Utilization of wireless sensor bolus technology: Evaluating rumen pH dynamics and production metrics in dairy cows subjected to SARA-inducing diets

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Abstract

With the intensification of the cattle farming system, a high amount of easily fermentable carbohydrates is being fed to increase production and fulfill the nutrient requirement of the cows. Feeding high amounts of easily degradable carbohydrates such as starch can reduce the rumen pH of cows, resulting in the condition subacute ruminal acidosis (SARA). SARA has a great impact on the production, health, and welfare of the cow, leading to a decreased economy for the farmer. Hence, the diagnosis of SARA is important in the early stages. Diagnosis of SARA is done on the basis of rumen pH. The technology used previously for the determination of rumen pH was highly expensive and invasive and did not provide accurate data on rumen pH. With the advancement of technology, a wireless sensor bolus was developed, which is non-invasive, farmer-friendly, and provides continuous monitoring of rumen pH. The efficacy of this technology and its ability to detect minute variations in rumen pH are still in question. Moreover, due to its battery lifespan and drift over time, the use of this technology in real farm conditions is highly expensive and unfeasible. Considering it, an alternative indicator of SARA must be found, which should be noninvasive, cheap, and accurate. Hence, this study aims to monitor the effect of SARA inducing diet on rumen pH using a wireless sensor bolus, together analyze the effect of inducing diet on milk yield (MY), dry matter intake (DMI), and milk parameters, such as milk fat, milk protein and milk fat to protein ratio.

A continous study was performed on 25 primiparous cows in 3 periods lasting for 52 days. Rumen sensor bolus was applied on the first day of the study. Then, a low concentrate diet containing a 48:52 concentrate-to-silage ratio was fed for the first 9 days (CONT1 period). Then, from day 10 to 32, the SARA inducing diet containing a 64:36 concentrate-to-silage ratio was fed (SAR2 period). Finally, the study was ended by feeding the same CONT1 diet from day 33 to day 52 (RECOV3). During the period, rumen pH was monitored every day throughout the period. Daily DMI and MY was measured throughout the period. A sampling of milk was done for the last two days of each period to analyze the milk parameters.

Variation in rumen pH across periods and fluctuations in rumen pH within a day was seen in this study. A significant drop in mean rumen pH and an increase in the mean duration of a cow with rumen pH 6 was reported in the SAR2 period, which instantly reverted back to normal when the low concentrate diet was fed during the RECOV3 period. Using the rumen pH metrics, cows that were likely to be in SARA were identified. No changes were observed in MY throughout the periods. Significant differences in results were observed for DMI and milk parameters. Considering the results of this study and the outcomes from other studies, it was concluded that the rumen sensor bolus was able to detect minute changes in rumen pH throughout the day, making it an efficient device for monitoring rumen pH in cows. Additionally, the use of other parameters, such as DMI, MY, and milk parameters, as indicators of SARA demands more studies that include the factors that have a direct effect on those parameters.

Keywords: Subacute ruminal acidosis, wireless rumen sensor bolus, rumen, milk production

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Abbreviations

DIM	Days In Milk
DM	Dry matter
DMI	Dry Matter Intake
ECM	Energy-Corrected Milk
FAs	Fatty Acids
FPR	Fat-to-Protein Ratio
IQR	Interquartile Range
MUFA	Monounsaturated Fatty Acid
MY	Milk Yield
NDF	Neutral Detergent Fiber
OECD-FAO	Organization for Economic Co-operation and Development – Food and Agriculture Organization
PUFA	Polyunsaturated Fatty Acid
RA	Ruminal Acidosis
SARA	Sub-acute Ruminal Acidosis
SFA	Saturated Fatty Acid
SHB	Swedish Holstein Breeds
SRB	Swedish Red Breeds
TDM	Total dry matter
TMR	Total Mixed Ration
UFA	Unsaturated Fatty Acid
VFA	Volatile Fatty Acids
VMS	Voluntary Milking System
pKa	Dissociation Constant

1. Introduction

With the increase in the global population, there is a concurrent rise in demand for food (van Dijk et al. 2021). According to the Organization for Economic Co-operation and Development and Food and Agricultural Organization (OECD-FAO), by 2032, per capita demand for meat will increase by 2% over the 2020-2022 base level, and by 2031, overall per capita demand for dairy will increase by 0.4%, 2% and 1.5% in high income, low-middle income, and low-income countries respectively over 2021 base level (OECD/FAO 2022). So, to increase productivity and fulfill the demand of the population, an intensified cattle farming system has been implemented. But with the intensified system of farming, challenges to meet the nutritional requirement of the cows also increases (Sundrum 2015). Hence, feeding high amounts of rapidly fermentable carbohydrates such as starch and other concentrates to meet the energy requirement of the highly-producing dairy cattle is not an unusual practice. However, feeding high concentrate diets may result in alteration in volatile fatty acid (VFA) production and ultimately results in a decrease in rumen pH, leading to conditions like ruminal acidosis (RA) and subacute ruminal acidosis (SARA) (Plaizier et al. 2008; McAuliffe et al. 2022).

SARA is one of the significant problems in the dairy industry, which has highly impacted the welfare of the cows and also accounts for the economic loss of approximately $400/cow/lactation, as observed in a study conducted on dairy farms in Central New York (Plaizier et al. 2008). Studies have reported the incidence of SARA in cows to be ranging between 14% to 28% in different parts of the world (Tajik et al. 2009; Stefańska et al. 2017; Vallejo-Timarán et al. 2020). Though SARA is an economically and epidemiologically significant metabolic disease, there is still no proper definition of SARA. It is being defined based on rumen pH but differs significantly between studies. Among all, it is widely accepted that SARA occurs when the rumen pH ranges between 5.5 to 5.8 for several hours a day (Beauchemin et al. 2003; Humer et al. 2018). Being subclinical, this disease is difficult to diagnose on farms. Moreover, the clinical signs seen are not specific to SARA (Krause & Oetzel 2005). So, SARA is taken as a syndrome with a mixture of many signs rather than a disease (Khafipour et al. 2009a). Usually, those signs are seen during the late stage of the disease when cows are already in bad health (Coppa et al. 2023). So many cows suffering from SARA go undetected.

Currently, the standard test to diagnose SARA is done by monitoring ruminal pH (Enemark 2008). Earlier, the pH of rumen fluids was monitored using rumenocentesis, rumen cannula technique, oral stomach tube technique, and indwelling rumen pH data logger method to access the rumen pH. These methods to determine the rumen pH are either highly invasive or inaccurate and highly impact the welfare of the cows. Except for the indwelling rumen pH method, other methods could not provide continuous data for rumen pH analysis (Danscher et al. 2015). Ruminal pH fluctuates along the day and varies according to the diet composition, feed intake rate, fermentation rate, VFA production and absorption rates, and gastrointestinal motility rate (Dijkstra et al. 2020). Individual variation between animals fed the same diet also hugely impacts the pH of the rumen (Brown et al. 2000). Considering the high variation of rumen pH within a day, taking a rumen fluid sample intermittently to determine the rumen pH may give biased results. So, continuous monitoring of rumen fluid must be done throughout the day to accurately determine the variation in rumen pH and diagnose SARA in the early stage (Enemark 2008; Celi et al. 2019). With the advancement in technology, continuous monitoring of the rumen pH is facilitated by using the wireless rumen sensor bolus, which can help identify the cows in SARA, however, studies on the efficacy of this sensor bolus in identifying the rumen pH variation are scarce.

The use of wireless rumen bolus to monitor the rumen pH is feasible in study and research farms, but its use on real farms is in question. This device has a low lifespan and drifts over time (Andersson et al. 2018; Villot et al. 2018; Han et al. 2022). So, the use of this sensor in commercial farms becomes economically expensive. Considering the economic aspect and feasibility of the farm, other alternatives to rumen pH must be found to diagnose SARA. Those indicators should be non-invasive, affordable, and reliable enough to diagnose SARA accurately. Studies have been done to relate the relationship between rumen pH and milk yield, milk constituents, and Dry matter intake (DMI) (Enemark et al. 2004; Ospina et al. 2010; Danscher et al. 2015) but those studies have incomplete or contradictory outcomes.

2. Aim and hypotheses

This study aims to monitor the effect of a SARA-inducing diet on rumen pH metrics of dairy cows using a wireless rumen bolus and identify the cows that are most likely to be in SARA. Additionally, determine the effects of dietary periods on milk yield (MY), milk constituents, and DMI. This study hypothesizes that feeding a SARA-inducing diet decreases the rumen pH of a cow below 6 and maintains it for several hours a day, resulting in the development of SARA in susceptible cows. It is further hypothesized that fluctuation in rumen pH caused by SARA inducing diet can significantly impact DMI, milk yield, and milk parameters.

3. Literature review

3.1 Rumen and ruminal pH

Rumen is the first and largest part of the fore stomach in ruminants, where the microbial fermentation of feed occurs. Adult cow's rumen has a capacity of holding approximately 90 - 180 liters according to the size of the cow. Normally, the rumen pH of cows fed with a grass diet ranges between 6 to 7, and the cows fed with a high amount of concentrate ranges between 5.6 to 6.2 (Church 1993; Grünberg & Constable 2009; Evans & Hooser 2010). In the rumen, microbial fermentation of feed occurs, which leads to the production of VFA and lactic acid in minor quantities. Major VFAs produced in rumen are acetic acid, butyric acid, and propionic acid. These VFAs causes a decrease in ruminal pH in cows. Failing in absorption of VFA by the rumen and the inability of the buffering system to buffer the rumen pH leads to a prolonged decrease in ruminal pH, leading to conditions like SARA and RA (Dijkstra et al. 2012).

The rumen has a complex microbial ecology. They consist of mixtures of archaea, bacteria, virus and eukaryotes as fungi and protozoos. This complex microbiome helps in the degradation of the feed and, as a final products of the anaerobic fermentation, provides the host nutrients that are easily absorbable. Acetate, propionate, and butyrate are the major final forms of dietary carbohydrate fermentation. The process of degradation of carbohydrates to VFAs is shown in Figure 1. Production of acetate is facilitated by cellulolytic bacteria, *Acetobacterium woodi,* and *Entodinium caudatum* facilitate acetate production, whereas *Butyrivibrio fibrisolvens* and some *Clostridium* spp facilitate butyrate production. *Selenomonas ruminantum* and *Megasphaera elsdenii* help produce propionate (Castillo et al. 2013). The ecology of rumen is highly dependent on the ruminal pH. Some fiber-degrading bacteria, such as *Fibrobacter spp, Ruminococcus spp,* and *Prevotella spp*, prefer to grow in a rumen pH above 6. In contrast, starch-degrading bacteria prefer a slightly acidic environment than fiber-degrading bacteria (Slyter et al. 1970). When cows are fed high amounts of easily fermentable carbohydrates such as starch, amylolytic bacteria proliferate; thus, the production of VFA increases, causing a decrease in rumen pH (Chen et al. 2016). If the decrease in rumen pH is not halted by the absorptive and buffering mechanism

of rumen, then it can lead to the condition SARA. The decrease in rumen pH favors the further growth of *Streptococcus bovis* and *Lactobacillus* spp. This bacteria activates the enzyme lactate dehydrogenase. Lactate dehydrogenase changes the route of VFA production to lactate production. Unlike VFAs, lactic acid is a stronger acid, and it can drop the pH of the rumen further below 5.5, causing peracute or acute RA in cows (Hernández et al. 2014). Some protozoa, *Megasphaera elsdenii* and *Selenomonas ruminantium*, can convert lactate to VFAs, but the production of lactate surpasses the utilization rate of lactate by these protozoa, causing failure to restrain the decreasing rumen pH (Aikman et al. 2011; Cabral & Weimer 2024).

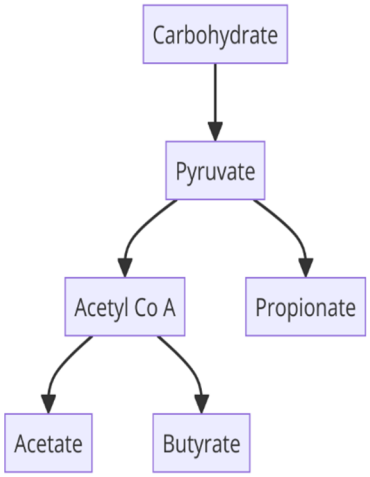


Figure 1. Degradation of Carbohydrate to VFAs in rumen (Sjaastad 2016)

3.2 Regulation of ruminal pH

pH of the rumen is determined by the rate at which the microbial fermentation of the feed occurs in the rumen and the rate at which the produced fermentation product, i.e., VFA, leaves the rumen, primarily by absorption from the rumen wall and some by passing to the rear gut, or by getting neutralized by buffer (Dijkstra et al. 2020). The mechanisms involved in ruminal pH regulation are illustrated in Figure 2. When production of VFA exceeds the rate at which VFA is removed from the rumen, accumulation of VFA in the rumen occurs, leading to a decrease in ruminal pH (Dijkstra et al. 2012). Decrease in ruminal pH is countered by removing the produced acids from the rumen or by buffering it (Kohn & Dunlap 1998; Yang & Beauchemin 2007; Dijkstra et al. 2020).

