Sveriges lantbruksuniversitet
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Vågmarkörer för proteinnedbrytning hos mjölkkor

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Innehåll

Sammanfattning

Tillförsel av kväve och fosfor i överskott till naturen leder till övergödning. En stor del av överskottet härstammar från lantbruket vilket leder till att kraven på lantbrukaren blir större. Att överutfodra korna är något man vill undvika då foderkostnaden är en av de större på gården. Överutfodring av protein innebär också en ökad miljöbelastning. Det finns flera tidigare studier som visar att man kan använda olika markörer för att övervaka proteinnedbrytningen hos kor. Syftet med denna studie var därför att se om det fanns ett samband mellan flyktiga komponenter i våmgas (vätesulfid, dimetylsulfid och metanetiol) och kvävebalansen i våmvätskan. Vår hypotes var att flyktiga komponenter i våmgas kan användas som markörer för proteinnedbrytningen i våmmen och i framtiden utnyttjas som ett verktyg för att undvika överutfodring av protein.

Experimentet utfördes i två delar. Det första experimentet utfördes som en romersk kvadrat med 6 kor och 3 olika nivåer av kalium (experimentet utfördes samtidigt som ett annat examensarbete, Johansson, 2014). I försöket utfodrades korna med grovfoder, kraftfoder, kalium och urea. Det andra experimentet utfördes som en 4x4 romersk kvadrat med fyra kor och två olika fodermedel med två olika givor. Korna utfodrades enbart med rapsmjöl eller åkerböna under experimentet och var fastande över natten innan mätningarna. Efter utfodring startade mätningen som varade i drygt 4 timmar med mätningar var tjugonde minut. Mätningarna utfördes med GA2000 Plusgas analyzer som bland annat mätte vätesulfidkoncentrationen i våmgas.

Resultaten från Experiment 1 visade tydligt att koncentrationen av vätesulfid ökar efter utfodring. Resultatet i Experiment 2 visade att vätesulfiden ökade snabbt efter utfodring och att den högsta koncentrationen var nådd inom 1-2 timmar. En signifikant ($P < 0{,}0001$) högre koncentration av vätesulfid uppmättes när rapsmjöl utfodrades jämfört med åkerböna. Det fanns även en signifikant skillnad i koncentrationen av vätesulfid när en hög och en låg giva utfodrades av samma fodermedel. Båda behandlingarna av rapsmjöl resulterade i en högre koncentration av vätesulfid jämfört med åkerböna. Den höga givan av åkerböna resulterade i en högre koncentration först och den högsta koncentrationen nåddes innan den högsta koncentrationen av rapsmjöl nåddes. Efter att toppen var nådd sjönk koncentrationen fort för åkerböna. Efter utfodring ökade även ammoniumkväve (NH3-N) ($P < 0.0001$) och alfa-aminokväve (AA-N) ($P < 0{,}0001$) i vomvätskan med en signifikant skillnad i koncentration beroende på fodermedel och vilken nivå som utfodrades. Koncentrationerna av NH_3-N och AA-N följde vätesulfidkoncentrationen. Andra potentiella markörer som identifierades i våmgas var bland annat dimetylsulfid och metantiol. Resultaten kompletterar tidigare resultat men mer forskning behövs för att förstå sambandet mellan koncentrationen av markörerna i våmgas och proteinnedbrytning i olika foder.

Abstract

A surplus of nitrogen and phosphorus in the environment leads to eutrophication. Since a large part of the surplus derives from the agricultural sector, farmers have an increased responsibility to avoid unnecessary losses. The agricultural sector has been rationalised during the last years with bigger herds. One of the greatest costs on the farm is the feed cost and to feed a surplus of protein is expensive and detrimental for the environment. Earlier studies indicate that there are several potential markers that can be used to monitor the protein degradation. The aim of this study was therefore to evaluate the relationship between volatile compounds (hydrogen sulfide, dimethyl sulfide and methanethiol) in rumen gas and in rumen liquor. Our hypothesis was that volatile compounds in rumen gas reflect protein degradation in the rumen and can be used as markers for protein degradation in rumen in order to avoid excessive feeding of protein. Two experiments were performed. The first experiment was designed as a 3x3 Latin square with 6 cows and 3 levels of potassium bicarbonate (another master project, Johansson, 2014, was carried out within the experiment). The cows were fed silage, concentrate, potassium bicarbonate and urea.

The second experiment was designed as a 4x4 Latin square where four cows were used, the treatment feed was arranged in a 2x2 factorial arrangement. Rapeseed meal and field beans were used at high and low levels of inclusion. The animals fasted during the night before treatments, and during measurements the following day cows only consumed rapeseed meal or field beans meal. After feeding, the rumen gas was measured during four hours with a measurement interval of twenty minutes. Gas measurements were performed with a portable gas analyser, GA2000 Plusgas analyzer. According to the results in Experiment 1 it was clear that the concentration of hydrogen sulfide increased after feeding. In Experiment 2, the concentration of hydrogen sulfide increased fast after feeding and the peak was reached after 1-2 hours. A significantly ($P < 0.0001$) higher concentration of hydrogen sulfide was measured from rape seed meal compared to field beans. There was also a significant difference in the concentration of hydrogen sulfide depending of amount of field beans or rapeseed meal fed ($P < 0.0001$). Both rapeseed treatments resulted in higher concentrations of hydrogen sulfide compared to field beans. Ammonium nitrogen (NH_3-N) and alpha-amino nitrogen (AA-N) also increased after feeding with a significant difference in concentration depending of feed and level ($P < 0.0001$). Field bean meal resulted in the highest concentration of AA-N and NH_3-N. Ammonium nitrogen and alpha-amino nitrogen followed the concentration of hydrogen sulfide. Several other potential markers such as dimethyl sulfide and methanethiol was found in rumen gas. Our results confirmed earlier results but more research is needed in order to understand the relationship between potential markers and protein degradation in different feeds.

Introduction

During the last years, there have been large changes in Swedish milk production. The number of dairy herds has decreased from 5697 in 2010 to 4230, in 2015 (LRF, 2015). There has also been a trend towards larger herds. To compensate for low milk prices, the dairy farms also need to be more efficient and cut costs. There is also a societal demand for reduced environmental impact. The structural changes in the sector, presumably leading to fewer man hours per cow, together with a need for improved nutrient efficiency, will probably lead to a higher demand for new management tools.

One of the main costs on the dairy farm is the feed. Feed costs make up 40% of total costs on Swedish dairy farms (Svensk Mjölk, 2012). Optimized feed rations, with balanced nutrient composition, are therefore of great importance. Feeding excessive amounts of protein leads to accumulation of ammonia in rumen liquor (McDonald et al., 2002) which will be absorbed through the rumen wall and transported to the liver where urea is formed. Urea is thereafter eliminated in the urine.

An excess of nitrogen and phosphorus in the environment will lead to eutrophication in water and on land (Lundström et al., 2008). A part of the nitrogen is volatile and will be lost into the atmosphere in the form of ammonia and contribute to global warming (Wright, 2003). According to FAO, livestock contributes to 80 % of the greenhouse gas emissions from agriculture (FAO, 2006). Emissions of ammonia have been estimated by Galloway et al. (2004) to be 47 million tons of nitrogen of which 94 % is produced by the agricultural sector. According to FAO (2006), the livestock sector contribution is 68 % of the agricultural share and consist mainly of deposited and applied manure. Therefore, it is of great interest to find ways to monitor the cow's nitrogen balance as a tool for improving nitrogen efficiency.

Previous studies have proposed different compounds in rumen gas as markers for protein degradation in the cow (Dewhurst et al., 2001; Dewhurst et al., 2007). Results have shown that sulfuric compounds, of which hydrogen sulfide occur in high concentrations, in rumen gas are correlated to the concentration of ammonia in rumen liquor (Dewhurst et al., 2001). Rustas et al. (2012) measured expired air from cows and found that dimetylsulfide, methylcyclopentane and hexane were correlated with crude protein concentration in the feed and urea in the urine. Some possible markers that need to be further investigated are dimethyl sulfide, hydrogen sulfide and also some hydrocarbons such as methanethiol.

These possible markers could eventually be used when evaluating nitrogen turnover in the cow. For example, if the composition of expired air from cows could be analyzed, volatile markers might be a tool for evaluating protein degradation in the rumen. Rations could be adjusted according to marker concentration in order to feed the cows with a proper amount and quality of protein.

The aim of this study was to evaluate the relationship between volatile compounds in rumen gas and exhaled air, such as hydrogen. sulfide, dimethyl sulfide and methanethiol, and protein degradation metabolites in rumen liquor.

Literature review

Protein utilization in rumen

Protein in feeds can be divided into protein and non-protein nitrogen (NPN) (McDonald *et al.*, 2002). Most of the degradable protein will be hydrolysed to peptides and amino acids, but amino acids could also be degraded to organic acids, ammonia and carbon dioxide in the rumen by rumen microbes (McDonald *et al..*, 2002).

Microbes use ammonia, peptides and free amino acids for protein synthesis (Sjaastad *et al..*, 2003; McDonald *et al..*, 2002). Rumen microbes can also convert NPN to protein. Non-protein nitrogen consists of amino acids, amides, amines, urea, ammonia and nitrate. The amount of NPN can be up to 30 % of total nitrogen in the feed. (McDonald *et al..*, 2002).

