Sveriges lantbruksuniversitet
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Faculty of Veterinary Medicine and Animal Science

The Effect of Ration Forage Proportions on the Microbial Ecology of the Rumen

Grovfoderandelens påverkan på den mikrobiella ekologin i vommen

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Sammanfattning

Grovfoder utgör en stor del av kons foderintag och dess struktur är viktig för kons hälsa. En foderstat som till största delen innehåller kraftfoder främjar tillväxten av exempelvis *Lactobacilli* och *Streptococcus bovis (S.bovis)* som i sin tur producerar laktat. Laktat sänker pH i vommen och kan leda till vomacidos. Laktatomvandlande mikroorganismer kan inte omvandla laktat till flyktiga fettsyror (VFA) i samma hastighet och utsträckning som *Lactobacilli* producerar laktat. Vid idissling främjas salivproduktionen, ett av kons buffertsystem. Det finns ingen direkt koppling till att enbart grovfoder- och kraftfoderproportionerna har en inverkan på den totala koncentrationen av flyktiga fettsyror. Vid jämförelse av två grovfoder gav det ena en högre koncentration av flyktiga fettsyror (VFA) vilket indikerar att grovfoder- till kraftfoderproportionerna inte är den enda betydande faktorn. Däremot tycktes det finnas en koppling mellan foderstatens-kraftfoderandel och proportionerna propionat:acetat:butyrat. En ökad mängd kraftfoder i dieten gav upphov till protozotillväxt vilken i sin tur ger en högre andel butyrat. Grovfodrets partikelstorlek och grovfodertyp (gröda, konserveringsmetoder etc.) tycktes även ha en inverkan på koncentrationen av flyktiga fettsyror.

Abstract

Forage constitutes a large part of the cow's feed intake and its structure is important for the cow's health. A ration that mainly contains concentrates promotes the growth of, for example, *Lactobacilli* and *Streptococcus bovis* (*S.bovis*), which in turn produces lactate. Lactate lowers the pH in the rumen and may lead to rumen acidosis. Bacteria specialized in fermenting lactate into VFA are unable to convert at the same rate and extent as *Lactobacilli* can produce lactate. During rumination, saliva production is promoted, one of the cow's buffer system. There is no connection that it is only the forage to concentrate ratio that has a direct effect on the total VFA concentration. When comparing two forage feeds, one gave a higher concentration of VFA which indicates that forage to concentrate ratio is not the only significant factor. However, the proportion of concentrate feed appeared to have an effect on the propionate:acetate:butyrate ratio. An increased amount of concentrate in the diet promotes the growth of protozoa which in turn give a higher proportion of butyrate. The particle size of the forage feed and which type of forage (crop, conservation method etc.) also appear to influence the VFA concentration.

Introduction

Feeding the cow, it is not the cow that is getting fed per se, it is the microorganisms that are utilizing the nutrients. Through the fermentation process there are various end products. The cow can utilize the end products from the microorganisms to gain the required dietary energy. The rumen's ecosystem consists of three major groups of microorganisms; Fungi, protozoa, bacteria (Sjaastad *et al.,* 2003). Each microorganism is specialized for certain substrates. Furthermore, microorganisms are the main protein source for the ruminant.

One of the most common metabolic diseases is rumen acidosis. Rumen acidosis may occur when the animal is fed too much concentrate and not given the opportunity to adapt to its new diet (Krause & Oetzel, 2006). There are naturally occurring buffers that may be used to buffer the pH in the rumen. One such being saliva. The production is promoted when the animal is given the opportunity to ruminate and the rumination time is largely dependent on the type of feed given to the animal (Sjaastad *et al.*, 2003). For example, fibrous feed will increase time spent ruminating.

The aim with this literature compilation is to examine how the volatile fatty acids (VFA) concentration and the molar proportions of different VFA will be affected if part of the forage is changed to concentrate and why it might be the case. Also, what other properties may affect the environment in the rumen. It is also of interest to examine possible disorders related to a low rumen pH and why they may occur, how it affects the ecology in the rumen, what mechanisms there are to keep a stable pH in the rumen and what sort of consequences there may be with an abnormal change in the rumen.

