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# Effect of changing from forage to high grain diet on ruminal pH, salivary pH and salivary buffer capacity in dairy cows

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Für meine Eltern, Brüder und Großeltern

Danke für eure unermüdliche Unterstützung!

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# 1 Introduction

## 1.1 Physiology and pathophysiology of selected topics concerning ruminal digestion

### 1.1.1 Anatomy and basic function of the rumen

The rumen is the first of four compartments of the digestive tract of the cow. It is located on the left side of the abdomen and reaches from the seventh intercostal space to the pelvis. The rumen of the calf starts to develop with the first intake of solid feed. In the adult ruminant its volume ranges from 60-100 litres, which is about 80 % of the entire volume of the ruminant's gastrointestinal tract. Basically, the rumen consists of a dorsal and a ventral compartment *(Saccus dorsalis and ventralis).* The compartments are divided through longitudinal grooves (*Sulci and Piliae ruminis).* These grooves define a cranial and a caudal ending of each compartment of the rumen *(Atrium ruminis, Recessus ruminis, Saccus cecus caudodorsalis and Saccus cecus caudoventralis).* The wall of the rumen is histologically described as an aglandular, cutaneous mucous membrane and has villi (papillae), that can reach up to ten millimetres. The villi are especially in the ventral compartment of the rumen and in the endings. There are no villi on the *pilae ruminis* (Salomon 2015).

The basic function of the rumen and the other pre-stomachs is to ferment plants and make its compounds digestible for the animal (Engelhardt 2015).

In the rumen the feed particles are divided into bigger particles, that are floating on top and need to be ruminated again, smaller particles, which are ready for fermentation and gas which needs to be released via belching. This leads to the specific stratification of the rumen (gas on top, solid fibre in the middle, fluid at the bottom). The rumen's main function is to ferment the feed compounds. Additionally, it sorts the feed into ingesta, which is ready to be digested in the hindgut and feed which needs to be ruminated again and to release the developing gas. Fermentation takes about 18-72 hours (Breves et al. 2015).

The rumen contracts regularly in stereotypical A- and B-Cycles to digest and to sort the feed. The contractions are regulated by the vagal nerve (Kaske 2015).

### 1.1.2 Rumen microbes and the ideal ruminal milieu

According to Breves et. al. (2015), ruminants are only able to digest plant fibre because they have a symbiosis with several microbes in their rumen. The main part of the microbes are bacteria and protozoa. Each of those groups weigh about as much as 10 % of the whole mass of the rumen. Furthermore, there are archaea and fungi.

Bacteria start to increase in the rumen from the first day of life of the animal. The calf gets in touch with the bacteria through other animals and its environment. A healthy adult cow has about $10^9$-$10^{11}$ $ml^{-1}$ bacteria in its rumen. They are mainly anaerobic bacteria. Feeding has a big influence on the dominating type of bacteria. High fibre rations lead to a higher number of cellulolytic species like *Ruminococcus albus, Ruminococcus flavefaciens* or *Bacteroides ruminocola.* Rations with a higher amount of starch lead to an increase of amylolytic bacteria, like *Streptococcus subspecies* or *Lactobacillus subspecies* (Breves et al. 2015).

Protozoa are divided into ciliates and flagellates. In a physiological rumen there are about $10^5$-$10^8$ $ml^{-1}$ ciliates and $10^3$-$10^4$ $ml^{-1}$ flagellates. They also get in the rumen through direct contact with other animals right after birth. The most important impact on the amount and the type of occurring protozoa species is the amount of concentrate in a ration and the frequency of feed intake (Breves et al. 2015).

The rumen microbes benefit of the regular provision of plant biomass and the steady milieu they face in the rumen. This milieu includes a static temperature of 38-42 °C, a redox potential between -300 and -350 mV and a constant pH of 6-7 (Theodorou and France 2005).

Therefore, it can be concluded that a steady milieu including the pH is very important to keep the ecosystem of the rumen working and the dairy cow healthy.

### 1.1.3 Carbohydrates and their digestion

Carbohydrates are a big chemical group made of carbon and hydrogen. They are classified in monosaccharides, disaccharides, oligo- and polysaccharides, depending on how many sugar molecules are connected. The most important carbohydrates for ruminants are the polysaccharides. Depending on their digestibility they can be divided in easily digestible starch and other non-starch polysaccharides, like celluloses, hemicelluloses, pectin, fructan or pentosan, which are harder to digest (Stangl et al. 2014).

Starch is built of amylose and amylopectin, the connections are an alpha glycosidic bond, which is highly branched. Non starch polysaccharides like cellulose are connected with a beta glycosidic bond. Furthermore, they are built in a straight way (Kues and Köckritz-Blickwede 2020). The chemical structure of starch, which is easily degraded by ruminal microbes, can explain why starch is more rapidly digested than cellulose and other non-starch polysaccharides.

The main part of the digestion of carbohydrates is done in the rumen. They are broken down to monosaccharides and further to pyruvate. From here there are different pathways the rumen microbes use to build various short chain fatty acids. The most important are propionate, butyrate and acetate. Starch and sugar mainly lead to the building of propionate and butyrate. Rations rich in fibre mainly lead to the building of acetate. Most of the short chain fatty acids are resorbed in the rumen and can be used for the energy metabolism of the cow (Stangl et al. 2014).

Concentration of short chain fatty acids can differ in the rumen from 60-180 mmol l-1. The concentration is increasing until several hours after eating. Easily digestible carbohydrates lead to a faster and higher increase of the concentration of short chain fatty acids. This leads to a decrease of ruminal pH (Breves et al. 2015).

### 1.1.4 Pathophysiology of ruminal acidosis

The main factors influencing the ruminal pH are:

- Concentration of short chain fatty acids
- Concentration of buffer substances like $NaHCO_3$ or $Na_2HPO_4$ from saliva
- Speed of absorption of the short chain fatty acids
- Speed of ingesta passage (Dirksen 2002)

Rations with a high amount of easily digestible carbohydrates lead to a fast increase of short chain fatty acids. Moreover, salivation rate is lower in these rations because there is less fibre and so the cows chew less. This leads to a lower concentration of buffer substances. These mechanisms lead to a decrease of ruminal pH. Ruminal pH can change physiologically from 5.5 to 7.0. In cows, ruminal pH decreases after the first feed intake in the morning and rises again about 8 hours later. Ruminal acidosis begins at a pH lower than 5.5 (Dirksen 2002).

Acidosis leads to lesions in the ruminal epithelium and to a malfunction of various transport mechanisms. Furthermore, it can lead to a change of the ruminal microbe community. Microbe's production of lactate increases by an increment of lactate producers in detriment of other microbes. The problem is that lactate is a stronger acid than the other short chain fatty acids and is resorbed a lot less. This leads to an even higher decrease in pH (Breves et al. 2015). Furthermore, the organism tries to compensate acidosis by pulling water in the ruminal lumen. This can lead to dehydration of the cow. Other complications associated with ruminal acidosis are metabolic acidosis and endotoxemia (Dirksen 2002).

