2 Feeding transition and pH

The transition period have been described as a period from 3 weeks pre calving to 3 weeks post calving (Grummer, 1995). It is characterized by marked change in endocrine status and a reduction of feed intake when nutrient demand for the conceptus development and the impending lactogenesis are increased. The alimentary transition between dry period and onset of milk production have to be realized in 3 weeks of time. This is the needed time to allow the adaptation of ruminal mucosa to absorption capacity of VFA as described earlier. Ruminal epithelium will encompass morphological adaptation with tissue proliferation as rumen papillae will extend in length and individual cells will see their function evolving (Gäbel and Aschenbach, 2007). The ruminal papillae surface is increasing during this period (Dirksen et al., 1985; Bannink et al., 2008).

The pH profile during this transition indicate usually a lowering of the mean pH and depending of the susceptibility of the cows pH can drop regularly and cause ruminal acidosis. Fig 16 and 17 illustrate this drop of pH from the parturition.



a) Daily mean reticulorumina pH from d7 prepartum to d8 after calving in 9 Simmental and 4 Brown Swiss cows

b) Classification of those animal in 2 groups, SARA susceptible '(pH<5.8 for longer than 330 min/d) and SARA tolerant (pH<5.8 for less than 330min/d).

Figure 16 : Reticuloruminal pH in peripartal cows (Humer et al 2015)

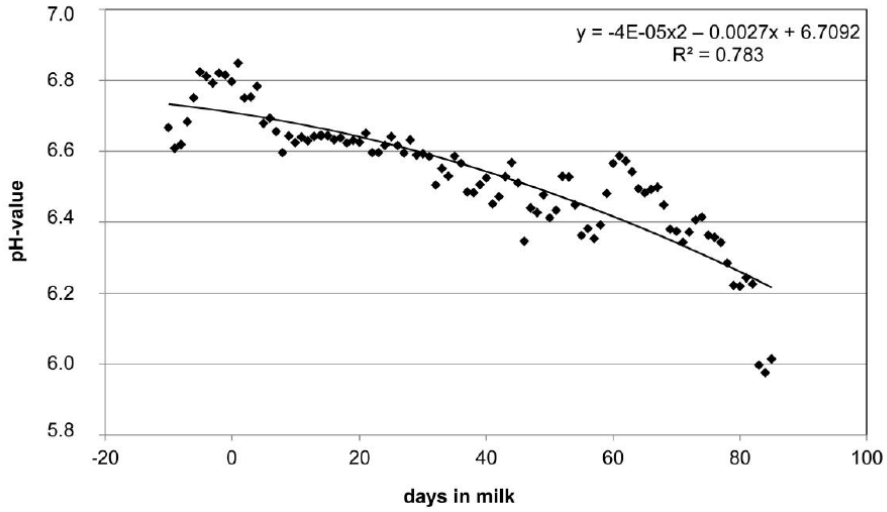


Figure 17: Herd level pH-data interpretation of ruminal pH measurement pre (d7) and postpartal (d80) period (Humer et al 2015)

During dry period the average pH is 6.7 while it drops at pH 6.1 at day 80. The ruminal pH is reaching its lowest in average at about 30 days after parturition to increase afterwards (Gasteiner and Guggenberger, 2013). Close to parturition the rumination is decreasing reducing ruminal buffer production.

Ruminal microflora is adapting to high energy intake and equilibrium have to be found between rapid production of VFA and its absorption. Once adapted the ruminal mucosa will be able to balance the pH by absorbing VFA and the mean pH will get back closer to physiological range. In case of failure to adapt to high concentrate nutrition and avoid the drop of mean pH cows will experienced period of acidosis. Considering the physiological adaptation, dairy cows are considered at risk of acidosis the first month of lactation (Gröhn and Bruss, 1990).

Ruminal acidosis have various forms and can be seen as an acute process or subacute even chronic in some cases. Those forms are connected as pathomechanism involve nutritional management for all of those 3 forms. The figure 25 present a gradation in health state and a correlation with the ruminal pH essential to integrate nutrition, physiology, microbial fermentation and external signs of acidosis.

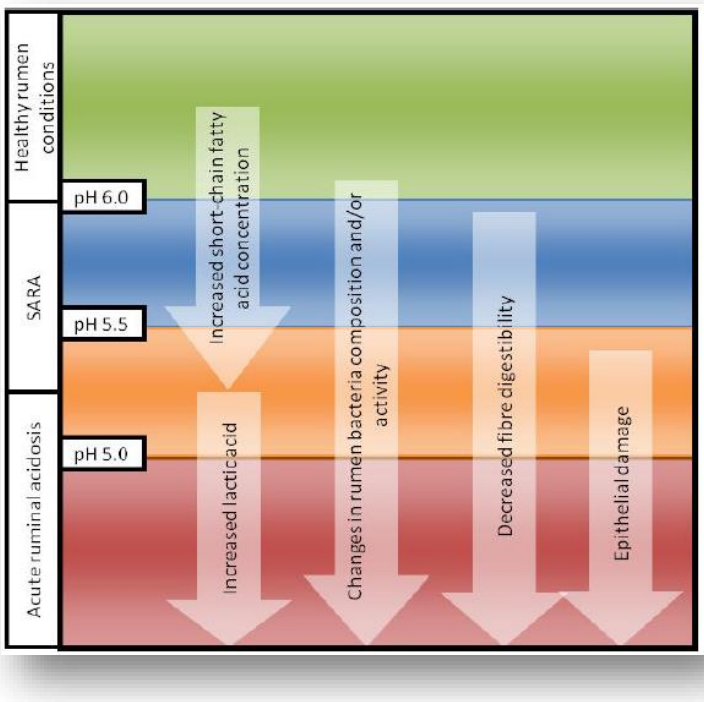


Figure 18 : Relationship between ruminal pH and the associated changes in the ruminal environement and ruminal epithelial function (reproduced from Penner and Beauchemin 2010)

1.3.2. Acute ruminal acidosis

Acute ruminal acidosis represent the condition where uncompensated drop of pH follows large intake of rapidly fermentable carbohydrate and is characterized by lactic acid concentration rise (Owens et al., 1998). This situation is seen in the Fig.19. The ruminal pH drop below 5 and is usually seen when cattle didn't have a proper feed transition to adapt to high concentrate (Radostits et al., 1994) (Cf fig. 19 (Gasteiner et al., 2009)).

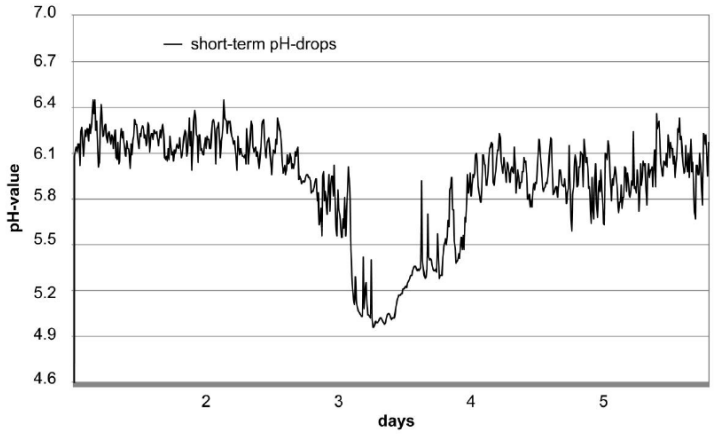


Figure 19: Effect of rapid intake of highly fermentable carbohydrate (Gasteiner et al 2009).

Acute ruminal acidosis is the consequence of the combination with the underdevelopment of viable population of lactic acid utilizing bacteria (*Megasphaera elderseni*) and the under development of ruminal papillae that are too short and unable to absorb the high quantity of VFA produced (Dirksen et al., 1985). Acute ruminal acidosis progress usually quickly due to its uncompensated status (inactivation or death of microflora that can overcome the pH drop and inability of buffers to restore a too depressed pH) and its pathophysiology include peracute rumenitis, rumen hyperosmolality, dehydration and systemic academia (Radostits et al., 1994; Owens et al., 1998).

Feed deprivation have also been identified as predisposing factor to acute acidosis due to inhibition of lactate utilizing bacteria that need slightly acidic pH to develop (cf fig. 20). The reintroduction of feed will produce massive amount of acid (VFA and lactic acid) that will drop the pH and worsen the situation by inhibiting the buffers of the pH.

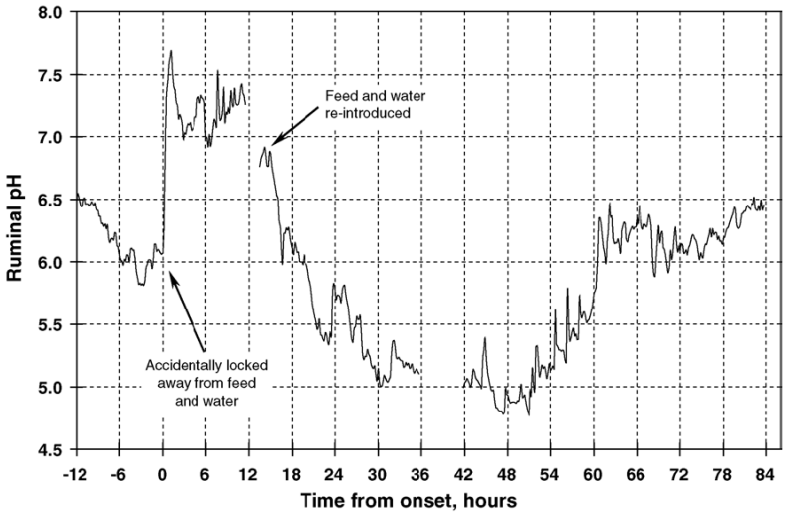


Figure 20: Ruminal pH following a period of feed deprivation and re-feeding in a Holstein steer. (Unpublished data Prentice et al 2000)

The clinical appearance of a cow in acute ruminal acidosis is characteristic and highly evocative with general signs as dehydration, hyperthermia, polydipsia, digestive signs with anorexia, absence of rumination and ruminal stasis, meteorisation, diarrheic faeces and nervous signs with shaking, unsteady position and coma (Guatteo, 2013). Those signs are clearly recognizable and associated easily with acidosis. Measuring the pH on field will not be necessary as it will have no particular diagnostic value unlike SARA as we are going to discuss in the next part.

1.3.3. SARA: context and diagnosis

Unlike acute acidosis, subacute ruminal acidosis in a compensated drop of pH. It is not marked by strong pH drop and rise of lactic acid concentration but essentially caused by accumulation of VFA in the rumen causing a transient drop of pH (Krause and Oetzel, 2006). Drop of pH between 6.0-5.5 is mostly due to overproduction and accumulation of butyric acid whereas accumulation of propionic acid will create a more serious acidosis with pH around 5.5-5.0 (Guatteo, 2013).

More than a simple drop of pH SARA is defined by a certain period of time during the day when pH drop below a certain pH value. Literature does not fully agree on the pH threshold to consider. Either being a depression of pH between 5.2 and 5.6 for at least 3h/day (Gozho et al., 2005), a drop below 5.8 for at least 490min/day or below 5.6 for at least 295 min/day (Danscher et al., 2015) or a drop below 5.8 for more than 330min/day (Humer, Khol-Parisini,

et al., 2015) it is agreed that SARA represent a period of transient pH drop below physiological range for a repeated time. SARA is not a well-defined diagnosis but more likely a syndrome or set of signs associated with low ruminal pH and poor ruminal health (Li et al., 2013).

SARA is not a health condition that will exhibit clear specific clinical signs (Krause and Oetzel, 2006). This repeated acidosis will be associated with a clinical picture directly linked pathological conditions. Here will be presented briefly those evocating signs of SARA.

- Decreased dry matter intake: Decreased appetite and feed intake is often regarded as indicator of SARA and 25% decrement in TMR has been observed in SARA induced period (Kleen et al., 2003). Decreased DMI is cyclic and alternation of high intake in a day and low intake the day after will be seen (Gozho et al., 2005). This will have clear consequences on the pH pattern (cf fig. 20). A decrease in the ruminal motility might also be responsible for the decrease of pH in the rumen (Guatteo, 2013).
- Laminitis: Subacute or chronic aseptic inflammation of the hoof dermal layer can be seen in cattle experiencing SARA. Some signs can be hoof discoloration, sole hemorrhages, sole ulceration and misshapen hooves (Nordlund et al., 1995). The prevalence of laminitis in herd subject to SARA can be higher to 10% (Enemark et al., 2003)
- Milk-fat depression: The link between milk fat depression and SARA is controversial and complex as other factors such as lactation state, breed or feed ration composition can affect this parameter (Enemark et al., 2003). Because pH changes in the rumen fermentative patterns will be upset and depression in milk fat will occur via a change in VFA ration modification (Kleen et al., 2003). However it is difficult to apply as herd diagnostic tool and cow side individual test are not always specific for SARA detection.
- Alteration of feces: Change in feces consistency, structure and pH is seen in cows affected by SARA. pH of feces of affected cows is lower and size of undigested particles may be larger than normal (Kleen et al., 2003). Feces are clearer, yellowish with a sour smell and liquid to diarrheic (Guatteo, 2013). Fibrin casts can also be seen. However modification of feces is not always very sensitive to SARA it can sometime be associated to severe acidotic conditions
- High-culling rate: in SARA affected herds the culling rate can be higher than normal. Indistinct and unexplained death, lameness, loss of body condition or non-responsive pathological conditions are probably the most important causes (Nordlund et

al., 1995; Kleen et al., 2003) however it can only give a tendency as this is not a specific sign.