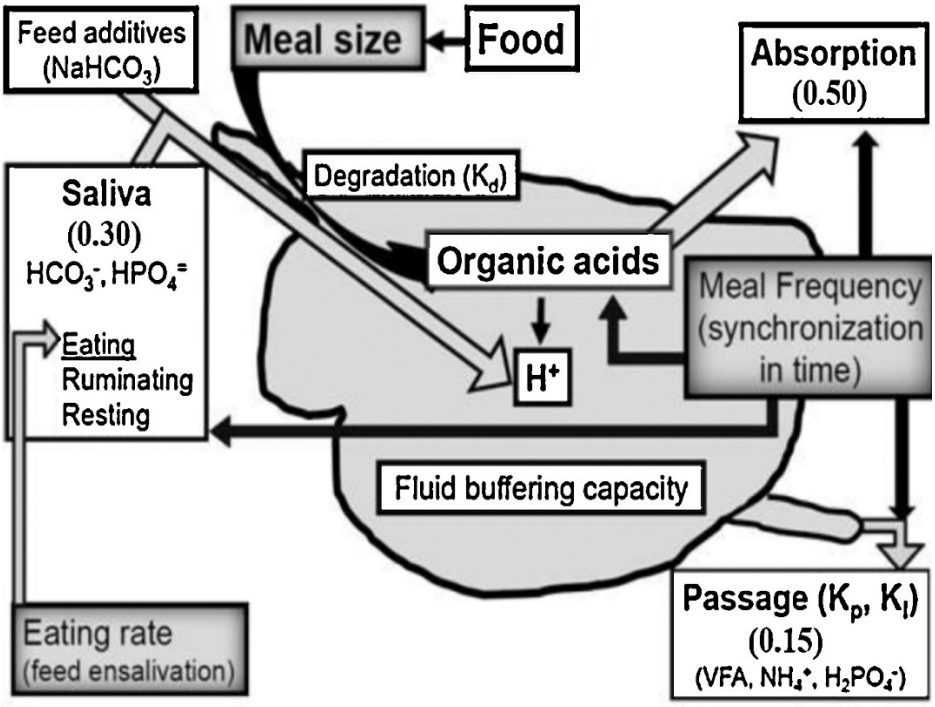


Figure 2. The mechanism involved in the regulation of rumen pH (González et al. 2012)

3.2.1 Regulation by removing VFA from rumen

Removal of VFA from the rumen occurs by various means. A major route for removal of VFA occurs by absorption of it from the rumen wall. In adult dairy cows, approximately about 60% to 70% of the VFA is absorbed from rumen walls, and other portions get moved toward the distal part of the gastrointestinal tract (Aschenbach et al. 2011; Stumpff 2018). Absorption of VFA in the rumen occurs by different mechanisms, such as passive diffusion, bicarbonate-dependent active transport mechanism, carrier protein-mediated active transport, and bicarbonate-independent transport mechanism (Dijkstra et al. 2012). Passive diffusion is the predominant mechanism for absorbing VFA in rumen.

The majority of the VFA produced in a rumen is in a dissociated form, which is usually absorbed by a bicarbonate-dependent transport mechanism. This mechanism involves the exchange of bicarbonate ions (HCO_3^-) present in the ruminal epithelial cell to VFA anions present in the rumen (Gäbel et al. 2002). This leads to the addition of bicarbonate to the rumen and a decrease of VFA from the rumen. Hence, this mechanism also plays an important role in the regulation of rumen pH, as added bicarbonate has a buffering capacity, which can buffer the acidic environment of the rumen. The bicarbonate-dependent active transport mechanism is regulated by rumen pH and ruminal VFA concentration. An increase in VFA concentration and a decrease in rumen pH enhances VFA absorption through this mechanism (Aschenbach et al. 2009). Another mechanism, which is the main route for the absorption of VFA, is passive diffusion. This mechanism

involves the movement of VFA from a higher concentration gradient to a lower concentration gradient across the rumen epithelium. Usually, undissociated VFA follows this mechanism to get absorbed through the rumen wall. Undissociated VFAs are very few in amount during the normal state of rumen pH. As the pKa value for VFA is around 4.8 when the rumen pH decreases below 5.8, the production of undissociated VFA increases (Oetzel 2007; Dijkstra et al. 2012). Hence, a concentration of undissociated VFA increases in the ruminal lumen, which causes an increase in concentration gradient across the rumen epithelium, leading to an increase in VFA absorption through this mechanism. Other mechanisms, such as carrier protein-mediated transport and bicarbonate-independent mechanisms, also add up to the absorption of VFA from the rumen, but compared to other methods, less VFA is absorbed from the rumen through this mechanism. Bicarbonate-independent mechanisms are important when VFA concentration is extremely high in rumen. This mechanism uses a system of transport that does not use bicarbonate as a co-transport ion. Rather, it uses chloride ions or sodium ions to exchange VFA anion from rumen (Aschenbach et al. 2011). Unlike other methods, protein-mediated transport of VFA occurs either by facilitated diffusion or active transport mechanism. This mechanism is facilitated by a special carrier protein. This protein assists in the transportation of dissociated VFA along the concentration gradient or against the concentration gradient from rumen towards ruminal epithelial cells. Usually, carrier protein works along with a bicarbonate-independent mechanism. Hence, the bicarbonate-independent system and the protein-mediated system move side by side to absorb and regulate VFA concentration in the rumen (Aschenbach et al. 2011).

Rumen epithelium is the site where all the mechanisms that are involved in the absorption of VFA occur. So, rumen epithelium is an important constituent for absorption and regulation of ruminal pH. The development of rumen epithelium is powered mainly by butyric acid (Kristensen 2005). Thus, providing a feed mixture that facilitates the production of butyrate can highly assist in the development of rumen epithelium (Odongo et al. 2006). Moreover, the higher incidence of SARA in primiparous cows compared to multiparous cows is also due to the underdeveloped rumen epithelium (Ismail et al. 2019). Overall, the developmental stage of rumen epithelium, the efficiency of the buffering system of the rumen, and the efficacy of transport mechanisms to remove VFA from the rumen regulate the overall ruminal pH of a cow.

3.2.2 Regulation by buffering system

Another mechanism to regulate the ruminal pH is the bicarbonate buffering system. It has an important role in buffering VFAs and regulating rumen pH. The process by which bicarbonate shows buffering ability is shown in the equation 4. The main source of bicarbonate in the rumen is saliva and the bicarbonate-dependent VFA

absorption mechanism. In a liter of cow saliva, approximately 7.32 g of bicarbonate and a small amount of phosphate are present (Dijkstra et al. 2012). An average of 150 liters of saliva is produced by lactating dairy cows daily (Allen 1997). The production of saliva depends mainly on the chewing and rumination activity of the cow, which increases when the cow chews and ruminates feed for a long time (Beauchemin et al. 2008). Additionally, per day, a cow spends about 3 to 8 hours chewing and about 10 hours ruminating (White et al. 2017). The chewing time and rumination are directly related to the fiber and size of the particle. Feeding a high amount of neutral detergent fiber (NDF) content feed with large particle size increases the chewing time and salivation in a cow (Beauchemin et al. 2008). Though one of the studies has reported that overall daily production of saliva is not influenced by chewing and ruminating activity (Maekawa et al. 2002), it is widely accepted that feeding high physically effective NDF content feed with higher particle size can lead to increased salivary production, which can buffer the rumen and protect the cow from RA and SARA (Yang et al. 2001; Krause et al. 2002). Among all the bicarbonate that enters the rumen, half is derived from saliva, whereas the other half comes from VFA absorption (Owens et al. 1998). The amount of bicarbonate entering the rumen from rumen epithelium increases when the proportion of concentrate in a feed is increased and reaches up to 65% of the total bicarbonate flow to the rumen (Dijkstra et al. 1993). Hence, considering the amount of bicarbonate that flows from the rumen epithelium to the rumen, it is clear that bicarbonate-dependent VFA absorption plays a significant role in maintaining and regulating the ruminal pH.

Other buffering activity in a rumen is shown by dietary components. The feed containing components such as calcium and magnesium has a natural buffering ability. They are usually in carbonate and bicarbonate form in feed (McBurney et al. 1983). The buffering ability of the calcium and magnesium is shown in equation 1 and 2 respectively. Moreover, the feed containing protein and peptide also acts as a buffering agent. They get converted to ammonia with the help of rumen microbiome. Ammonia reacts with VFA to produce ammonium salts, leading to buffering of rumen pH (Owens et al. 1998).

Buffering mechanism of calcium:

$$CaCO_3 + 2R\text{-}COOH \rightarrow Ca_2^{+} + 2R\text{-}COO^{-} + CO_2 + H_2O \quad (1)$$

Buffering mechanism of magnesium:

$$MgCO_3 + 2R\text{-}COOH \rightarrow Mg_2^{+} + 2R\text{-}COO^{-} + CO_2 + H_2O \quad (2)$$

Buffering mechanism of ammonia:

$$NH_3 + R\text{-}COOH \rightarrow R\text{-}COONH_4 \quad (3)$$

Buffering mechanism of bicarbonate

$$R\text{-}COOH + HCO_3{-} \rightarrow R\text{-}COO^{-} + CO_2{+}H_2O \quad (4)$$

Overall, the produced acids in the rumen decrease the rumen pH. In contrast, the cow has various mechanisms, such as an absorptive and buffering mechanism, to regulate the rumen pH to not go low below the threshold and safeguard the animals from conditions like RA and SARA.

3.3 Ruminal acidosis

Ruminal Acidosis is one of the common problems in the intensified cattle production systems. Ruminal acidosis occurs when the pH in rumen decreases below a certain level for a certain time. The drop in rumen pH usually occurs when a large amount of easily fermentable carbohydrates is included in the feed of a cow. It occurs in various forms ranging from peracute to subacute form (Jaramillo-López et al. 2017). The peracute and acute form of ruminal acidosis occurs when the pH of the rumen drops below 5.2 (Nagaraja & Lechtenberg 2007). It is a severe form of ruminal acidosis with prominent signs. In this form of ruminal acidosis, a significant amount of lactic acid is produced, which facilitates an extreme decrease in rumen pH (Hernández et al. 2014). The steps by which ruminal and metabolic acidosis are initiated are shown in Figure 3. Unlike SARA, peracute and acute ruminal acidosis is life-threatening, causing sudden death of the animal (Hernández et al. 2014). The peracute and acute form of RA acidosis is mainly seen in feedlot cattle (Castillo-Lopez et al. 2014).

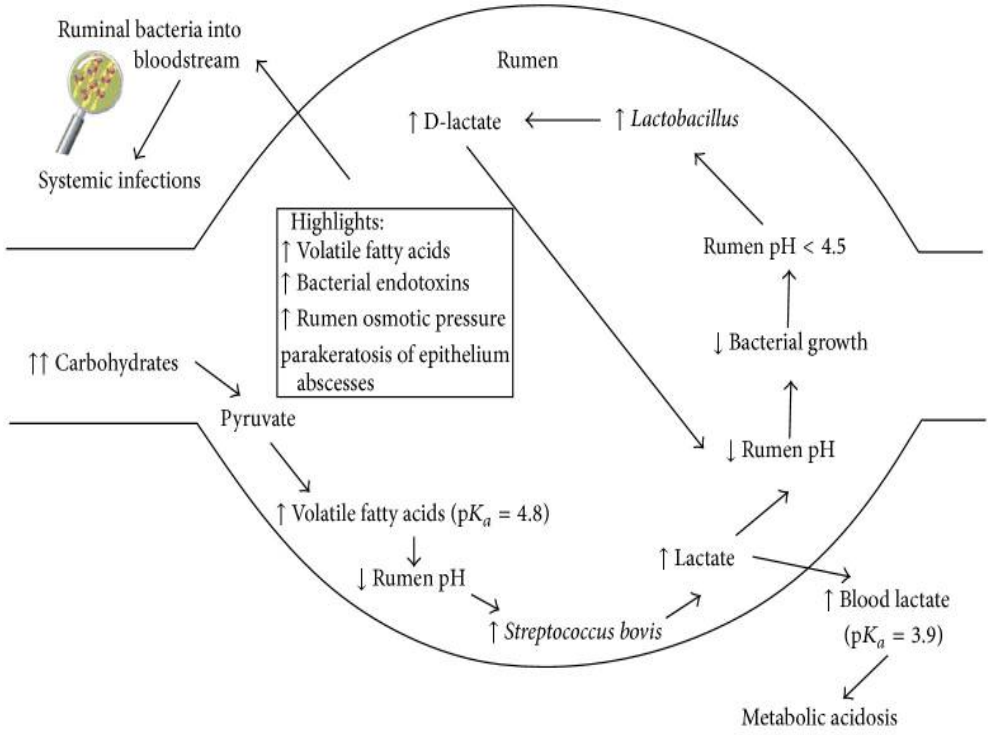


Figure 3. A cascade of events for metabolic acidosis (González et al. 2012)

3.4 Sub acute ruminal acidosis (SARA)

SARA is the sub acute form of ruminal acidosis. Compared to peracute and acute forms, this form of ruminal acidosis does not severely decrease the rumen pH. Moreover, the production and pooling of lactic acid in rumen are not evidenced in SARA (Krause & Oetzel 2006). SARA is defined mostly based on rumen pH, where the pH of the rumen decreases below the suboptimal range for several hours a day, after feeding a cow with high concentrate feed. This part of the definition is agreed by most of the studies, but disagreement is presented in defining the suboptimal pH range and time spent by cows under that pH (Abdela 2016). Zebeli et al. (2008) used a threshold of pH 5.8 that lasted for more than 5.2 hours to define SARA. Similarly Beauchemin et al. (2003) used a threshold of 5.8 to define SARA. Gozho et al. (2007) used a threshold of 5.6, lasting more than 3 hours a day, to define SARA. Whereas Plaizier (2004) and Neubauer et al. (2018) used threshold 6 to define SARA. Moreover, some studies have defined SARA with a low pH threshold of 5.5 (Garrett et al. 1999). Variations in the definition of SARA across studies could be due to the techniques used in rumen pH analysis and the location of the sensor in the rumen that can significantly vary the pH measured within the rumen (Sato et al. 2012).