### 1.1.5 Overview of the various factors influencing the ruminal pH

As already mentioned, the major factors of ruminal pH regulation are removal of the short chain fatty acids and neutralising the acids through buffer systems. The most important buffer system of the ruminants is the bicarbonate buffer (Dijkstra et al. 2012, Dirksen 2002). Other types of buffering systems are the phosphate and the protein buffer system (Bardow et al. 2000).

Short chain fatty acids can either be removed through a passage to the lower digestion tract in the liquid compartment of the rumen or be absorbed through the rumen wall. There are three pathways by which the short chain fatty acids can be absorbed. First, they can be absorbed undissociated through passive diffusion. Second, they can be absorbed dissociated as an exchange with bicarbonate (Breves et al. 2015, Dijkstra et al. 2012). Third, there has been an investigation showing dissociated absorption, which is bicarbonate independent. It is estimated that about half of all short chain fatty acids are absorbed through the second pathway (Penner et al. 2009).

The secretion of bicarbonate through the rumen wall as an exchange with short chain fatty acids is a very important factor in stabilising ruminal pH. Besides saliva this is the most important mechanism providing bicarbonate to the cow (Cassida and Stokes 1986, Erdman 1988).

An important aspect for the passage and absorption of the acids is the proper stratification on the rumen. Longer fibre particles create a solid fibre mat in the rumen, which stimulates contraction activity of the rumen. This activity is very important to mix the ruminal contents and keep the passage and absorption on a high level. Therefore, ruminal acidosis can be prevented (Yang and Beauchemin 2006).

### 1.1.6 General aspects of saliva in ruminants

Saliva is an important fluid for digestion. For all mammals, saliva is important for the protection of the mucous membrane in the mouth and for making the feed easier to swallow. Especially for ruminants saliva has an essential role in the digestive system for buffering the ingesta (Breves 2015).

A cow can produce up to 250 litres of saliva a day. There are two different types of saliva. The first type is isotone to the plasma and the components are independent from the excretion rate. There are always high bicarbonate and phosphate concentrations. It is excreted by the *glandula parotis* and the *glandulae buccales.* The second type has a lower proportion on the whole saliva rate, than the first one. It is in basal excretion rate hypotone to the plasma with low bicarbonate and phosphate concentrations. This type of saliva is stimulated with feed intake. It can change its concentrations of ions massively when it is stimulated. Higher salivation rates lead to higher concentrations of sodium, chloride and bicarbonate. It is excreted by the *glandula mandibularis,* the *glandulae sublinguales* and *glandulae labiales.* Depending on the concentration of mucin the saliva can be serous, mucoserous or mucous. Furthermore, saliva contains nitrogen as a part of the ruminohepatic circulation (Breves 2015).

## 1.2 Current issues regarding high yielding cows and nutrition

### 1.2.1 Development of dairy cows and resulting challenges

Milk yield increased immensely over the last years. The graph below shows an example of the average milk yield relative to one dairy cow in Germany over the last century. It shows that from 1990 to 2020 the average milk yield of a dairy cow increased by nearly 80 %. Though the number of dairy cows reduced over the years, the amount of produced milk stayed the same (BLE 2021).

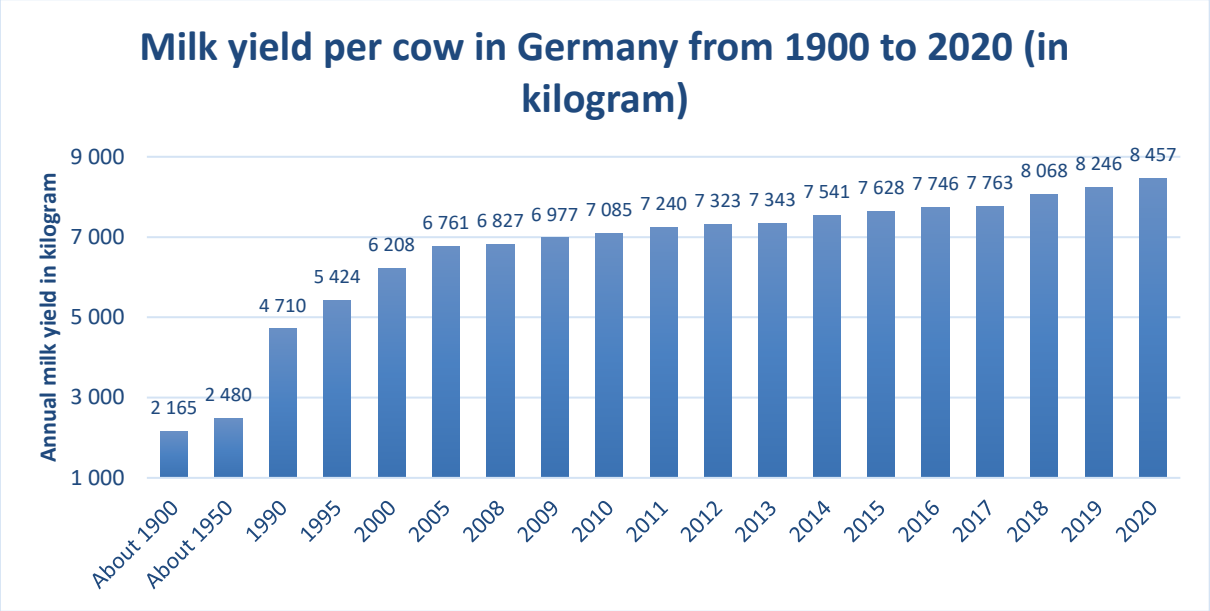


*Figure 1 Milk yield per cow in Germany from 1900 to 2020 (in kilogram) (BLE 2021)*

A big factor for increasing milk yield is the rising genetic potential of the high yielding breeds. High yielding dairy cows have been bred to be bigger, more angular and are able to have a higher feed intake. Their genetic potential gives them the ability to produce a higher amount of milk with less loss of their body fat (Reynolds 2004).

According to the rising milk yield and the high genetic potential of dairy cows in intensive production systems appropriate feeding has become a challenge. Dairy cows face very high metabolic and energetic challenges. Especially in early lactation they need a lot of energy through the fast increase of their milk production combined with stress of birth and metabolic changes. In the first period of lactation, the milk yield rises, even if the cow is facing a negative energy balance. Common diseases according to this situation are ketosis, fatty liver syndrome and parturient paresis (Blowey 2016, Stangl et al. 2014).

To prevent this lack of energy and the following disorders attention needs to be paid to feed the dairy cows appropriately especially in the first period of the lactation. It is a challenge to provide the cow enough energy at this stage of lactation. In the first weeks after calving feed intake of the cows is 20-25 % less than usual. Furthermore, the change of diet is a problem for the metabolism of the rumen and excessive easily digestible carbohydrates can lead to acidosis (Spiekers and Potthast 2004).