Ruminant digestion

Ruminants differentiates from monogastrics. They have a forestomach consisting of three compartments; rumen, reticulum and omasum (Sjaastad *et al.,* 2003). The rumen is the primary site for microbial digestion for the breakage of the beta-glycosidic bonds in the fibers. Fermentation require an absence of oxygen which is achieved through bacteria adhered to the surface of the reticulo-ruminal epithelium utilizing the oxygen that is diffused from the bloodstream, creating an anaerobic environment within the rumen (Sjaastad *et al.*, 2003).

The digestion of fibrous feed requires the combination of rumination and microbial digestion (Sjaastad *et al.,* 2003). Rumination allows the ruminant to transport feed from the forestomach back to the mouth, increasing the time spent digesting the feed and reduce the particle size. Reducing the size of the particles allows further bacterial fermentation. The time spent to ruminate is based on the digestibility and structure of the feed. Larger particles will take longer to pass through the rumen.

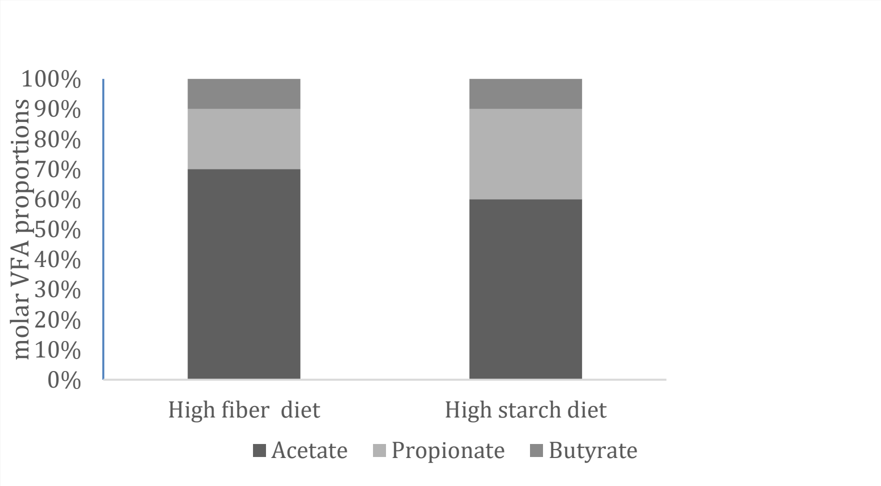

Figure 1: How the molar proportions of VFA differentiates from a high fiber diet to a high starch diet. Modified from Sjaastad *et al.*, (2003).

The habitants of the rumen

To feed a ruminant, the rumen ecology must be considered. The three main groups of rumen microorganisms are bacteria (50-90 % of microbial mass), protozoa (10-50 %), fungi (5-10 %) but there are other groups such as the archaea family (Sjaastad *et al.*, 2003). They are affected by a combination of diet and the host (Henderson *et al.*, 2015). Seven bacterial groups appear to be part of the core microbiome; *prevotella, butyrivibrio, ruminoccocus, lachnospiraceae, ruminococcaceae, bacteroidales and clostridiales* (Henderson *et al.*, 2015). These groups are likely required for the digestion of cellulose, hemicellulose, pectin, starch, fructans, organic acids and protein.

Methanogens (part of the archaea family) is the main group which dispose the hydrogen from the fermentation process by converting carbon dioxide (CO_2) and hydrogen (H_2) to methane (McAllister & Newbold, 2008). There is essentially a competition in the rumen which group of microorganisms may utilize the H_2. If there is an absence of methanogens other groups may begin utilizing the H_2 and keep the partial pressure below the threshold for what other competitors require to be able to utilize the H_2 (Van *et al.*, 1998). Reductive acetogens are also able to utilize H_2 and CO_2 fermenting it into acetate (Yang *et al.*, 2015).