Ammonia has an important role in protein metabolism. Deficiency of nitrogen in the rumen will lead to depressed digestion. This is the result of low concentration of ammonia which limits the growth of microbes and hence the degradation of carbohydrates (McDonald *et al..*, 2002). An excess of protein will lead to an accumulation and a surplus of ammonia in rumen liquor. A high concentration of ammonia leads to ammonia being absorbed through the rumen wall and transported in the blood to the liver. In the liver, ammonia will be converted to urea (detoxification). A small part of the urea will be recirculated through blood and saliva to the rumen but most of it will be excreted in the urine (McDonald *et al..*, 2002). Protein that has not been degraded in the rumen and microbial protein will continue to the small intestine (Sjaastad *et al..*, 2003).

Sulfur

Sulfur exists in different forms in the animal's body, mainly in the amino acids methionine and cysteine (McDonald *et al..*, 2002). Other compounds in the body that also contains sulfur are thiamin and biotin (vitamins), insulin and also coenzyme A. Most of the sulfur in herbage is contained in methionine and cysteine within proteins (Havlin *et al.*, 2004). However, the proportion of sulfur contained in amino acids might differ between feeds. In rape seed meal, about two thirds of total S is amino acid S whereas in soy bean meal, the proportion is close to one (Norfor, 2014). Some feeds contain considerable amounts of inorganic S. For example, when ethanol is produced from grain, sulfuric acid is added in the process and part of that inorganic sulfur end up in the byproduct, distillers dried grain.

Utilization of sulfur

Organic forms of sulfur, such as methionine and cysteine, fed to the animals can be degraded to sulfide by the rumen microbes and incorporated into microbial protein (Onodera, 1993) or degraded and form methanethiol (Bray & Till, 1975; Zikakis & Salsbury, 1969). Methanethiol can further on be converted to both hydrogen sulfide and dimethyl sulfide (Onodera, 1993).

Ruminants can also transform inorganic sulfur to organic compounds (Kandylis, 1984). There are two different types of sulfate reducers in the rumen. In dissimilatory sulfate reduction sulfate is used as an electron acceptor in an anaerobe environment to yield energy and form hydrogen sulfide (Willey, 2008). In assimilatory sulfate reduction, bacteria reduce sulfur to sulfide which is used in protein synthesis. Sulfate undergoes reactions with adenosine 5´-phosphosulfate (aps) and phosphoadenosine 5´-phosphosulfate (paps) in order to reduce sulfate to sulfide (see Figure 1 below).

Sulfate → Adenosine 5´phosphosulfate (aps) → Phosphoadenosine5´phosphate(paps)
→ Sulfide → Hydrogen sulfide

Figure 1. Pathway for reducing sulfate by assimilatory sulfate reducing bacteria (Willey, 2008).

Utilization of sulfur depends on the rate of sulfide production, the uptake of sulfide by the microorganisms but also the loss of sulfide from the rumen (Kandylis, 1984). Further on, Anderson (1956) means that a low amount of sulfate in the feed, will lead to a slower conversion of sulfate to sulfide. This will result in a higher proportion of sulfide being used by the microbes since the amount absorbed through the rumen epithelium will be smaller (see major pathways below in figure 2).

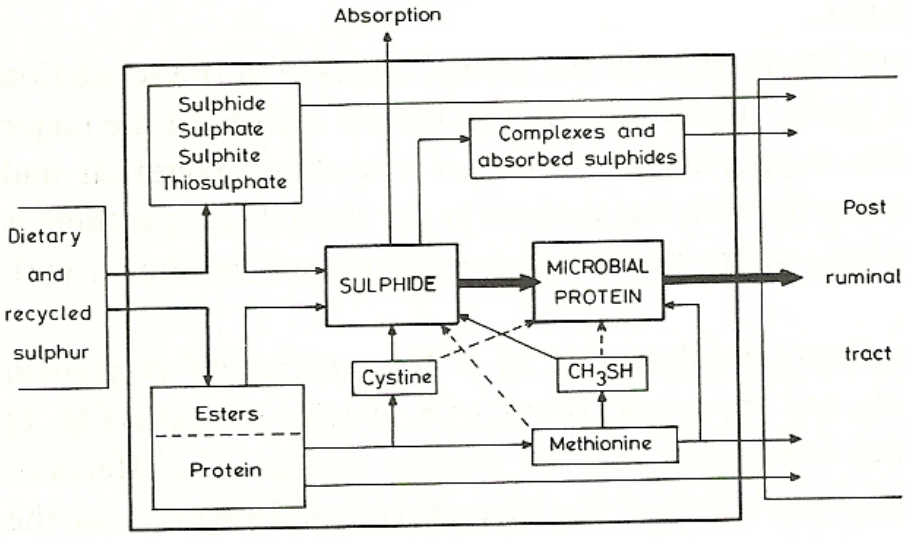

Figure 2. Major pathways in sulfur degradation and metabolism in rumen (Bray and Till, 1975).

Hydrogen sulfide pathways

Hydrogen sulfide that is absorbed through the rumen epithelium is further on transported by the blood (Bray & Till, 1975). Sulfide oxidizes in blood and in the liver and form sulfate; sulfate is thereafter recycled to the large intestine for synthesis of microbial protein and some is excreted in feces as sulfide or is excreted in the urine.

Another recycling pathway for sulfate is transportation to the rumen through the saliva where sulfate is available for synthesis of microbial protein. (Bray & Till, 1975) (see figure 3). According to Kandylis (1984), both inorganic and organic compounds can be excreted in the urine. Urine excretion is regulated by the concentration of inorganic sulfate in plasma, the capacity of reabsorption and filtration rate in kidneys.

Absorption of hydrogen sulfide through the rumen epithelium is related to rumen pH (Bray & Till, 1975). Hydrogen sulfide (H_sS) will disassociate in the rumen, and form sulfide ions (HS^- & S^{--}), the equilibrium between the different forms of sulfide being dependent of pH in the rumen. Below pH 6.8, approximately 50% of the hydrogen sulfide will be dissociated as hydrogen sulfur ion (HS^-) and hydrogen ion (H^+). With higher pH (> 6.8), the concentration of

hydrogen sulfur ion will increase and the amount of hydrogen sulfide will decrease. Hydrogen sulfide is absorbed faster than hydrogen sulfur ions (HS^-).

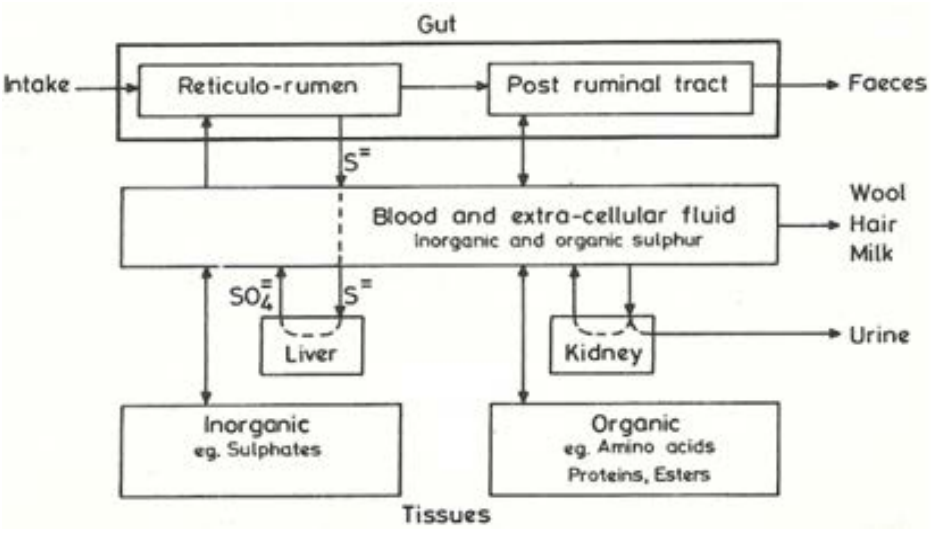

Figure 3. Pathways of sulfur in ruminants (Bray and Till, 1975).

Dimethylsulfide

Dimethyl sulfide consists of two CH_3-groups and one S. The formula is $CH_3S - CH_3$ (Salsbury & Merricks, 1975). Formation of dimethyl sulfide occurs when organic sulfur is degraded but can also be formed during methylation of sulfide and methanethiol (Taylor & Kiene, 1989). Dimethyl sulfide and methanethiol can be formed from sulfur amino acids by rumen microbes during incubation (Salsbury & Merricks, 1975). Feeding cysteine instead of methionine resulted in a larger amount of produced methanethiol. The amount of produced dimethyl sulfide was similar regardless of which amino acid that was fed (Salsbury & Merricks, 1975).

Carbon hydrogen compounds

Feeding sulfur amino acids such as S-methyl-L-cysteine and methionine to ruminants will lead to formation of methanethiol in the rumen (Salsbury and Merricks, 1975 & Zikakis & Salsbury, 1971). Methionine is transferred to S-methyl-L-cysteine and later on converted to methanethiol by rumen microbes (figure 4) (Zikakis & Salsbury, 1971).