### 1.2.2 Common digestive disorders associated with high concentrate diets

Feeding high amounts of easily digestible carbohydrates leads to a fast increase of short chain fatty acids in the rumen, resulting in decreases of ruminal pH. If the pH of the rumen stays under 5.6 for 3-5 hours this clinical situation is called subacute ruminal acidosis (SARA). SARA leads to various subsequent diseases like reduced fibre digestion, diarrhoea, liver abscesses or laminitis. Additionally, SARA affected cows have a decrease in milk yield and milk fat percentage. In combination with higher costs for veterinary services and higher replacement rates these circumstances lead to higher productive costs. It is assumed that these costs are about 400 US Dollars per cow and lactation (AlZahal et al. 2007, Plaizier et al. 2008).

Cows in early and mid lactation are predisposed to SARA (Garret et al. 1997, Plaizier et al. 2008). It has been discovered that the prevalence of SARA over all cows in dairy herds in Germany is 20 %, in Denmark 22 % and in Italy even 33 % (Enemark and Jørgensen 2001, Kleen et al. 2013, Morgante et al. 2007). Clinical signs are a decrease in ruminating and irregular feed intake. Cows hardly show any symptoms when dealing with ruminitis or liver abscesses (Dirksen 2002).

According to this economic and animal welfare issues it is obvious that the conclusion needs to be, that preventing SARA and doing more research on ruminal pH and feeding is necessary.

A diet with a big amount of starch and other fermentable carbohydrates can also lead to an insufficient digestion in the rumen. Carbohydrates pass from the rumen to the hindgut and are fermented there. An excess of short chain fatty acids and other acids can lead to a decrease in pH and furthermore, to hindgut acidosis (Gressley et al. 2011). Hindgut acidosis can lead to a change of the microbial ecosystem and a damage of the epithelium (Plaizier et al. 2018).

Another disorder which may occur in dairy cows in early lactation is metabolic acidosis. This can be caused by an inappropriate diet or as a result of ruminal and hindgut acidosis (Enemark et al. 2002).

### 1.2.3 Important guidelines for dairy cattle nutrition

There are various parameters which need to be optimised to feed the cows appropriately and prevent ruminal acidosis and its resulting problems. First, cows need enough fibre in their rations. Fibre stimulates ruminating and therefore, saliva flow. Saliva brings buffering substances in the rumen, which help to stabilize the ruminal pH. A general recommendation is that dairy cows should not have less than 18 % of fibre in their ration (Dirksen 2002).

Another factor influencing ruminal pH is processing the various kinds of feed. For example, a study of Yang and Beauchemin has shown, that a higher grade of processing barley leads to a higher availability of the starch in the rumen. This resulted in a higher increase of acids in the rumen and a higher risk for ruminal acidosis (Yang et al. 2001). Concerning forage less processing is also an advantage to prevent acidosis. As pointed out before, longer fibre supports the formation of a solid fibre mat, which stimulates ruminal contractions and therefore absorption and passage of the acids (Yang and Beauchemin 2006).

According to Dirksen concentrate is best fed divided in small portions all over the day alternately with roughage. Total mixed rations (TMR) are beneficial, but it’s important not to break up the roughage too much, so the cows are still stimulated to chew sufficiently. Ideally dairy cows are divided into performance groups and each group can be fed with their appropriate TMR.

Furthermore, adaptation is very important for the microbes of the rumen and its mucosa. Ideally the proportion of concentrate is increased slowly over two to four weeks. This gives the microbes time to adapt to the ration and the mucosa can proliferate its surface, which leads to a higher resorption rate of the short chain fatty acids. Cows around their date of birth eat less and often prefer the concentrate, while leaving the roughage. This leads to drastic decreases in ruminal pH. That is why it is important to feed the dry cows an adaptation ration for two to three weeks. The proportion of concentrate should be raised slowly and they should get small amounts of their ration after giving birth (Dirksen 2002).

Another method for preventing acidosis is to feed additional exogen buffer substances to the cows, like sodium bicarbonate or magnesium oxide. These products stabilise the ruminal pH either through raising buffer capacity or through direct neutralising of the arising acids (Zamarreño et al. 2003).

## 1.3 Current research on pH and buffer capacity of saliva

### 1.3.1 Saliva as a buffering substance

Saliva plays a major role for buffering short chain fatty acids produced in the rumen. According to different sources saliva provides 30 % to 90 % of the whole buffer capacity in the rumen (Allen 1997, Dijkstra et al. 2012, Kay 1966).

It is generally assumed that fibre intake increases chewing activity and ruminating of the cows. This leads to a higher salivation rate and to a higher amount of buffering substances in the rumen. Therefore, including appropriate fibre concentrations in a diet is important for acidosis prevention (Krause and Oetzel 2006).

This rationale is also supported by a study done by Yang and Beauchemin in 2007. According to the study, an increased proportion of forage in a diet leads to better conditions in the rumen including a higher pH. The reasons are an increase in chewing activity, different feed intake behaviour and a decrease in the production of short chain fatty acids (Yang and Beauchemin 2007).

Another study supporting this relationship is a study done by Castillo-Lopez et al. (2021). It has been shown that the buffer substances of saliva are very important factors depending pH regulation in the rumen. Cows consuming high concentrate diets for short periods are able to keep the ruminal pH higher through higher chewing and salivation rates, compared to cows dealing with those rations for a longer time. It has been shown that those cows consuming a high concentrate diet for a longer time gain a higher buffer capacity and phosphate concentration in stimulated saliva. This might be an adaptation to the diet. However, they have a higher risk to develop ruminal acidosis due to a decrease in chewing and insalivation. It is suggested to focus research on strategies to increase salivation of cows consuming high concentrate diets to prevent ruminal acidosis (Castillo-Lopez et al. 2021).

Nonetheless, there are results from several studies showing controversial results. A higher amount of fibre or a higher particle size of the roughage in high concentrate diets did not affect the total daily saliva production in those studies as an example. Cows consuming a diet with more fibre showed more time ruminating and eating. The saliva rate during eating and ruminating was 1.3-2.2 times higher than while resting. However, the total daily saliva production only increased minimally compared to control cows (Jiang et al. 2019, Maekawa et al. 2002). Similar results were also achieved in a study done by Zebeli et. al. (2007). Cows fed with a high grain diet showed more ruminating with increased particle size of roughage. However, higher rate of ruminating and chewing did not lead to an increase in ruminal pH. An explanation could be that the buffer system of saliva is not able to compensate the high amounts of short chain fatty acids being produced with high grain diets (Zebeli et al. 2007).

This conclusion is also supported by Yang and Beauchemin (2007). In high concentrate diets with low forage amount, the low proportion of effective fiber is not sufficient to increase particle size of the total mixed ration. The effect on chewing and ruminating is small, when the amount of forage in a diet becomes relatively too small. Therefore, it is concluded that from a certain point chewing behaviour is not able to compensate the arising acids anymore (Yang and Beauchemin 2007). The study by Castillo-Lopez et al. (2021) showed that cows fed with a high concentrate diet for a longer time showed less insalivation of their feed. It is suggested that saliva as a buffering substance gets less important in those diets compared with the different resorption mechanisms of the short chain fatty acids (Castillo-Lopez et al. 2021).