Unlike acute and peracute ruminal acidosis, SARA does not have particular signs to diagnose it. Hence, many of the cows go undetected on a farm. Ruminal pH is the only indicator that is being used to diagnose SARA in the early stage (Enemark 2008). Some of the studies have rejected the use of rumen pH as an indicator of SARA. They defined SARA as a syndrome with combinations of many signs. Hence, using rumen pH alone as an indicator of SARA cannot be justified (Khafipour et al. 2009a). However, currently, as there are no best alternatives to rumen pH to diagnose SARA in the early stages, ruminal pH is used as a standard test to diagnose SARA in the early stages.

3.4.1 Consequences of SARA

SARA has caused huge economic losses to farmers due to a decrease in production and an increase in the culling/death rate of animal (Gozho et al. 2007). SARA has shown effects on DMI, digestion of fiber, and milk compositions of a cow. It can cause diarrhea, lameness, laminitis, liver abscess, rumenitis, parakeratosis, inflammation, and loss of body condition (Plaizier et al. 2008; Abdela 2016). Signs of SARA are the result of a sequence of events that occur after inflammation of the rumen, i.e., rumenitis. Hence, most of the signs of SARA are seen when cows are already in bad health. Unlike the abomasum, the rumen does not have a protective

mucosal covering, and a drop in rumen pH can affect the ruminal epithelium, leading to ulcers and inflammation of the rumen. Additionally, a decrease in ruminal pH activates the production of proinflammatory cytokines, which also adds up to initiating inflammation of the rumen (Zhao et al. 2018). This can lead to the adherence of microorganisms to the lesions, causing the leaking of microorganisms from the rumen to portal circulation to the liver, causing liver abscesses. Microorganisms can further leak into the systemic circulation and get colonized to the various organs of the body, causing infection and inflammation of those organs (Abdela 2016).

3.4.1.1 Effects on DMI

During SARA, the intake of feed may decrease up to approximately 25% (Krajcarski-Hunt et al. 2002) and it is also taken as one of the important signs of SARA. It is believed that DMI decreases due to the rise in osmolarity in the rumen, decrease in fiber digestibility, increase in propionate proportion, and decrease in rumen motility (Allen 2000) but the exact cause of this depreciation of DMI is still unknown. DMI reduction was reported when cows were induced SARA with high concentrate diet, whereas when SARA was induced by feeding alfalfa, a decrease in DMI was not reported (Khafipour et al. 2009a). In both conditions, the amount of VFA produced and rumen osmolality have been the same. Studies have reported that SARA induced by feeding a high-concentrate diet has increased the label of acute phase protein in the systemic circulation, which is an indicator of multiple organ inflammation in cows (Gozho et al. 2007; Abdel Raheem et al. 2018). Inflammation of multiple organs in animals causes a decrease in appetite, ultimately reducing DMI in cow (Weingarten 1996; Gozho et al. 2007). Impaired digestion of the fiber also causes a decrease in DMI in cows.

Cellulolytic bacteria, *Fibrobacter spp, Ruminococcus spp,* and *Prevotella spp* prefer slightly near-neutral pH above 6 to survive and grow (Sari et al. 2017). When the pH of the rumen drops below 6, those bacteria struggle to survive, leading to decreased degradation of fiber (Slyter et al. 1970; Zhang et al. 2017) causing accumulation of undigested fiber in the rumen. A higher accumulation of undigested fiber in the rumen activates stretch receptors, signaling the brain that the rumen is full. As a feedback mechanism, the brain suppresses the appetite, reducing DMI in a cow (Pereira et al. 2023). Impairment in the digestion of fiber in the rumen can significantly affect the health and production of the cows.

3.4.1.2 Effects on milk yield and its composition

While SARA can affect milk yield and composition, studies have reported controversial results. Krause & Oetzel (2006) reported a decrease in milk yield, whereas Kmicikewycz et al. (2015) reported an increase in milk yield when SARA was induced in cows. Similarly, one of the studies reported no change in milk yield

(Danscher et al. 2015). Moreover, in SARA cows, an increase in milk protein and a decrease in milk fat and milk yield were observed (Khafipour et al. 2009a). Gozho et al. (2007) did not observe any changes in milk fat. Another study found a decrease in milk fat, whereas milk yield and milk protein remained unchanged (Kitkas et al. 2019).

Various theories have been proposed to define the drop in milk fat with respect to the high-concentrate feed. One of the theories is based on the glucogenic-insulin theory, which explains the drop in milk fat based on the increase in propionate production (Fairfield et al. 2007). Propionate is produced in high amount when starch-rich feed is fed to the cow. The propionate acts as a precursor for the synthesis of glucose in the liver. Increased glucose synthesis in the liver leads to the high release of insulin from the pancreas, leading to hyperinsulinemic conditions in cows. During hyperinsulinemic conditions, the lipolysis in adipose tissue decreases, causing a decrease in milk fat production (Bauman & Griinari 2003). This theory is true when the cows are in a negative energy balance, but when cows are in a positive energy balance, the dependence of cows on mobilized fatty acids, produced from lipolysis of adipose tissue, for the production of milk fat remains very low (Kenéz et al. 2015). Considering it, this theory cannot fully satisfy the decrease in milk fat with respect to the high-concentrate diet.

Another theory for the decrease in milk fat is based on a decrease in acetate production. When SARA is induced with low fiber-rich feed, the production of acetate decreases. Usually, acetate is produced by cellulolytic bacteria, and fiber acts as a substrate for those bacteria. A decrease in rumen pH inhibits the growth and activity of cellulolytic bacteria, causing decreased production of acetate in the rumen (Castillo et al. 2013). Acetate is one of the major precursors for the de-novo synthesis of fatty acids. Acetate with the help of acetyl Co-A synthetase converts to acetyl Co-A in the cytoplasm. The produced acetyl Co-A acts as a primer for the synthesis of fatty acids. Produced fatty acids get incorporated into triglycerides, forming fat in milk (Shingfield et al. 2012). Hence, decreased production of acetate in rumen can affect milk fat. This theory is controversial. Studies have reported that the production of acetate in the rumen does not decrease when high-concentrate feed is fed. It was found that a decrease in the molar proportion of acetate was observed due to the high production of propionate (Sutton et al. 2003; Matamoros et al. 2022). Overall, considering no change in acetate production in the rumen due to high concentrate, this theory cannot justify the decrease in milk fat with a high concentrate diet.

Lastly, another theory is based on biohydrogenation. Among all theories, this theory is mostly accepted. The decrease in ruminal pH leads to incomplete biohydrogenation of dietary fatty acids. This results in the production of trans -10 cis 12 conjugated linoleic acid. These fatty acids have inhibitory action in milk fat production, causing decreased milk fat in milk (Baumgard et al. 2001; Bauman &

Griinari 2003; Maxin et al. 2011). Milk protein was found to be increasing when SARA was induced in dairy cows (Khafipour et al. 2009a; Li et al. 2012; Villot et al. 2018). SARA increases the permeability of the rumen wall, leading to the leaking of lipopolysaccharides into the portal system (Khafipour et al. 2009b; Zhao et al. 2018). To defend against these endotoxins, the cow's defense mechanism triggers an acute phase response in the liver. This response stimulates the production of acute-phase proteins, which are released into the bloodstream to improve immunity, neutralize pathogens, and repair tissues. During systemic inflammation, the permeability of the mammary gland epithelium increases as other vascular barriers. The increase in permeability of mammary gland epithelium leads to the leak of acute phase protein into the milk, adding up the value of protein in milk (Plaizier et al. 2012). The fat-to-protein ratio is one of the emerging parameters of milk constituents, which is found to be a more reliable indicator of SARA, which decreases when SARA is induced (Li et al. 2012; Villot et al. 2018).

3.4.1.3 Effects on the health of a cow

Laminitis and lameness are the signs seen in SARA. Laminitis stands for inflammation of laminae, a soft tissue that connects pedal bone to the hoof wall (Bergsten 2003). The exact cause of laminitis is still not clear. However, it is assumed that a decrease in ruminal pH favors the growth of gram-negative bacteria, which produces endotoxins (lipopolysaccharides) in the rumen. With the decrease in the integrity of the rumen barrier due to the decreased rumen pH, these toxins leak into the systemic circulation, causing systemic inflammation. In response to systemic inflammation, the body moves the blood to the central circulation. This causes a decrease in peripheral blood circulation, and without proper blood flow to the laminae of the hoof, it gets inflamed and weak. As a result, lameness occurs in cows (Danscher et al. 2010; Kofler et al. 2023).

Liver abscess is another sign that is usually seen in SARA cows. This occurs due to the leaking of rumen microbiome from the rumen to the portal system due to the compromised barrier of rumen epithelium. The barrier of the rumen gets weak due to the altered rumen environment during SARA. A decrease in ruminal pH activates the production of proinflammatory cytokines, which also adds up to initiating inflammation of the rumen and makes the rumen wall weak and ulcerative (Zhao et al. 2018). Weak rumen barrier and ulcers in the rumen facilitate the rumen microorganisms to adhere to the lesions and leak to the portal circulation causing liver abscesses. This microorganism can further cross the hepatic barrier and get into systemic circulation. Once microorganism gets to the systemic circulation, the organisms can get lodged in various organs of the body, causing inflammation and infection of that organ (Abdela 2016).

Diarrhoea is commonly seen in cows with SARA. Diarrhea in SARA occurs due to altered ruminal motility, rumination, digestion, and other physiological

disruptions that are associated with SARA (Liu et al. 2023). Due to the decrease in ruminal pH, cellulolytic bacteria cannot degrade fiber in a rumen, resulting in the degradation of a large amount of undigested feed material in the hindgut. An increase in hindgut fermentation decreases the pH of the hindgut, resulting in the corrosion of the lining of the large intestine. To protect the corrosive area from infection, the large intestine secretes mucus and fibrins. The damage to the lining of the large intestine impairs its ability to absorb nutrients and water effectively, which leads to increased fluid in the intestine, causing diarrhea in cows (Liu et al. 2023). The feces of SARA cows are foamy and yellowish and contain high amounts of mucin and fibrin (Nordlund et al. 2004).

3.5 Methods used for measuring rumen pH

The study of the rumen dates back to the mid-1920s when scientists understood the important role of rumen pH in feed digestion (Han et al. 2022). Rumen pH has a crucial role in assessing the rumen health and productivity of a cow. For a long time, many methods have been used to measure the rumen pH of a cow. The most common methods used for rumen pH analysis are done either by direct sampling of rumen fluid or by using a continuous monitoring system. Both systems have their pros and cons for use. In the direct sampling method, rumen fluid is collected using rumenocentesis, oral stomach tube methods, or rumen cannula technique. Whereas in a continuous monitoring system, pH sensors are directly lodged into the rumen, allowing continuous and real-time monitoring of changes in rumen pH over time. This method uses either an indwelling rumen pH data logger or a wireless rumen sensor smart bolus to measure the real-time rumen pH (Geishauser 1993; Nordlund & Garrett 1994; Duffield et al. 2004; Danscher et al. 2015).

3.5.1 Rumenocentesis method

Rumenocentesis is a method where rumen fluid is withdrawn through the left paralumbar fossa of the cow using a rumenocentesis needle. In this method, the site for needle insertion is aseptically prepared, and the site for puncture is locally anesthetized. The site selected for needle insertion is the left paralumbar fossa. Then, about 12.5 cm long and 16-18 gauze sterile stainless steel needle is inserted into the rumen, and about 5 ml of rumen fluid is collected using a sterile syringe. After collecting fluid, its pH is measured using a pH meter (Nordlund & Garrett 1994). This method was one of the commonly used methods by veterinarians and researchers to take rumen samples in real farm conditions. This method helps to analyze rumen pH effectively, as the rumen environment is not disturbed by outer influences. However, this method is highly invasive and needs the proper supervision of a veterinarian when performed. Improper and aseptic withdrawal of

rumen fluid can cause an abscess, hematoma, and even peritonitis in a cow, which can impact the production and health. Another disadvantage of this method is that it cannot provide continuous real-time variation of rumen pH with time (Atkinson 2017). Hence, this method is not practiced widely.

3.5.2 Oral stomach tube method

The oral stomach tube method is one of the common methods to analyze the rumen pH in farm conditions. In this method, the cow is properly restrained, and a probe is inserted from the mouth to the rumen of the cow. After that, with the help of a suction pump, the rumen fluid is drawn out for pH analysis (Geishauser 1993). The major disadvantage of this method is that the sample collected can get contaminated by saliva, leading to an inaccurate measurement of ruminal pH. The contamination of saliva can be reduced by taking the sample 1 hour before feeding or 4 hours after feeding (Muizelaar et al. 2020). Contamination of rumen fluid with saliva can increase the rumen pH (Duffield et al. 2004). Similar to rumenocentesis, this method also cannot provide continuous real-time variation of rumen pH over time.