- Loss of body condition: it can be suggestive for SARA when thin cows can be detected while feeding high energy diet (Nordlund et al., 1995; Kleen et al., 2003) but not always linked with it.
- Rumenitis-Caudal Vena Cava syndrome complex: Some clinical signs such as rumenitis, rumen parakeratosis, liver abscesses and pulmonary bacterial emboli are detectable at the time of autopsy and shows previous periods of acidosis.

Repeated bouts of low ruminal pH during SARA in cattle might also be associated with depression of cellular immunity (Enemark, 2008; Plaizier et al., 2008; Sato, 2015). This suppression of immunity will have direct influence on dairy herd health as peripartal disorders like mastitis can be enhanced in an immunocompromised animal. Inflammatory state of cows will be also high due to ruminal LPS translocation into blood circulation (Kleen et al., 2003; Khafipour et al., 2009; Chaidate et al., 2014). Indeed lowering of ruminal pH will have negative effect on bacteria and their death will release LPS that are susceptible to be translocated to blood circulation due to the loss of rumen wall integrity. Once in the circulation pro-inflammatory cytokines and acute phase protein will maintain a constant inflammatory alert throughout the body.

Abomasal displacement can be a possible consequence of SARA (Plaizier et al., 2008) and a subsequent loss of ruminal contractility. LDA have been described by Gasteiner et al (2015) with long period of pH below 5.8, more drinking acts, lower milk production and random pH fluctuation not linked with feeding act. Abomasoruminal reflux can also be observed lowering regularly the ruminal pH (cf fig.21).

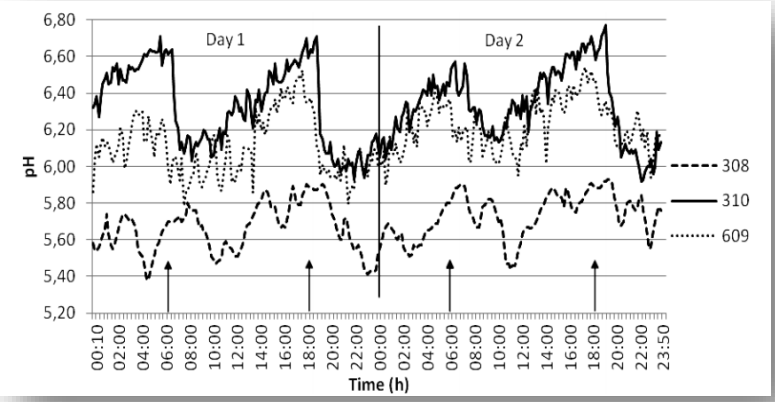


Figure 21: 48h reticuloruminal pH variation. Arrows indicate feeding act. Cow 308 present the pH variation during a LDA (Gasteiner et al 2015).

SARA have also been reported to predispose to Haemorrhagic Bowel Disease in cattle (Tajik et al., 2010).

The measurement of pH is considered as the definite diagnostic of SARA. All signs of SARA appears later than the first decrease of pH. Monitoring of ruminal pH is the solution of choice to detect it. The measurement of ruminal pH and temperature simultaneously have allowed to established a negative correlation between temperature and pH variation during episodes of SARA (AlZahal et al., 2011).

Through this variety of detrimental health effect SARA is known to have deleterious financial consequences. It have been estimated that in the New-York state (USA) SARA cause between 400 and 475US$ loss of income per year. SARA would be detected in about 19-20.1% of early and at peak of lactation (Garrett et al., 1999). In Netherland, Ireland and Australia the prevalence of SARA have been reported between 10-13.8% of dairy cows (Kleen et al., 2003; Bramley et al., 2008; O'Grady et al., 2008) and in Italy SARA is seen in about one third of dairy farms (Morgante et al., 2007). SARA also increase treatment cost, decrease productivity with suboptimal cows performance and increase feed cost due to bad feed efficiency and poor fiber digestion (Beauchemin et al., 2006) This highlight the necessity of monitoring ruminal acidosis and develop strategies to prevent it.

In the upcoming part we will develop the technical aspect of ruminal pH measurement and how we have developed diagnostic tools that are precise enough to be reliable in term of measure itself but also its location within the rumen.

II-THE MEASURE OF PH IN THE RUMEN

2.1. How to measure the pH?

2.1.1. Technical aspect of pH measurement.

2.1.1.1. The electrode

Measurement of pH is done on liquids via a pH probe. It is given as the $-Log^{10}$ ($[H_3O^+]$=concentration of hydronium ions = proton concentration). Direct transcription of pH and acidity is then appraise. To measure the pH a glass electrode and a reference electrode is needed as the pH measurement is based on a potential difference of between those two points (see hereafter Fig.22). For more convenient pH measurement most probe utilized nowadays are single combined probes. Portable indwelling ruminal probes are designed with those types of compact combined electrodes. Reference solution are systematically used to calibrate the pH meter. Those reference solutions are two solutions with known pH values of 4 and 7. The great majority of experiment we will discuss later are ruled by this calibration in order to validate results. Calibration is a requirement for the validation of measures. With time measures value can deviate from the references settled; this is the standard deviation. All measurement technics needs frequent recalibration in order to reduce this standard deviation. In some experiences like with the wireless ruminal pH sensor, Lohölter et al (2013) measurement device can be recalibrate and the pH drift is registered in order to be able to forecast a duration of measuring accuracy along time and the possible standard deviation.

Being sensitive element probes can be damaged by environmental factors (corrosion, oxidation and contact with heavy metal…) (Gasteiner et al., 2009) and deteriorate the measure.

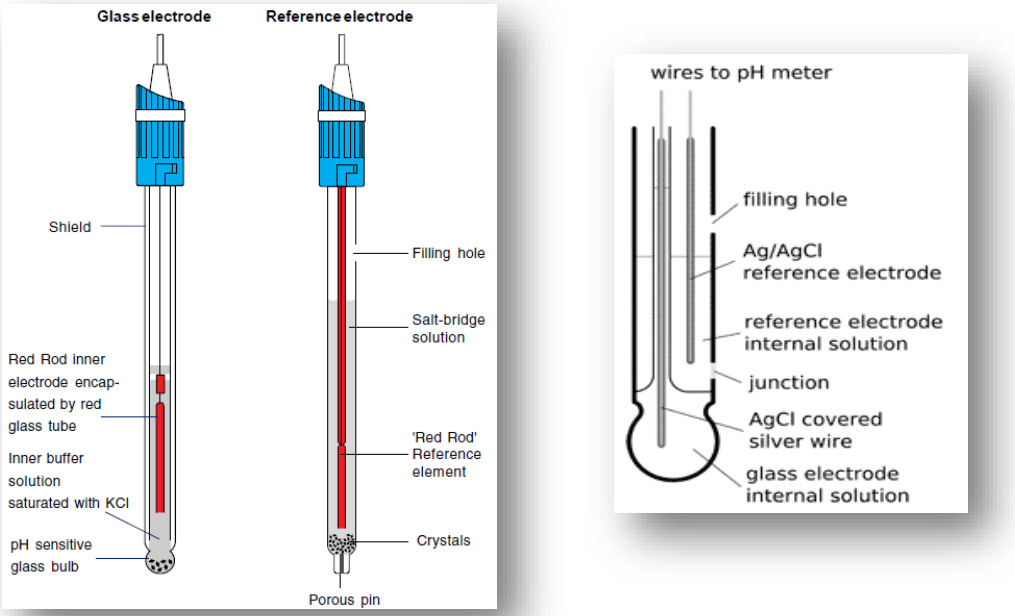


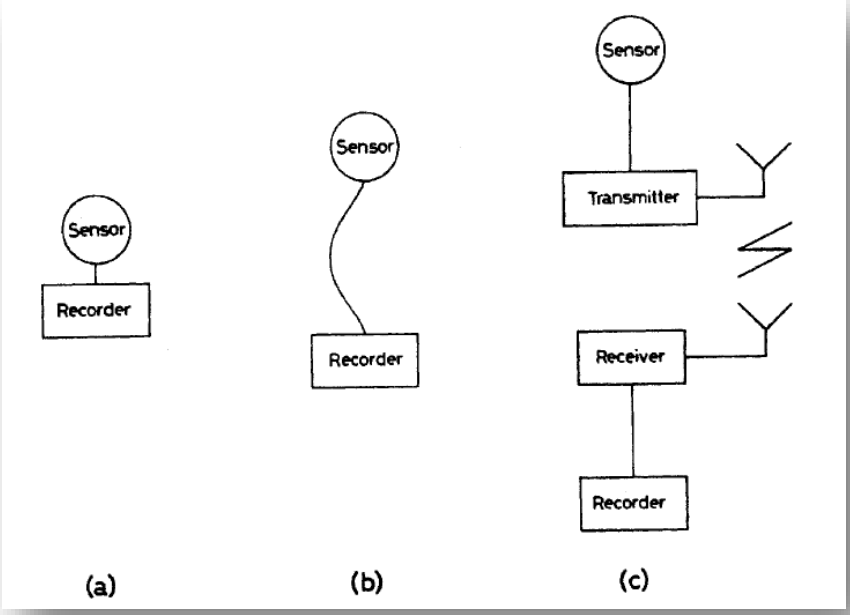
Figure 22: Typical electrode construction (pH Theory and Practice-Radiometer analytical Ltd leaflet)

Component of electrodes is considered to be relevant (AgCl electrode and KCl saturated solution) as the use of pH probe should not be armful or cause any hazard to the animal (Gasteiner et al., 2009).

2.1.1.2. The transmission system

Ruminal pH measurement can be done on Ruminal fluid collected from the rumen or directly in situ. With ruminal fluid collection the measure is done directly with the fluid. In the case of in situ measurement Blight et al (1974) began the reflexion of in situ telemetry wired or wireless measuring system (Bligh and Heal, 1974). This represented the possibility to realize distant measurement of physiological parameter (Temperature, pH, gas composition…). In the Figure 23 hereafter present the different measuring and recording strategies that will be discussed later in the case of pH measurement. In a) we have a representation of classic measurement where the recorder register on site the signal of the sensor. To be able to realize small distance measurement we can have a probe link to the recording unit via a wire allowing to measure physiological parameters .We record it without being too invasive in the organism and take the risk to cause stress that could alter targeted physiological parameter to measure. Wireless system are composed of a sensor/transducer that will convert the physiological signal

and generate an impulse proportionally to the physiological parameter. This impulse will be received translated and recorded by a distant station. There is no physical connection between the *in situ* measurement and the distant recording allowing a great freedom of movement for studied organism. We can measure and follow one or more physiological parameters in normal breeding condition to assess the herd health or in artificially simplified condition during experiment to study influencing factors on physiological constant.



a) the sensorand recorder are constructed as a single unit with a fixed and close relation between them
(b) thesensor is at a distance from the recorder, but connected to it by a flexible cable
(c) there is no cable
connection between sensor and recorder and information is transmitted from the one to the other by radio

Figure 23: A schematic representation of the relations between sensor and recorder
(BLIGH AND HEAL, 1974)

We will now approach the particular case of ruminal pH measurement in cattle where all of those three sensor/recorder system have been develop and studied to bring along precise tool for animal welfare and breeding technology

2.1.2. The particular case of the Rumen.

Being a huge compartment with distinct anatomical spaces, the rumen and the reticulum have a certain physico-chemical variation of its content. The ingesta have a sequential movement within the rumen and reticulum due to wall contraction. Those contractions cause

currents of ingesta within the rumen thus creating a heterogeneity in the distribution of food particles and liquid.

The ruminal pH is given by the following equation: $pH_{Rumen} = 7.74 + \log([HCO^{3-}] / pCO_2)$ (Kohn and Dunlap, 1998). Gaseous environment and proton concentration is determinant and will be mentioned later.