Methionine → S-methyl-L-cysteine → *methanethiol*

Figure 4. The pathway of methanethiol (Zikakis & Salsbury, 1971).

Toxic effects of sulfur

Feeding an excess amount of sulfur could lead to a toxic condition (McDonald et al., 2002). It is important to consider total sulfur intake to avoid a toxic effect. The sulfur level in silage is low to moderate while by-products from the industry often contain more sulfur. In table 1, sulfur content of common feeds is listed. Another cause of sulfur toxicity could be high concentrations in water (Wagner, 2013).

Table 1. Contents of sulfur in feed stuffs (Norfor, 2014).

Feed	CP	Sulfur	AA-S[1]	Cysteine	Methionine
	g/ kg DM	g/ kg DM	%	g/100 g DM	g/100 g DM
Silage[2]	149	2	0.38	0.78	1.43
Rape seed meal	389	7.3	0.58	2.49	2
Soy bean meal	487	4	0.85	1.5	1.42
Field bean seed	302	1.7	0.92	1.31	0.8
Peas seed	236	1.5	0.93	1.44	0.98
Barley seed	123	1.4	0.85	2.5	1.4
Wheat	126	1.2	0.89	2	1.5
Wet brewers´ grain	215	3	0.71	2.1	2.04
Wet distillers´ grain	320	3.9	0.66	1.81	1.52

[1] *Amino acid sulfur*
[2] *1-50 % Clover*

Ruminants eructate gas and it is believed that inhalation of a large amount of hydrogen sulfide from the rumen gas can cause a toxic condition. Inhaled hydrogen sulfide is absorbed in the pulmonary system and transported to the brain where it can cause neurotoxicity in the animal (Gould *et al*., 1997 & Limin Kung *et al*., 1998). Bird (1972) studied ruminal infusions of sulfate in sheep, and after a couple of days several sheep refused to eat or drink when the amount of infused sulfate was over 6 grams per day. With continuous infusions of sulfur, the rumen activity and motility decreased, and according to Bird (1972), the reason could be due to problems with rumen emptying caused by either sulfide ion in the hypothalamus or a local reaction with hydrogen sulfide disturbing the nerves in the rumen walls. Another reaction after ruminal infusions was muscle twitching, collapsing and respiratory distress (Bird, 1972; McDonald *et al*., 2002). When the infusions ended, animal conditions were restored, with most of the animals recovering after the infusions were stopped, except for one sheep, which seemed to have recovered but was blind.

Polioencephalomalacia (PEM), often called polio, is a disease where the cause still is unclear; the disease is probably caused by excessive feeding of sulfur, lead poisoning, or from thiamin deficiency (vitamin B1) (SVA, 2017; Gould, 1998). Microbes that produce thiaminase exist naturally in low concentrations in the rumen (SVA, 2017). Thiamin deficiency can occur from an abrupt change from forage to concentrate (Merck, 1993). The reason is that an abrupt change can lead to a shift in rumen microflora resulting in a decrease of thiamin since thiaminas producing microbes such as Gram positive bacilli, Gram negative cocci and coccobacilli predominate produce more thiaminas than optimum. Deficiency of thiamin could lead to necrosis in cerebral cortex and affect visual cortex. Ruminal pH has a major role in production of hydrogen sulfide which means that the amount of digestible carbohydrates in the feed could be of great importance to prevent PEM (Gould, 1998). Since carbohydrates are fermented rapidly, they generally produce a more acidic environment than fiber and a more acidic environment in the rumen leads to an increased level of hydrogen sulfide (Sjaastad *et al.*, 2003; Bray and Till, 1975).

There are two different types of PEM (SVA, 2017; Niles *et al*., 2002), the first leads to ataxia (problem to coordinate muscle movements), sudden death, deep unconsciousness and it is an acute condition (SVA, 2017). Cattle affected by acute PEM often do not respond to treatment

and dies. In the other type, the animals show symptoms like low appetite, low vision and tremor in ears and head. Cattle can in some cases recover from this type but they are often affected by brain damage caused by the disease.

Rumen gas

Microorganisms are essential for ruminants because they degrade fiber during fermentation. This is the reason why ruminants can digest feed that monogastric animals cannot. When the microorganisms utilize the feed, gas is formed (Sjaastad *et al*., 2003). The gas is located in the upper part of the rumen. The gas consists mainly of carbon dioxide (~ 60 %) and methane (~35 %) (Dougherty, 1968). A smaller proportion of the gas consist of nitrogen, oxygen and hydrogen sulfide. Dewhurst *et al.* (2001) found that beyond hydrogen sulfide rumen gas also consist of a considerable proportion of methyl sulfide and dimethyl sulfide.

Experimental arrangements to evaluate the effect of sulfuric compounds on hydrogen sulfide in rumen gas

A few other studies have investigated the relation between rumen gas concentrations and different sulfur sources (Dewhurst *et al*., 2007 & Fonseca et al., 2013). Both lactating and non-lactating cows have been used in these trials. The cows were fasted before treatment, in order to start with a hydrogen sulfide concentration close to zero at treatment onset. For example, in a trial by Dewhurst *et al*. (2007), the cows were fasted four hours before collection. All the cows in the trials were fitted with rumen cannulae. The cows were fed portions of different protein feeds (Fonseca *et al*, 2012), or pure amino acids (Dewhurst *et al*, 2007) and if not all was consumed in a short time, the remainder was inserted directly into the rumen via the cannula. Hydrogen sulfide collection started thirty minutes after feeding and continued until 2.5-4 hours after feeding at thirty minute intervals (Dewhurst *et al.*, 2007; Fonseca *et al.*, 2012). Collection of gas was performed using a vacuum pump with a liquid trap. Rumen gas was collected in plastic bottles and thereafter analyzed (Dewhurst *et al*., 2001).

In Dewhurst *et al*. (2007) and Fonseca *et al*. (2012), hydrogen sulfide concentration was measured after collection of rumen gas with an instrument equipped with a hydrogen sulfide sensor (Crowcon Triple + meter, Crowcon detection Instruments Ltd, Oxfordshire, UK). Samples with higher concentration than 700 parts per million (ppm) were diluted with CO_2 and thereafter reanalyzed. The concentration of hydrogen sulfide in the rumen varied and changed fast. Rumen concentrations rapidly declined from 500-1000 ppm to 0 ppm a few hours after feed was removed (Dewhurst *et al*., 2007).

Hydrogen sulfide and sulfur source

Different trials have been conducted to investigate the relation between hydrogen sulfide and different dietary sources of sulfur. The effect of cysteine, methionine and sodium sulfate on produced hydrogen sulfide was evaluated in a Latin square design trial by Dewhurst *et al.* (2007). Dewhurst et al. found that there were differences both in level and rate of change in concentration between different sulfur sources. The highest concentration of hydrogen sulfide was obtained with cysteine. Cysteine also had the most rapid increase in hydrogen sulfide concentration compared to methionine and sodium sulfate.

The effect of increasing amounts of fed cysteine has been evaluated (Dewhurst *et al*., 2007). Cows were given amounts varying between zero and twelve gram cysteine. The amount of hydrogen sulfide produced was higher with a higher amount of cysteine given. The results indicated that at eight grams of cysteine, degradation was saturated since twelve gram of

cysteine remained at a higher hydrogen sulfide concentration for a longer period. The hydrogen sulfide peak was reached after thirty minutes.

Brasche *et al.* (2012) fed beef cattle different sources of sulfur, dry distiller's grain, condensed distillers solubles, sulfuric acid, sodium sulfate and calcium sulfate. Gas measurements were done six hours after feeding. Brasche *et al*., (2012) couldn't find any differences in hydrogen sulfide concentration that depended on which sulfur source the animals were fed.

Hydrogen sulfide and protein feed

Fonseca et al. (2012) conducted two experiments. The aim was to evaluate the relationship between initial stages of protein degradation and concentration of hydrogen sulfide. In the first experiment, three protein sources that differed in nitrogen degradation rates were evaluated. Sources were maize gluten feed, sun flower seed meal and soybean meal. Maize gluten feed resulted in the highest concentration of hydrogen sulfide in the rumen, followed by sun flower seed meal.

In the second experiment, the relationship between hydrogen sulfide concentration and sulfur degradation was investigated. Four different batches of maize gluten meal were used and the different batches represented different degrees of heat damage. The batches were scored by their color, since color is assumed to relate to the degree of heat damage and, hence, protein degradability of the feed. Thereafter, a color analysis was performed) with a colorimeter (CR 400, Minolta, Osaka, Japan). The in sacco technique measured DM, nitrogen and sulfur degradation. Nitrogen degradation was performed with 5 grams of feed in each bag. The bags were incubated in the rumen during 1, 2, 4, 8, 12, 16, 24 & 48 hours. Sulfur degradation was performed in a similar way, the bags were incubated at 1, 2, 4, 8, 16, 24 & 48 hours. Bags where incubated and thereafter washed, dried and thereafter analyses of nitrogen (Kjeldahl) and sulfur content were performed. In Experiment 2, the batch containing the highest amount of sulfur resulted in the highest concentration of hydrogen sulfide at 1-2 hours after feeding. The hydrogen sulfide peak was also reached later in Experiment 2 and the concentration of hydrogen sulfide was also higher in this experiment.