3.5.3 Rumen cannula technique

In rumen fistulated cattle, the rumen fistula is prepared surgically in the left paralumber fossa. This method of measuring rumen pH is highly expensive and invasive and needs the help of a professional, such as a veterinarian surgeon, to perform the surgery (de Assis Lage et al. 2020). From the fistula, rumen fluid is collected in a container to measure the rumen pH (Duffield et al. 2004). The environment of the rumen is anaerobic, and frequent opening and closing of the lid can disturb the anaerobic condition of the rumen by ingressing atmospheric air into the rumen. The ingress of atmospheric air can also occur continuously from the gaps between the skin and the cannula (Castillo & Hernández 2021). Disruption of anaerobic conditions can facilitate the growth of aerobic and facultative anaerobic microorganisms rapidly, resulting in a decrease in anaerobic bacteria in the rumen. This can cause impact on the fermentation process and ultimately alter the pH of the rumen (Russell & Rychlik 2001). Another drawback of this method is that the sample must be taken in frequent intervals to analyze the variation of pH with time properly. If the sampling is done sporadically, they fail to provide accurate results. Additionally, it is not acceptable to fistulate the cows in real farm conditions, so this method is mainly used in studies and research.

3.5.4 Continuous monitoring system

The indwelling rumen pH method is a commonly used method to monitor the continuous rumen pH on research projects as fistulated cattle. Similar to the rumen cannula technique, the early rumen sensors were primarily used in rumen fistulated

cow, and those sensors were attached with some weight and placed in a ventral sac of the rumen (Danscher et al. 2015). The first introduction of a continuous rumen pH monitoring device was done in 1993 (Dado & Allen 1993). Unlike other methods, this method provides continuous variation of rumen pH throughout the day. The use of indwelling sensors was limited to restrained or tethered cows due to the use of external cable (Dado & Allen 1993), limiting its use to tie-stall housing systems and research studies. Moreover, this system of pH measurement needs frequent calibrations and needs to be removed frequently (Dado & Allen 1993; Sato et al. 2012).

With the advancement of technology, wireless rumen sensor boluses have been developed. The first idea for a wireless rumen bolus was presented in 2008 (Mottram et al. 2008). These boluses are the improved version of the old indwelling rumen probe. This sensor has the ability to continuously monitor the rumen pH and temperature of the cows and send the data wirelessly to the user. These devices are farmer-friendly and do not need any special invasive method to implant them into the rumen. Additionally, the farmer can have access to data anytime, anywhere, with the use of the particular application of the manufacturer (Zabasta et al. 2019). Being wireless, these sensors are the only ones that can be used in loose housing systems (Sato et al. 2012). The main problem with this device is its battery life span and drifting over time (Kaur et al. 2010), which decreases the accuracy of the measurement with the time and makes it highly expensive for farmers to use. However, the improvement in the battery system and the development of low power range bolus has been made which increased the lifespan of the bolus from 6 months to 5 to 6 years (Kim et al. 2018) but monitoring of pH by bolus is still confined to a 3-month period (Han et al. 2022). The wireless rumen bolus is less invasive compared to other methods. The installation of the rumen bolus in cows is done by bolusing it orally with a balling gun, and it stays a lifetime inside the cow unless surgically removed. Sato et al. (2012) reported that the most common site where this bolus resides is the reticulum rather than in the rumen. Hence, the lodging place of this bolus should be considered as rumen pH varies greatly within various sites in the rumen (Duffield et al. 2004).

3.6 Variation of pH within reticulorumen

Variation in pH at various sites of the rumen and reticulum has been discussed and reported in studies. Reticular pH, compared with ruminal pH, was found to be higher by approximately 0.2 pH units (Sato et al. 2012; Falk et al. 2016; Neubauer et al. 2018). The rumen is the primary compartment where intensive fermentation occurs, leading to a lower pH than the reticulum. Additionally, the content of the reticulum is more often diluted by the saliva and ingested feed, causing a rise in pH (Falk et al. 2016). The pH variation has not only been observed between these two

compartments of the ruminant's stomach but also across the different sites within the rumen (Dias Batista et al. 2021). In the cranial and caudal regions of the rumen, the pH is highest, while the central and ventral rumen have the lowest pH (Shen et al. 2012).The pH levels in different parts of the rumen vary due to differences in fermentation rates and VFA production. Additionally, variations in absorption and buffering rates in each site also affect the pH levels (Dijkstra et al. 2020). The variation significantly affects the diagnosis of SARA, as different methods for monitoring rumen pH depend heavily on the sensor placement or rumen fluid collection site. Therefore, defining the threshold pH for diagnosing SARA must take into account the sensor placement or rumen fluid collection site.

In this study, wireless rumen sensor bolus (Moonsyst cattle monitoring, Moonsyst international, Ireland) was used for monitoring rumen pH. Unlike old indwelling rumen pH meters, which used to be attached with weight and lodged in a ventral sac of the rumen, this wireless rumen sensor bolus can get lodged in any part of a rumino-reticular wall, but majorly in the reticulum (Mottram et al. 2008). Among all definitions, it is widely accepted that SARA occurs when the rumen pH ranges between 5.5 to 5.8 for several hours a day (Beauchemin et al. 2003; Humer et al. 2018). Hence considering this statement and reticular pH being 0.2 units higher than ruminal pH, a threshold of pH 6 was set in this study. Additionally, to be considered as SARA, the cow must spend more than 3 h/d under this threshold pH.

4. Materials and methods

This study is part of a project that aims to find biomarkers of SARA and develop a method to detect SARA quickly and robustly. The study was conducted with proper ethical approval (DNR 5.8.18-13494/2023).

4.1 Animals, housing, and feeding

For this study, 25 primiparous cows (8 Swedish Holstein (SHB) and 17 Swedish Red (SRB)) were selected from the herd of cows at the Swedish Livestock Research Center in Lövsta, Uppsala, and housed separately in an experimental barn on the same farm. The inclusion criteria for these cows was that they should be primiparous cows in early to mid lactation. Moreover, they should not have been treated with any antibiotic after calving and should not have had any history of mastitis or any metabolic diseases. Cows were housed in a free-stall housing system with free access to the feeding, water and resting area. The experimental barn consists of a resting area, feeding area, and milking area. Each area was connected with each others either with a one-way passage gate or by an automatic passage selection gate. From the resting area, the cows were either directed to the milking area or toward the feeding area with the help of an automatic selection gate. After milking, the gate gets opened towards the feeding area. From the feeding area, the cows get access to the resting area through a one-way passage gate. The layout of the farm is shown in Figure 4. The resting area consisted of individual cubicles. The floor of the cubicles was rubber beds with wood shaving Manure was scrapped several times per day, with the help of a wire-driven scrapper.

Adlibitum water and feed were provided to the cows. In an individual feed trough, a total mixed ration (TMR) was provided, whereas clean water was provided in a common water trough. The concentrate (Table 1) was pelleted and provided both in the milking unit and in a feed trough. In the feeding trough, the concentrate pellet was mixed with TMR. About 0.5 kilogram of concentrate was provided to each cow per day in a milking unit, and it was provided in a fixed amount throughout the study period. Meanwhile, the amount of concentrate that was to be mixed with TMR varied according to the the different treatment periods within the study. A mineral block was placed in a feeding area to supplement the cows with minerals. The feed was delivered twice a day (7:30 and 15:00) in a feed

wagon, from where it was distributed to the feed trough. The feed wagon was attached to the conveyor belt for collecting and further distributing the feed.

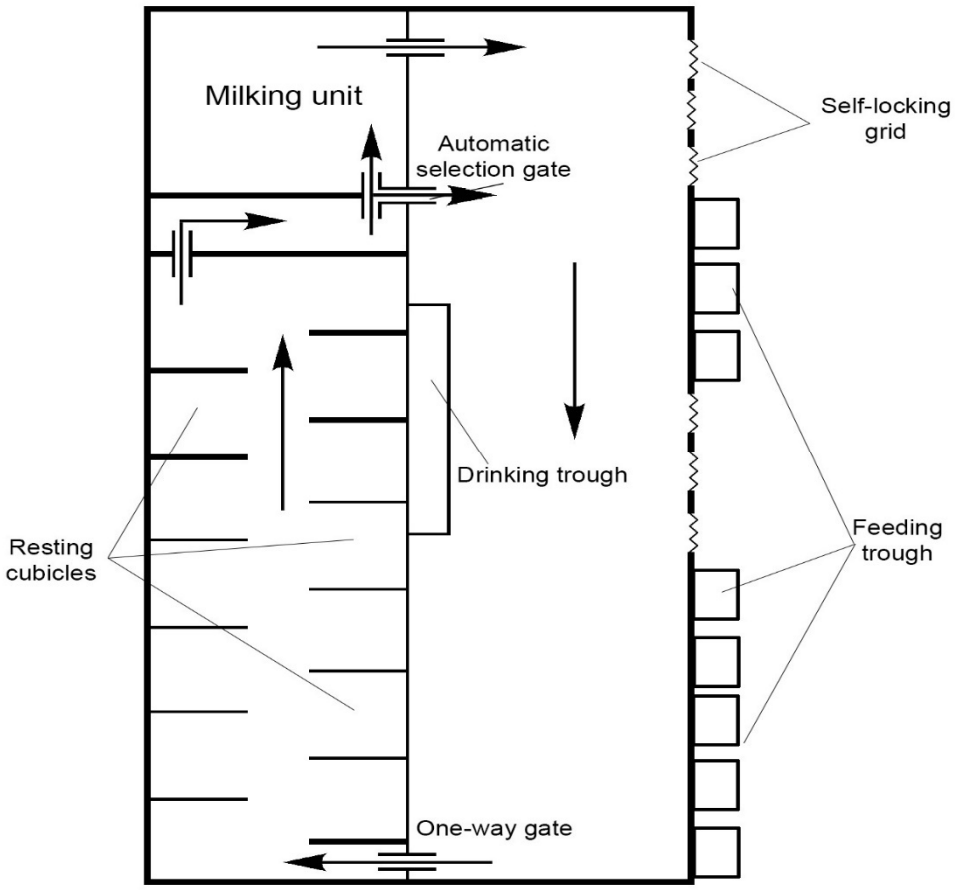


Figure 4. Layout of the cow barn

4.2 Experimental design

The study was performed as a continuous treatment where the treatments were applied in a sequence as follows: low concentrate diet (CONT1)-high concentrate SARA inducing diet (SAR2)-low concentrate diet (RECOV3), also referred as periods 1, 2, and 3. The first period was a control, which lasted for 9 days (CONT1). The diet given during this period was the same as what the cows typically had on the farm before selection, so the duration for the first period was relatively short compared to the others. In this period, cows were fed a low concentrate diet where the proportion of concentrate to silage was 48:52 on a dry matter basis. The next period was the SARA induction period (SAR2), in which the proportion of concentrate was increased to 64% of TMR. The SAR2 period lasted for 23 days. The increase in concentrate proportion was done gradually over the time of 6 days.

Increasing the concentrate proportion gradually is more ethical as it reduces the risk of acute distress or complications that can arise due to sudden changes in diet (Bevans et al. 2005). Moreover, increasing concentration gradually helps monitor changes in rumen pH with respect to changes in concentrate-to-silage ratio. Lastly, the third period was a recovery period, which lasted for 20 days (RECOV3). In this period, cows were fed the same feed as in the first period. The transition of feed from the SAR2 diet to the RECOV3 diet was done instantly. Different periods and time spent on that period are shown in Figure 5.

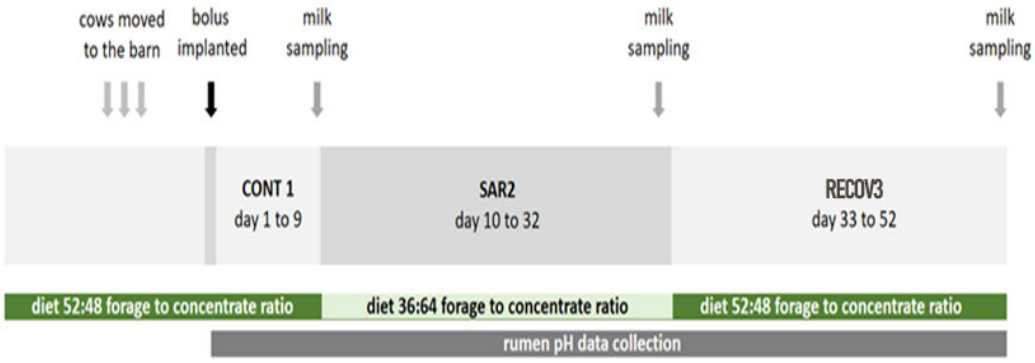


Figure 5. Timeline showing the different diet trial periods

Table 1. Nutrient composition of concentrate (Nova 190 from Johan Hansson AB)

Component	Amount per kg DM
Energy	13.6 MJ ME
Protein	190 g
Fat	57 g
Starch	305 g
Neutral Detergent Fiber (NDF)	245 g
Ash	70 g
Calcium	8 g
Phosphorus	5.4 g
Magnesium	4.6 g
Potassium	6.2 g
Vitamin A	6000 IU
Vitamin D	2000 IU
Vitamin E	40 mg
Copper	7 mg
Selenium	0.4 mg

MJ ME: Megajoules of Metabolizable Energy; IU: International Unit; g: Grams; mg: Milligrams

At the beginning of the study, on day 0, one pH bolus (Moonsyst cattle monitoring, Moonsyst international, Ireland) was administered orally to each cow with the help

of a balling gun. For the administration of bolus, cows were locked in a self-locking grid. The head of a cow was restrained manually by the help of a person. Before administrating, the precalibrated boluses were activated and checked for connection of the bolus to the base station. Then, it was cross-referenced with the individual cow's ear tag and administered to the cow. The moonsyst rumen bolus automatically collected data on rumen pH, at 10 minute intervals, throughout the periods and transmitted data to the dedicated cloud server through a communication gateway installed in the barn. With the help of the moonsyst application, the data of rumen pH was downloaded from the cloud server and then was used for further statistical analysis.