The most common category of protozoa in the forestomach is ciliates (Sjaastad *et al.*, 2003). Ciliates are so called obligate anaerobes, meaning that they are unable to grow in the presence of oxygen. The density of ciliates is also affected by the amount of carbohydrates starch and fiber, where a higher proportion of fiber will result in a lower density. Ciliates can reduce the risk of acidosis by delaying fermentation in the rumen through the digestion of whole starch granules and small feed particles. Protozoa may be removed voluntarily with the use of three common methods; Separating the lamb from its mother, using chemicals or emptying the ruminal content and thereafter treating it (Jouany *et al.*, 1988). Protozoa is not a necessity for the survival of the animal and the removal may give rise to the bacterial- and fungi population and affect the ruminal digestion and methanogenesis. Where one example of the ruminal digestion being affected is the digestion of fiber, which is somewhat affected by defaunation.

The least common microorganism in terms of microbial mass is the fungi (Sjaastad *et al.*, 2003). Its key role in the ecosystem is the digestion of plant fibers. A feed with a short retention time will lead to the absence of fungi in the rumen. An absence of anaerobic fungi does not affect ciliates protozoa nor the bacteria population (Gordon & Philips, 1993). However, an absence of fungi in the rumen decreases fiber digestibility and voluntary feed consumption.

Rumen carbohydrate fermentation patterns

Bacteria can attach to the surface of the plant where thereafter the enzymes produced by the bacteria can begin the breakdown process (Sjaastad *et al.*, 2003). The first step is hydrolysis. The chemical bonds are broken up, releasing glucose, other monosaccharides and short-chained polysaccharides into the ruminal fluid. The compounds are transported into the bacteria where glycolysis is performed, creating two molecules of pyruvate per each molecule of glucose. The environment within the rumen is anaerobic and therefore the end- and waste product produced through fermentation is VFA. The three main VFA:s are acetate, propionate and butyrate. The process is illustrated in Figure 2. Volatile fatty acids are used as the main source of dietary energy for the host.

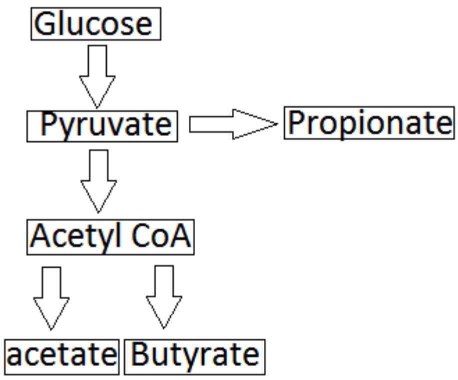

Figure 2: Based on Sjaastad *et al.*, 2003. The production of propionate, acetate and butyrate in the reticulorumen and large intestine.

A diet high in concentrate (mainly starch and sugars) will promote the growth of *Streptococcus bovis (S. bovis)* (Krause & Oetzel, 2006*).* It ferments glucose into lactate rather than VFA, reducing the pH in the rumen and giving the *Lactobacilli* a more suitable environment to grow. Lactate increases the risk of developing rumen acidosis. Most of the lactate produced can be metabolized into VFA, mainly propionate and acetate by *Megasphaera elsdenii* and *Selenomonas ruminantium* (Chen & Wolin, 1977). The VFA:s are easily absorbed by the animal. As the pH falls below 5.0 the environment becomes too acidic and the growth of the bacteria are inhibited. The environment will favor the growth of *S.bovis*, upping the population (Krause *& Oetzel,* 2006*)*. The fermentation of lactate into VFA is a slow process. As the population of S.*bovis* increases. Glucose may be fermented into lactate at a faster rate than what *Megasphaera elsdenii* and *Selenomonas ruminantium* may ferment lactate into VFA.

How different proportions of forage affects VFA and pH

In the study by Russell (1998) a diet containing 100 % forage was compared to a diet containing 90 % concentrate. Ruminally fistulated, nonlactating cows were given each feed 12 times per day. The pH and acetate:propionate ratio were lower and the propionate and total VFA production were higher for cows fed the concentrate diet compared to the 100 % forage diet. Kessel & Russell (1996) fed fistulated cows an all forage diet containing exclusively timothy hay, resulting in a constant pH of 6.7-6.9 in the rumen. In another study by O'Grady *et al.* (2008) with grazing Irish cattle whose main diet consisted of perennial rye grass supplemented with <2 kg concentrate, subacute ruminal acidosis (SARA) (pH ≤5.5) was prevalent in 11 % of the herd while 42 % showed signs of marginal (pH 5.6-5.8) acidosis and 47 % was normal (pH >5.8).