Fonseca et al., (2012) showed that hydrogen sulfide concentration was ranked in a similar way as sulfur degradation and in sacco nitrogen degradability (see Table 2). Fonseca et al., (2012) suggest that there could be differences in sulfur degradability that are not reflected in the apparent in sacco sulfur degradation. Soluble protein could disappear from the bags without being degraded while still considered as degraded. This means that the in sacco technique is better for feeds with low proportions of soluble protein. Besides, it is also difficult from a practical point of view to evaluate a feed with a high degradation rate since it is difficult to handle bags with short incubation time. Therefore, measuring hydrogen sulfide production could be a more accurate technique.

Table 2. The table shows the result from an experiment by Fonseca et al. (2012) evaluating the rumen degradability compared to measuring the hydrogen sulfide concentration. Nitrogen degradation was performed with 5 grams of feed in each bag. The bags were incubated in the rumen 1, 2, 4, 8, 12, 16, 24 & 48 hours. Sulfur degradation was performed in a similar way, the bags were incubated at 1, 2, 4, 8, 16, 24 & 48 hours. Bags where incubated and thereafter washed, dried and thereafter analyses of nitrogen and sulfur content were performed. MGF 1-4 correspond to four different batches of maize gluten feed with different degrees of heat damage.

Experiment 1	**Protein feed**	**Hydrogen sulfide (ppm)**	**N degradability (%)**
	Maize gluten feed	116	79
	Soyabean meal	55	72
	Sun flower seed meal	15	65,2

Experiment 2	**Batch**	**Hydrogen sulfide**	**S degradability (%)**
	MGF 1[1]	377	74,4
	MGF 2[1]	253	68,9
	MGF 3[1]	182	63,9
	MGF 4[1]	188	52,2

[1]Maize gluten feed

Hydrogen sulfide and forage proportion

The relationship between forage proportion in the diet and hydrogen sulfide has been investigated in studies with beef cattle. The aim has been to find ways to prevent the disease polioencephalomalacia (PEM) (Vannes *et al*., 2009a & Vanness *et al*., 2009b). In the studies by Vanness *et al*.. (2009a; 2009b), pH was measured by wireless pH probes in the rumen. Hydrogen sulfide collection was done during the last day after several days of adaption to the diet. Gas collector devices were inserted through the rumen cannulae and gas was collected after feeding (Vanness *et al*., 2009a; Vanness *et al*., 2009b). After collection, gas was analyzed with a spectrophotometer (Vanness *et al*., 2009b).

One experiment evaluated steers fed wet distiller's grain with varying proportions of roughage to investigate the amount of hydrogen sulfide produced (Vanness *et al*., 2009a). The ration consisted of hay, corn and wet distiller's grain. Four different diets were fed, all consisted of 50% wet distiller´s grain. Diet 1 consisted of 35 % hay and 10 % corn, diet 2 consisted of 25 % hay and 20 % corn. The third diet consisted of 15 % hay and 30 % corn. The finishing diet consisted of 50 % wet distiller´s grain, 7.5 % hay and 37.5 % corn. Gas collection and measurement was performed the last day with a gas collection device inserted through the rumen cannulae.

A ration with low proportion of roughage in the diet had a higher concentration of hydrogen sulfide in the rumen compared to a diet with more roughage (Vanness et al., 2009a). The other experiment fed the animals with copper or iron in order to prevent PEM (Vanness et al., 2009b). Copper and iron may react with the sulfur and prevents high concentration of hydrogen sulfide. They also measured pH with ruminal pH probes. Ruminal pH and ruminal hydrogen sulfide were weakly correlated and the authors conclude that pH not is a good indicator of increased concentration of hydrogen sulfide (Vanness *et al*., 2009b). The weak correlation could be due to large individual animal variations in hydrogen sulfide levels (Vanness *et al*., 2009a; Vanness *et al*., 2009b). However, using mean values for pH and hydrogen sulfide Vanness *et al*. (2009a) concluded that rumen pH is correlated to hydrogen sulfide.

Other factors that can influence hydrogen sulfide in the rumen

Dewhurst *et al.* (2007) compared perennial ryegrass and white clover regarding hydrogen sulfide concentrations in rumen head space. Perennial ryegrass had a higher concentration of hydrogen sulfide compared to white clover. The peak was reached 1.5 hours after feeding. White clover contains more cysteine but still it did not result in a higher concentration of hydrogen sulfide compared to perennial ryegrass. Dewhurst *et al.* (2007) speculated that the low amount of produced hydrogen sulfide could be due to cyanogenic compounds that occur in white clover. During cyanogenesis, plants synthesize cyanogenic glycosides which release hydrogen cyanide during hydrolysis. Cyanide detoxification takes place in the rumen when sulfur is present. The sulfur is consumed and rumen microbes convert hydrogen cyanide to thiocyanate; thiocyanate is thereafter secreted in the urine (Onwuka *et al*., 1992).

Aim

From the above, it is obvious that the development over time of H_2S concentrations in rumen head space gas can be related to protein degradation characteristics.

However, in a practical feeding situation, several factors might affect H_2S production in the rumen. Amino acid composition is important as more H_2S is produced from cysteine compared with methionine. Hydrogen sulfide is also produced from other organic and inorganic sulfur sources. A dairy cow ration is generally composed of several feeds and they might contain different forms of S.

Rumen conditions, mainly pH, might also affect the amount of H_2S that is released into the head space gas. Hydrogen sulfide is also used in microbial synthesis as it is incorporated in S-amino acids. Production rate of microbial protein would therefore affect how much H_2S that is emitted into rumen gas.

To investigate the relation between rumen gas concentration of H_2S and protein degradation two experiments were carried out. In the first experiment, two common protein sources for Swedish for dairy cows, field beans, with a high proportion of soluble protein, and rape seed meal, with a low proportion of soluble protein, were examined. Close to 100% of S in field beans is contained in amino acids, whereas in rapeseed meal, a considerable portion is non-amino acid S (Table 1). In the second experiment, H_2S production was monitored over time in cows fed a standard ration distributed over the day. This was done within an experiment evaluating the effect of $KHCO_3$ on protein metabolism (Johansson, 2014). We expected the H_2S concentration to fluctuate in relation to the feeding occasions and that the treatment (KHCO3) through its effect on rumen pH, would affect H_2S.

Materials and methods

Gas analysis

Gas measurements were done with a handheld GA2000 Plus gas analyser (Geotechnical Instruments, Royal Leamington Spa, UK). The instrument measured oxygen, carbon dioxide and methane in percent, it also measured hydrogen sulfide and carbon monoxide in parts per million (ppm, corresponding to µL/L). All of these parameters were registered but the hydrogen sulfide was the one of major interest. Table 2 and 3 show technical information from the GA2000 Plus gas analyser. The analyzer drew the gas sample into the instrument and measured the gas in the flow (Keison Manual).

Table 3. Technical data from the GA2000 Plus gas analyzer, the table shows the measuring range, measuring time and how the measurements are performed.

Gas	Range	Time (sec)	Measured by
H_2S	0-500ppm	<60	Internal electrochemical cell
CO	0-2000 ppm	<60	Internal electrochemical cell
CH_4	0 -70%	<20	Dual wavelength infrared cell
CO_2	0 -60%	<20	Dual wavelength infrared cell
O_2	0-25%	<20	Internal electrochemical cell

Table 4. Technical data from the GA2000 Plus gas analyzer, accuracy during measurements.

Accuracy	0-5%	5-15%	15 %-100 %
Hydrogen sulfide (H2S)	± 10.0 %		
Carbon monoxide (CO)	± 10 % of reading or 15 ppm, whichever is greater		
Methane (CH4)	± 0.5 %	± 1.0 %	± 3.0 %
Carbon dioxide (CO2)	± 0.5 %	± 1.0 %	± 3.0 %
Oxygen (O2)	± 1.0 %	± 1.0 %	± 1.0 %

Gas collection

Composition of rumen head space gas was measured with the gas analyzer in samples taken directly from the rumen. In order to avoid oxygen leakage during measurements, the rumen cannula was modified during Experiment 1. The first version that was used in order to collect gas consisted of a tube connected to a hole in the rumen cannulae lid. Inside the rumen, a small plastic bottle (50 ml) was attached to the end of the tube. The aim of the plastic bottle was to keep the opening of the tube above the rumen content to avoid rumen fluid coming into the tube. One problem was that the rumen headspace was small in some cows and almost

disappeared during rumen contractions. This resulted in rumen liquid entering the tube which made the machine turn off. This was an automatic response to prevent fluid from coming in to the sensors. However, the floating object did not prevent fluid from entering the tube and therefore a better gas collection tool and the rumen cannulae lid had to be developed.

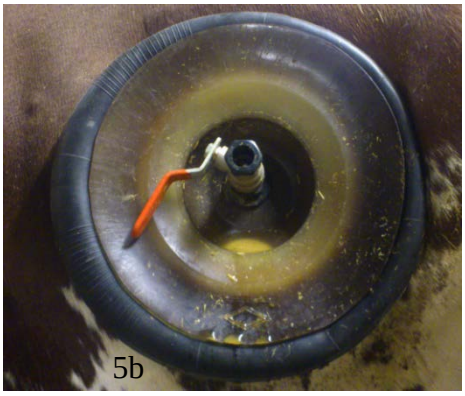

Figure 5A. The measuring tool and Figure 5b show rumen cannulae after development.