Other studies show that mean ruminal pH is only slightly affected by increasing fibre length, but there are differences in the pH fluctuations over the day. For example, the mean ruminal pH of a diet with low particle length only decreased by 4 %, but the time ruminal pH was under 5.8 was two to three times higher than diets with higher particle lengths (Beauchememin and Yang 2003, Krause et al. 2002, Yang et al. 2001).

### 1.3.2 General research on saliva

In veterinary medicine, the main focus on research of saliva has been, to make saliva samples of animals potentially useful as a diagnostic tool. For example there have been studies showing that the proteome of saliva might be useful to detect bloat in cattle (Rajan et al. 1996). A current study by Franco-Martínez et al. (2021) also pointed out that cows with mastitis showed significant differences in the proteome of saliva, which might be useful for the diagnostic of this disease in future (Franco-Martínez et al. 2021).

There have been studies in pigs showing that saliva might be a useful tool to detect pregnancies via progesterone measurement, inflammation via the C-reactive Protein or even viral infections of the porcine respiratory and reproductive disease virus and influenza (Gutiérrez et al. 2009, Moriyoshi et al. 1996, Prickett and Zimmerman 2010). Furthermore, it is hypothesized, that the proteome of saliva could be useful to detect malnutrition (Lamy and Mau 2012).

In ruminants a study by Palma-Hidalgo et al. (2021) shows that saliva seems to play another useful role in ruminal ecosystem besides being a buffering substance. It is suggested that there are some components in saliva which are able to modulate the activity of microbes in the rumen (Palma-Hidalgo et al. 2021).

Other studies found that mucin in saliva of cattle is able to disperse foam and that lysozyme showed in vitro microbe modulating and methane lowering effects (Bartley and Yadava 1961, Biswas et al. 2016).

In the study done by Castillo-Lopez et al. (2021) various aspects of unstimulated saliva have been investigated concerning changes with the duration of a high concentrate diet. The result was that pH, buffer capacity, bicarbonate and phosphate concentration, total protein, lysozyme concentration and activity and mucin concentration did not change. The osmolality of the unstimulated saliva changed significantly, which has been suggested to be possibly used for diagnosis of acidosis. Furthermore, it has been shown that stimulated saliva has a higher buffer capacity and phosphate concentration than unstimulated saliva in general and that a higher saliva flow leads to a higher concentration of phosphate and bicarbonate. The pH of stimulated saliva decreased with a longer duration of high concentrate feeding while buffer capacity and phosphate concentration increased (Castillo-Lopez et al. 2021).

In human medicine, there is a lot of research conducted on saliva, its pH and buffer capacity. The main reason is the importance of saliva and its buffer capacity to prevent dental caries (Stamford et al. 2005, Yeh et al. 2012). The buffer capacity correlates with the concentration of bicarbonate and the flow rate of saliva, but also with gender (Bardow et al. 2000, Wikner and Söder 1994). Another study showed that stimulated saliva shows a significantly higher buffer capacity than unstimulated saliva (Moritsuka et al. 2006). In humans, pH measurements have been done on tooth plaques. They are regarded to be modified by saliva (Roth and Calmes 1981). After eating pH rises in the first 5 minutes. About 15 minutes later pH decreases to 6.1 or lower. Then pH returns slowly back to resting pH of 6-7 (Bibby et al. 1986, Edgar 1976, Rugg-Gunn et al. 1975).

Because of the important role of saliva in animal health and gut function the objectives of this study were to evaluate the effect of changing from a forage diet to a high concentrate diet on ruminal pH, salivary pH and salivary buffer capacity of Holstein dairy cows.

# 2 Material and Methods

## 2.1 Animals

The experiment was conducted at the Vet Farm (Kremesberg 13 2563 Pottenstein) from the 10th of June 2019 to the 27th of June 2020. Nine ruminally-cannulated Holstein Friesian cows were used for the feeding experiment. The cows were moved to the experimental pen three days before the experiment started, to give them time to adapt to the conditions. Each cow was adapted to always use the same feedbunk, which it could enter with a chip. In this way data of dry matter intake could be taken from every cow individually. The number of the approval for this animal experiment is BMBWF-68.205/0003-V/3b/2019.

## 2.2 Diets

Each run of the experiment lasted six weeks. In the first week the cows received an only forage diet. The second week was an adaptation week. The grain in the diet was raised every day until they received a diet with 65 % grain and 35 % forage on the last day of the adaptation week. This high-grain diet was fed for four more weeks. Table 1 shows the details of the two different diets.

***Table 1 Details of Rations***

| | Diet | |
|---|---|---|
| Item | Forage | 65% concentrate |
| Ingredients, % | | |
| Grass silage | 45.00 | 26.25 |
| Corn silage | 45.00 | 8.75 |
| Grass hay | 10.00 | 0.00 |
| Concentrate mix[1] | 0.00 | 65.00 |
| Chemical composition | | |
| DM, % fresh matter | 34.0 ± 1.06 | 47.1 ± 1.84 |
| Crude protein, % | 11.0 ± 0.18 | 17.8 ± 0.38 |
| Neutral detergent fiber (NDF), % | 55.0 ± 1.80 | 32.0 ± 1.90 |
| Acid detergent fiber (ADF), % | 34.2 ± 0.23 | 21.7 ± 0.85 |
| Starch, % | 17.3 ±1.75 | 28.8±1.90 |
| Ether extract, % | 1.66 ± 0.25 | 3.23 ± 0.48 |
| Non-fiber carbohydrates, % | 22.9 ± 1.62 | 39.3 ± 2.21 |
| Ash, % | 6.70 ± 0.10 | 6.76 ± 0.27 |
| Particle fraction (% as-is retained)[2] | | |
| Long (>19.0 mm) | 64.6 ± 3.55 | 28.6 ± 3.14 |
| Medium (8.0 to 19.0 mm) | 21.3 ± 3.00 | 29.1 ± 4.63 |
| Short (1.18 to 8.0 mm) | 13.6 ± 0.52 | 40.1 ± 5.00 |
| Fine (<1.18 mm) | 0.51 ± 0.02 | 2.13 ± 1.23 |
| Physically effectiveness factor | 0.85 ± 0.005 | 0.58 ± 0.06 |
| peNDF[3] >8 mm | 49.3 ± 1.53 | 18.5 ± 1.48 |

[1]The concentrate mixture contained: wheat (30.36%), triticale (18.06%), bakery by-product (23.02%), rapeseed meal (23.94%), molasses (2.99%), mineral-vitamin premix for dairy cattle (1.53%), limestone (1.0%). The chemical composition and particle size of the 40% concentrate diet was estimated from data measured for the forage and high concentrate diets.
[2]Particle fractions determined with the Penn State Particle Separator with a 19-mm screen (long), 8-mm screen (medium), 1.18-mm screen (short), and a pan (fine) according to Kononoff et al. (2003).
[3]Physically effective NDF.

This feeding process was repeated four times, which leads to four runs. Each run lasted six weeks, between the runs, there were washout-periods. The washout-periods lasted for four weeks. In this way every cow was used as its own control.

