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Examensarbete 317
30 hp E-nivå

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Abstract

Ruminants produce methane during the fermentation of feed in the rumen. This release of methane represents not only an energetic loss for the animal but also contributes to the global warming because methane is released to the atmosphere. To mitigate the methane production from ruminants, and in particular from cows, feeding strategies need to be studied. The objective of the thesis was to evaluate the quantity of methane produced in diets with or without protein concentrate combined with two silages differing in protein content (17% vs. 13%). An experiment using a Latin squares design with six Swedish Red cows in two orthogonal blocks was performed to study three different treatments: treatment AC with silage A (2/3 early harvested silage + 1/3 red clover) and cereal, treatment AP with silage A, cereal and protein supplement and treatment BC with silage B and cereal. Each period lasted three weeks with two weeks adaptation to the diet and last week as a measurement period. There were no differences in methane production in absolute terms between treatments, and the average methane production of the cows was 473 g/d. Milk production and methane production per kg milk did not differ between treatments. Significant differences were found only between treatments AP and BC in methane production per kg of protein intake (104.7 g/kg vs. 203.3), per kg of MJ intake (1.6 g/kg vs. 2.0) and per kg of starch intake (492.1 g/kg vs. 228.5).

Introduction

Agriculture is one of the activities that has the highest anthropogenic emissions of green house gases. It is estimated that agriculture contributes to the anthropogenic emissions of carbon dioxide ($C0_2$), methane (CH_4) and nitrous oxide (N_2O) with about 21-25, 60 and 65-80% respectively (Moss et al. 2000). The release of methane from agricultural activity is shown in Table 1. At the same time, agriculture is the basis for human survival and cannot be replaced. For this reason, it is especially interesting to study methods to modify production and/or change the production profile with the objective to decrease the green house gases emissions.

Methane (CH_4) is one of the most important greenhouse gases in the atmosphere. Methane affects not only the energy balance on earth (because of its radiative forcing properties) but also ozone, hydroxyl radicals, carbon monoxide concentrations, and stratospheric chlorine and ozone chemistry (Harper et al., 1999). Methane is released into the atmosphere both by natural (for example wetlands) and anthropogenic sources (rice fields, biomass, ruminants, etc).

The contribution to the methane emission of monogastric animals such as pigs, poultry, rabbits, etc., is very low compared with the ruminant contribution. It can be concluded that it is important to study and try to decrease the emissions from cattle because ruminant livestock can produce between 250 and 500 L of methane per day (Johnson et al., 1995). When these methane emissions are applied to the number of cattle in the world the total emissions from cattle is equivalent to about 15% of global methane emissions (Lassey et al., 1997), so a good way to reduce the global methane emissions is to decrease the emissions from cattle and particularly from cows. This is becoming more and more important each day because of the increasing demand of meat and milk due to the increasing world population and the improved standard of living in many countries.

Table 1. Methane emission rates from agriculture sources (Watson et al, 1992)

Agricultural sources	Methane emission rates (million tonnes per year)
Enteric fermentation	80
Paddy rice production	60-100
Biomass burning	40
Animal wastes	25
Total	**205-245**

Concerning ruminants, methane is formed during the fermentation of the feed in the rumen and the amount is dependent on the quality and quantity of the diet. The loss of ingested energy as eructated methane in cattle is around 6% (Johnson et al., 1995) so if it were possible to find feeding strategies that decreases methane emissions this would not only reduce the emissions of this greenhouse gases to the atmosphere but also improve the efficiency in terms of feed energy utilization in cattle production systems.