To enable better control over the tube in the rumen the plastic tube was replaced by a metal tube, formed to make it easier to keep the end of the tube above the rumen content (figure 5a). To avoid rumen solids coming into the tube, the end of the pipe was covered with a nylon cloth.

Since some of the cows had a lot of rumen content, the gas cap was small and therefore it was sometimes difficult even with this arrangement to avoid rumen liquid to enter the tube during collection. A liquid trap was therefore added to the tube. A bottle with two tubes in the lid was used. One tube was used as entrance for gas and fluid from the rumen and the other as an exit for gas. The exit tube led gas to the analyzer while rumen fluid stayed in the bottle. Additionally, one more water trap was added to the measurement tool. This water trap was a filter located just before the inflow of the instrument, if liquid reached the filter the instrument turned off automatically.

Rumen cannulae

During initial measurements of rumen gas, high concentrations of oxygen was found, indicating leakage through the cannulae. Since the oxygen level in the rumen of intact cows is expected to be close to zero, we considered the oxygen concentrations found (5-10 %) to indicate leakage through the cannulae. To reduce leakage, a tube, normally used for tires on wheelbarrows, was placed around the rumen cannulae. This solution worked and a low oxygen level of leakage was reached. The cannulae lid was fitted with a hole where a valve with a lever was attached. With the lever it was easy to start measuring and close after measurement (see figure 5b).

Experiments

The experimental work was carried out at the Swedish Livestock Research Centre, SLU. The work included two experiments and since Experiment 1 was performed together with the Johansson (2014) experiment, potassium bicarbonate was used in this experiment.

Lactating cows of Swedish Red breed (SR) with rumen cannulas were used in the experiment; 6 cows in Experiment 1and 4 cows in Experiment 2. The animals were tied up. Before the experiment started, the cows were weighed and body conditions scored. The cows were milked twice a day, in the morning (06:00) and in the afternoon (17:00).

Experiment 1

The aim of experiment one was to investigate how hydrogen sulfide concentrations in rumen gas relates to feed intake and protein degradation over the day. The experiment was designed as a 3x3 latin-square design with three periods and three treatments (levels of potassium bicarbonate). The length of period was two weeks and gas measurements were performed during the last four days of each period.

Three levels of potassium bicarbonate were given: low (no potassium bicarbonate), medium (24 g/kg DM) and high (36 g/kg DM). Potassium bicarbonate were mixed in the forage and fed twice a day. The silage was a clover-grass crop ensiled in round bales and before feeding, bales were decomposed in a mixer wagon and were then fed twice a day. The feed ration was adapted to each cow based on individual productio before the experiments started. The cows were fed four times a day with a concentrate (Solid 620, Lantmännen, Malmö). The feeding scheme is presented in table 4 below. Table 5 shows chemical analysis of feeds used in the experiment.

Table 4. Feeding scheme, Experiment 1

Time	Feed
05.45	Silage + Potassium bicarbonate (50 %)
06.00	SOLID (25 %) + urea (50 %)
09.00	SOLID (25 %)
13.00	SOLID (25 %) + urea (50 %)
16.45	Silage + potassium bicarbonate (50 %)
17.00	SOLID (25 %)

Measurements were performed on three cows per day in order to collect data with a short measurement interval. Measurements were performed both in the morning and in the afternoon. The cows were given restricted amounts of silage aiming at no or small amounts of leftovers. Gas was measured during approximately five consecutive hours per day.

In Experiment 1, measurements of hydrogen sulfide were done in sequence with 15-20 min intervals for each cow. All cows were fed simultaneously and to make the measurements comparable in relation to feeding time, values were linearly interpolated to the nearest hour or half hour by using the FORECAST function in MS Excel 2010. Similar interpolations were applied to the hourly ruminal liquid measurements of pH in experiment two (figure 12).

Experiment2

This experiment had a 4x4 Latin-square design with four one-day periods, four lactating cows and four treatments. Treatments were either rape seed meal or field beans administered at two levels, corresponding to 0.34 and 0.68 kg CP/d, in a 2×2 factorial arrangement. The aim with this experiment was to evaluate the effect of level and source of CP on hydrogen sulfide production in the rumen, and also to investigate rumen fluid composition and its relation to rumen gas. Roughages was not fed during the experiment.

The feed was ground in a Wiley mill with a 2 millimeter sieve and was given to the cows through the rumen cannulae. The cows fasted during the night before treatment so that the hydrogen sulfide level should be low in the morning. In the morning, the cows were offered the experimental feed and not until after the measurements were finished they got more feed. The measurement of rumen gas started twenty minutes after that the feed was administered into the rumen through the cannula. Measurements started before feed was administered and continued with twenty minute intervals during approximately 4 hours after feed administration. Directly after each rumen gas measurement, a rumen fluid sample was collected into two 10-ml plastic bottles per cow. Directly after sampling, pH was measured on the fluid samples with a pH meter (pHenomenal pH 1000 H, VWR Int., Leuven, Belgium).

One hour after feed administration, rumen gas was collected in bags. Collection of gas was done 100 seconds after the ordinary measurement. The gas analyser were disengaged and a rigid plastic tube made of polyvinyl chloride (PVC) was connected to the metal tube going into the rumen. Gas collection was made during three minutes and approximately 1½- 2 liters were collected in the bag with a peristaltic pump. This sample was sent for analysis to SP Kemi & Material in Borås, Sweden.

Sampling and analysis

In experiment 1individual feed residues (concentrate and silage) were collected, weighed and frozen daily during experiment. Silage was collected and frozen daily during the experiment. The frozen forage from the different periods was thereafter ground in a meat grinder with a 10-mm screen. A smaller amount of the forage was collected for extraction. The forage was mixed with distilled water (proportion 1:1, fresh weight basis) over night and thereafter pressed in a hydraulic press. The liquid was analyzed for fermentation products (HPLC), N (Kjeldahl, soluble crude protein) and pH was also measured. The liquid was also analyzed with an auto analyzer system for ammonia and α-amino nitrogen (AAN).

Feed samples were pooled to one sample per experimental period. Pooled samples of silages were dried at 60°C for 18 h in a forced-air oven for DM determination and further analyses. Silage samples were analysed for ash, acid insoluble ash (AIA), minerals, ash free neutral detergent fiber (NDF), water soluble carbohydrates, N (Kjeldahl) and rumen in vitro organic matter digestibility by the VOS-method. Metabolisable energy (ME) in silage was calculated from VOS-values according to Spörndly (2003).

Concentrate samples were dried overnight (60ºC) and thereafter ground and analyzed. The concentrate was analyzed for Kjeldahl nitrogen, dry matter, ash, AIA, NDF, minerals, buffer soluble nitrogen (BSN) and α-amino nitrogen. Rumen fluid was analyzed with an auto analyzer for ammonia nitrogen and alpha amino nitrogen.

Two different methods were used at SP Kemi & Material (Borås) to analyze the rumen gas. Hydrogen sulfide was analyzed with an OFCEAS-instrument (Optical Feedback Cavity Enhanced Absorption Spectrometer) which is based on IR spectrometry. Dimethyl sulfide,

methyl mercaptane, ethanol, methyl cyclopentane and hexane was analysed with gas chromatography with a FID-detector (flame ionisation detector).

Table 5. Composition of feeds used in the experiments (standard deviation within brackets).

	Silage	**Concentrate**	**Rapeseed meal**	**Field beans**
DM, %	55.8 (2)	88.3 (0.4)	91.8 (0.35)	92.5 (0.21)
Metabolisable energy, MJ/ kg DM	11 (0.41)	13.2[4]	12.2[3]	12.9[3]
CP, /kg DM	106 (4.2)	175 (0.5)	35 (0.28)	33 (0.50)
NDF, g/ kg DM	537 (16.3)	232 (11.2)	-	-
INDF[1], g/ kg NDF	226 (34.1)	-	-	-
EPD[2, 3],	-	-	54.9 %	88.5 %
Ash, g/ kg DM	58.3 (3.16)	64.3 (0.4)	77	39
S, g/ kg DM	2.9	3.86	7.9	1.5
S[5] from amino acids, g/kg DM	1.1	-	4.6	1.4

[1]Indigestible neutral detergent fibre.
[2]Effective protein degradation, Spörndly, 2003.
[3]Spörndly, 2003.
[4]According to Lantmännen.
[5]Calculated value from table 1.

Calculations

All data was compiled in a Microsoft Excel document. To calculate the proportion of S from amino acids in relation to total S, values on amino acid contents from the Norfor Feedtable (2014) was used together with analyzed or tabulated crude protein contents and the proportion of S in cysteine and methionine. The formulas below and mole weights in Table 6 were used in order to calculate the amount of amino acid sulfur (AAS) in the feed.