Total daily feed consumed by individual cows was recorded automatically with the help of a weighing cell that was attached to the feeding trough (CRFI, BioControl Norway A/S, Rakkestad, Norway). DM content (%) of silage (38.5%) and concentrate (90%) was obtained from the Eurofins Agro and Johan Hansson AB respectively. TMR for CONT1 and RECOV3 was prepared by mixing silage and concentrate at the proportion of 72:28 on a feed basis and for the SAR2 period, TMR was prepared by mixing silage and concentrate at the proportion of 56:44 on a feed basis. Hence DM of TMR was calculated to be 53% for CONT1 and RECOV3 feed and DM of SAR2 feed was calculated to be 61%. The daily feed intake (D) for each cow was retrieved from the weighing cell, and by using the below formula, DMI was calculated for each cow for each day.

$$\text{DMI (kg/d)} = \text{D} \times \text{DM of TMR (\%)}$$

Milking was carried out voluntarily in a single-station voluntary milking system (VMS, DeLaval International AB, Tumba, Sweden). Daily milk yield was measured using a milk meter (MM25, DeLaval International AB, Tumba, Sweden), and samples of milk were taken with the use of a milk sampler (DeLaval Milk Sampler, DeLaval International AB, Tumba, Sweden). Sampling was done at the last 2 days of each period. Milk samples were preserved with Bronopol and stored in a refrigerator at 2-4 ^{O}C until further analysis. Milk sample analysis was done at the laboratory of the Swedish University of Agricultural Sciences, Uppsala, within 3 days of collection. Mid-infrared Fourier transform spectroscopy methods (CombiScope FTIR 600 HP, Delta Instruments B.V., Drachten, the Netherlands) were used to measure fat, protein, lactose, and fatty acids. Energy-corrected milk (ECM) (4%) was calculated according to Sjaunja et al. (1990).

$$\text{ECM (kg / day)}: \text{MY(kg / day)} \times (383 \times \text{fat\%} + 242 \times \text{protein\%} + 157 \times \text{lactose\%} + 20.7)/3.140$$

5. Statistical analysis

All the data handling and statistical analysis were done using R Studio Version 4.3.2 and Microsoft Excel 2311. The analysis was performed using collected data from 22 cows, as 3 cows were removed midway from the study due to illness. Initial data was checked for inconsistency, duplicate data and for missing data using linear methods to maintain the time series integrity of the data. Test for normality was performed using the Shapiro-Wilk test. Descriptive statistics such as mean maximum and minimum rumen pH, mean milk yield, mean constituent of milk, duration under pH 6, and mean DMI were calculated for each period.

The study was conducted using a continuous treatment approach, where treatments were applied sequentially. The model was designed to consider feed period as a fixed variable, individual cows as a random variable, and rumen pH, duration a cow had rumen pH below 6 (h/d), DMI, and milk parameters as dependent variables. Repeated measure ANOVA was performed in normally distributed data to assess the effect of dietary periods on mean maximum rumen pH, mean minimum rumen pH, DMI, milk fat, milk protein, milk lactose, ECM, SFA, palmitic acid, stearic acid, and myristic acid. Further Post Hoc tests (Tukey's HSD test) were performed on significantly different variables to analyze the pairwise difference between the variables. For non normally distributed data friedman test was performed to find the effect of dietary period on duration below pH 6 (h/d), UFA, MUFA, PUFA, Oleic acid, and fat-to-protein ratio. Further Post Hoc tests (Wilcoon Signed-Ranked Test) were performed on significantly different variables to analyze the pairwise difference between the periods. The level of significance was adjusted using the Bonferroni correction.

A mixed-effect model was performed to see the effect of a dietary period and individual variation on mean ruminal pH, considering the effect of diet as a fixed effect, individual cows as a random effect, and rumen pH as a dependent variable.

The Pearson correlation coefficient was calculated to determine the relation between mean rumen pH/duration under pH 6 value with daily milk yield, daily dry matter intake, and milk parameters. Finally, a linear regression model test was used to model the relationship between effects and outcomes. Rumen pH and duration below pH 6 (h/d) are considered fixed effects, and daily DMI, milk yield, milk fat, and milk fat: protein ratio are dependent variables. Statistical findings were considered significant at level $p \leq 0.05$.

6. Results

Data on rumen pH metrics are presented in Tables 2 and 3. There were significant differences between mean ruminal pH across all three periods. The SAR2 period had the lowest mean rumen pH compared to the RECOV3 period and CONT1 period. Also, the effect of individual differences on rumen pH was found (Table 3). Moreover, the average time each cow spent below pH 6 per day was also found to be highest in the SAR2 period, whereas in the RECOV3 period, the cows spent least time below rumen pH 6. Data concerning mean maximum rumen pH showed no difference between the periods. Mean minimum rumen pH was found to be higher in the CONT1 and RECOV3 periods compared to the SAR2 period.

Table 2. Effect on ruminal pH and DMI levels in dairy cows fed diet with different concentrate to forage rations (48:52 for CONT1 and RECOV3; and 64:36 for SAR2).

Parameters	**CONT1**	**SAR2**	**RECOV3**	**SEM[1]**	**P value**
Mean max rumen pH	7.1	7.1	7.2	0.02	0.16
Mean min rumen pH	5.8^{b}	5.6^{c}	5.9^{a}	0.08	< 0.001
Time below pH 6 (h/d)	0.5^{b}	3.3^{a}	0.1^{c}	0.07	< 0.001
Mean DMI (kg/d)	16.1^{b}	18.4^{a}	15.5^{b}	0.40	< 0.001

[1]SEM: standard error of the mean; DMI: Dry matter intake

[a,b,c,] Values within a row with different superscripts differ significantly at $P < 0.05$

In the CONT1 period, the median line of the ruminal pH of cows aligned in a narrow range, and the variation between rumen pH was less, but in the SAR2 period, the variation in rumen pH was high, and the median pH line shifted down in the SAR2 period. In the RECOV3 period, the median line of rumen pH shifted toward the CONT1 period. The variability of rumen pH in this period was lower compared to the SAR2 period. Among all periods, the most extreme value of maximum and minimum rumen pH (max pH: 7.95; min pH: 5.3) was recorded in the SAR2 period, whereas a more narrow variation in the maximum and minimum rumen (max pH: 7.38; min pH: 5.71) were observed in the CONT1 period and RECOV3 period (max pH: 7.66; min pH: 5.49) (See Figure 6).

Table 3. Effect on ruminal pH levels in dairy cows fed with different diets (concentrate to forage rations at 48:52 for CONT1 and RECOV3 and 64:36 for SAR2)

Periods	Mean pH	SD[1]	Effect estimate	P value	REV[2]	P value for REV[2]
CONT1	6.4	0.24	0	1	0.009	0.05
SAR2	6.3	0.27	-0.12	< 0.001	0.009	0.05
RECOV3	6.5	0.21	+0.11	< 0.001	0.009	0.05

[1]SD: Standard deviation; [2] REV: Random effect Variance

Table 4. Longest time a cow had rumen pH below 6 in a period where different diets were fed (concentrate to forage rations (dry matter basis) at 48:52 for CONT1 and RECOV3 and 64:36 for SAR2)

CowID	CONT1 (h/d)	SAR2 (h/d)	RECOV (h/d)
2524	2.7	14.3	0.3
2542	2.5	16.7	0.5
2544	2.5	10.3	0.2
2545	3	10.2	1.7
2547	4	10	0.7
2549	0	3.8	5.7
2551	3	5	0
2554	1.3	13.8	1.5
2557	4.5	14.3	3.5
2559	2.7	5.5	0.7
2565	0.7	10.5	7.7
2566	1.3	5.7	1
2567	0.5	2	0.7
2575	2	6.5	0
2577	1.2	10.3	0
2578	0	0.8	0
2579	5.2	4.5	2.3
2580	0.5	6.3	0
2584	0.3	2.7	0
2592	0.2	7	0
2602	0.2	1.2	0.2
2607	1	10.2	0

As seen in Table 4, most of the cows had rumen pH below 6 for a longer period of time in the SAR2 period. Except for four cows, all the cows had more than 3 h of time below rumen pH 6. The longest time spent by a cow under rumen pH 6 was recorded in cow number 2542, where the cow spent 16.7 h/d, and the lowest time, 0.8 h/d, was observed in cow 2580. In comparison between periods, the longest time where rumen pH was below 6 was observed in the SAR2 period, except for cow 2579. Moreover, in the RECOV3 period, most of the cows were found to have

a low or decreased duration of time spent under rumen pH 6 compared to the SAR2 period, except for cow 2549, which had a rumen pH below 6 for a longer time in the RECOV3 period.

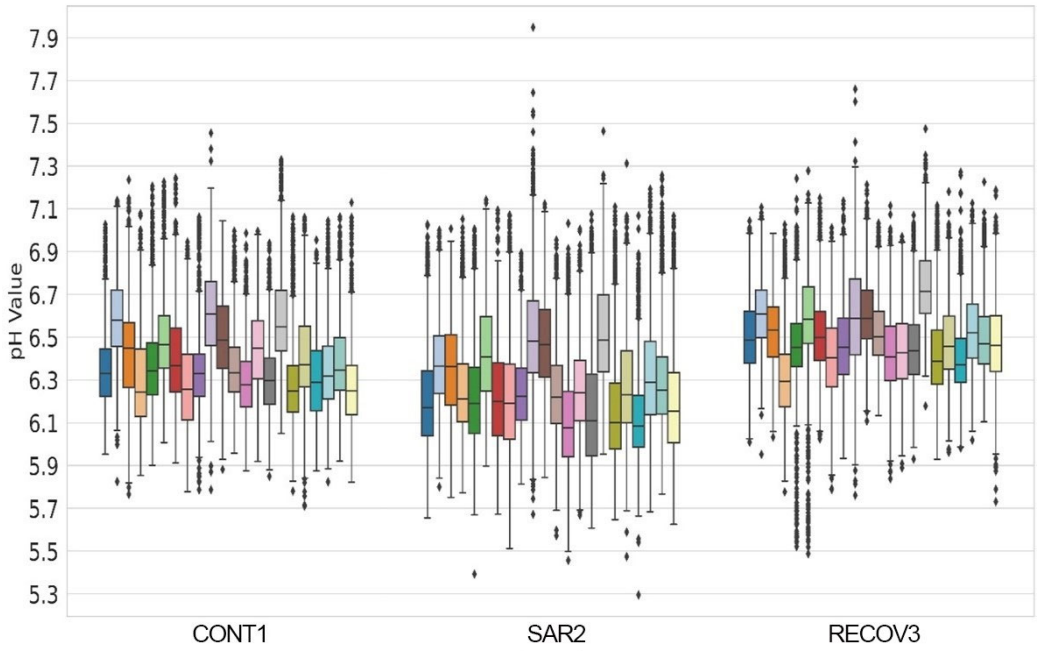


Figure 6. Rumen pH value across three different periods for individual cows. In the box plots the each differently colored box represents an individual cow, and it shows an interquartile range (IQR) of rumen pH. The line present inside the box represents the median value of rumen pH. Maximum and minimum rumen pH values within 1.5 times IQR beyond quartiles are represented by the whiskers. Individual rumen pH, which falls beyond the whiskers, is represented by outliners.