Parts of rumen is distinguished anatomically with the dorsal and ventral rumen. They have both cranial and caudal part. Cranially we find the reticulum that communicate with both rumen and omasum. The ingesta represent a bulk with fiber (and grain depending on the feeding) soaked in a mix of ruminal juice (water of feed, drinking water and saliva). During rumination cycle and food processing movement contraction of reticulum and ruminal wall spreading this soaky bulk throughout the reticulum and rumen causing distinct microenvironment. Ruminal microenvironements are characterized with different composition (particle size, microorganism) and pH.

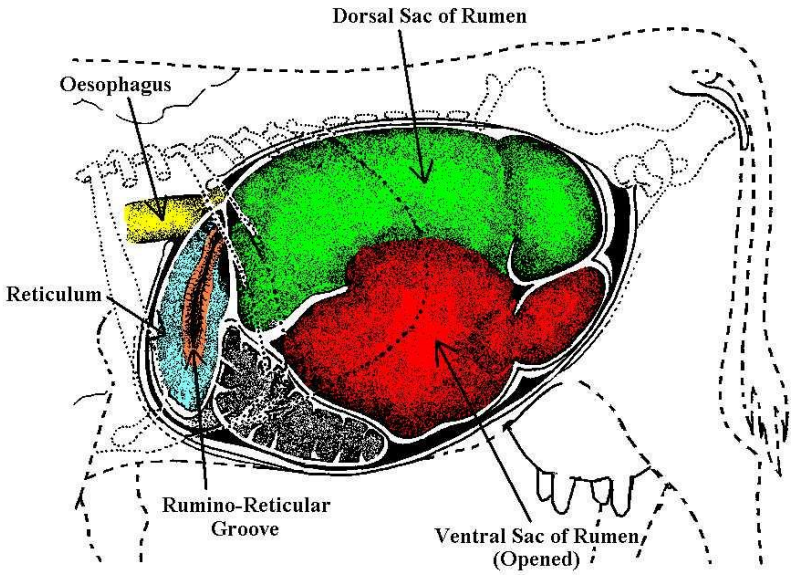
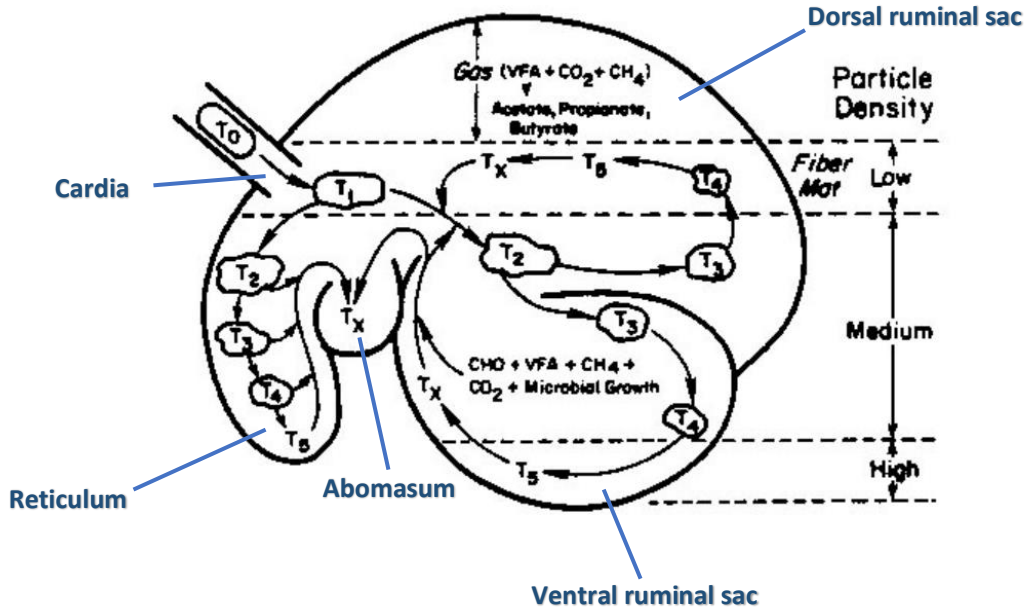


Figure 24:Left aspect of rumen and anatomical delimitation. (University of Bristol 1999)

There are two major distinctions. First of all pH can have on average 0.5 pH variation from the top to the bottom of the rumen (Gasteiner et al., 2009). The fig 25 help to realize that pH could be lower in dorsal rumen as more gas are dissolved included VFA that causes acidity. Dado et al (1993) did point out the importance of location for pH value and settled that pH at

the vicinity of ruminal wall is 0.7pH point higher than in the ventral sac due to rapid absorption of VFA or high concentration of ammonia (Dado and Allen, 1993).



T0 T1 T2 T3 T4 T5 correspond to the particle size that is reduce during its travel within the rumen and Tx represent the size of particle that can be processed further via omasum and omasal opening

Figure 25: Rumen, particle path and density. (J.E. Huston and W.E. Pinchack, 1991)

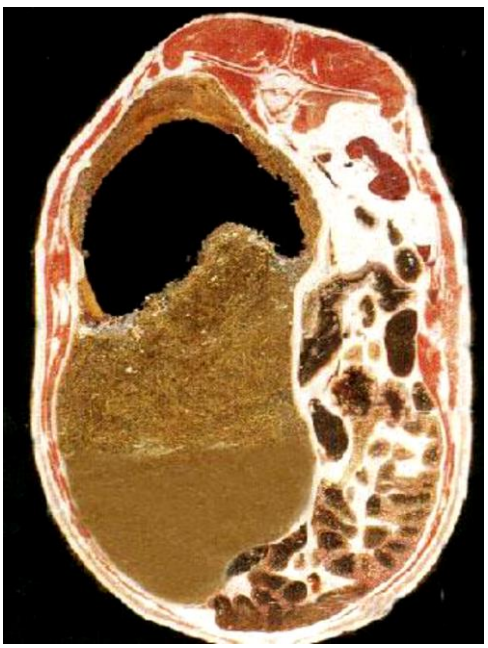


Figure 26: Cranial view of a transversal section of cattle digestive tract (at the level of ventral sac of the rumen) (www.ecow.co.uk, 2016)

The second origin of the pH variation is explained by Klevenhusen et al (2014) and concern the repartition of liquid and solid phase within the rumen (Klevenhusen et al., 2014). Indeed the rumen content is divided in vertical ruminal mat and Free Ruminal Liquid (**FRL**) in ventral rumen. The Fig. 26 gives a good indication of the division of ruminal content with ruminal mat lying on the ventral rumen and orientated vertically within the entire rumen. The ruminal mat (bulk part) is dense enough to create a barrier that retain small undigested potentially degradable particles and VFA (Storm and Kristensen, 2010). There is less exchange of VFA with PARL and FRL causing lower pH in the Particle Associated Ruminal Fluid (**PARL**) (Tafaj et al., 2004).

The location of sample of ruminal fluid or of residual measurement with in situ sensor mechanism is then determinant to get precise results and to interpret it of measures (Mottram et al., 2008; Kilic, 2011). Adjustment and evaluation to correlate location and the pH is needed to have meaningful and exploitable field measures.

2.2 The measures of the Ruminal pH

We are going here to make a review of all the technics that have been developed since couple of decades to measure ruminal pH and follow its variation through time. The point will be to discuss the advantages and drawbacks of each technics to have key elements to understand measuring result when reading pH value during an experiment. A table is presented in annex (cf Table 2) and mention the advantages and disadvantages of existing technics for pH measurement.

2.2.1. Measure on ruminal fluid sample

The collection of ruminal fluid is the externalization of fluid from the subject animal in order to realize measurement on site or in lab. Three technics will be discussed here.

2.2.1.1 The ororuminal tube

The sampling of fluid is made via a long flexible tube with small holes at the extremity for fluid to be sucked in and inserted orally (Duffield et al., 2004) (Cf Fig 27). It have been settled that according the length the place of sampling in the rumen differs. Largely discussed in the literature the earliest device have been described by Pouden (1954). When a tube is inserted at about 180cm the sampling site is considered to the cranial-dorsal and at a depth of 200cm we reach the ventral rumen (Shen et al., 2012). Geshauser et al (1993) developed an ororuminal tube with a suction pump used for ruminal fluid sample. This tool can be used for ruminal fluid collection but also for transfaunation or intra-ruminal water soluble drug application.

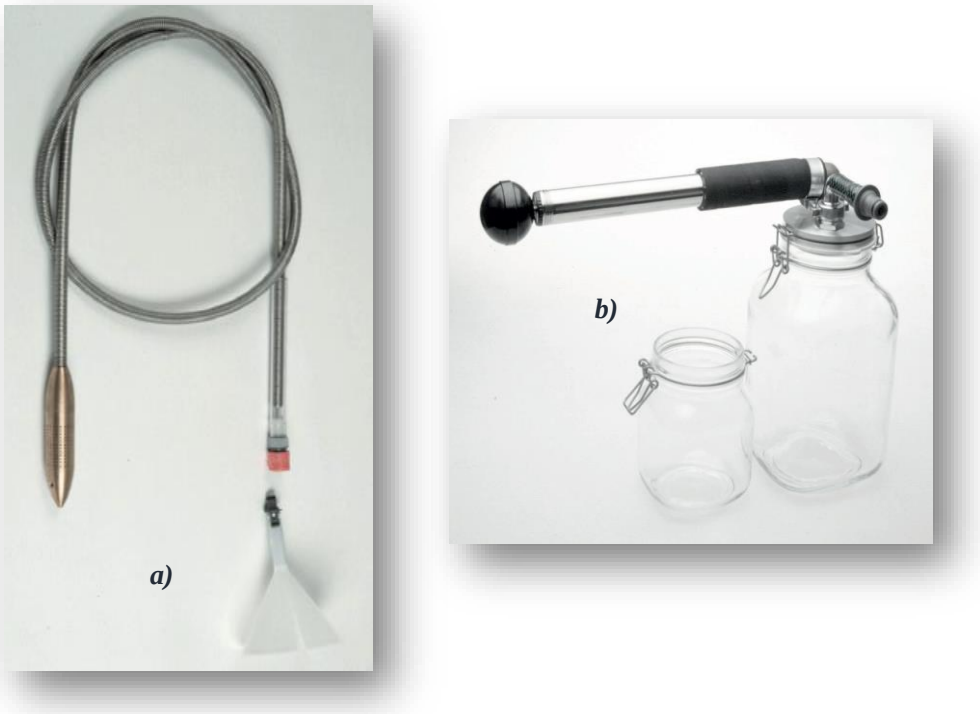


Figure 27: Oro-ruminal tube (a) and its suction pump and contain (b)

For the collection of ruminal fluid the tube is inserted in the mouth and 180cm to 225cm should be inserted using the incisive as landmark. Stomach tube is inserted caudally as far as the $9^{th\ rib.}$. Animal have to be restrained and the head is pulled by a nose leader. I case of blockage of collecting holes during suction we withdraw the tube for about 30cm to 100cm and push it back. Time of sampling is important and Geishauser (1993) suggested to sample ruminal fluid before morning feeding and 3-4H after feed intake.

Variety of system have been designed and they all comparable results with -0.02 to +0.09 pH point variation even for simple system more accessible as water hose with a gag (Steiner et al., 2014)

Because of oral application of this sampling technic the salivary contamination is of major importance. This will increase the pH of collected fluid of about 0.10-0.14 because of contamination of the head of the probe (Shen et al., 2012). Duffield et al (2004) recommend to discard the 200 first mL before to sample required amount for pH measurement (25-30mL). Time of sampling is also important and the longer the oro-ruminal tube stay in place, the more saliva will be produce having potential action on the pH (Dirksen, 1975).

The density of ruminal mat can hinder the penetration of the head of the probe down to the ventral rumen. As a consequence the measure via the probe stop to cranial-dorsal rumen and central rumen (Duffield et al., 2004).

2.2.1.2. Rumenocentesis

Rumenocentesis or ruminal puncture is a technic that consist to the sample of ruminal fluid via a transcutan needle puncture straight into the rumen through the abdominal wall. First rumenocentesis have been documented in 1984 in German literature (Hollberg, 1984; Nordlund et al., 1995; Enemark, 2008).

We realize the puncture in the ventral rumen on the left side, at the junction of the last rib (the 18^{th}) and a horizontal line that cross the hind limb at the level of the patella (or preinguinal skin fold). The Fig 28 present the location of the rumenocentesis. The caudo-ventral part of the rumen is used as ventral rumen contains the most liquid. A 1.5mm gauge epidural needle with mandarin (Hofirek et al., 2001) or 18 gauge/100-120mm long needle (Garrett et al., 1999) is used to collect 3-5mL . The puncture is realized under local anaesthesia (Lidocain injecti SC or IM) and scrubbing of the puncture area is need and realized with a disinfectant (povidone iodine or chlorhexidine). Animal should be restrained and rear legs tied together. Timing of sampling depend on the needed evaluation. The post feeding pH measurement can be done 5-8H post morning feeding in TMR fed herds and 4H post feeding in concentrate feeding (Nordlund et al., 1995; Kleen et al., 2003).