McDonald *et al.* (2011) compiled experiments with different forage:concentrate proportions and summarized the total VFA concentration (measured as mmol/l) and the molar proportion of each individual VFA (shown in the Figure 3). A feed high in hay yielded a higher total VFA concentration (97 mmol/l) in comparison to the feed high in concentrate (70 mmol/l VFA concentration). McDonald *et al*. (2011) also compiled different ratios of forage:concentrate in sheep and as they were given a higher concentrate feed the total VFA concentration production was reduced, illustrated in Figure 6. As the feed contain a higher concentrate ratio, the VFA produced through fermentation shows a steady decline. In the study of Patel *et al.* (2011), lactating dairy cows were fed according to their individual energy requirements throughout the experiment (based upon their starting requirements and kept constant throughout it) and with diets consisting of 500/500, 700/300 or 900/100 g kg^{-1} forage (grass silage in the form of early first cut timothy and meadow fescue)/concentrate (mixture of oats, barley, peas, rapeseed cake, beet fiber, wheat bran, rapeseed, minerals and vitamins). Cows were fed DMI ranging from 15-16 kg (kg day-^{1}). There was no difference between the total VFA (mM) across the three diets but there was a difference in the molar proportions. The proportion of butyrate was higher in the 500/500 diet. The ruminal pH was lowest in the 500/500 diet (pH 6.08) and highest in the 900/100 (pH 6.26) with a $p=0.003$. Butyrate has been suggested to be linked to the number of protozoa in the rumen which mainly produce acetate and butyrate.

The maturity and type of forage (ryegrass, silage, pelleted or not pelleted etc.) also influences VFA production and the VFA molar proportion (McDonald *et al.*, 2011). Both Figures 4 and 5 illustrates the difference in the proportions. Feed containing pelleted hay had a higher total VFA concentration (140 mmol/l) compared to long hay (96 mmol/l). Sheep fed young ryegrass herbage had a total VFA concentration of 107 mmol/l while cattle fed mature ryegrass herbage had a total VFA concentration of 137 mmol/l. In a study by Visser *et al.* (1998) the maturity of silage and the addition 0, 2 and 4 kg of flaked corn starch was tested to see how it affected the molar proportions of VFA and the VFA concentration. The Dutch Holstein-Friesian dairy cows were fed with either early cut- or late cut silage with a combination of 0, 2 or 4 kg of corn starch. The maturity of the feed did not appear to influence the VFA molar proportions (very minor difference when corn starch was excluded). When incorporating flaked corn starch the molar proportion of propionate saw an increase with the early cut silage, but not with the late cut. Rinne *et al*. (1997) compared silage from four stages of maturity of timothy-meadow fescue sward, all harvested one week apart, and reported that silage NDF content and rumen pH increased, rumen butyrate proportion decreased, and rumen propionate proportion was unaffected as they matured. All which the authors linked to the decreased number of protozoa in the rumen. As the plant matures, the NDF content increases but the digestibility goes down (Rinne *et al*., 1997). This is due to the increased proportion of lignin in the cell wall (McDonald *et al*., 2011). The anaerobic bacteria's in the rumen has difficulty breaking down lignin which is most likely due to the low oxygen content in lignin.

In a comparison of 23 different studies where rumen pH was measured on lactating dairy cows fed pasture (Kolver & Veth, 2002), it was concluded that no single dietary variable can be used to predict the ruminal pH within a fresh pasture diet. The results were different between the in study and the across studies. For example, within study forage NDF (%DM) had a significant (p=0.005) effect on the ruminal pH while across study NDF (%DM) was not significant (p=0.187). The same difference can be seen when comparing linearly related dietary variables to pH. Within study only NDF and NDF concentration were the only variables that was significant. Across studies only starches and sugars (NSC) was significant. However, neither of these two could potentially be used to predict the pH in further studies.