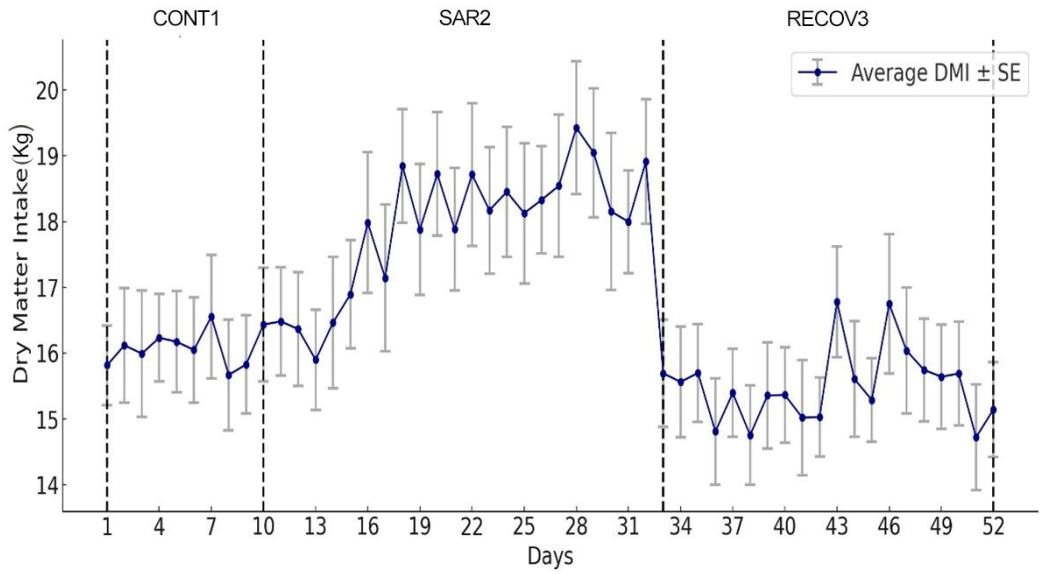


Figure 7. Dry matter intake (DMI) over the study periods, 52 days in total

Table 5. Effect of the rumen pH metrics on the milk parameters and dry matter intake (DMI).

Parameters	Pearson correlation coefficient	Linear Regression Model			P value
		Coefficient	Intercept	R^2	
Average pH with Milk yield	-0.28	-12.77	111.65	0.08	< 0.001
Average pH with DMI	-0.41	-10.97	86.82	0.17	< 0.001
Average pH with Milk fat	0.35	1.86	-7.20	0.12	0.004
Average pH with Milk fat: protein ratio	0.35	0.43	-1.63	0.12	0.004
Duration under pH 6 with Milk yield	0.20	0.55	29.23	0.04	< 0.001
Duration under pH 6 with DMI	0.35	0.56	15.90	0.12	< 0.001
Duration under pH 6 with Milk fat	-0.42	-0.15	4.85	0.18	< 0.001
Duration under pH 6 with Milk fat: protein ratio	-0.44	-0.04	1.20	0.19	< 0.001

R^2: Coefficient of determination

In the CONT1 and RECOV3 periods, no significant difference in DMI was observed (Table 2). Average DMI in cows increased rapidly when high-concentrate feed was fed in the SAR2 period. When the low-concentrate diet was provided without any transition from the SAR2 period to RECOV3, a sharp decrease in DMI was observed. (See Figure 7).

Daily mean rumen pH was found to be negatively weakly correlated to MY ($p < 0.001$). The correlation was further confirmed by the value of the linear regression coefficient (Table 5), which indicates that every unit increase in rumen pH can decrease the MY by 12.77 units. Moreover, as the model R^2 value is 0.079, the variation in MY caused by rumen pH is low. Similarly, the daily duration of time a cow had rumen pH below 6 was positively weakly correlated with MY. According to the R^2 value, the duration of rumen pH < 6 has less impact on the variation of MY. For other parameters, a moderate correlation was observed with average pH and duration of rumen pH < 6. Milk fat and milk fat-to-protein ratio (FPR) were positively correlated with average rumen pH. In contrast, milk fat and milk FPR were negatively correlated with the duration of rumen pH < 6. The results were further confirmed by the linear regression coefficient value. The R^2 value, which is above 0.1 (Table 5), indicates a more pronounced effect of rumen pH and duration

of time < 6 on milk parameters. Regarding DMI, a moderately stronger correlation can be seen. Daily DMI had a negative correlation (p: -0.414) with rumen pH, whereas a positive correlation (p: 0.348) with a duration of time < 6 was recorded.

Overall, the effect of rumen pH seems to be less on the milk yield and has more effect on the DMI and milk parameters. Though the relation between rumen pH and DMI seems to be stronger, the effect of duration of time < 6 shows a stronger correlation with milk fat ((p: -0.422) and milk FPR (p: -0.437) with a higher coefficient of determination (milk fat: 0.178; milk FPR: 0.191). The effect of rumen pH/duration under pH < 6 on DMI, MY, and its parameter can be witnessed in figure 8 and 9.

Table 6. Effect on milk parameter levels in dairy cows fed diet with different concentrate to forage rations (48:52 for CONT1 and RECOV#; and 64:36 for SAR2).

Parameters	**CONT1**	**SAR2**	**RECOV3**	**SEM[1]**	**P value**
Milk yield(L/d)	30.33	30.14	29.30	0.67	0.20
ECM (kg/d)	33.26	33.17	33.18	0.827	0.99
Fat (g/100g milk)	4.77^{b}	4.23^{c}	4.97^{a}	0.14	< 0.001
Protein (g/100g milk)	3.98^{b}	4.11^{a}	4.07^{b}	0.04	< 0.001
Fat to protein ratio	1.20^{a}	1.02^{b}	1.22^{a}	0.02	< 0.001
Lactose (g/100g milk)	4.80	4.80	4.79	0.03	0.98
Fatty acid profile (g/100g milk)					
SFA	3.13^{b}	2.79^{c}	3.29^{a}	0.10	< 0.001
UFA	1.35^{a}	1.21^{b}	1.40^{a}	0.03	< 0.001
MUFA	0.99^{a}	0.87^{b}	1.04^{a}	0.03	< 0.001
PUFA	0.17^{a}	0.15^{b}	0.17^{a}	0.01	< 0.001
Palmitic acid (C16.0)	1.30^{a}	1.16^{b}	1.32^{a}	0.05	< 0.001
Stearic acid (C18.0)	0.54^{b}	0.46^{c}	0.69^{a}	0.02	< 0.001
Oleic acid (C18.1C9)	0.79^{a}	0.67^{b}	0.80^{a}	0.02	< 0.001
Myristic acid (C14.0)	0.63^{a}	0.55^{b}	0.61^{a}	0.02	< 0.001

[1]SEM: standard error of the mean; SFA: Saturated Fatty Acids; UFA: Unsaturated Fatty Acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids; ECM: Energy corrected milk; ^{a,b,c,} Values within a row with different superscripts differ significantly at P < 0.05

There was no difference in milk yield, ECM, and lactose content between periods. Milk fat content was found to decrease in the SAR2 period compared to the CONT1 and RECOV3 periods. A slight increase in milk fat was observed in RECOV3 periods compared to the CONT1 period. Milk protein increased in SAR2 cows. Regarding fatty acid profile, a significant drop in SFA and UFA in milk was recorded in the SAR2 period. In contrast, between the CONT1 and RECOV3 periods, UFA had no significant differences, and SFA increased during the RECOV3 period compared to the CONT1 period. Moreover, PUFA and MUFA decreased during the SAR2 period. Specific milk fatty acids, palmitic acid, stearic acid, oleic acid, and myristic acid, dropped in the SAR2 period. The differences in

palmitic acid, oleic acid, and myristic acid were not different between CONT1 and RECOV3, but stearic acid was found to be higher in the RECOV3 period. The fat-to-protein ratio was also found to be decreased in the SAR2 period. No difference in fat-to-protein ratio was observed between the CONT1 and RECOV3 periods (Table 6).

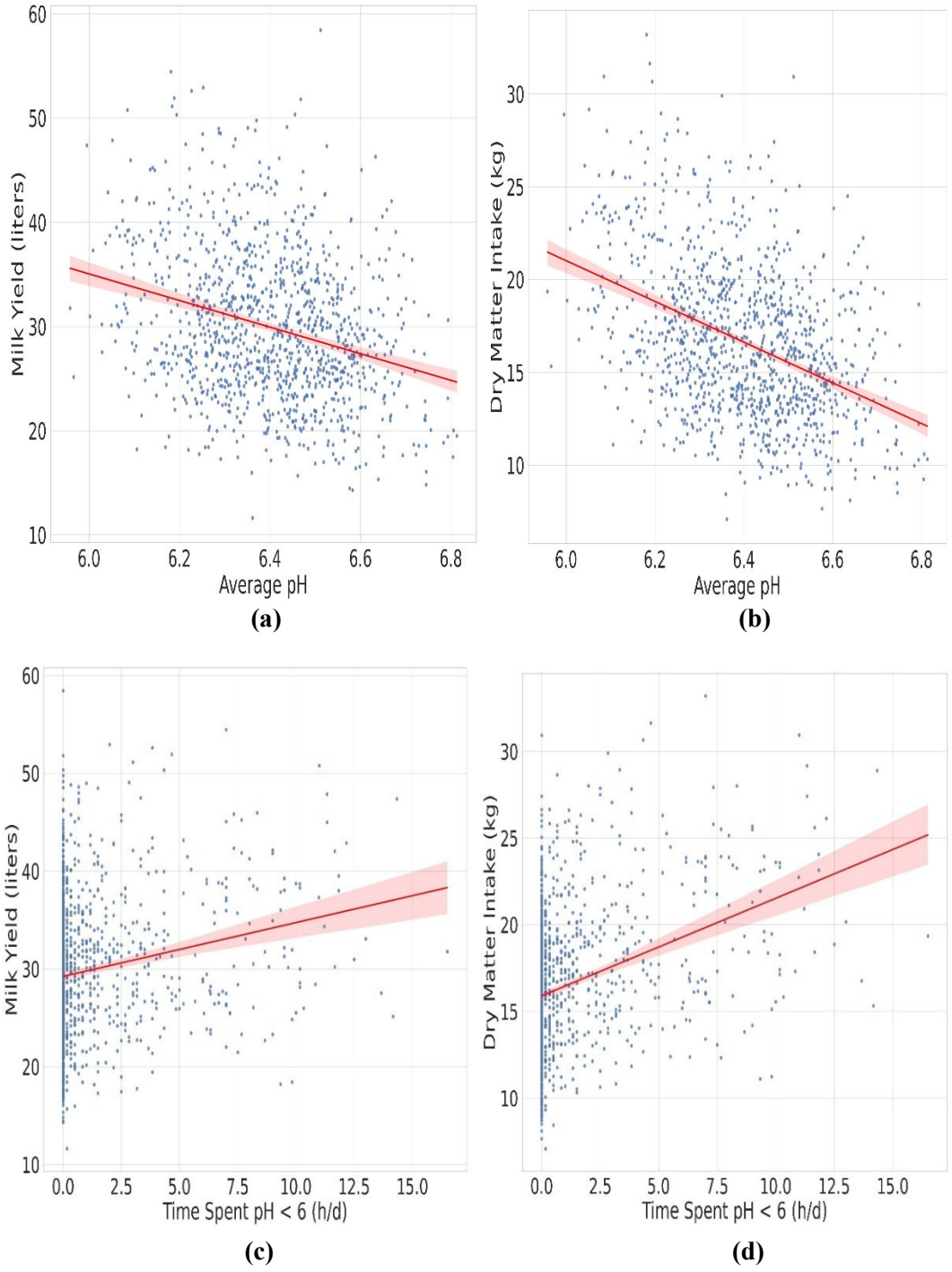


Figure 8. Scatter plot with trendline showing the relation of average rumen pH with (a) daily milk yield (liters) and (b) daily dry matter intake (DMI) in kilograms and time under pH 6 with (c) daily milk yield (liters) and (d) daily dry matter intake (DMI) in kilograms. A dark red line indicates the

regression line and a faint red color around the regression line indicates the confidence interval. Blue dots indicate each data point.