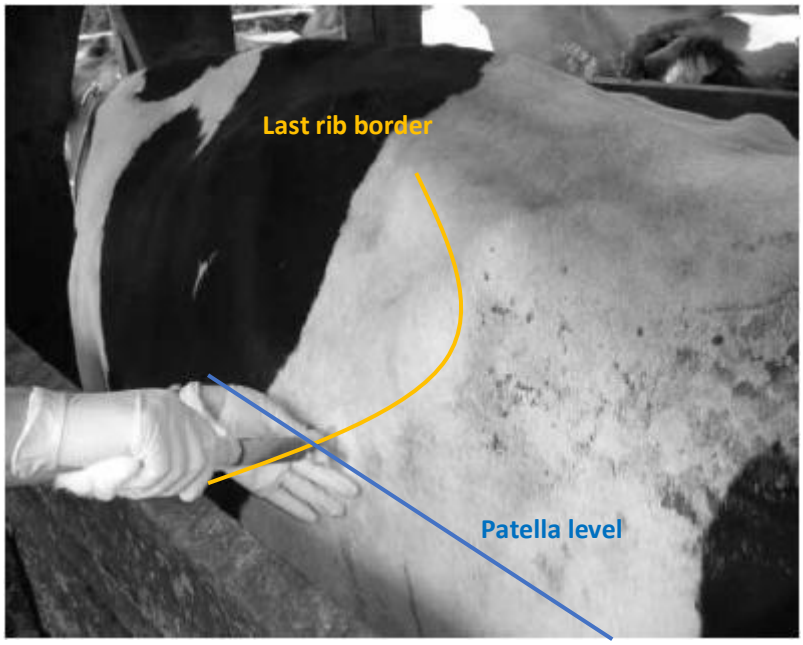


Figure 28: Picture of surgical rumenocentesis (Noro et al 2013).

Rumenocentesis is considered to be the reference diagnostic tool for routine examination and ruminal acidosis diagnosis (Duffield et al., 2004).

Complication as hematomas or abscesses at puncture site and septic peritonitis have been reported (Kleen et al., 2003) but if realize in good condition its incidence stay pretty low (0.5-12.24%) (Enemark, 2008; Morgante et al., 2007). Small needle size, deep local anaesthesia, local disinfection and small volume of collected volume are factors that decreases post puncture complications (Garrett et al., 1999).

PH measured by rumenocentesis have a positive linear relationship with pH measured through a ruminal cannula and rumenocentesis sample were about 0.28 pH unit lower than simultaneous sample taken from rumen cannula (Garrett et al., 1999). This could be explained by the retension of VFA in the ruminal mat present in the ventral rumen.

It is considered to be the best field test for ruminal pH determination and the definite diagnosis for SARA by Duffield et al (2004). The variation between the pH measured via ruminal tube and rumenocentesis varies from 0.28 (Garrett et al., 1999) to 0.76 (Enemark et al., 2003) or 1.1 (Nordlund et al., 1995). The sampling method and sample handling are crucial. Variation with other methods can be explained by the sampling technics of the oro-ruminal tubing or a bad keeping of samples and a too long time between sampling and measure.

2.2.1.2 Fistulated/Canulated cows

In some experiment ruminal fluid have been collected via a fistula situated in paralumbal fossa of left flank (cf Fig 29). It have been used since a long time in order to collect ruminal content and read the pH from its fluid (Monroe and Perkins, 1939) and more recently in studies to validate the localization of stomach probe location after oral application (Enemark et al., 2003) and study the variation of pH within the rumen space (Duffield et al., 2004)(Geishauser and Gitzel, 1996).

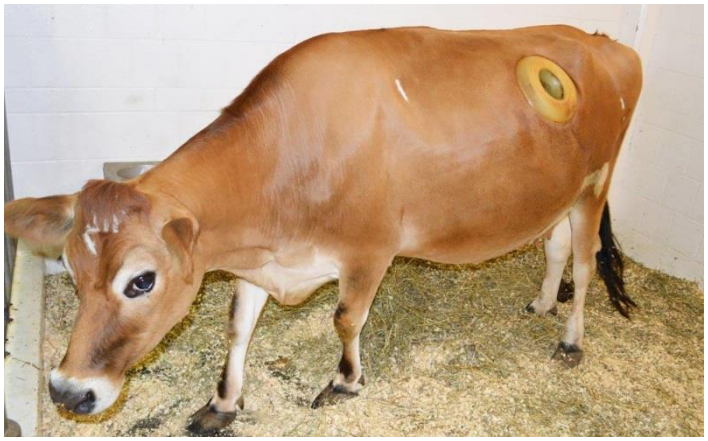
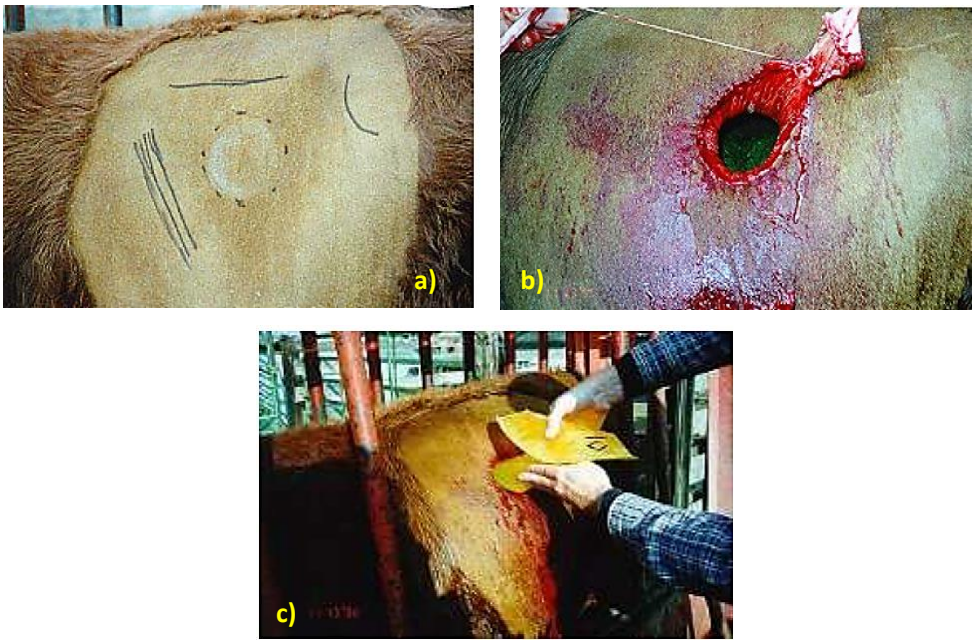


Figure 29: Fistulated jersey cow. (Cornell University of veterinary medicine)

A fistula is realized on the left side of the animal. It is a surgically realized under paravertebral local anesthesia. During the surgery a cannula is settled in place into the fistula to warranty a continuous access to the ruminal environment. The fig. 30 sum up the steps of the installation of a ruminal canula.



a) hair clipped surgical area with transverse process of lumbar vertebrae dorsally, caudal border of 13th rib cranially and tuber coxae caudally,
b)rumenotomy transabdominal with fixation of ruminal wall and mucosa to abdominal wall,
c) placement of long-term cannula. (Bovine Surgery for Fistulation of the Rumen and Cannula Placement BAR DIAMOND™

Figure 30: Surgical installation of a ruminal cannula. (Bar diamond inc, 2011)

Through the cannula ruminal content can be collected and further investigation can be done. It is no need to mention the major role of cannulated cattle in nutrition and digestive physiology research. Transfaunation from healthy cows to animal suffering of microflora disbalance can also be done easily with cannulated cattle (Laflin and Gnad, 2008) as the access to large amount of ruminal content and fluid (up to 2L) is ensured. About all sites of the rumen can be sampled through a ruminal cannula (cf Fig.31) (Monroe and Perkins, 1939; Duffield et al., 2004). This method of manual sampling have been used to evaluate the correlation coefficient between different measuring technics like (AlZahal et al., 2007)(Klevenhusen et al., 2014)(Sato, Kimura, et al., 2012).

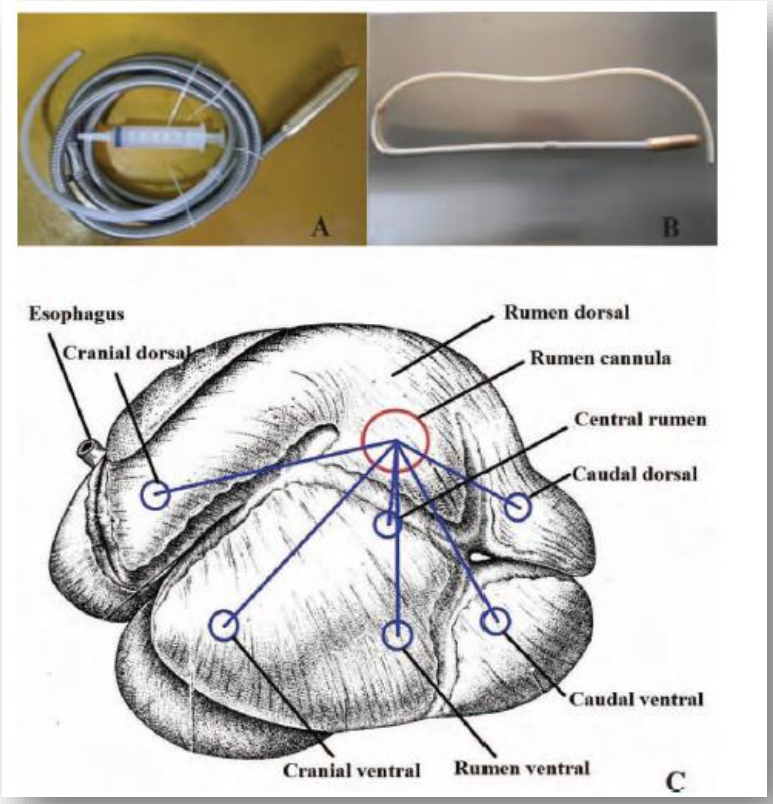


Figure 31A) Oral stomach tube with 60-mL syringe, (B) probe used for ruminal fluid collection from rumen cannula, and (C) different sampling sites (rumen picture reproduced from Chen, 2010, with permission of China Agricultural University Press, Beijing).

Those 3 technics are effective on direct sampling of ruminal fluid. In order to measure the pH in the best conditions it have to be done in a reasonable time after sampling. Indeed ruminal flora and fauna can alter the physico-chemical values of ruminal fluid with higher VFA concentration due to bacterial activities that will increase acidity or elimination of acid by diffusion of CO2 (Aschenbach et al., 2011). As a consequence, pH measured on ruminal fluid collected tend to have a slightly higher pH than *in situ* measures underestimating the level of acidosis of the rumen (Smith, 1941; Dado and Allen, 1993; Garrett et al., 1999; Enemark et al., 2003).

Another important matter of those point sampling methods is that they are subjective of a particular sampling time and highly dependent on the day time. Literature describe different sampling protocol like before morning feeding and then 1 to 7 hours post feeding (Monroe and Perkins, 1939; Geishauser, 1993; Nordlund et al., 1995; Kleen et al., 2003; Chaidate et al., 2014). To have a follow up of pH within a day multiple measures have to be done at precise

intervals (Monroe and Perkins, 1939; Enemark et al., 2003; Duffield et al., 2004; Chaidate et al., 2014).

Marden et al (2005) developed a pump that can be placed between those 2 principles (presented in Fig 32). Indeed the system is composed of on inner part with a tube and a collecting head inserted in the rumen through a cannula and an outside pumping system that allow to suck out regularly a small amount of ruminal fluid and measure directly targeted parameters. The measure is not continuous but the collection can be considered as long-term action.