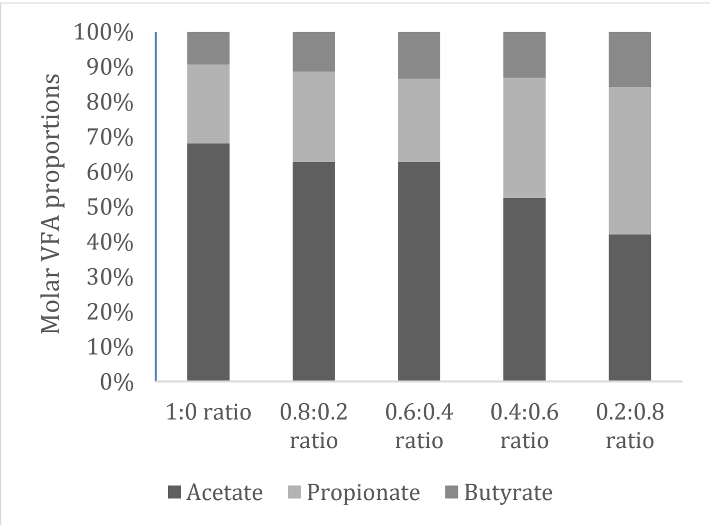

Figure 3: A comparison between different ratios of forage (hay): concentrate and how it affects the different VFA molar proportions in sheep. Data taken from McDonald *et al.,* (2011).

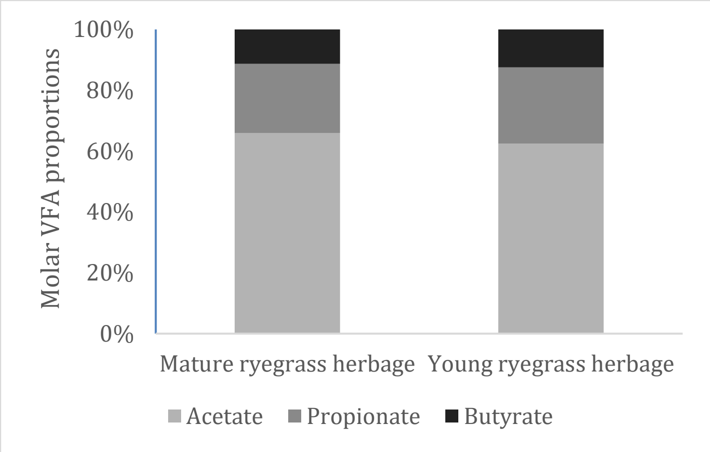

Figure 4: Comparing the VFA proportions for different stages in maturity. Data taken from McDonald *et al.*, (2011).

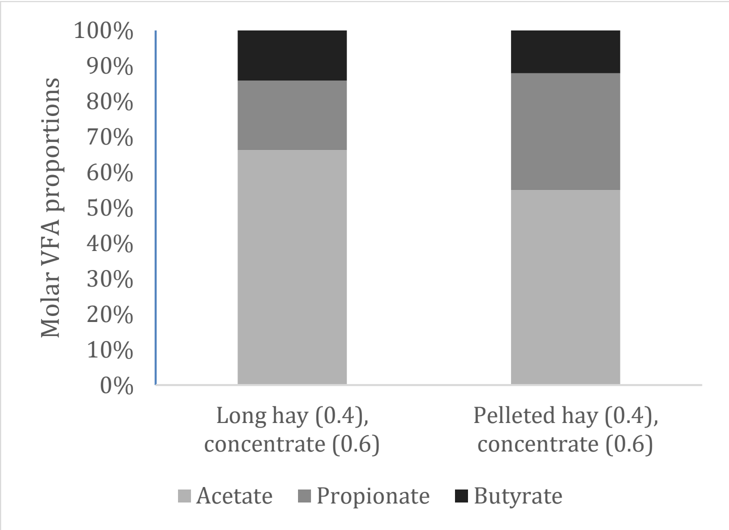

Figure 5: Comparing VFA proportions between two different forage feeds in a 40:60 forage: concentrate ratio for cattle. Data taken from McDonald *et al.*, (2011).