## 2.3 Measurements of ruminal pH

Ruminal pH was monitored using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; DASCOR Inc., Oceanside, CA, USA) and followed the methodology described by Penner et al. (Penner et al. 2006). The pH systems were calibrated in pH 4 and 7 prior to inserting the sensors, through the ruminal cannula into the ventral sac of the rumen and after removal. Proper communication with the computer for data collection and download function were verified. Ruminal pH data were measured every 15 minutes and the data were downloaded on a weekly basis. At the end of each sampling period, the pH systems were placed in a container with warm water and the data were downloaded. The appropriate location of probes was confirmed at the moment of retrieval (Castillo-Lopez et al. 2014).

## 2.4 Sampling of saliva

The saliva samples were collected once a week before the morning feeding. The samples were collected in the week of forage diet and in the four weeks of high grain diet. This way five saliva samples were collected from each cow in one run. They were taken from the mouth from the space between teeth and cheek using a vacuum-pump. Immediately afterwards they were frozen at -20 °C. Every sample consisted of about 8 ml saliva.

## 2.5 Analysis of pH and buffer capacity of saliva

In the laboratory, the saliva samples were thawed 2-4 hours. Afterwards, they were centrifuged to divide the fluid from the solid parts of the sample. The saliva was pipetted with a piston-operated pipette (Eppendorf Research, Eppendorf SE, Germany). One ml of every sample was pipetted twice into measuring vessels. Every sample was done twice to raise precision.

For the pH measurements, the pH meter Mettler Toledo Seven Multi (Mettler-Toledo LLT, United States of America) was used to receive more precise results. An alternative would have been the Mettler Toledo Seven Go (Mettler-Toledo LLT, United States of America) which is faster, but less accurate.

After calibration of the pH meter the electrode was flushed with WEK water and dried with paper. Then the pH of the samples were measured (→ pH before). Between every measured sample the electrode was flushed and dried again.

For the evaluation of the buffer capacity 3 ml of 0.005 mol/l hydrochloric acid was added to the 1 ml saliva samples after the first pH has been taken. The solution was stirred with a magnetitic stirrer (Heidolph MR Hei Standard, Heidolph Instruments GmbH & Co. KG, Germany) at about 260 rounds per minute set speed for 10 minutes. After 10 minutes the second measurement was done (→pH after).

The number of mol of HCl used for each sample was calculated. Then buffer capacity of saliva was calculated and expressed in mol of HCl used for every unit that salivary pH changed. A higher value indicates an improved ability of saliva to regulate ruminal pH in the presence of acids.

## 2.6 Statistical analysis

Data were analysed statistically with the PROC Mixed procedure of SAS with experimental period and type of diet (forage vs. high concentrate) as fixed effects, and cow within period as random effect. Data from different times (weeks) from the same cow in the same treatment were processed as repeated measures with first order variance-covariance structure matrices taking into account that the variance-covariance decays time. The largest standard error of the mean (SEM) was reported. Statistical significance was declared when $P \leq 0.05$ and tendency was mentioned and discussed if $0.05 < P \leq 0.10$.

Regression analysis was conducted with SAS using Proc reg to evaluate the association between salivary pH and salivary buffer capacity.

# 3 Results

## 3.1 Ruminal pH

Figure 2 shows the mean ruminal pH in the week of forage feeding and the four weeks of high grain feeding. As expected, the ruminal pH was highest in the week of forage feeding ($P<0.05$). The mean ruminal pH of all cows was 6.5. After the adaptation week ruminal pH dropped to a mean value of 6.05. In the following weeks the pH slightly increased again to mean values of 6.1-6.15. The superscripts show that the decrease from the first week of forage feeding to the following weeks of high concentrate feeding is significant, while the variations between the weeks of high grain feeding are not significant.

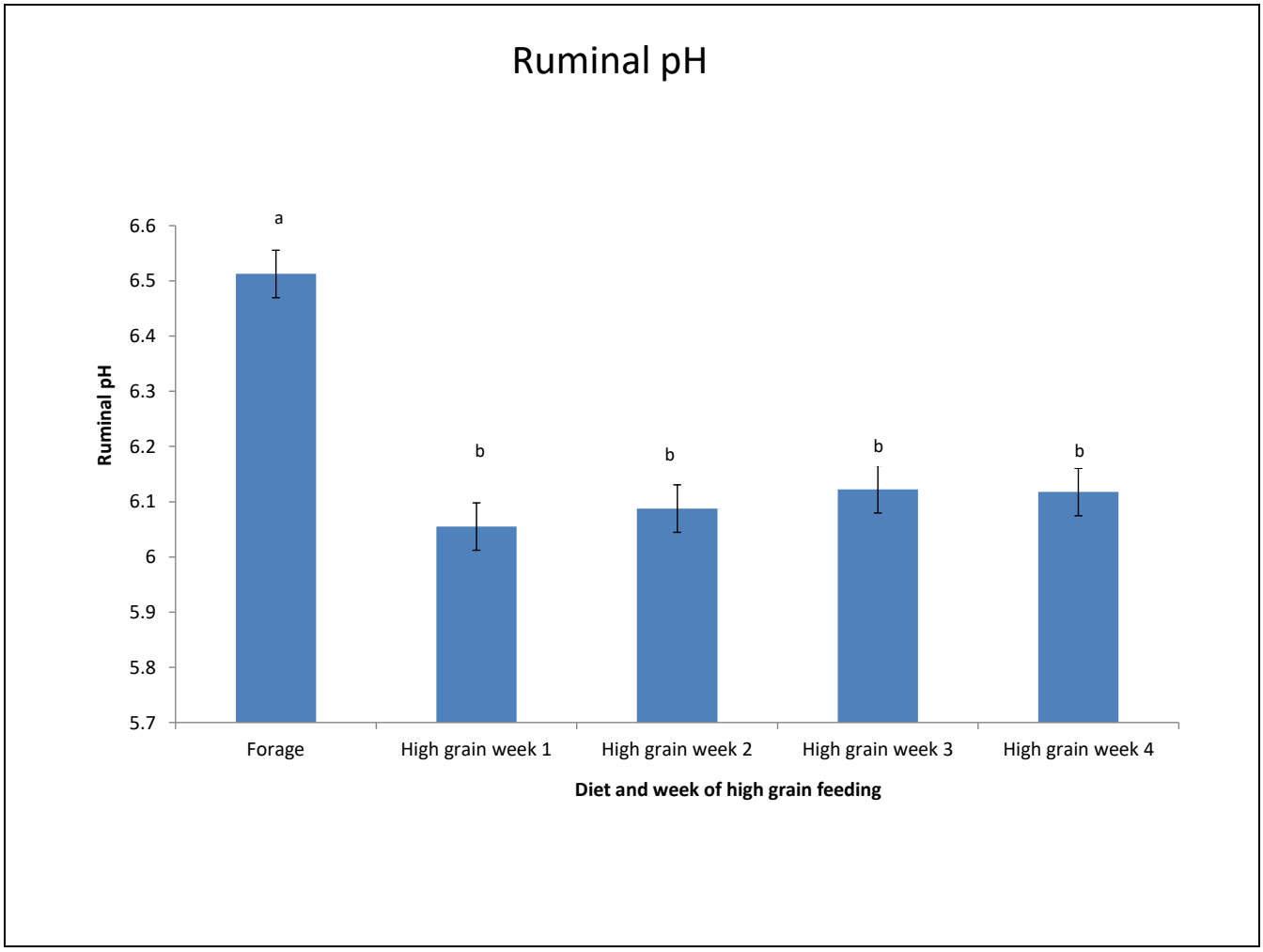


*Figure 2 Mean ruminal pH in the week of forage feeding and four weeks of high concentrate feeding*