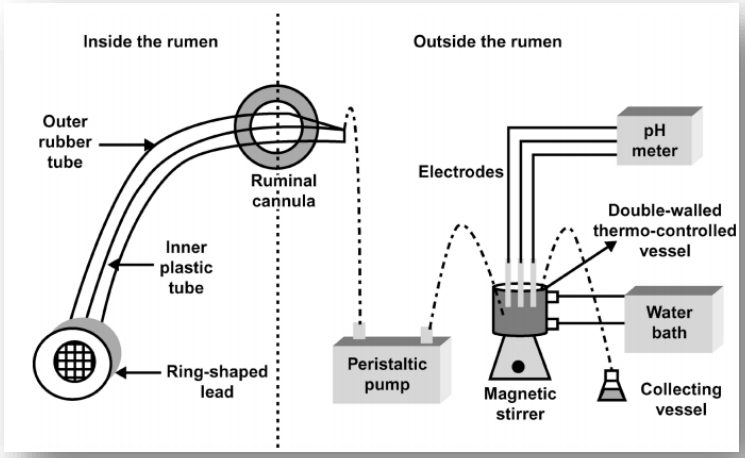


Figure 32: method of continuous sampling and measurements with elimination of gaseous contamination. **(MARDEN ET AL., 2005)**

In order to be the most accurate and have a clear follow up continuous long term in situ method have been developed

2.2.2. Indwelling intra-ruminal sensor

Real time measurement of physiological constant have revolutionized the world of animal experiment and allow to consider the dynamic of homeostasis. That is why since nearly half a century scientist works on developing indwelling intra-ruminal sensor that measure intraruminal pH and transmit the signal to receptor via a wire located externally to the animal. Sensor is inserted through a cannula previously performed and is placed in the rumen, mostly

on the left side The pH is recorded continuously in the time giving a direct look to the physiological stage of the animal and its rumen.

In the majority of the experiments measures of pH by indwelling pH probe is done on cows kept tied in stale. Probes are inserted through a ruminal cannula and it have to be weighted in order to sink into the ventral rumen just below the cannula (Johnson and Sutton, 1968; McArthur and Miltimore, 1968; Penner et al., 2006). pH probe is inserted into a metal or plasic tube to be protected and the extremity consist of a perforated head that allow the bulb of the sensor to be in contact with ruminal fluid but avoid plug of fiber to form and hinder fluid to move freely around the bulb (Johnson and Sutton, 1968; Penner et al., 2006). Some state that the absence of protection is also a solution to avoid aggregation of organic matter and plug formation (Nocek et al., 2002).

The first appearance of indwelling pH sensor measures via ruminal cannula in the literature is in 1968 with a british and a canadian research team (Johnson and Sutton, 1968; McArthur and Miltimore, 1968) (cf Fig 33). They gave the major principle that are to be developed later.

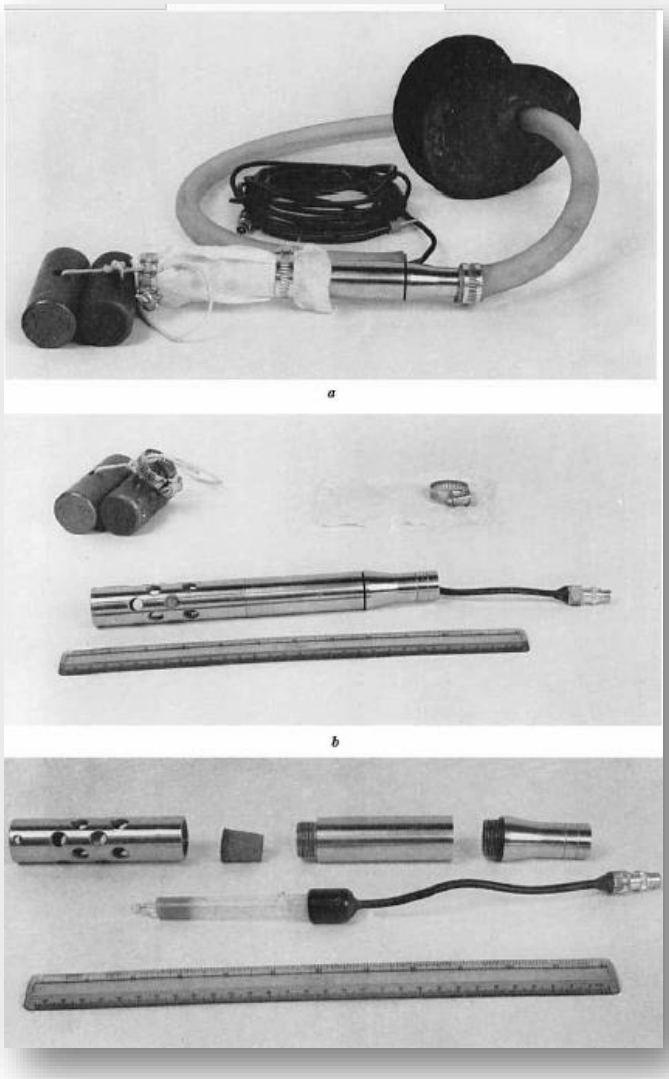


Figure 33: Intra-ruminal indelling continuous wired pH sensor. (Mc Arthur et al, 1968)

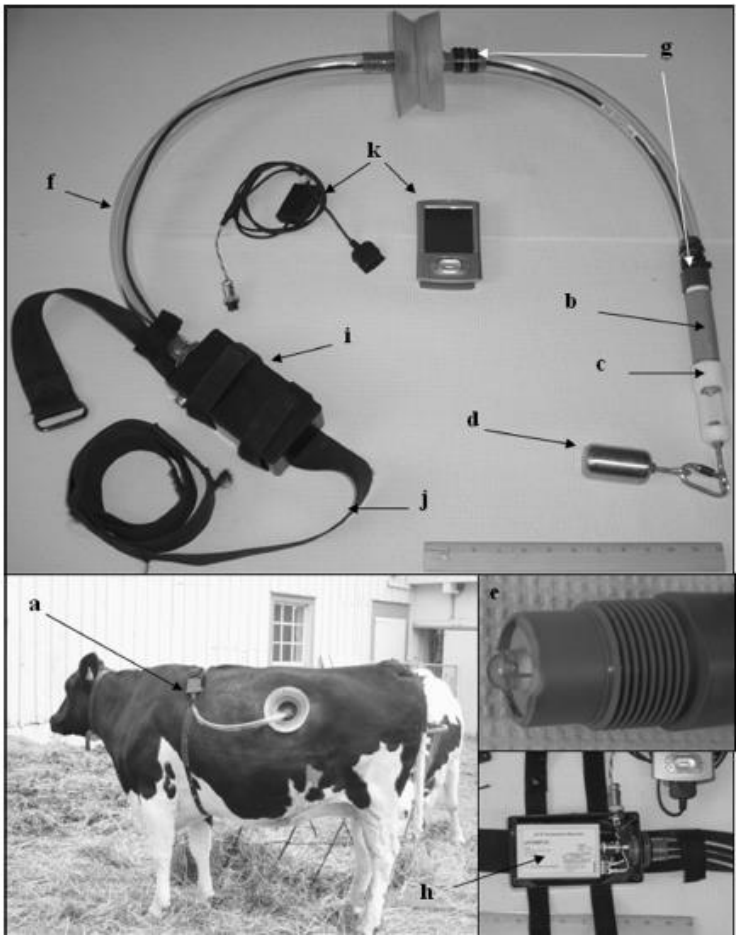
In another communication, Dado et al (1993) described an experiment with tied cows in a barn, the *Sensorex Combi pH system*. This system will be used later in many other experiences (Nocek et al., 2002; Cottee et al., 2004; Duffield et al., 2004; Rustomo et al., 2006).

Used in nutrition experiment (Penner et al., 2006) a system using sensorex pH electrode have been designed: The *LRCpH system* (Guide, 2007). This system is used as reference for testing and elaboration of new tools as portable device (Falk et al., 2016). A PVC pipe version of this *LRCpH system* have been used for few years (Penner et al., 2006, 2007) in experiments that study the severity of ruminal acidosis (cf fig 34).

Figure 34: Take appart and assembled LRCpH PVC pipe system (Penner et al, 2006, 2007)

In 2007 AlZahal et al presented their system. In their work, the indwelling probe is linked to the external monitor which is attached to the cow's back (cf Fig 35) which represent a great advantage in the freedom of movement of the cow. Temperature is also measured by their device allowing to state on the relation between pH and Temperature.

Research was crucial to develop those indwelling ruminal sensor. The validation of measures and location of sensors have been realized by measuring the correlation coefficient between hand-made sampling and pH measurement in spot sampling experiment and continuous measurement(Dado and Allen, 1993; Penner et al., 2006; AlZahal et al., 2007). The high correlation between the Spot sampling and continuous pH measurement with rumen fistulated animal allows to validate the accuracy of the continuous indwelling pH measurement. Same kind of experiment have been done on cannulated small ruminant and measures done by spot sampling bench pH meter and by continuous indwelling sensor showed strong correlation coefficient (Reis et al., 2014).



a) recording unit on the animal during exercise; b)pH probe assembly; c)weight connector; d) stainless weight, e)pH electrode; f) protective tube; g) plastic connectors; h) data logger; i) logger housing, j) belt; k) personal digital assistant and its cable.

Figure 35: RTB (Ruminal Telemetric Bolus)-Portable intra-ruminal indwelling pH measurement system **(AlZahal et al., 2007).**

AlZahal et al (2011) developed a reflexion that can link the temperature and the pH of the rumen. They established a negative correlation between the 2 parameters leading to a rise of temperature while the pH is dropping. Temperature appear to be a tool for estimating ruminal pH.

In all systems a calibration of pH electrode is need prior to measurement period in standard solution pH 4 and pH 7 before to be inserted into the rumen. Calibration have to be done regularly to maintain accuracy in the measures and the frequency of calibration depend upon experiment and systems

2.2.3. Indwelling wireless intra-ruminal sensor

Wireless probe have been developed in order to measure the pH of the rumen of cattle without any invasive intervention (surgical or physical) and is used in free range animal. The minimum intrusion, manipulation and constraint toward animals are beneficial considering animal welfare and limit stress that could alter the measures.

Variety of wireless intra-ruminal sensor have been designed and developed those past 10 years. Each of them have their specific characteristics and are listed in the table (cf Table 3 in Annex).

All system are composed of:

- The indwelling intraruminal wireless sensor
    - A pH probe calibrated with reference solution
    - A processing unit that read and register th pH signal
    - A converter that convert pH signal in radio frequency
    - A battery for autonomous measurement
- The receiver and the operating system
    - A receiver that receive the radio signal
    - A converter to translate the signal
    - A software that will help to treat all measurements

The range for transmission of bolus signal is limited and varies with systems. Receiver/operating system are not always in the vicinity of the bolus which means that data collection are done in various ways. Data collected by the sensor are either transmitted in real time to the operating system (Lohölter et al., 2013) or can be stored for few hours or few days and be downloaded at fixed time or when needed (Mottram et al., 2008). The site for downloading can be strategically chosen like milking parlor, milking robots or any places regularly visited by animal at the farm.

The ruminal boluses are applied orally to animals. We aim to insert it in the rumen. The question of the location of the bolus after insertion and during the experiment have been studied with interest and is still the object of experiment as from the placement of the bolus depend the entire value of measured and transmitted pH. When the ruminal sensor is inserted manually in the rumen via a cannula (Klevenhusen et al., 2014) to spot sampling of ruminal fluid in the area

where the bolus lies (Mottram et al., 2008; Kaur et al., 2010; Zosel et al., 2010; Sato, Kimura, et al., 2012; Lohölter et al., 2013) experiment have been realized to be able to establish the link between the pH measured by the bolus and the spot sampling pH of known place thus giving us an indication on the location of the bolus.

However it have been largely discussed in literature. It is well known that the ruminal content influence the location of the bolus as the ruminal mat is dense and boluses can be retained dorsally in the rumen (Gaughan, 2010). The desired location is the ventral sac and in order to have the bolus sunken down in the ventral sac of the rumen a minimum average density of 2.3 g/cm^3 have to be reached (Fallon and Rogers, 2001) which represent about 300g minimum. Enemark and Peters (2003, 1997), and Li et al stated on the tendency of the ruminal probe to be pushed further to the reticulum due to the reticuloruminal contraction cycle (Enemark et al., 2003; Li et al., 2013).

The variation between ruminal fluid and ruminal probe measurement lead to talk about the *reticulo-ruminal pH* (Enemark et al., 2003; Mottram et al., 2008; Gasteiner et al., 2009; Khol-Parisini et al., 2015)(Klevenhusen et al., 2014) while some are distinguishing *ruminal pH* and *reticular pH* (Falk et al., 2016). Reticular pH will be slightly more stable and higher than ventral rumen pH due to salivation (0.7pH point higher). Some other are considering those 2 pH as equivalent enabling to appreciate one with the help of the other (Gasteiner et al., 2012; Sato, Kimura, et al., 2012). It appears that in the case of small ruminant the bolus measure the pH of ventral sac in the majority of the cases (Penner et al., 2009)

Whether the bolus is lying in the rumen or in the reticulum is a detail that we should not neglected. If we settle cut off values we need to define the place where those measures are originated. Falk et al (2016) presented a comparison between ruminal and reticular pH measurement (cf fig. 36). A difference between pH measured in those 2 locations is seen and this have to be considered when elaborating any diagnostic limits. Falk et al found a mean difference of 0.24 pH +/- 0.08 pH unit difference between the 2 with reticular pH being higher. This difference can go up to 0.7 pH unit in acidotic cows (Sato, Ikeda, et al., 2012).