1) $Proportion\ of\ S\ in\ each\ AA = \frac{Mole\ weight\ sulfur}{Mole\ weight\ of\ AA}$

2) $Sulfur\ from\ AA = (proportion\ of\ amino\ acid \times crude\ protein\ content) \times proportion\ of\ S\ in\ AA$

3) $Proportion\ amino\ acid\ sulfur = \frac{Sulfur\ in\ AA}{total\ sulfur}$

Table 6. Values used in the AAS calculations. (Haynes, 2014)

Mole weight	g/mole
Sulfur	32.065
Methionine	149.21
Cysteine	121.16

Hydrogen sulfide concentration was adjusted to zero oxygen content using the following calculation: H_2S/ ((21- % of O_2)/21) in order to obtain comparable values throughout the experiments. Twenty-one was used since there is 21 % oxygen in the air (NCAR, 2014). The adjustment was done because of air leakage in the different measurements.

Statistical analysis

Data from Experiment 1 is presented descriptive in figures.

Data from sequential measurements and samplings in Experiment 2 were analyzed by Procedure Mixed in SAS 9.3. Treatment, sampling time and their interaction were considered as fixed factors while period and cow were considered as random factors. To analyze the composition of rumen gas collected in bags in Experiment 2, the previously described model was used except that time was excluded. Due to missing values, all results are presented as least squares means (LS means).

Some values from the analysis made by SP Kemi & Material were below detection limits and these values were therefore set equal to the detection limits for the statistical analyses.

Result

Experiment 1

Results from Experiment 1 are shown graphically below (figure 6, 7, 8 & 9). Figure 6 shows the different treatments effect on hydrogen sulfide. It is clear that the hydrogen sulfide level was low before feeding and rose after feeding. The hydrogen sulfide peak was reached approximately two hours after feeding.

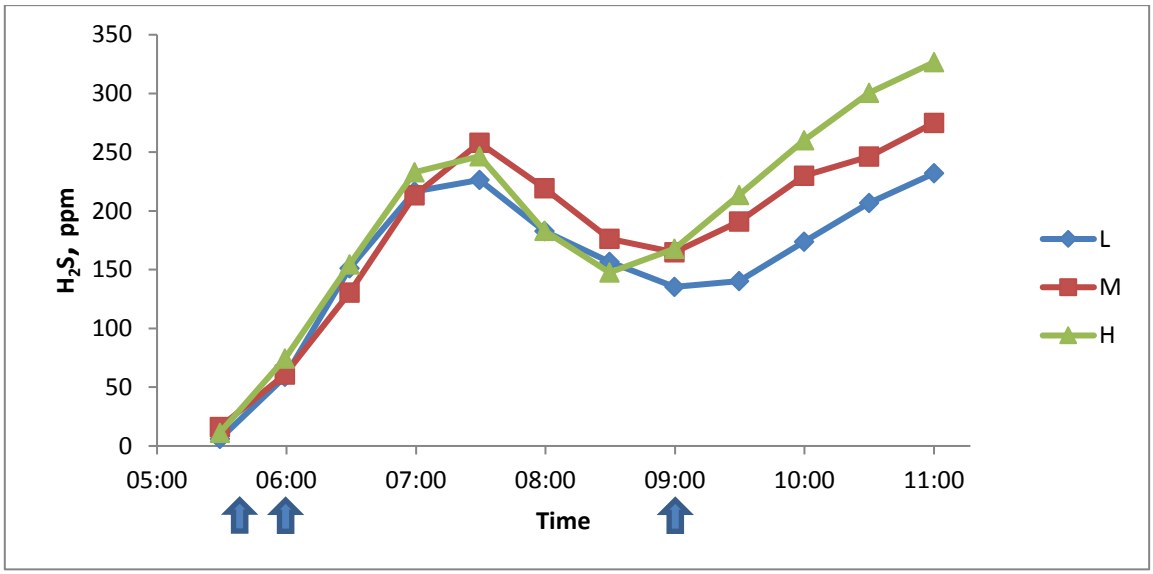

Figure 6. The figure shows treatment effects on hydrogen sulfide concentration in rumen headspace gas at different times (L, low, M, medium or H, high levels of potassium bicarbonate). The blue arrows indicate feeding times (5:45 Silage and potassium bicarbonate, 6:00 & 9:00 Concentrate and urea).

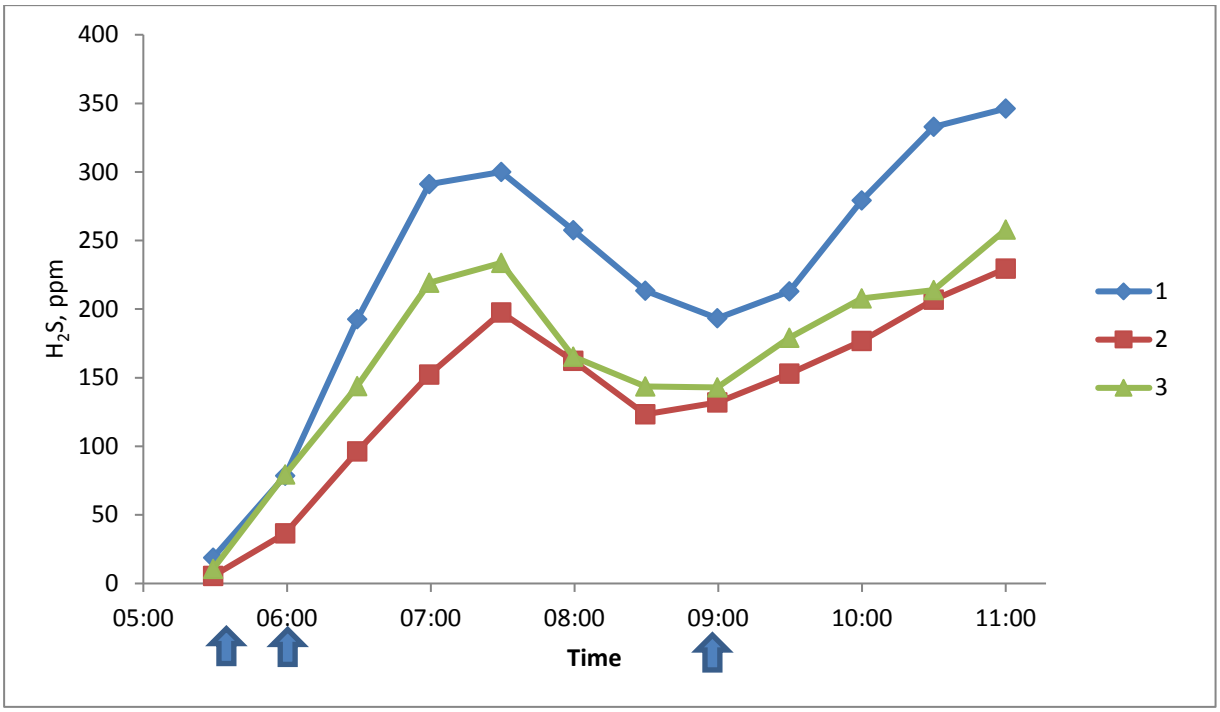

Figure 7. Mean value from period 1, 2 and 3. The figure shows treatment effects on hydrogen sulfide concentration in rumen headspace gas (adjusted) at different times. The blue arrows indicate feeding times (5:45 Silage and potassium bicarbonate, 6:00 & 9:00 Concentrate and urea).

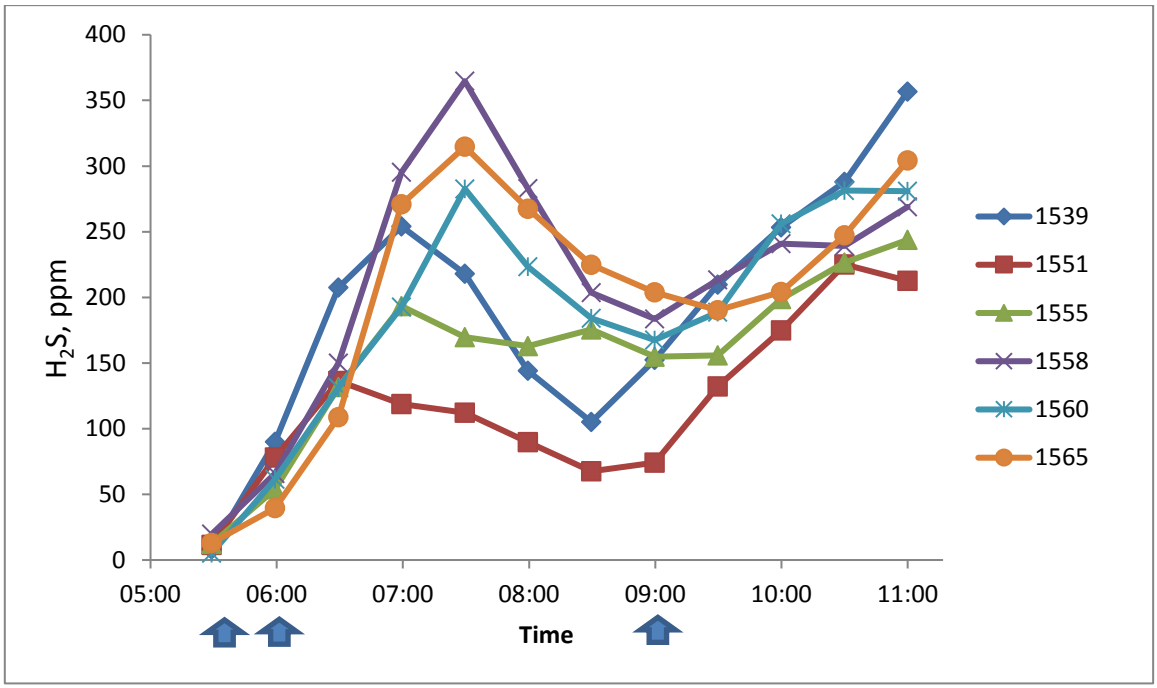

Figure 8. Individual cow hydrogen sulfide concentration (period means) at different times. The blue arrows indicate feeding times (5:45 Silage and potassium bicarbonate, 6:00 & 9:00 Concentrate and urea).