## 3.2 Dynamic fluctuation of ruminal pH

Figure 3 shows the variation of ruminal pH over one day of a cow consuming the forage diet compared to a high grain diet. The blue line visualizes the fluctuation of a cow consuming the forage diet. It is shown that ruminal pH is highest in the morning at a value of 6.8 and tendentially decreases with various small peaks throughout the day. Ruminal pH is lowest at night at 00:15 with a value of 6.37. Afterwards it increases again more and more.

The red line visualizes the fluctuation of ruminal pH of a cow consuming the high concentrate diet. As expected, all values are in a lower level, than in the week of forage feeding. It is clearly seen that the lowest value is at 5.72 in the evening at 19:15. Overnight pH also increases and reaches its maximum value of 6.4 at 6:30 in the morning. Due to the first feeding ruminal pH rapidly drops again to 6.12 at 7:45. Over the day the pH further decreases with small peaks.

The main differences between the two pH curves are, that the pH curve of the forage feeding is on a higher level. Especially the minimum values show an enormous difference with 6.37 in the forage diet week and 5.72 in the week with high concentrate diet. Another clear difference is that the difference between the maximum and the minimum value in the forage feeding is 0.43, while it is 0.67 in the high concentrate diet. This suggests that pH fluctuation over the day is higher with high concentrate diet than forage diet. Also important to notice is that cows consuming the high concentrate diet had a pH lower than 6 for 13 hours per day, while the cows consuming forage did not reach this threshold at all.

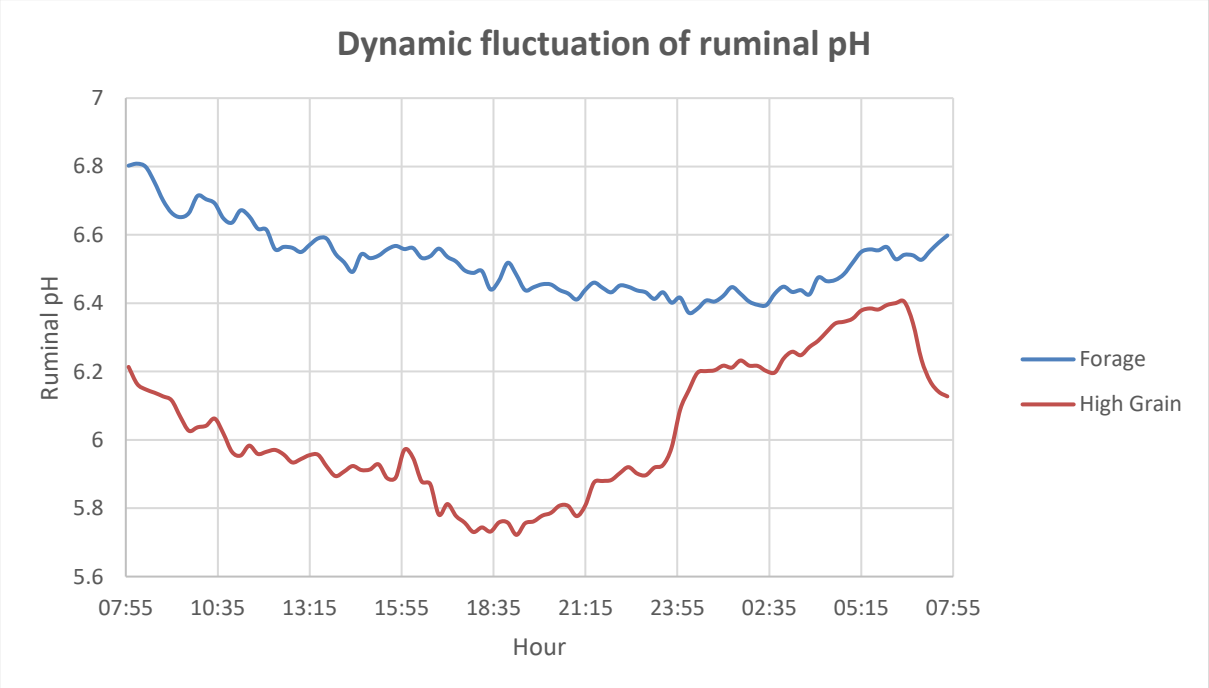


*Figure 3 Dynamic fluctuation of ruminal pH*

## 3.3 Salivary pH

In figure 4 the mean salivary pH of the cows depending on the diet and week of high grain feeding is shown.

In the first week with only forage diet, the mean salivary pH was 8.87. After the dietary adaptation week, the mean salivary pH dropped to 8.81 in the first week of high grain feeding. This is the minimum value of all measurements ($P < 0.05$). In the second week of high concentrate feeding the pH increased again to 8.90. The maximum mean pH was measured in the third week of high grain feeding with 8.95. In the fourth week the pH slightly decreased again to 8.92. SEM was calculated 0.03644 for all values.

The superscripts show that the difference of salivary pH between the week of forage feeding and the first week of high concentrate feeding is not significant. However, there was a significant difference between the first week of high concentrate feeding and the following weeks with high concentrate diet.

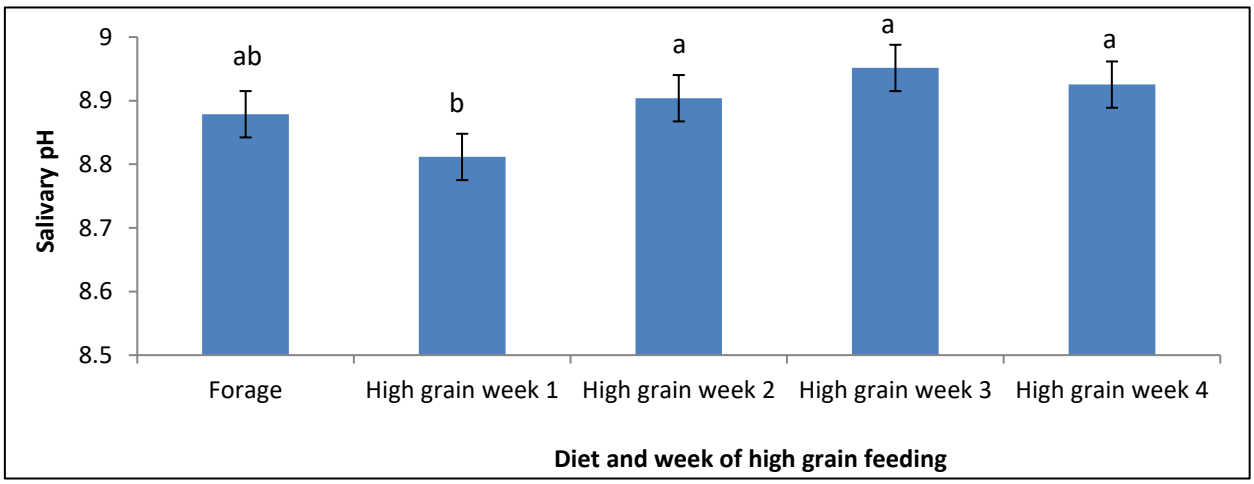


*Figure 4 Effect of changing from forage to high grain diet on salivary pH in dairy cows*

## 3.4 Salivary buffer capacity

In figure 5 the mean salivary buffer capacity of the cows depending on the diet and the week of high grain feeding is shown.