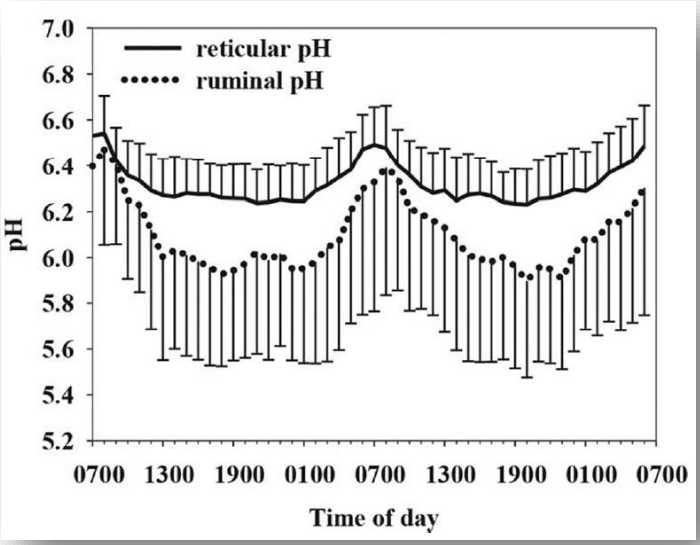


Figure 36 : Average pH profile of 6 cows in lactation period withe simultaneous reticulum pH measurement (eCow) and ruminal pH measurement (LRCpH)

Wireless ruminal boluses are considered to be reliable since publications are concluding on positive results considering the correlation between wireless boluses and calibrated laboratory pH probe measurement (Dado and Allen, 1993; Beauchemin et al., 2006; Goopy and Woodgate, 2009; Penner et al., 2009).

CONCLUSION

The evaluation of ruminal pH is a simple and very useful diagnostic tool. With the addition of the temperature measurement with AlZahal et al (2011) and some wireless indwelling pH probes the detection of febrile state can help to detect potential health issues. As body temperature is estimated we can detect some infection. It have been seen to detect temperature change that follows inflammatory processes, Bovine Respiratory Disease (BRD) or even Bovine Viral Diarrhea (BVD) (Dye et al., 2007). Peripartal period is a period of great physiological and nutritional changes that are detectable by pH and temperature changes. The announcement of the parturition could be a future field research in order to compare the reliability and to settle reference values to be able to interpret measures.

The rumen is a heterogeneous space with more or less variation in the microenvironment from a location to another. We have seen that research did study and deepen this variation its understanding is fundamental. Indeed a variation of pH due to a local change within the rumen have no diagnostic value whereas a variation of pH of this location from physiological standard is informative. A clear knowledge of those local variation is a prerequisite for the interpretation of any experiment result. Where we are? What do we measure? Why do we measure this? And What are we expecting? Are question we have to answer while considering an experiment.

Precious tool for continuous measurement of ruminal pH the wireless indwelling devices might be of major importance in the coming years. Precision Livestock Farming (PLF) in a modern breeding vision of farming. The choice of objective and simple parameters to be evaluated either in an automatic or in a continuous way will allows to monitor herd health on an automatized that will help to detect health issues earlier and on all animals. Measuring pH of dairy cows is considered to be a key strategy monitor dairy herd health as SARA is the most common trouble for them. The idea could be to equipped all animals of the farm with a bolus in order to be as reactive that possible to detect those SARA episodes and restore normal ruminal conditions to avoid any reproductive, metabolism or health complication.

It is worthwhile to mention that the continuous measurement of ruminal pH have always been used in nutrition research. The impact of different feeding strategies have been studied by mean of ruminal pH change. Modern nutrition have designed highly efficient feeding strategies that help cow to cope with the industry's demand. Those Feeding strategies have considerably lowered the average pH of a dairy cows. Today's average of a dairy cow is mostly under known

physiological range whereas we can find in the literature that cattle ruminal pH value were even seen in alkaline range, around 8.89 (Monroe and Perkins, 1939). Thereby a need to monitor the ruminal pH is common nowadays in order to be able to detect acidifying consequences of concentrate feeding and avoid SARA or "High concentrate syndrome" (Calsamiglia et al., 2012).

Modern wireless sensor brings also new opportunities to veterinarians. Specialized consultation with ruminal boluses can allow to work with farmers on herd or individual health.

Follow the pH in real time and on site with breeding condition of free range animals can bring very important and indicative information that will help the veterinarian in an individual or herd diagnosis. In case studies, Mottram and Gasteiner and their respective team show a great example of onsite studies that give priceless information linking breeding to information that have a physiological meaning (Mottram, n.d.; Gasteiner et al., 2009). New diagnostic tool and opportunities are available onsite.

The notion of breed interspecific differences is a source of variability. Dairy cattle breeds show differences in production traits and also health status sensitivity. Jersey cows are well known for their good milk protein content but have also been identified to tolerate better grain challenge (Luan et al., 2016). Other breeds like the Swiss Brown or the Simmental are known to have a lower susceptibility to SARA (Humer, Ghareeb, et al., 2015). Even if nutrition management have some influences on health status of animals the robustness of cow breed is in direct relation with the low incidence of SARA and other production disorders. As physiological response to feeding strategies can varies between breeds it's important to determine the evolution of health indicators, like ruminal pH, specific to each breeds to settle clear cut off values for that particular breed. This is the only way we can compare objectively the same parameter among different breeds and continuous measurement ease considerably this breed qualification (Khol-Parisini et al., 2015).

Measurement and follow-up of ruminal pH by autonomous wireless sensor is used easily on field. Devices are now reliable, we only need to define the field of use of those tools in farming and the potential limit of it.

ANNEXES

- *Table 2 :: Advantages and disadvantages of pH measurement technics*

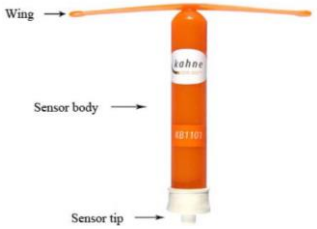
- *Table 3: Wireless pH bolus, its apparition in the litterature/experiment and measurement*

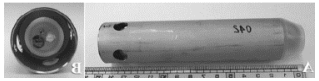
Table 4 :: Advantages and disadvantages of pH measurement technics

TECHNICS		ADVANTAGES	DISADVANTAGES
RUMINAL FLUID COLLECTION	ORO-RUMINAL PROBE	-Least invasive technics for collection or ruminal fluid and easily applicable on field -Large quantity of fluid (up to 2L) can be collected for multiple application (microbial analysis, complementary exam in clinical examination, Ruminal fluid transfer) -Application of water soluble drug and other oral substances	-Uncomfortable for cattle -Position of the suction head cannot be fully directed -Saliva contamination -Fluctuation of pH results depend on operator's skill (Garrett et al., 1999; Sato, Ikeda, et al., 2012)
	RUMENOCENTESIS	-Easily repeatable from animal to animal (ventral rumen) -Minimal post sampling modification of ruminal fluid (fluid collected in a syringe) -Better tolerated by animals -Few negative effect on animal health (Nordlund et al., 1995; Bramley et al., 2008)	-Surgical procedure have to be done by an experienced person and anaesthesia (veterinarian) -Can have some post-operative complication (intramural abscesses, hematoma, peritonitis) if protocol not followed accurately (hair clipping, desinfection…) -Relatively small collected amount of fluid
	RUMINAL CANNULA	-Direct access to different chambers of the rumen -Precision of location for sampling -Large quantities of ruminal content can be sampled	-Invasive technics the present some risks and is usually not reversible -Surgical procedure that required professional skills and anaesthesia (veterinarian)
INDWELLING INTRA-RUMINAL SENSOR		-Animals are allowed close to normal activity during recording -Continuous data offer an excellent tool for monitoring and analyzing ruminal pH -Other parameters can also be measured like temperature (AlZahal et al., 2009)	-Can only be used on fistulated animal that makes it impracticable for commercial use in farms -Ruminal cannula represents risk for animal health and question animal welfare -Animal have to be tied in a stall
WIRELESS INDWELLING RUMINAL SENSOR		-Real time and continuous pH measurement -Animals are free of movement -Only the oral introduction of the sensor is a technical act	-Location of the sensor have to be precise for valuable results

Table 5: Wireless pH bolus, its apparition in the litterature/experiment and measurement

WIRELESS PROBE	ARTICLES/RESEARCH TEAM	EXPERIMENT	MEASURES
E-cow rumen analyzer 	Holman et al (2013) Poster-University of Kentucky Department of Animal and Food Sciences, Lexington, KY	Acidosis challenge in 4 crossbred milking cows	
	Danscher et al (2015)	Probe in the ventral sack Weighted by 1kg stainless steel	Measures every min Averaged over 5 min Daily mean, minimum, maximum, time under curve below 6.0-5.8-5.6-5.2
SMAXTEC 	Gasteiner et al (2009)	-36 non rumen cannulated cows: 80 d starting 7 d prior calving -"off feed syndrome" -Rapid intake of high grain based concentrate - TMR vs separate offered feedstuff -DIM and adaptation of milking feed -Concentrate feeding -Diurnal reticulo-ruminal pattern.	Interval of 10min Daily mean, minimum, maximum, time below 6.3-6.0-5.8-5.5
	Gasteiner et al (2012)	Feeding trial with Holstein friesien dairy cows	40 days with 144 datasets/cow/day
	Klevenhusen et al (2014)	Manual insertion to ventral rumen via cannula pH adaptation to concentrate 50 day in use warranty Comparison of : **Indwelling wireless sensors**, **FRL via ruminator**, **PARL manually collected** via cannula at 4 moment of adaptation to concentrate (d0, d7=end of adaptation 60% concentrate, d14 and d34)	pH and tp° every 10 min
	Humer et al (2015) (Humer, Khol-Parisini, et al., 2015)	30 early lactating cows Measure taken until d80 postpartum	pH and tp° every 10 min Tme below 5.8-6.0 Tp° used for diurnal changes

	Humer et al (2015) (Humer, Ghareeb, et al., 2015)	9 Simmental – 4 Brown swiss Measure d7 prepartum to d8 postpartum	pH and tp° every 10 min then daily mean pH
	Khol-parisini et al (2015)	Feed experiment on Holstein dairy cattle	
Khane Ltd Ruminal Probe 	Kaur et al (2010)	Rumen fistulated sheep 3 series of 10d pH measurement with comparison (pH drift) betwen ruminal fluid collection+measurement and Khane Ltd ruminal probe	Measure every 20 min (ruminal fluid every 4h) Measure of Tp°-pH-Pressure Low pearson correlation + minor level of agreement
	Lin et al (2010)	9 rumen fistulated non-lactating Holstein*Jersey cows Comparison of feeding regime Measuring of pH drift	
	Kilic et al (2011	Review of wireless probe and Khane Ltd Probe	
	Lohölter et al (2013))	Measures of Ruminal pH, tp° and pressure via wireless device and comparison with manual measurement technic (cannulated Holsteins dairy cows) Evaluation of pH drift +	2 days of measures
WELL Cow system  **WELL Cow system**	Mottram et al (2008)	4 fistulated steers with feeding trial Comparison with hand collection of ruminal fluid (every hour)	Measure of Reticular pH Tp°>31°C=switch "ON" Callibration effective for 4 weeks measurement then 0.1pH point drfit per 30 days Tp° and pH every minute + averaged every 5 minutes
	Mottram et al – Case study	-Case of concentrate induced acidosis -High sugar grass acidosis -TMR feeding	On site diagnosis

	Mottram et al – Case study	-Robot milking feeding pattern -Traditional milking cycle with concentrate and grass -Rumen buffer supplementation	
	Zosel et al (2010)	Behaviour of the sensor during callibration + measures with fistulated cow free ranging dairy cow. Additional weight on the probe allow it to stay in the ventral sac of the rumen measuring only the ruminal pH. Evaluation of pH drift for validation of long-term pH measurement	
	Falk et al (2016)	Comparison of pH measurement of ebolus and LRCpH in ventral ruminal sac. 6 ruminally cannulated multiparous Holstein dairy cows LRCpH for ruminal pH Ebolus for reticular pH	
S.Sato's Wirless pH sensor 	Sato et al (2012)	Wirelless pH sensor + data measurement receiver + relay unit + PC with software for exploitation 4 non lactating rumen fistulated Holstein dairy cows with different diet strategies (influence of feeding strategies) + one non fistulated dry Holstein cow for long-term ruminal pH (peripartal pH measurement: 2 weeks before to 5 weeks after parturition) Comparison between spot sampling of ruminal fluid and continuous recording at the ventral sac of the rumen.	Spot sampling every 3h Continuous recording every min during the day for 2 weeks Good correlation between the 2 measurement strategies
"Free Range animal pH probe"	Peters et al (1997b)	Measure on gastric pH of 4 pengouins for 10 days	
	Enemark et al (2003)	Measure of pH in the antrum ruminis/reticulum in 2 cannulated non pregnant dairy cows Comparison point sampling (syringe) and continuous sampling and check of location of the probe 8 days experiment + calibration and check of eventual drift of measures	Measure pH and temperature every 30 second in continuous measurement + averaged every 15 min (before ruminal fluid sampling) Location of the probe=bottom of the reticulum.
SRS	Penner et al (2009)	-One rumen cannulated Friesian wether sheep to compare in vivo pH measurement SRS vs portable pH meter. -30 non cannulated female german merino sheep.	