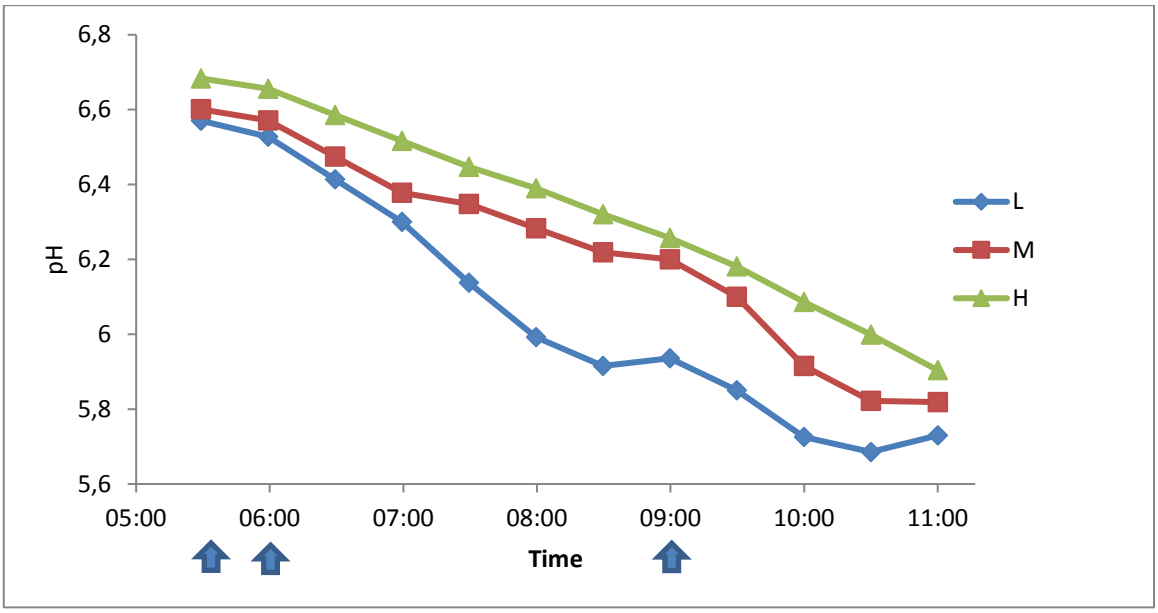

Figure 9. Shows the pH mean values effect of different treatments over time. The blue arrows indicate feeding times (5:45 Silage and potassium bicarbonate, 6:00 & 9:00 Concentrate and urea).

Experiment 2

Hydrogen sulfide

In Experiment 2 rape seed meal resulted in a higher concentration of hydrogen sulfide compared to field beans (table 7 & figure 10). The treatment with the highest level of rape seed meal resulted in the highest concentration of hydrogen sulfide, followed by the low level of rape seed meal. The lowest concentration of hydrogen sulfide came from the lowest level of field beans.

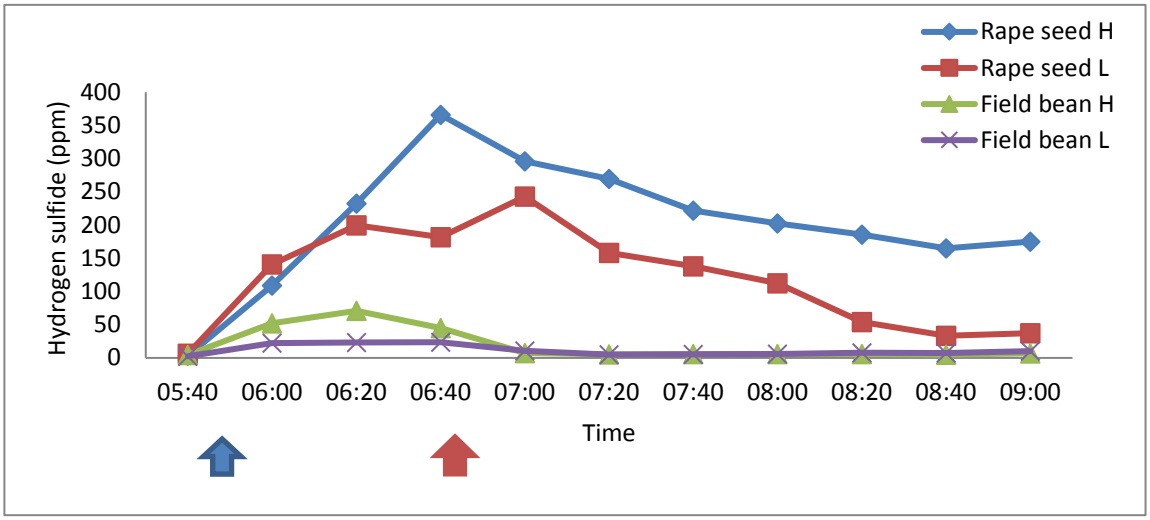

Figure 10. The effect of rape seed meal or field beans on rumen concentration of hydrogen sulfide in Experiment 2. The blue arrow indicates feeding time. The red arrow indicates collection of rumen gas.

Ammonia nitrogen and alpha amino acid nitrogen

The concentration of ammonium nitrogen in the rumen increased after feeding and the treatments with field beans resulted in the highest concentration of ammonium nitrogen (see figure 11). The level of ammonium nitrogen concentration was similar for the treatments with rape seed meal while there were clear differences between the field beans treatments.

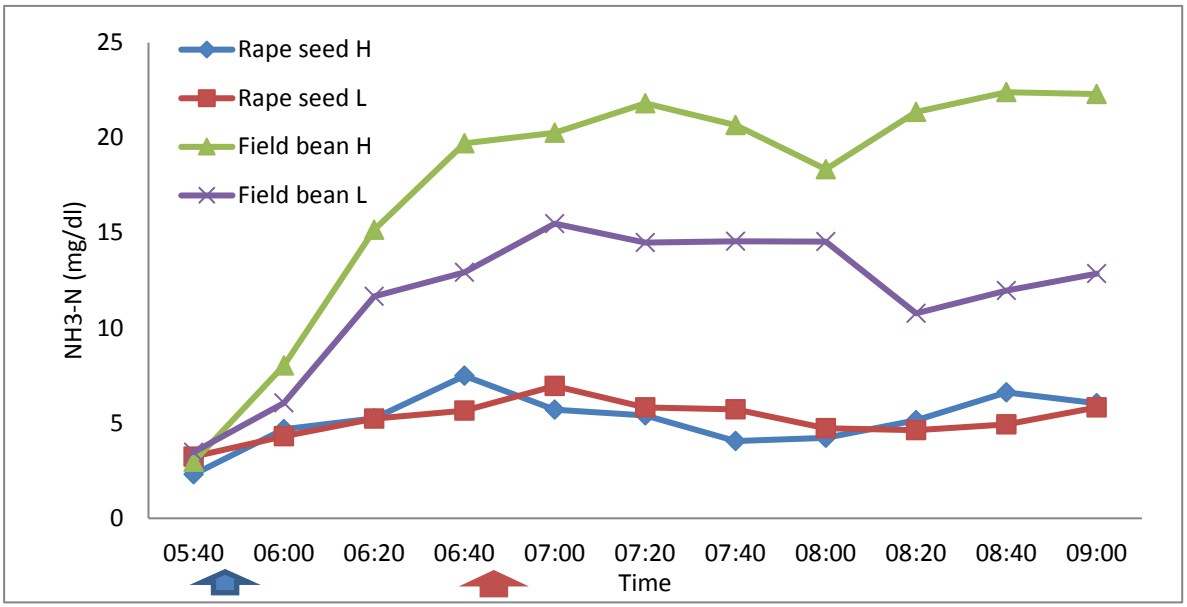

Figure 11. The concentration of ammonium nitrogen over time with the different treatments of field beans or rape seed meal. The blue arrow indicates feeding time. The red arrow indicates collection of rumen gas.

The concentration of amino acid nitrogen was highest for field beans (high), while the other treatments resulted in similar concentrations other than at 6:20 (figure 12).