It is clearly shown that the buffer capacity of the saliva raised ($P<0.05$) with increasing proportion of concentrate in the diet. In the first week, where the cows received an only forage diet, the mean buffer capacity of saliva was 0.0126 (SEM 0.00038), which is the minimum value of all measurements.

In the first week of high concentrate feeding the buffer capacity reached its maximum value of 0.0140 (SEM 0.00039). Afterwards buffer capacity decreased again. In the second week of high concentrate feeding the mean salivary buffer capacity was 0.0133 (SEM 0.00038) and in the third week it was 0.0135 (SEM 0.00039). In the last week of high grain feeding, the buffer capacity slightly increased again to 0.0138 (SEM 0.00038). Overall, it can be concluded that buffer capacity increased significantly due to the change in diet. Moreover, it maintained high during the feeding of the high concentrate diet. Therefore, results demonstrate an effect of the proportion of concentrate in a diet on the buffer capacity in saliva.

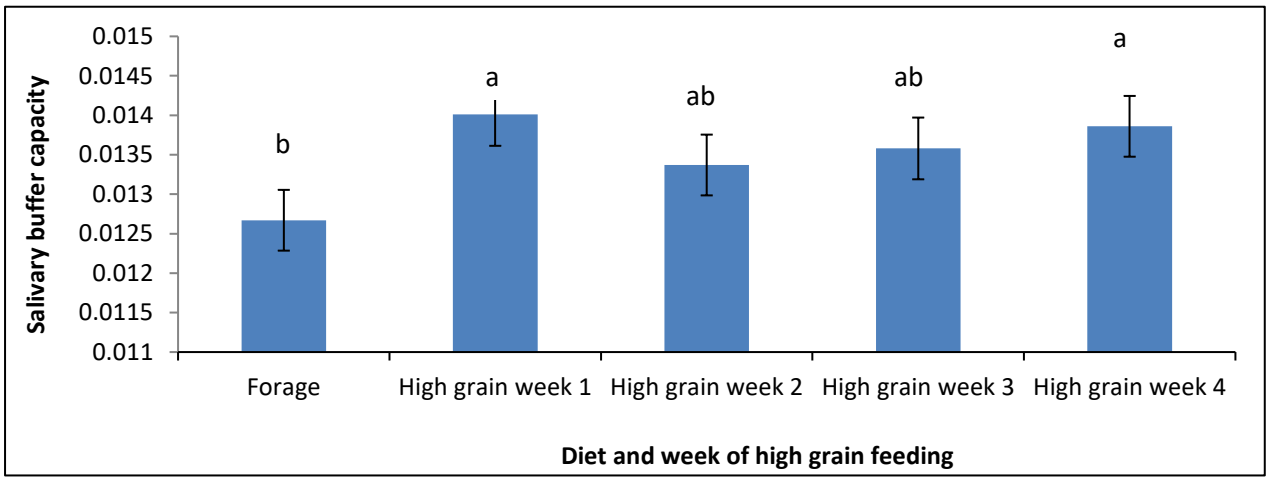


*Figure 5 Effect of changing from forage to high grain diet on salivary buffer capacity in dairy cows*

## 3.5 Correlation between pH and buffer capacity

Figure 6 shows the regression analysis of the salivary buffer capacity and salivary pH. It shows that there is a low correlation between those two variables. The p-value below 0.01 indicates that there is a significant positive correlation. However, the $R^2$ value of 0.273 shows that the association is low.

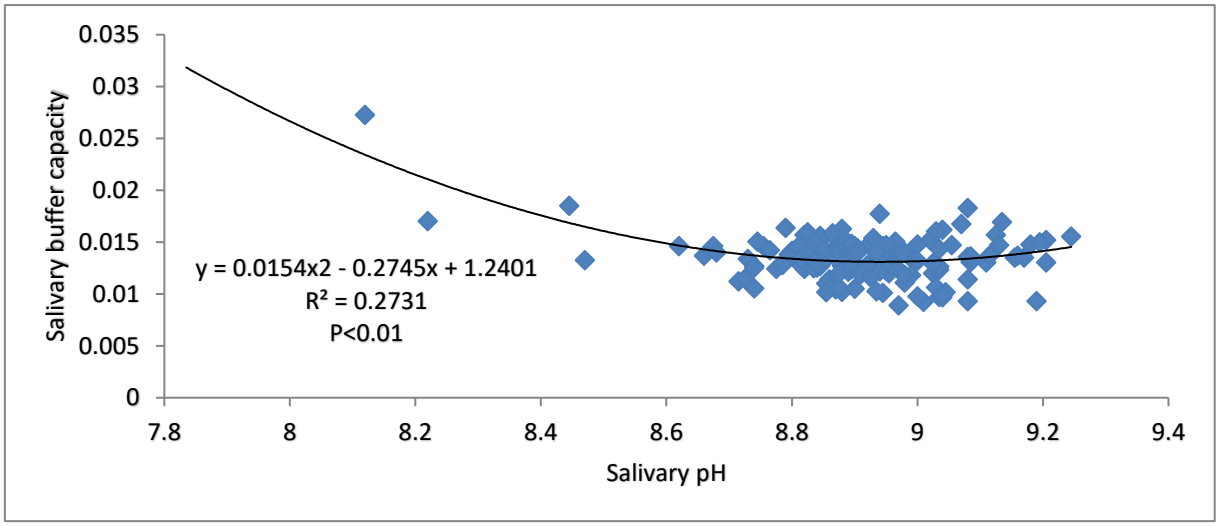


*Figure 6 Regression curve for salivary pH and buffer capacity*

# 4 Discussion

The objectives of this study were to evaluate the effect of changing from a forage diet to a high concentrate diet on ruminal pH, salivary pH and salivary buffer capacity of dairy cows.