Moow Rumen Bolus 	Hungary	Brand new: no literature/no constructor information	Long term measurement of ruminal pH and temperature

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BIBLIOGRAPHY

ALZAHAL, O., H. ALZAHAL, M.A. STEELE, M. VAN SCHAIK, I. KYRIAZAKIS, T.F. DUFFIELD, and B.W. MCBRIDE. 2011. The use of a radiotelemetric ruminal bolus to detect body temperature changes in lactating dairy cattle. *Journal of dairy science* 94: 3568–3574.

ALZAHAL, O., B. RUSTOMO, N.E. ODONGO, T.F. DUFFIELD, and B.W. MCBRIDE. 2007. Technical note: A system for continuous recording of ruminal pH in cattle. *Journal of animal science* 85: 213–217.

ALZAHAL, O., M.A. STEELE, E.V. VALDES, and B.W. MCBRIDE. 2009. Technical note: The use of a telemetric system to continuously monitor ruminal temperature and to predict ruminal pH in cattle. *Journal of dairy science* 92: 5697–5701.

ASCHENBACH, J.R., G.B. PENNER, F. STUMPFF, and G. GÄBEL. 2011. Ruminant Nutrition Symposium: Role of fermentation acid absorption in the regulation of ruminal pH. *Journal of animal science* 89: 1092–1107.

BANNINK, A., J. FRANCE, S. LOPEZ, W.J.J. GERRITS, E. KEBREAB, S. TAMMINGA, and J. DIJKSTRA. 2008. Modelling the implications of feeding strategy on rumen fermentation and functioning of the rumen wall. *Animal Feed Science and Technology* 143: 3–26.

BEAUCHEMIN, K.A., W.Z. YANG, and G. PENNER. 2006. Ruminal acidosis in dairy cows: Balancing effective fiber with starch availability. *In* Proc. of the 41st Annual Pacific Northwest Animal Nutrition Conference, Vancouver, BC, 5–17. Available at: ftp://s173-183-201-52.ab.hsia.telus.net/Inetpub/wwwroot/DairyWeb/Resources/PNWANC2006/Beauchemin.pdf [Accessed September 8, 2016].

BELBIS, G. 2007. Flore du rumen, origine, évolution, composition, conséquences physiopathologiques. École vétérinaire de Maisons-Alfort. Available at: http://www.theses.fr/2007PA123038 [Accessed October 15, 2016].

BLIGH, J., and J.W. HEAL. 1974. The use of radio-telemetry in the study of animal physiology. *Proceedings of the Nutrition Society* 33: 173–181.

BRAMLEY, E., I.J. LEAN, W.J. FULKERSON, M.A. STEVENSON, A.R. RABIEE, and N.D. COSTA. 2008. The Definition of Acidosis in Dairy Herds Predominantly Fed on Pasture and Concentrates1. *Journal of Dairy Science* 91: 308–321.

CALSAMIGLIA, S., M. BLANCH, A. FERRET, and D. MOYA. 2012. Is subacute ruminal acidosis a pH related problem? Causes and tools for its control. *Animal Feed Science and Technology* 172: 42–50.

CHAIDATE, I., C. SOMCHAI, N. JOS, and H. HENK. 2014. A cow-level association of ruminal pH on body condition score, serum beta-hydroxybutyrate and postpartum disorders in Thai dairy cattle. *Animal Science Journal* 85: 861–867.

COTTEE, G., I. KYRIAZAKIS, T.M. WIDOWSKI, M.I. LINDINGER, J.P. CANT, T.F. DUFFIELD, V.R. OSBORNE, and B.W. MCBRIDE. 2004. The effects of subacute ruminal acidosis on sodium bicarbonate-supplemented water intake for lactating dairy cows. *Journal of dairy science* 87: 2248–2253.

DADO, R.G., and M.S. ALLEN. 1993. Continuous Computer Acquisition of Feed and Water Intakes, Chewing, Reticular Motility, and Ruminal pH of Cattle. *Journal of Dairy Science* 76: 1589–1600.

DANSCHER, A.M., S. LI, P.H. ANDERSEN, E. KHAFIPOUR, N.B. KRISTENSEN, and J.C. PLAIZIER. 2015. Indicators of induced subacute ruminal acidosis (SARA) in Danish Holstein cows. *Acta Veterinaria Scandinavica* 57: 1.

DIRKSEN, G. 1975. Eine lenkbare Sonde zur gezielten Entnahme von Pansensaft beim Rind. *Tierarztliche Umschau*. Available at: http://agris.fao.org/agris-search/search.do?recordID=US201302759104 [Accessed November 6, 2016].

DIRKSEN, G.U., H.G. LIEBICH, and E. MAYER. 1985. Adaptive changes of the ruminal mucosa and their functional and clinical significance. *Bovine Pract* 20: 116–120.

DUFFIELD, T., J.C. PLAIZIER, A. FAIRFIELD, R. BAGG, G. VESSIE, P. DICK, J. WILSON, ET AL. 2004. Comparison of techniques for measurement of rumen pH in lactating dairy cows. *Journal of Dairy Science* 87: 59–66.

DYE, T.K., C.J. RICHARDS, L.O. BURCIAGA-ROBLES, C.R. KREHBIEL, and D.L. STEP. 2007. Efficacy of rumen temperature boluses for health monitoring. *In* JOURNAL OF DAIRY SCIENCE, 255–256. AMER DAIRY SCIENCE ASSOC 1111 N DUNLAP AVE, SAVOY, IL 61874 USA.

ENEMARK, J.M. 2008. The monitoring, prevention and treatment of sub-acute ruminal acidosis (SARA): A review. *The Veterinary Journal* 176: 32–43.

ENEMARK, J.M.D., G. PETERS, and R.J. JØRGENSEN. 2003. Continuous monitoring of rumen pH–a case study with cattle. *Journal of Veterinary Medicine Series A* 50: 62–66.

FALK, M., A. MÜNGER, and F. DOHME-MEIER. 2016. Technical note: A comparison of reticular and ruminal pH monitored continuously with 2 measurement systems at different weeks of early lactation. *Journal of dairy science* 99: 1951–1955.

FALLON, R.J., and P.A.M. ROGERS. 2001. Evaluation of Rumen Boluses as a Method of Electronic Animal Identification. *Irish Journal of Agricultural and Food Research* 40: 161–168.

GÄBEL, G., and J.R. ASCHENBACH. 2007. SCFA, protons and ruminal epithelium: The good, the bad, the barrier. *Production Diseases in Farm Animals. M. Fürll, ed. Universität Leipzig, Leipzig, Germany*291.

GARRETT, E.F., M.N. PEREIRA, K.V. NORDLUND, L.E. ARMENTANO, W.J. GOODGER, and G.R. OETZEL. 1999. Diagnostic Methods for the Detection of Subacute Ruminal Acidosis in Dairy Cows. *Journal of Dairy Science* 82: 1170–1178.

GASTEINER, J., M. FALLAST, S. ROSENKRANZ, J. HÄUSLER, K. SCHNEIDER, and T. GUGGENBERGER. 2009. Measuring rumen pH and temperature by an indwelling and wireless data transmitting unit and application under different feeding conditions. *In* Proc. Fourth Eur. Conf. Prec. Livestock Farming, Wageningen, the Netherlands, 127–133. Available at: http://www.wageningenacademic.com/doi/pdf/10.3920/978-90-8686-663-2#page=128 [Accessed September 8, 2016].

GASTEINER, J., and T. GUGGENBERGER. 2013. Long-term measurement of reticuloruminal pH-value in dairy cows under practical conditions by an indwelling and wireless data transmitting unit. *In* 22th International Scientific Symposium on Nutrition of Farm Animals' Zadravec-Erjavec Days',, Radenci, 14-15 Nov 2013, Kmetijsko gozdarska zbornica Slovenije, Murska Sobota (Slovenia); Kmetijsko gozdarski zavod, Murska Sobota (Slovenia). Available at: http://www.smaxtec-animalcare.com/fileadmin/user_upload/documents/Gesamte_Publikationsliste/smaxtec_Publikationsliste.pdf [Accessed September 8, 2016].

GASTEINER, J., T. GUGGENBERGER, J. HÄUSLER, and A. STEINWIDDER. 2012. Continuous and Long-Term Measurement of Reticuloruminal pH in Grazing Dairy Cows by an Indwelling and Wireless Data Transmitting Unit. *Veterinary Medicine International* 2012: . Available at: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3504470/ [Accessed September 8, 2016].

GASTEINER, J., T. GUGGENBERGER, L. VARGA, and R.D. OLLHOFF. 2015. Continuous and long term measurement of reticuloruminal pH in crossbreed dairy cows in Brazil by an indwelling and wireless data transmitting unit. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia* 67: 622–626.

GAUGHAN, J. 2010. Report to Kahne Limited, Evaluation of the KB1000 Series Bolus. *School of Animal Studies, The University of Queensland, Gatton QLD, Australia*.

GEISHAUSER, T. 1993. An instrument for collection and transfer of ruminal fluid and for administration of water soluble drugs in adult cattle. *Bovine Practitioner* 27: 38–38.

GEISHAUSER, T., and A. GITZEL. 1996. A comparison of rumen fluid sampled by oro-ruminal probe versus rumen fistula. *Small Ruminant Research* 21: 63–69.

GOOPY, J.P., and R. WOODGATE. 2009. Short-term validation of a rumen indwelling pH meter. *In* Recent dvances in Animal Nutrition—Australia. 20th Biennial Conference, University of New England, Armidale, NSW, Australia, 175.

GOZHO, G.N., J.C. PLAIZIER, D.O. KRAUSE, A.D. KENNEDY, and K.M. WITTENBERG. 2005. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *Journal of Dairy Science* 88: 1399–1403.

GRÖHN, Y.T., and M.L. BRUSS. 1990. Effect of Diseases, Production, and Season on Traumatic Reticuloperitonitis and Ruminal Acidosis in Dairy Cattle. *Journal of Dairy Science* 73: 2355–2363.

GRUMMER, R.R. 1995. Impact of changes in organic nutrient metabolism on feeding the transition dairy cow. *Journal of animal science* 73: 2820–2833.

GUATTEO, R. 2013. Acisdose aiguë et chronique du rumen. Polycopié d'enseignement ONIRIS, UV82, **11**, 22pp.

GUIDE, D.LRC.U. 2007. DASCOR™ DATA LOGGER DATA LOGGERS. Available at: http://anscitech.com/new/uploads/down/a13b9d0ca77603f3b2b1242b27c29856.pdf [Accessed September 8, 2016].

HARTNELL, G.F., and L.D. SATTER. 1979. Determination of rumen fill, retention time and ruminal turnover rates of ingesta at different stages of lactation in dairy cows. *Journal of Animal Science* 48: 381–392.

HOFIREK, B., D. HAAS, and OTHERS. 2001. Comparative studies of ruminal fluid collected by stomach tube or by puncture of the caudoventral ruminal sac. *Acta Veterinaria Brno* 70: 27–33.

HOLLBERG, W. 1984. Vergleichende Untersuchungen von mittels Schambye-Soerensen-Sonde oder durch Punktion des kaudoventralen Pansensacks gewonnene Pansensaftproben. *Deutsche Tierärztliche Wochenschrift*. Available at: http://agris.fao.org/agris-search/search.do?recordID=DE19850100768 [Accessed November 22, 2016].

HUMER, E., K. GHAREEB, H. HARDER, E. MICKDAM, A. KHOL-PARISINI, and Q. ZEBELI. 2015. Peripartal changes in reticuloruminal pH and temperature in dairy cows differing in the susceptibility to subacute rumen acidosis. *Journal of dairy science* 98: 8788–8799.