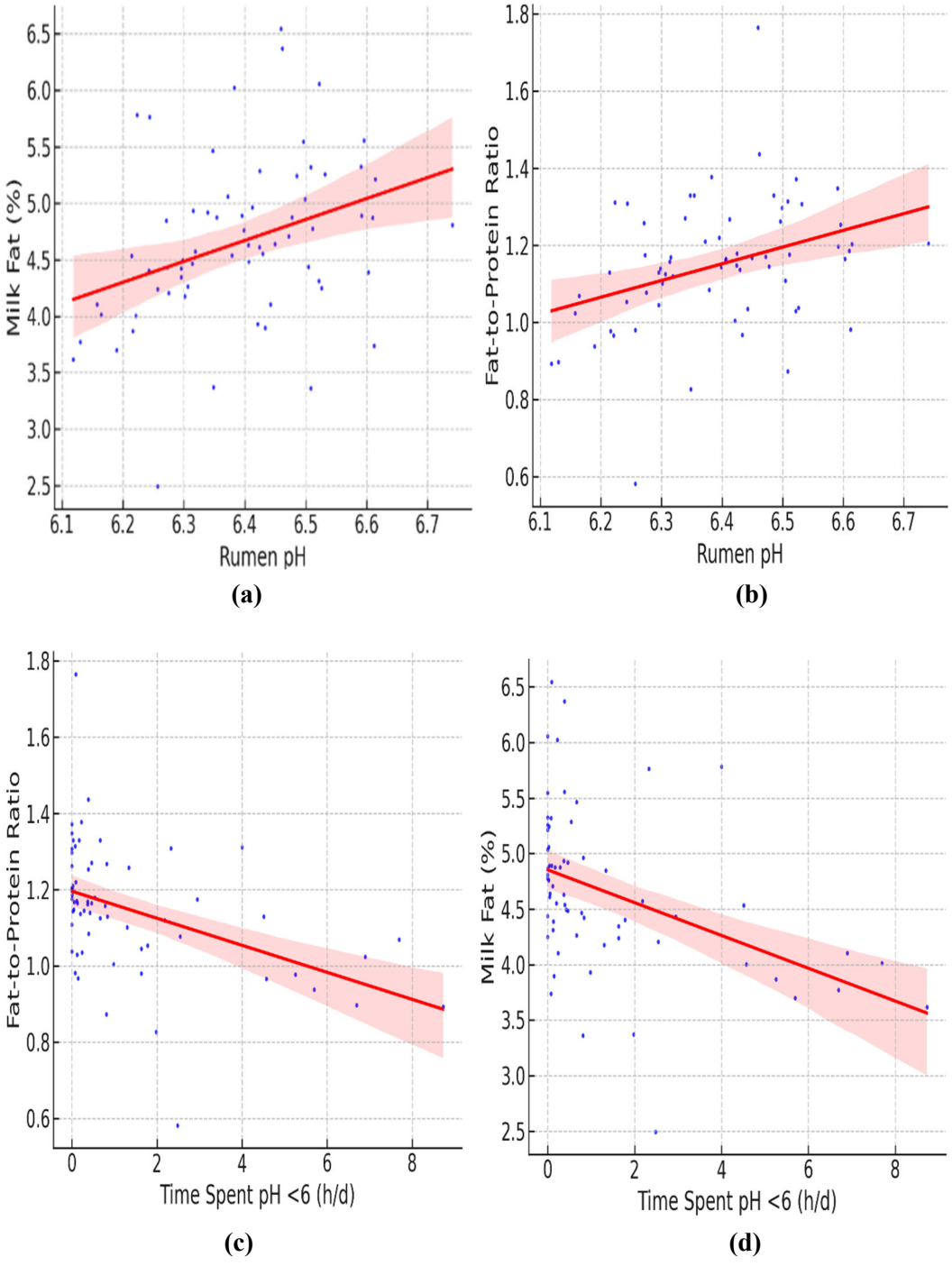


Figure 9. Scatter plot with trendline showing the relation of average rumen pH with (a) milk fat and (b) fat-to-protein ratio and time under pH 6 with (c) fat-to-protein ratio and (d) milk fat. A dark red line indicates the regression line and a faint red color around the regression line indicates the confidence interval. Blue dots indicate each data point.

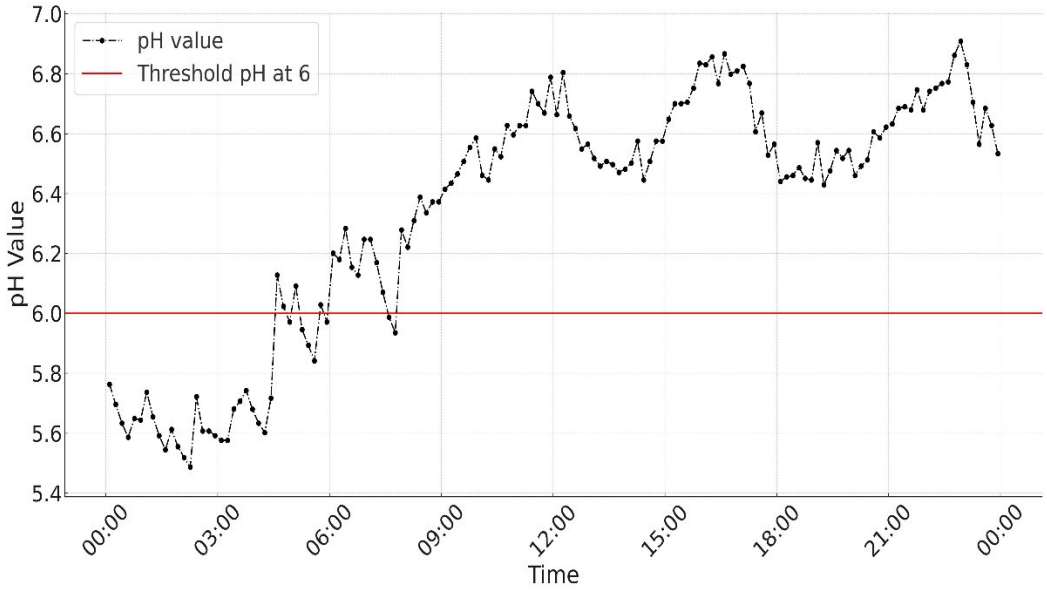
7. Discussion

SARA has been defined based on rumen pH for a long time, and it is being used as a gold standard test to diagnose it (AlZahal et al. 2007). In this study, to be considered as SARA, the rumen pH should be below 6 for 3 hours a day. A significant decrease in rumen pH and an increase in time duration for rumen pH below 6 was observed in SAR2 period. These changes/responses to high concentrate diets were reported in other studies as well (Danscher et al. 2015; Villot et al. 2018). The mean time duration of a cow with rumen pH below 6 was 3.3 h/d (Table 2) and many cows spend several hours below rumen pH 6 in a day. Acute ruminal acidosis is considered to occur when rumen pH is below 5.2 (Plaizier et al. 2008). In this study, no cows were recorded to have rumen pH below that threshold level. Moreover, the cows did not show any signs of acute ruminal acidosis. Hence, cows did not experience acute ruminal acidosis in this study. When cows were fed with the high concentrate diet (SAR2), the longest duration a cow had rumen pH below 6 was 16.2 h/d, and the shortest duration was 0.83 h/d. Out of 22 cows, half of the cows spent more than 10 hours under pH 6 in a day, and about 18 cows spent more than 3 hours under pH 6 in a day (Table 4). Considering the condition of the rumen pH threshold and time under the threshold pH, SARA was likely to occur in 18 cows out of 22 cows. This results also indicated the variation between the individual cow's responses towards the same diet. A similar variation was reported by Danscher et al. (2015). The individual variation in animals could be related to the differences in individual cows' feeding behavior, rumen fermentation capability, the rate at which VFA gets removed from the rumen, buffering capacity, and digesta passage rate (Brown et al. 2000; Dijkstra et al. 2020).

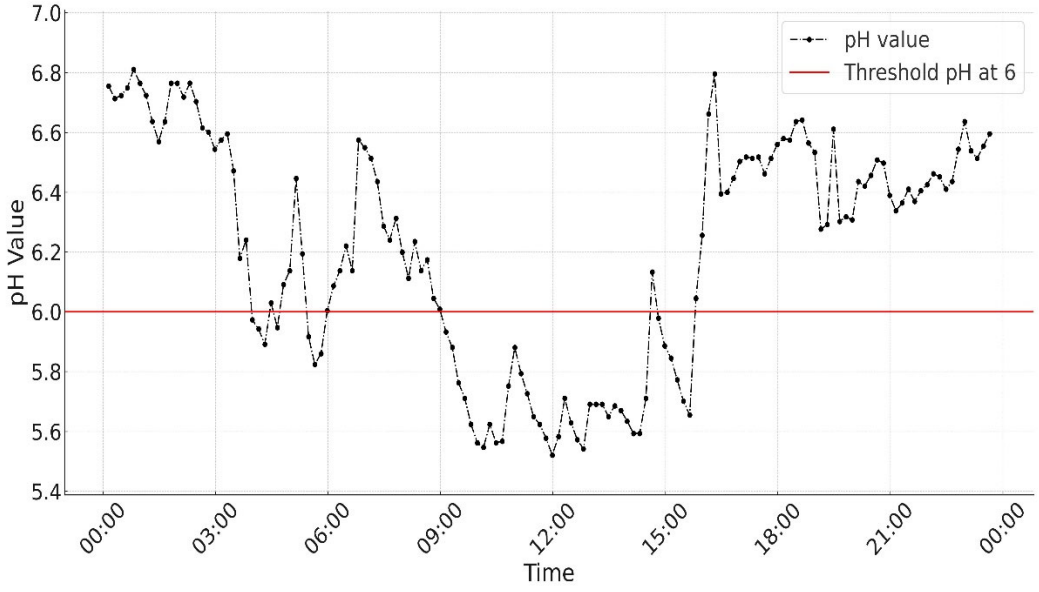
The rumen pH values significantly deviated from the median pH in the SAR2 period, which is shown by notable spikes both above and below the median value (Figure 6). The extreme difference in maximum and minimum rumen pH was also observed in SAR2 period (max pH: 7.95; min pH: 5.3). Usually, in normal cows, a shift of rumen pH can occur up to 1 pH unit in 24 hours (Nocek et al. 2002). However, in this study, in CONT1-fed cows, a shift in rumen pH was observed up to 1.5 pH units. This could have happened due to the variation in the lodging position of the monitoring device used in the study (Neubauer et al. 2018). In SARA-challenged cows, a fluctuation of about 2.1 pH unit was observed in a day. Between periods, fluctuation was significant for minimum rumen pH rather than

maximum rumen pH (Table 2). The decrease in mean minimum rumen occurs due to the feeding of high-concentrate feeds in the SAR2 period (Ramos et al. 2021). All the changes in rumen pH and its metrics that were observed in SAR2 cows went to normal or near normal soon after feeding the cows with a low concentrate diet in the RECOV3 period. This return of rumen pH to the normal range was also reported by other studies (Danscher et al. 2015; OGATA et al. 2019). Diurnal variation in rumen pH was observed throughout the day in this study. Based on the time of day, a similar pattern in variation of rumen pH was not observed between individual cows (Figure 10). However, one of the studies has reported an increase in rumen pH in the morning (Nocek et al. 2002), most of the study agrees that the variation of rumen pH within a day occurs due to the feeding time, amount of feed intake, and proportion of concentrate in feed (Beauchemin 2000; DelCurto-Wyffels et al. 2021). Considering all the points, the rumen sensor boli used was able to detect the variation in rumen pH throughout the day and period (Appendix 1).

Primiparous cows were used in this study to increase the chances of induction of SARA. These cows are more susceptible to SARA feed than multiparous cows (Krause & Oetzel 2006). Primiparous cows are believed to have underdeveloped and less rumen papillae than multiparous cows. Moreover, the rumen microbial ecology is less adapted to the high-concentrate diet (Penner et al. 2007). Primiparous cows consume less feed compared to multiparous cows resulting in lesser chewing time (DeVries et al. 2011). Moreover, the transition of feed from a high forage diet to a high concentrate diet leads to lesser availability of fiber in the feed, and decreased fiber portion in feed leads to decreased overall chewing time in cattle which can ultimately decrease the production of saliva, impacting the buffering capacity of the rumen (Maekawa et al. 2002; Yang & Beauchemin 2007). Studies have reported that the primiparous cows had rumen pH under pH 6 for a longer time than multiparous cows (Humer et al. 2015).



(A)



(B)

Figure 10. An example of diurnal variation registered in rumen pH in two different cows throughout the day, A=cow 2549 and B=cow 2565

Unlike most of the other studies, in this study, a significant increase in DMI intake was seen in SAR2 cows (Table 2) (Krajcarski-Hunt et al. 2002; Antanaitis et al. 2015; Danscher et al. 2015). Similarly, it was found that rumen pH has a moderate correlation with DMI, and duration of pH under 6 has a positive moderate correlation with DMI. The decrease in DMI in other studies was described on the basis of osmolarity in the rumen, decreased fiber digestibility, propionate concentration, and altered feed passage rate (Allen 2000). In contrast, Li et al.

(2014) reported no change in DMI in SARA goats that was induced using the AlfaAlfa hay, corn silage and concentrate. Moreover, in another study, the alfalfa pellets were used to initiate SARA with similar ruminal osmolarity and VFA concentration. Still, there was no change in DMI in cows (Khafipour et al. 2009a). Some other studies reported a similar result as this study, where an increase in DMI was observed (Pourazad et al. 2016; Khalouei et al. 2021). There is a high conflict between studies regarding DMI in SARA cows. The severity of SARA in cows can have an effect on DMI. A decrease in DMI in cows was observed in severe forms of SARA, whereas an increase in DMI was observed in mild forms of SARA (Plaizier et al. 2008; Pourazad et al. 2016; Khalouei et al. 2021). Another reason for the increase in DMI in cows could be their feed-sorting habit. Cows prefer to eat an easily fermentable concentrate diet over high-fibrous forages (Miller-Cushon & DeVries 2017). So, the preference of cows for a high-concentrate diet and its availability in the SAR2 period can be a reason for increased DMI in SARA-challenged cows in this study. Considering the conflicts between results in studies and the calculated low coefficient of determination with rumen pH and duration under pH 6 (See Table 5), it can be concluded that besides rumen pH and duration under pH 6, other factors do have a great role in the regulation of DMI in cows.

As expected, milk fat decreased significantly in SAR2 cows (Table 6). The effect of rumen pH on milk fat was found to be moderately positively correlated, and the impact of duration under pH 6 was found to be moderately negatively correlated with milk fat (Table 5). The result of this study is in agreement with other studies done to see the effect of SARA on milk fat (Danscher et al. 2015; Sandri et al. 2020; Morar et al. 2022). The depreciation of milk fat in SARA is usually associated with a higher production of glucogenic propionic acid. This propionic acid causes higher production of insulin, causing the inhibition of de novo fatty acid synthesis in the mammary gland. Leading to decreased fatty acid in milk (Plaizier et al. 2008). Another theory regarding decreased milk fat is based on the production of trans 10 cis 12 conjugated linolenic acids that occur due to a decrease in ruminal pH. Trans 10 cis 12 conjugated linolenic acids have strong inhibitory action in milk fat synthesis (Maxin et al. 2011). Milk fatty acid is influenced by many factors in a dairy cow farm. Milk fat percentage varies according to DIM, breed of a cow, season, feed constituents, milking frequency, etc. (Palmquist et al. 1993; Rémond et al. 2009; Maurice-Van Eijndhoven et al. 2011; Nateghi et al. 2014; Van et al. 2020). Though most of the studies have shown a significant decrease in milk fat in SARA cows, still all the factors that can influence the fat% in cow milk should be considered. Like milk fat, the fat-to-protein ratio (FPR) is another parameter that decreased significantly in SARA-challenged cows. In this study, a significant decrease in milk fat and an increase in milk protein were observed (Table 6), which is the main constituent of FPR. The decrease in fat and increase in protein in SARA has been confirmed by other studies as well (Fairfield et al. 2007; Khafipour et al.