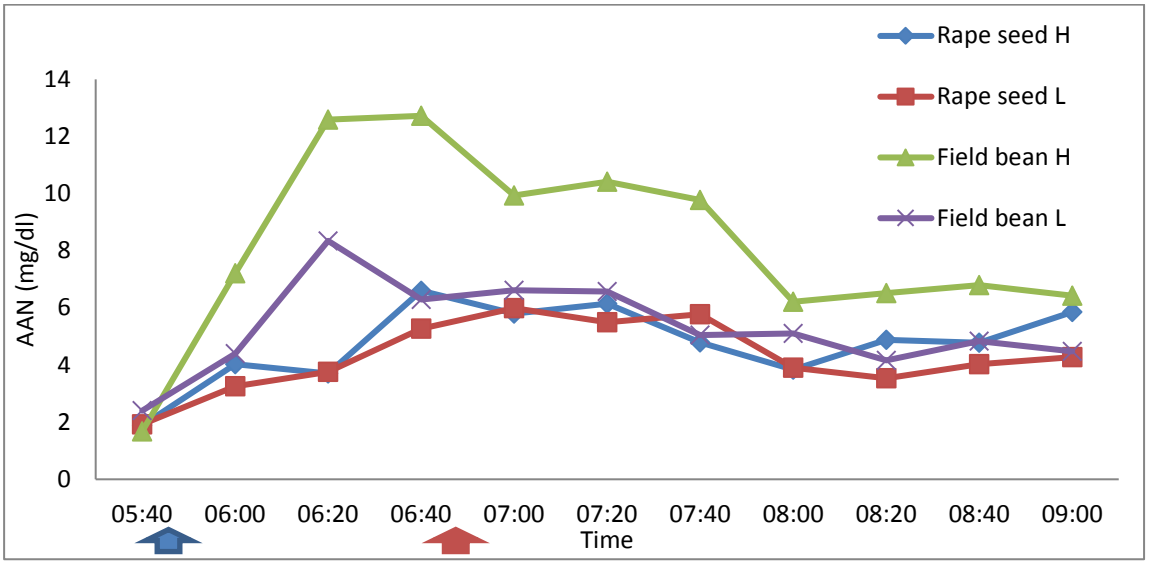

Figure 12. The concentration of amino acid nitrogen over time with the different treatments of rapeseed meal or field beans meal. The blue arrow indicates feeding time. The red arrow indicates collection of rumen gas.

Table 7. Average concentrations of H_2S in rumen gas. Concentrations of alpha amino nitrogen- and ammonium nitrogen in rumen liquid.

Item	Rapeseed meal		Field beans		SED			
	High	Low	High	Low		Time	Feed	Level
Hydrogen sulfide	202	119	20	11	9.5	<0.0001	<0.0001	<0.0001
Alpha amino-N	4.8	4.2	8.3	5.3	0.85[a]	<0.0001	<0.0001	<0.0001
Ammonium-N	5.2	5.2	17.5	11.7	1.30	<0.0001	<0.0001	<0.0001

[a] *Largest SED-value for treatment difference (P….)*

Other Volatile compounds, analyzed from gas in bags

Other analyzed compounds found in the bags were dimethyl sulfide, ethanol, methanethiol, 2-methylpentane, 3-methylpentane and pentane (see table 8). Feed type and feeding level had a significant effect on concentration of hydrogen sulfide.

The LS mean values of methanethiol, 2-methylpentane and 3-methylpenthane were negative at the high level of field beans.

Table 8. The table shows LS mean value, SED and P-value of different volatile compounds found after analysis of rumen gas. pH-values did not differ between treatments or feeds. Gas was collected one hour after feeding.

Item	Rapeseed meal		Field beans		SED	P-value, effect of		
	High	Low	High	Low		Feed	Level	Feed*Level
H_2S (PPM)	238	125	38	11	18.6[a]	0.004	0.019	0.049
Dimethyl sulfide (mg/m^3)	305	136	8	5	67[a]	0.067	0.28	0.29
Ethanol (mg/m^3)	2.8	5.3	2.0	1.6	2.18[a]	0.27	0.52	0.42
Methanethiol (mg/m^3)	127	52	-	8.2	25.9[a]	0.048	0.25	0.15
Pentane (mg/m^3)	6.2	9.2	8.1	7.7	1.71[a]	0.89	0.31	0.24
pH	6.7	6.7	6.8	6.7	0.08	0.63	0.35	0.91

[a] *Largest SED-value*

Discussion

Hydrogen sulfide concentration after feeding

Hydrogen sulfide concentration changed fast after feed intake in a similar way that Dewhurst et al. (2007) and Fonseca et al. (2012) found in their experiments. The concentration of hydrogen sulfide was very low before feeding and increased almost immediately after feeding. The hydrogen sulfide peak was reached after approximately two hours after feeding in Experiment 1 and at one hour after feeding in Experiment 2. It seems that there are differences in how fast the H_2S peak is reached. Dewhurst et al. (2007) noticed that a peak was reached later with added cysteine as compared to perennial ryegrass. In Experiment 2 the high level of rape seed meal sustained a high concentration for a longer time compared to the low level of rape seed meal, which reached a low concentration at the end of the measuring period. Dewhurst et al. (2007) showed similar results when feeding the highest level (12 g) of cysteine compared to 8 g. The result is probably because of the rumen microbes that convert cysteine are saturated.

In Experiment 1 the cows were fed silage and concentrate, irrespective of the treatments which only differed with respect to level of potassium bicarbonate. Even though the cows consumed different proportions of feed in Experiment 1, it seemed like there were differences in the quantity of produced hydrogen sulfide. Both Dewhurst et al. (2007) and Vanness *et al.* (2009a; 2009b) mentioned that there are variations in the quantity of produced hydrogen sulfide both per cow and day but also differences among cows fed the same diet. An explanation could be differences in the microbial flora which might affect efficiency of protein degradation in the rumen.

Hydrogen sulfide and different feed stuffs

In Experiment 2 it was clear that a larger amount of hydrogen sulfide was produced when animals were given rape seed meal compared with field beans. There are several possible reasons for this difference. One reason could be due to the higher sulfur content in rape seed meal, but another reason could be the lower proportion of sulfur from amino acids in rape seed meal, compared to field beans. Even though there are differences in the amount organic sulfur, there is also a large difference in total amount of sulfur in rape seed meal, compared to field beans. This probably also contributes to the difference in the amount of hydrogen sulfide produced. Another difference is also that there is more cysteine in rape seed meal and according to Dewhurst *et al.* (2007) and Fonseca *et al.* (2013), cysteine results in a higher concentration of hydrogen sulfide. Brasche *et al. (2012)*, however, suggest that similar amounts of sulfur results in the same amount of produced hydrogen sulfide level, regardless of which sulfur source that has been consumed.

Other volatile compounds collected from gas bags

Gas collection was performed in Experiment 2 and the analysis showed that, besides hydrogen sulfide, dimethyl sulfide, ethanol, methanethiol, 2-methylpentane, 3-methylpentane and pentane can be found in rumen gas. Several of these compounds have earlier been detected in rumen gas. Dewhurst *et al.* (2001) found that, except for the major gases carbon dioxide and methane, hydrogen sulfide, methyl sulfide and dimethyl sulfide can be found in the rumen at considerable concentrations. Several of the compounds detected: pentane, hexane, ethanol, have been found earlier in exhaled air from cows (Rustas *et al..*, 2012).

The ammonium nitrogen increased after feeding and declined slightly after a couple of hours. Field beans resulted in a higher concentration compared to rape seed meal. Dewhurst *et al. (*2001*)* showed that sulfur compounds such as hydrogen sulfide followed the ammonia concentration in rumen liquor. In our experiment, the ammonium nitrogen in the rumen fluid increased after feeding and thereafter decreased similar to what Dewhurst et al. (2001) Showed. However, Dewhurst et al., (2001) could not show an increase in the ammonium nitrogen or hydrogen sulfide immediately after feeding. The reason is probably because the highest peak of hydrogen sulfide and ammonium nitrogen was reached before the first measurements were taken.

When evaluating pH, no significant results were found in our experiment and pH was similar regardless of feed and level of feeding. Dewhurst *et al.* (2001) fed grass and concentrates and showed a significant difference in pH over time after feeding in his study, with pH increasing after feeding. Rumen pH can differ depending on amount of starch in the feed which means that feeding a high share of concentrate will lead to a lower pH in the rumen. A ration with a low proportion of roughage also produced more hydrogen sulfur compared to a ration consisting of more roughage (Vanness et al., 2009b).

Source of error

The concentration of hydrogen sulfide measured with GA2000 Plus Gas analyzer was adjusted in relation to the oxygen percentage because of varying proportions of oxygen in the rumen during the first experiment. The problem decreased when the rumen cannulae were sealed but most measured values still had to be adjusted in order to obtain uniform results.

The results from experiment one was presented graphically due to differences in the time of each single measurement but also due to differences in number of measurements per cow and day among periods. The reason was that, as the experiment continued, the measurment technique was improved which resulted in more measurements per day. In the beginning of the study, there were a lot of problems with rumen fluid and oxygen leakage during the measurements. Instead of performing several measurements early in Experiment 1, it would have been better to develop the technique before the whole experiment started.

Conclusion

The results in this experiment showed that the concentration of hydrogen sulfide increased after feeding and it is also clear that the concentration changes were rapid. This experiment, together with previously performed experiments, shows that hydrogen sulfide could be an interesting marker for protein degradation in the rumen in the future. Other potential markers are methanethiol and dimethyl sulfide.

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