HUMER, E., A. KHOL-PARISINI, L. GRUBER, J. GASTEINER, S.M. ABDEL-RAHEEM, and Q. ZEBELI. 2015. Long-term reticuloruminal pH dynamics and markers of liver health in early-lactating cows of various parities fed diets differing in grain processing. *Journal of dairy science* 98: 6433–6448.

JOHNSON, V.W., and J.D. SUTTON. 1968. The continuous recording of the pH in the bovine rumen. *British Journal of Nutrition* 22: 303–306.

KAUFMANN, W., H. HAGEMEISTER, and G. DIRKSEN. 1980. Adaptation to changes in dietary composition, level and frequency of feeding. *In* Digestive physiology and metabolism in ruminants, 587–602. Springer. Available at: http://link.springer.com/chapter/10.1007/978-94-011-8067-2_28 [Accessed October 31, 2016].

KAUR, R., S.C. GARCIA, A. HORADAGODA, and W.J. FULKERSON. 2010. Evaluation of rumen probe for continuous monitoring of rumen pH, temperature and pressure. *Animal Production Science* 50: 98–104.

KHAFIPOUR, E., D.O. KRAUSE, and J.C. PLAIZIER. 2009. A grain-based subacute ruminal acidosis challenge causes translocation of lipopolysaccharide and triggers inflammation. *Journal of Dairy Science* 92: 1060–1070.

KHOL-PARISINI, A., E. HUMER, Ö. SIZMAZ, S.M. ABDEL-RAHEEM, L. GRUBER, J. GASTEINER, and Q. ZEBELI. 2015. Ruminal disappearance of phosphorus and starch, reticuloruminal pH and total tract nutrient digestibility in dairy cows fed diets differing in grain processing. *Animal Feed Science and Technology* 210: 74–85.

KILIC, U. 2011. Use of wireless rumen sensors in ruminant nutrition research. *Asian Journal of Animal Sciences* 5: 46–55.

KLEEN, J.L., G.A. HOOIJER, J. REHAGE, and J.P.T.M. NOORDHUIZEN. 2003. Subacute Ruminal Acidosis (SARA): a Review. *Journal of Veterinary Medicine Series A* 50: 406–414.

KLEVENHUSEN, F., P. POURAZAD, S.U. WETZELS, M. QUMAR, A. KHOL-PARISINI, and Q. ZEBELI. 2014. Technical note: Evaluation of a real-time wireless pH measurement system relative to intraruminal differences of digesta in dairy cattle. *Journal of animal science* 92: 5635–5639.

KOHN, R.A., and T.F. DUNLAP. 1998. Calculation of the buffering capacity of bicarbonate in the rumen and in vitro. *Journal of Animal Science* 76: 1702–1709.

KRAUSE, K.M., and G.R. OETZEL. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Animal Feed Science and Technology* 126: 215–236.

LAFLIN, S.L., and D.P. GNAD. 2008. Rumen Cannulation: Procedure and Use of a Cannulated Bovine. *Veterinary Clinics of North America: Food Animal Practice* 24: 335–340.

LETTAT, A., P. NOZIÈRE, M. SILBERBERG, D.P. MORGAVI, C. BERGER, and C. MARTIN. 2010. Experimental feed induction of ruminal lactic, propionic, or butyric acidosis in sheep. *Journal of animal science* 88: 3041–3046.

LI, S., A.M. DANSCHER, and J.C. PLAIZIER. 2013. Subactue Ruminal Acidosis (SARA) in dairy cattle: new developments in diagnostic aspects and feeding management. *Canadian Journal of Animal Science* 94: 353–364.

LOHÖLTER, M., R. REHAGE, U. MEYER, P. LEBZIEN, J. REHAGE, and S. DÄNICKE. 2013. Evaluation of a device for continuous measurement of rumen pH and temperature considering localization of measurement and dietary concentrate proportion. *Applied Agricultural and Forestry Research*61. (63) 61-68

LUAN, S., K. COWLES, M.R. MURPHY, and F.C. CARDOSO. 2016. Effect of a grain challenge on ruminal, urine, and fecal pH, apparent total-tract starch digestibility, and milk composition of Holstein and Jersey cows. *Journal of dairy science* 99: 2190–2200.

MACKIE, R.I., and F.M. GILCHRIST. 1979. Changes in lactate-producing and lactate-utilizing bacteria in relation to pH in the rumen of sheep during stepwise adaptation to a high-concentrate diet. *Applied and environmental microbiology* 38: 422–430.

MARDEN, J.P., C. BAYOURTHE, F. ENJALBERT, and R. MONCOULON. 2005. A new device for measuring kinetics of ruminal pH and redox potential in dairy cattle. *Journal of dairy science* 88: 277–281.

MCARTHUR, J.M., and J.E. MILTIMORE. 1968. Continuous recording of the in vivo rumen pH in fistulated cattle. *Canadian Journal of Animal Science* 48: 237–240.

MONROE, C.F., and A.E. PERKINS. 1939. A Study of the pH Values of the Ingesta of the Bovine Rumen. *Journal of Dairy Science* 22: 983–991.

MORGANTE, M., C. STELLETTA, P. BERZAGHI, M. GIANESELLA, and I. ANDRIGHETTO. 2007. Subacute rumen acidosis in lactating cows: an investigation in intensive Italian dairy herds. *Journal of Animal Physiology and Animal Nutrition* 91: 226–234.

MOTTRAM, T., J. LOWE, M. MCGOWAN, and N. PHILLIPS. 2008. Technical note: A wireless telemetric method of monitoring clinical acidosis in dairy cows. *computers and electronics in agriculture* 64: 45–48.

MOTTRAM, T.T.F. 8.1. Dairy farm evaluation of rumen pH bolus data: identifying the benefits. *edited by: Ilan Halachmi*293.

NOCEK, J.E., J.G. ALLMAN, and W.P. KAUTZ. 2002. Evaluation of an Indwelling Ruminal Probe Methodology and Effect of Grain Level on Diurnal pH Variation in Dairy Cattle. *Journal of Dairy Science* 85: 422–428.

NORDLUND, K.V., E.F. GARRETT, and G.R. OETZEL. 1995. HERD-BASED RUMENOCENTESIS-A CLINICAL APPROACH TO THE DIAGNOSIS OF SUB ACUTE RUMEN ACIDOSIS. Available at: http://en.journals.sid.ir/ViewPaper.aspx?ID=134047 [Accessed November 7, 2016].

O'GRADY, L., M.L. DOHERTY, and F.J. MULLIGAN. 2008. Subacute ruminal acidosis (SARA) in grazing Irish dairy cows. *The Veterinary Journal* 176: 44–49.

OWENS, F.N., D.S. SECRIST, W.J. HILL, and D.R. GILL. 1998. Acidosis in cattle: a review. *Journal of animal science* 76: 275–286.

PENNER, G.B., and J.R. ASCHENBACH. 2011. Mechanisms of acid absorption in the rumen and impacts on subacute sumen acidosis. Available at: http://www.diss.fu-berlin.de/docs/servlets/MCRFileNodeServlet/FUDOCS_derivate_000000002305/9penner.pdf [Accessed September 8, 2016].

PENNER, G.B., J.R. ASCHENBACH, G. GÄBEL, and M. OBA. 2009. Technical note: Evaluation of a continuous ruminal pH measurement system for use in noncannulated small ruminants. *Journal of animal science* 87: 2363–2366.

PENNER, G.B., K.A. BEAUCHEMIN, and T. MUTSVANGWA. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. *Journal of Dairy Science* 89: 2132–2140.

PENNER, G.B., K.A. BEAUCHEMIN, and T. MUTSVANGWA. 2007. Severity of Ruminal Acidosis in Primiparous Holstein Cows During the Periparturient Period. *Journal of Dairy Science* 90: 365–375.

PLAIZIER, J.C., D.O. KRAUSE, G.N. GOZHO, and B.W. MCBRIDE. 2008. Subacute ruminal acidosis in dairy cows: The physiological causes, incidence and consequences. *The Veterinary Journal* 176: 21–31.

RADOSTITS, O.M., D.C. BLOOD, and C.C. GAY. 1994. Acute carbohydrate engorgement of ruminants (rumen overload). *Veterinary Medicine. A textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 8th ed. Baillière Tindall, London*262–269.

REIS, L.F., A.H. MINERVINO, C.A. ARAÚJO, R.S. SOUSA, F.L. OLIVEIRA, F.A. RODRIGUES, E. MEIRA-JÚNIOR, ET AL. 2014. Comparison of rumen fluid pH by continuous telemetry system and bench pH meter in sheep with different ranges of ruminal pH. *The Scientific World Journal* 2014: . Available at: http://www.hindawi.com/journals/tswj/2014/195782/abs/ [Accessed September 8, 2016].

RUSTOMO, B., O. ALZAHAL, J.P. CANT, M.Z. FAN, T.F. DUFFIELD, N.E. ODONGO, and B.W. MCBRIDE. 2006. Acidogenic value of feeds II. Effects of rumen acid load from feeds on dry matter intake, ruminal pH, fibre degradability and milk production in the lactating dairy cow. *Canadian journal of animal science* 86: 119–127.

SATO, S. 2015. Subacute ruminal acidosis (SARA) challenge, ruminal condition and cellular immunity in cattle. *Japanese Journal of Veterinary Research* 63: S25–S36.

SATO, S., A. IKEDA, Y. TSUCHIYA, K. IKUTA, I. MURAYAMA, M. KANEHIRA, K. OKADA, and H. MIZUGUCHI. 2012. Diagnosis of subacute ruminal acidosis (SARA) by continuous reticular pH measurements in cows. *Veterinary research communications* 36: 201–205.

SATO, S., A. KIMURA, T. ANAN, N. YAMAGISHI, K. OKADA, H. MIZUGUCHI, and K. ITO. 2012. A radio transmission pH measurement system for continuous evaluation of fluid pH in the rumen of cows. *Veterinary Research Communications* 36: 85–89.

SEHGAL, P., and K. SINGH. ANAEROBIC RUMEN FUNGI: POTENTIAL AND APPLICATIONS. 2009. Available at: http://users.aber.ac.uk/gwg/pdf/Nagpal-AnaerobicFungiRev08.pdf [Accessed October 16, 2016].

SHEN, J.S., Z. CHAI, L.J. SONG, J.X. LIU, and Y.M. WU. 2012. Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows. *Journal of dairy science* 95: 5978–5984.

SMITH, V.R. 1941. In Vivo Studies of Hydrogen Ion Concentrations in the Rumen of the Dairy Cow. *Journal of Dairy Science* 24: 659–665.

STEELE, M.A., O. ALZAHAL, S.E. HOOK, J. CROOM, and B.W. MCBRIDE. 2009. Ruminal acidosis and the rapid onset of ruminal parakeratosis in a mature dairy cow: a case report. *Acta Veterinaria Scandinavica* 51: 1.

STEELE, M.A., J. CROOM, M. KAHLER, O. ALZAHAL, S.E. HOOK, K. PLAIZIER, and B.W. MCBRIDE. 2011. Bovine rumen epithelium undergoes rapid structural adaptations during grain-induced subacute ruminal acidosis. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 300: R1515–R1523.

STEINER, S., A. NEIDL, N. LINHART, A. TICHY, J. GASTEINER, K. GALLOB, W. BAUMGARTNER, and T. WITTEK. 2014. Randomised prospective study compares efficacy of five different stomach tubes for rumen fluid sampling in dairy cows. *Veterinary Record*vetrec-2014-102399.

STORM, A.C., and N.B. KRISTENSEN. 2010. Effects of particle size and dry matter content of a total mixed ration on intraruminal equilibration and net portal flux of volatile fatty acids in lactating dairy cows. *Journal of Dairy Science* 93: 4223–4238.

TAFAJ, M., B. JUNCK, A. MAULBETSCH, H. STEINGASS, H.-P. PIEPHO, and W.D.P.D. H.C. 2004. Digesta characteristics of dorsal, middle and ventral rumen of cows fed with different hay qualities and concentrate levels. *Archives of Animal Nutrition* 58: 325–342.

TAJIK, J., G.R. MOHAMMADI, M. RAD, and A. BARATI. 2010. Hemorrhagic bowel syndrome in dairy cattle in Iran: a case report. *Iranian Journal of Veterinary Research* 11: 180–183.

ZOSEL, J., H. KADEN, G. PETERS, M. HOFFMANN, P. RUDISCH, L. JÄKEL, G. LAUCKNER, A. GODRIAN, U. GUTH. 2010. Continuous long-term monitoring of ruminal pH. *Sensors and Actuators B: Chemical* 144: 395–399.

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