The mean pH of the cows fed with the forage diet is not only higher but does also not show as high fluctuations as the pH of cows fed with the high concentrate diet. Regarding Plaizier et al. in 2008 SARA is described as the condition when the ruminal pH stays under 5.6 for 3-5 hours. Cows consuming the high concentrate diet showed a higher risk for SARA, as they stayed 13 hours per day with a ruminal pH lower than 6. Cows consuming the forage diet did not reach this threshold at all. This is for sure explained by the higher amount of easily digestible carbohydrates, which leads to a fast increase in short chain fatty acids in the rumen and a fast decrease in ruminal pH. This context might also support the hypothesis that cows are able to compensate ruminal pH changes to a certain extent due to their buffer systems in saliva. However, when the amount of concentrate gets too much, the cows are not able to compensate the arising acids in the short time anymore. Buffer systems of the rumen get relatively more important (Allen 1997). If the amount of easily digestible carbohydrate gets too much and buffer systems can not compensate the arising acids anymore, pH drops and SARA occurs.

Contrary to the findings of Castillo-Lopez et al. (2021), the present study shows that buffer capacity significantly increases with the transition from forage to high concentrate diet. Additionally, buffer capacity maintains high during the whole period of the high concentrate diet. This could be attributed as an adaptation mechanism of the cows. Due to the increase of the salivary buffer capacity more buffer substances can be provided to the rumen to prevent ruminal acidosis.

Regarding the salivary pH, an increase proportional to the raising buffer capacity was expected. This positive correlation could not be proven. It is important to point out that there are a lot of different components which have an enormous influence on salivary pH. For example, the concentration of bicarbonate, phosphate and the protein buffer system has not been measured in this study. It is possible that changes in the concentration of any of those salivary components could be an explanation for the pH fluctuations. Additionally, there are a lot more different factors influencing salivary pH. For example, a study done by Heintze et.al. (1983) showed that the buffer capacity of saliva in humans is influenced by various factors such as gender and alcohol or nicotine consumption (Heintze et al. 1983).

This shows that there needs to be done more research on saliva in ruminants, and more especially in dairy cows, to determine other influencing factors on salivary pH and buffer capacity.

Another factor potentially influencing the pH measurements in the present study is the processing of the saliva samples. It is possible that the freezing to –20 °C and the thawing might have affected the measurements of saliva. It is suggested to measure the pH immediately after collection on the farm in the future to evaluate these effects.

However, there was a significant difference of salivary pH between the first week of high concentrate feeding and the last three weeks of high concentrate feeding. It can be suggested that the cows have been able to raise the salivary pH through the higher buffer capacity in saliva.

The higher salivary buffer capacity from the second to the fourth week of high concentrate feeding could have been expected to contribute to an increase of the ruminal pH. However, this was not the case in this study. Similar results were achieved by a study done by Dohme et al. (2008). Cows fed with a high concentrate diet had a higher risk for SARA with advanced days of feeding the diet (Dohme et al. 2008). Possible explanations might be that cows challenged with a low ruminal pH for a longer time have increasing health problems such as ruminitis, disbalances in ruminal microbiome and potentially also metabolic acidosis. Possibly the adaptability of cows decreases with a worse general condition or can at least not be increased.

According to the great importance of ruminal acidosis in high-yielding dairy cows it is obvious that there needs to be done a lot more research concerning various aspects. Especially saliva and its buffer capacity should be more focused in future research.

# 5 Conclusion

Ruminal pH showed an enormous decrease with increasing proportion of concentrate. The duration of feeding the high grain diet did not affect ruminal pH significantly. Dynamic measurement on ruminal pH over the day showed that cows on a forage diet did not only have a higher level of ruminal pH, but also maintained more stable over the day, while cows on a high concentrate diet showed more fluctuation and more time in a lower level of ruminal pH.

Changing a forage diet to a high concentrate diet of 65 % concentrate resulted in various effects on salivary pH, buffer capacity and ruminal pH. Buffer capacity of saliva increased significantly due to the change in diet. Contrary to that, salivary pH did not change significantly from the week of forage feeding to the first. However, there was a significant increase in salivary pH from the first week of high grain feeding to the following three weeks.

In conclusion it can be said that buffer capacity of saliva is an interesting aspect for ruminant nutrition in the future. The challenge will be to provide high yielding dairy cows with enough energy and keep the ration appropriate for the requirements of cows as ruminating animals. There is more research necessary on saliva, its components, and its buffer capacity. Possibly this leads to opportunities to keep dairy cows healthier. Eventually, it is also debatable if the raising milk yield might reach a limit in future and other health aspects should be focused more on breeding.

# 6 Zusammenfassung

Die immer weiter steigende Produktivität in der Milchwirtschaft führt zu Herausforderungen hinsichtlich Fütterung und Gesundheit intensiver Milchviehherden. Die Fütterung dieser hochleistenden Tiere führt zu einem Spannungsfeld zwischen dem Zuführen der notwendigen Energiemengen und den damit einhergehenden zunehmenden Gesundheitsproblemen, wie subakuter Pansenazidose.

In diesem Sinne wurde in der vorliegenden Diplomarbeit ein Fütterungsversuch mit Holstein-Friesen Kühen durchgeführt. Die Kühe wurden zuerst mit einer reinen Raufutter Ration gefüttert, welche anschließend innerhalb einer Adaptations-Woche auf 65 % Kraftfutter gesteigert wurde. Diese Kraftfutter-Ration wurde für vier Wochen gefüttert und es wurden der pH-Wert des Pansens, der pH-Wert des Speichels und die Pufferkapazität des Speichels ermittelt.

Der pH-Wert des Pansens zeigte wie erwartet einen massiven Abfall mit steigender Konzentration an Kraftfutter. Dynamische pH Messungen des pH-Wertes im Pansen über den Tag zeigten bei der Raufutter-Ration nicht nur insgesamt ein höheres Niveau, sondern auch einen deutlich stabileren Verlauf. Bei der Kraftfutter-Ration hingegen, wurde nicht nur ein niedrigerer pH Mittelwert festgestellt, sondern auch größere Unterschiede zwischen den Messwerten und vor allem eine längere Zeit auf niedrigem pH Niveau.

Die Pufferkapazität des Speichels nahm durch die Umstellung der Ration signifikant zu. Der pH-Wert des Speichels zeigte keinen signifikanten Unterschied von der Raufutter-Ration in Vergleich zu den Messungen der Kraftfutter-Rationen. Dennoch konnte von der ersten Woche mit Kraftfutter-Ration auf die folgenden drei Wochen ein signifikanter Anstieg nachgewiesen werden. Dies könnte als Folge der gesteigerten Pufferkapazität gewertet werden.

Das Potential einer gesteigerten Pufferkapazität des Speichels, die mit einem zunehmenden Kraftfutter-Gehalt einhergeht, sollte in weiteren Studien untersucht werden. Möglicherweise kann dies ein Beitrag sein trotz bedarfsgerechter, energiedichter Rationen eine bessere Pansengesundheit für Milchkühe in der Zukunft sicherzustellen.

# 7 List of figures

# 8 List of Tables

# 9 List of abbreviations

| | |
|---|---|
| TMR | total mixed ration |
| SARA | subacute ruminal acidosis |
| SEM | standard error of the mean |

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