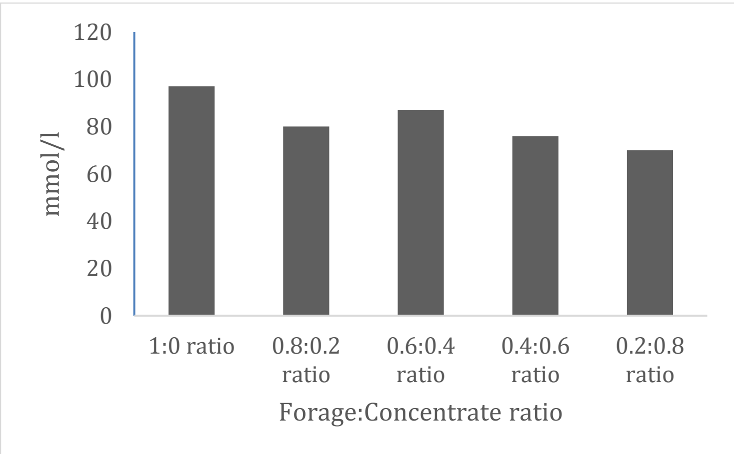

Figure 6: A comparison between different ratios of forage (hay): concentrate and how it affects the total VFA concentration in sheep. Data taken from McDonald *et al.*, (2011).

Rumen acidosis

Rumen acidosis can be categorized as acute or subacute (Russell & Rychlik, 2001). Krause & Oetzel (2006) defined subacute ruminal acidosis as a "period of moderately depressed ruminal pH from about 5.5 to 5.0". An increase of *Lactobacillus spp* and *S. bovis* population in the rumen has also shown an increased risk of developing lactic acidosis (Krause *et al.,* 2013). Furthermore, incorporating a higher proportion of fermentable soluble starch in the diet upped the population of said bacteria (Krause *et al.,* 2013), both which are specialized in fermenting starch and producing lactate instead of VFA, lowering the pH in the rumen (Russell & Rychlik, 2001).

Acute ruminal acidosis occurs when rapidly fermentable carbohydrates are fed in a large quantity (Krause & Oetzel, 2006). Consuming a large quantity will lead to a sudden drop in the ruminal pH which in turn increase the lactic acid concentration. To what degree the cow is affected is correlated to previous diet. If given the opportunity to adapt to a high grain diet, the risk for developing acute rumen acidosis is reduced.

Subacute ruminal acidosis (SARA is defined as *"periods of moderately depressed ruminal pH (about 5.5–5.0) that are between acute and chronic in duration"* (Krause & Oetzel, 2006). Unlike acute rumen acidosis, SARA is not caused by an increased lactic acid concentration but rather an increase in the total VFA accumulation. In dairy cows, SARA rarely happens outside lactation cycles. If the fiber content of the ration is low chemical buffers may be added to prevent SARA (Enemark, 2008). One chemical buffer used in North America is sodium bicarbonate which depresses the growth of lactic tolerant *Lactobacilli*. Besides aiding to treat and prevent subacute ruminal acidosis (SARA), sodium bicarbonate may increase total milk yield, feed intake and milk fat percentage.

Discussion

Part of the purpose of this literature study was to examine how the total VFA concentration and the proportions of different VFAs were affected by different forage to concentrate proportions. All studies found showed different results, indicating that it is not just the forage to concentrate ratio that influences VFA concentration. The type of concentrate may be one such factor, depending if it is NSC or NDF based. The VFA molar proportions was following a similar trend throughout all the forage to concentrate figures and studies. The proportion of butyrate was always the highest in the diets highest in concentrate. Concentrate feed tend to favor the growth of protozoa who mainly produce butyrate (Patel *et al*, 2011). Beside butyrate being the highest in concentrate, the acetate:propionate ratios were always the lowest. Something that was somewhat expected due to concentrate feed giving raise to *S.bovis* which in the end of it all mainly end up producing acetate and propionate.