2009a; Sandri et al. 2020). The association between FPR and SARA has been established by Zschiesche et al. (2020). Similar to Zschiesche et al. (2020), this study found a positive correlation between average rumen pH and FPR and a negative correlation between duration under pH 6 and FPR (Table 5). Among all parameters observed, the coefficient of determination for FPR was found to be highest with duration under pH 6, indicating a better prediction of the duration a cow spent under rumen pH 6 by FPR. However, consideration must be done to include all the factors affecting fat and protein in milk. Along with a drop in milk fat percentage, a drop in all other fatty acids was observed in this study (Table 6). Cui et al. (2023) have contradicting results to the fatty acid profiles analyzed in this study. Moreover, AlZahal et al. (2009) reported increased PUFA when low fiber was fed to the cows. In normal conditions, the rumen microbiome such as *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica* actively break the lipids from feed and bio hydrogenate the dietary PUFA to MUFA and SFA (Jenkins et al. 2008). During the SAR2 period, the rumen pH levels reach the sub-optimum level, where this microorganism struggles to survive and cannot perform biohydrogenation properly (Fuentes et al. 2009). Incomplete biohydrogenation leads to the decreased conversion of feed PUFA to MUFA and SFA. Hence, the decreased production of MUFA and SFA in rumen can be the reason for the low amount of MUFA and SFA in SARA-challenged cows in this study.

Studies have reported different results regarding milk yield in cows. Khafipour et al. (2009) witnessed an increase in milk yield and Krause & Oetzel (2006) and Khafipour et al. (2009) witnessed a decrease in milk yield when SARA was induced in cows. This study observed no significant difference in milk yield and ECM across the periods (Table 6). Similar results were observed in other studies as well (Krause & Oetzel 2005; Danscher et al. 2015; Kitkas et al. 2019). The variation in results can be explained on the basis of the coefficient of determination found between milk yield with average pH and duration of pH below 6. Extremely low values for the coefficient of determination were determined in this study (Table 5), which indicates that milk yield is less dependent on the rumen pH and duration under pH 6. The majority of the changes in milk yield are dependent on other factors like feed quality, breed, milking frequency and time, lactation number, DIM, the health of a cow and udder, temperature, stress, etc (Fleischer et al. 2001; Vijayakumar et al. 2017; Liu et al. 2019; Craig et al. 2022; Adriaens et al. 2023).

8. Conclusion

In conclusion, the wireless rumen sensor bolus used was able to detect variations in rumen pH metrics within individual cows with respect to changes in diet across periods. Moreover, it was able to monitor the rumen pH continuously in detail and provide detailed data regarding the minute fluctuation of rumen pH throughout the day to identify the cows that are most likely in SARA. The use of this device for a long run on the farm should be done, considering its battery lifespan and the drift of the sensor over time. Additionally, to efficiently use this device, a study must be done to identify the exact lodging position of this sensor in the rumen.

In addition, SARA inducing diet decreased the rumen pH and increased the duration of time the cow had a rumen pH below 6. It showed a significant effect on the DMI, milk fat and fatty acid profile, milk protein, and milk FPR. The variation in effect seen in individual cows shows that SARA does not affect all cows in the same manner. Some cows may experience severe drops in rumen pH for a longer time, while others may show minimum or no effect. This indicates the individual variation between the cows in terms of how they can cope with the changes in the rumen environment. The variations in the outcomes of DMI, MY, and milk parameters observed in various studies, including those found in this study, can be attributed to several factors. These factors include environmental conditions, diverse feeding practices, overall health of the cow, stage of lactation, cow breed, and farm management practices. Moreover, along with these factors, the use of different methodologies and measurement tools in studies can contribute to variations in findings between studies. Hence, considering the influence of various factors on DMI, MY, and milk parameters and the variation in results between studies, these parameters cannot stand alone as indicators of SARA.

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Popular science summary

Old technologies that were used for monitoring rumen pH to detect SARA were invasive, expensive, and not accurate. New technology, such as wireless rumen sensor bolus, claims to accurately monitor the rumen pH continuously. However, the efficacy of this technology is still in question due to its various mechanical limitations. This limitation makes its use very expensive in commercial dairy farms. Hence, studies have been done to find alternatives to rumen pH, which are cheap, noninvasive, and accurately identify drops in rumen pH in cows. Studies have been done to see the effect of rumen pH on various parameters such as DMI, MY, and milk parameters, but they show contradicting results. Hence, this study focuses on finding the efficacy of rumen sensor bolus. Along with it, find the impact of rumen pH metrics on DMI, MY, and milk parameters. The result shows that the sensor bolus was able to effectively identify the minute fluctuation in rumen pH throughout the day. Moreover, the effect of rumen pH metrics on production parameters was seen, but the use of this parameter as an indicator of SARA needs further study, incorporating all the factors that can directly influence those parameters in real-life farming condition.

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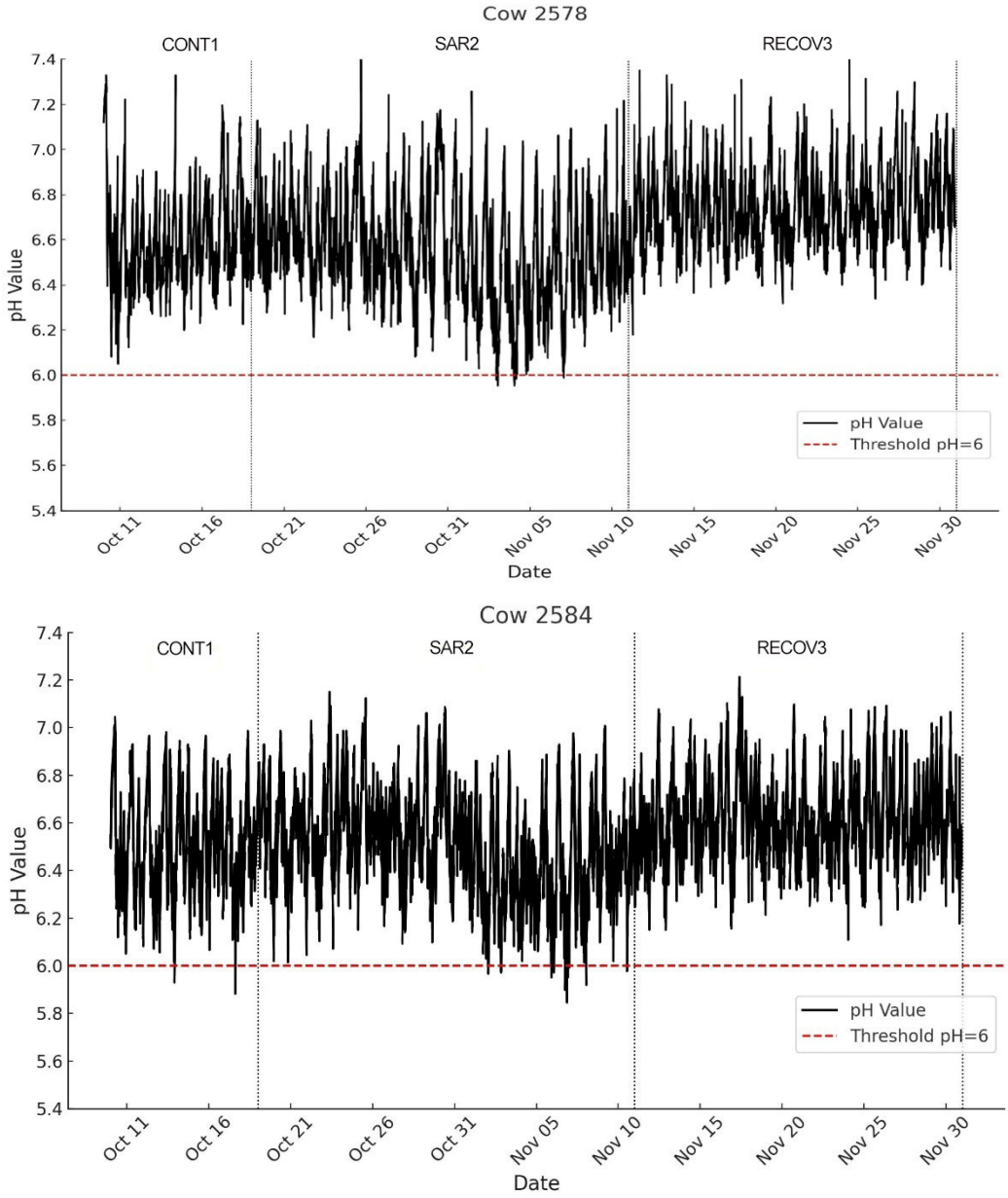
Appendix 1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Date
pH Value
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SAR2
RECOV3
pH Value
Date
pH Value
Threshold pH=6

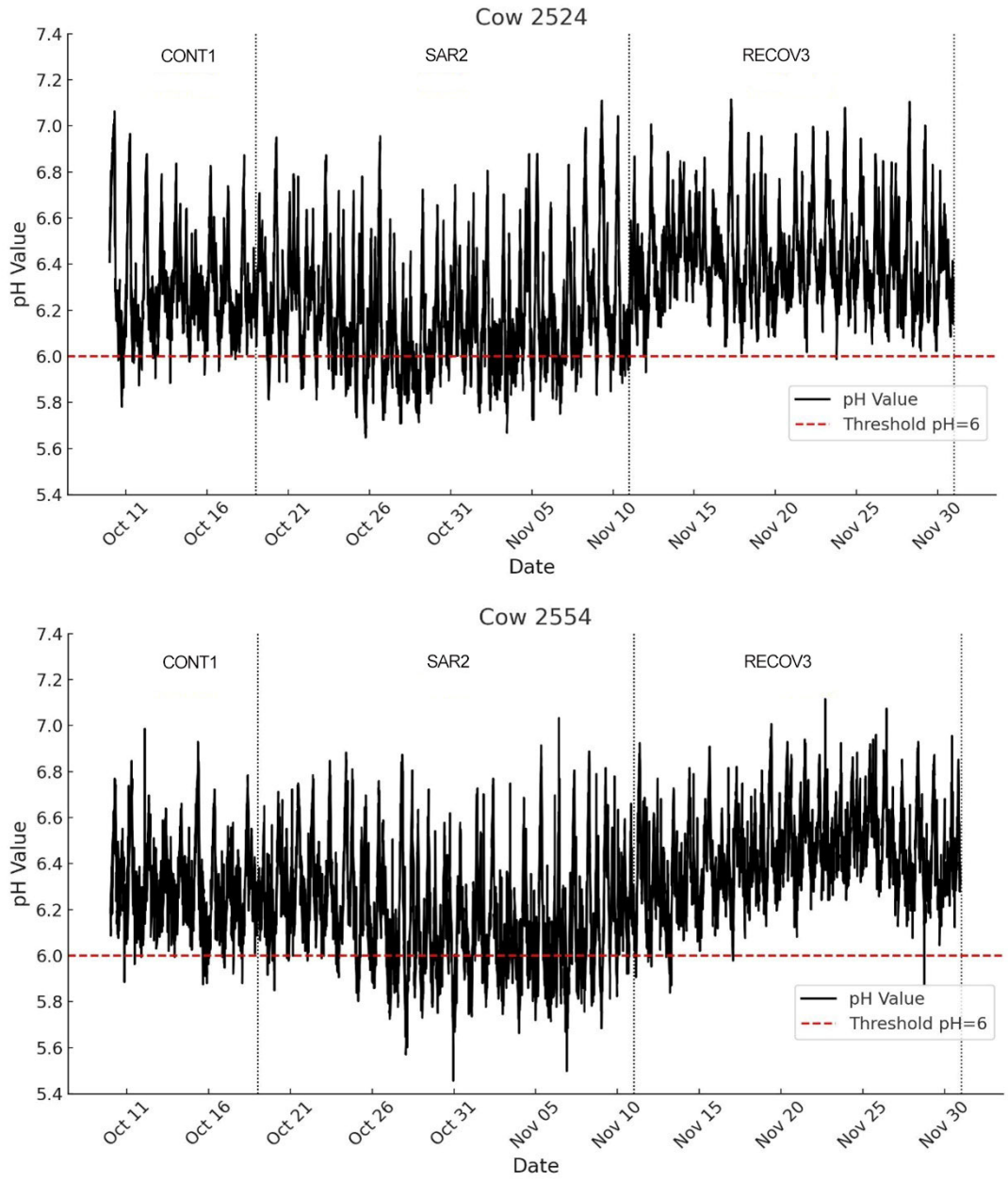

Figure 11. An example of the variation registered in rumen pH in two cows along the whole experiment.

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