The comparison between young and mature ryegrass was surprising. Although it was silage that was compared, NDF digestibility is higher in early cut silage than in the late cut. (Arieli & Adin, 1994). It could therefore be expected the young ryegrass diet should have the highest total VFA production, which was not the case. It was not specified when they were harvested. One possible explanation could be that the carbohydrate content is higher during spring and fall (Gately, 1984) and the mature one was harvested around that time. It could also be that the data used were taken from different studies as the molar proportion of butyrate did not go in line with Rinne *et al*. (1997) study. If that is the case the studies could had been performed in different countries, which affects the nutritional composition (Danielsson *et al*., 2012). The comparison was also most likely affected by the fact the total VFA production in the young ryegrass was measured in sheep and mature ryegrass was measured in cattle. However, the results from Visser *et al*. (1998) study yielded a different result as well from Rinne *et al.* (1997). Rinne *et al.* (1997) used cut from the same place while the same may not be true for Visser *et al*. (1998). This could had affected the quality of the feed which may influence the results. The DMI difference between the two feeds in the study by Visser *et al*. (1998) may also had played a role and if they were normalized there could have been a noticeable difference and the results could had fallen in line with the expected result.

The results for the pH was also consistent across all the studies where the diets highest in concentrate yielded the lowest pH. However, a high pasture diet does not necessarily mean that the pH will be kept at a normal level as there have been cases where cattle had a low ruminal pH (Kolver & Veth, 2002). It is important for the animal to have a stable environment in the rumen as a reduction in the pH may induce SARA. It is most likely due to concentrate feed generally consists of more starch and sugars than forage which have been linked to an increased population of *Lactobacilli* and *S. bovis* (Russell & Rychlik, 2001). It could also be because pH is at its lowest after feeding and it steadily rises again afterwards or that the animal has more time to adapt to the reduction of pH. SARA generally occurs if the pH is kept at a low level, which may not be the case in the pure pasture diet. A sudden increase in DMI and feeding frequency (less time between feeding, more meals per day) are both linked to a lowered ruminal pH (Krause & Oetzel, 2006). This detail could also be used to explain why early- and mid-lactating cows are the groups most likely to develop SARA because during the lactation period they are increasing their DMI to increase the energy consumption needed for a high milk yield. The total ration NDF content and forage NDF content are both positively related to the pH in the rumen (Kolver & Veth, 2002). It is most likely due to forage generally having a longer rumination time compared to concentrate feed. If the animal is fed a low forage diet and a low amount of functional fibers the animal will be unable to stimulate saliva production in the extent required to buffer the rumen (Bergsten, 2003; Krause & Oetzel, 2006). However, the proportion of forage does not alone help predict the rumination time. NSC in the diet has been shown across studies to reduce the time ruminating and reduce the buffer capacity of the feed (Kolver & Veth, 2002). Beside these factors, according to the results from Figure 5 forage processing also appears to play a role. The difference could be due to the time spent ruminating and the particle size of the feed. Smaller particle forage feed has been linked to an increased VFA concentration in the rumen (Krause *et al.,* 2002). There is no single accurate measurement tool for SARA and multiple indicators must be accessed to be able to give a potential diagnosis for SARA (Danscher *et al*., 2015).

Figure 6 is very much worth discussing on its own. The results given contradicts the other studies that were examined in this literature compilation, although most were about dairy cows. An increase in the concentrate ratio should had given a higher total VFA concentration even in sheep, as shown in Siciliano-Jones & Murphy (1989) study. One possibility is that there is a misprint in the literature and it may be fixed in a later revision.

Conclusion

There were several factors that affected the VFA concentration and the ratios between individual VFA:s. When comparing two different forage feeds with one another there was an apparent difference in VFA concentration. Two factors appear to be the type of forage (crop, conservation method etc) and the particle size. Increasing the amount of concentrate feed will yield a higher proportion of propionate, lower the pH and increase butyrate.

The ecology of the rumen does not just seem to be influenced by the forage to concentrate ratio but rather the chemical composition, the maturity of the feed and the type of feed.

Diets high in concentrate will probably reduce the pH in the rumen and increase the risk of developing rumen acidosis. If the pH is ≤ 5.5, it is defined as SARA. The reduction of pH will yield a more suitable environment for *Lactobacilli* and *S.bovis* which further reduces the pH and increases the risk of developing rumen acidosis. It is highly recommended that the majority of a cow’s ration consists of forage. Forage feed increase time spent ruminating which promote saliva production and other buffer systems. Chemical buffers may be added to prevent SARA. Forage play an important role for the ruminant’s welfare.

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