It is known that a higher proportion concentrate in cow's rations is a way to increment propionic acid production thus decreases methane production are obtained (Leng, 1993). Many experiments that have compared methane emissions on diets with different proportions of concentrates and roughages were performed with roughages that had a higher fibre content and a lower digestibility compared with the high quality roughages that have become more and more common during the last decades. Therefore, there has been an interest in studying the effect of different proportions concentrates and forages on methane emissions using the high quality grass silage that is commonly used in many temperate regions today. Although a number of experiments have been performed there is a need to study the methane production of cows under different feeding regimes further. The present methane study has its focus on diets of particular interest for organic milk producers. The research question is related to the fact that organically produced high protein concentrate feeds are expensive. Therefore, it has been suggested that it may be economical to feed the cows only with cereals as concentrates and combine the cereal with top quality silage containing both a high protein and energy content. Even though a certain decrease in milk production can be expected on a diet without protein rich concentrate supplements, the economical benefit of decreased feed costs may outweigh the decrease in milk revenues as the organically produced protein supplements are expensive. An ongoing production experiment with 40 cows will evaluate the production

effects of two concentrates (cereals only vs. conventional concentrates) combined with two types of grass/clover silages (17% vs 13% crude protein), both with a high energy content.

The present methane experiment has been designed as a 3*3 latin square to study possible differences in methane emissions from cows fed three of the four diets used in the production experiment:

1. cereal only with high protein silage
2. cereal only with low protein silage
3. conventional concentrates with high protein silage.

In the experiment, methane emissions were estimated using the SF_6 tracer technique. This technique has proved useful and sufficiently accurate for estimation of methane emissions on different diets in feeding experiments (Grainger et al., 2007).

The objective of the thesis was to evaluate the quantity of methane produced when protein concentrate is removed and two quality silages are fed.

Literature review

Organic milk production

In 2008, 7.8 million of hectares in the EU27 were covered by organic farming (Eurostat, 2010), as is shown in table 2, which is 7.4% more than in the previous year. The milk production is not an exception and the organic dairy farms are increasing not only in Europe but also in Sweden were in 2006, 6% of the dairy cows (24 141 heads) were in organic production (Eurostat, 2010). In 2009 the total amount of milk produced in Sweden was approximately 2900 thousand tonnes (Swedish Dairy Association, 2010). The proportion of organically produced milk has increased from approximately 5% in 2006 to approximately 8% in 2009 (Swedish Dairy Association, 2010). The large increase in percent is due to an increase in the amount of organically produced milk and a simultaneous decrease in the total amount of milk produced in Sweden during these years.

The organic production aims at producing high quality products in a sustainable way. In Sweden, the organ that regulates the organic production is KRAV that publishes standards that the farmers have to follow if they want to be labelled as organic producers and received the additional payment that is associated with organically produced milk. Swedish rules are sometimes more restrictive than European laws, and are focused mainly in housing, feeding, management and medical care. Up to now, methane production studies have mainly been performed with diets used in conventional milk production. Therefore, it is important to study how methane production is influenced by diets with the larger proportions of roughage that are mainly used in organic milk production. As earlier mentioned, organic cows have to be fed following the KRAV standards. The most important of these feed standards are the following:

- The feed should have a good hygienic quality
- All feeds and feed components must be 100% KRAV-certified
- Genetically modified feeds are not allowed
- The proportion of home-grown feed has to be at least 50% of annual feed intake
- Roughage should be at least 50% of the dry matter intake for ruminants during the first three months of lactation and at least 60% seen over the entire lactation
- The percentage of concentrates cannot be more than 40% of the daily dry matter intake (DMI) seen over the whole lactation.

As mentioned above, the concentrates must also be certified as organic. Most cereals can be organically produced at a comparatively low cost on the farm but the protein supplements are often purchased at a high price which increases the total feed price.

Table 2. Organically cultivated acreage in Europe (Modified from Eurostat, 2010)

	Organically cultivated		Increase in organically cultivated acreage	
	1000 ha, 2008	% of total utilized agricultural area, 2007	2008/2007 (%)	2008/2005 (%)
EU-27	7765	4.1	7.4	-
EU-25	7608	-	7.4	20.6
Sweden	336	9.9	9.1	51.0

Energy losses and methane production in the digestive tract of ruminants

The synthesis of the methane in ruminants reflects energy lost and it is due to the reduction of the carbon dioxide by methanogenic archea. After the feed is digested in the rumen, some of the energy is lost in the form of heat or methane, giving a production of methane utilizing between 11 and 13% of the digestible energy (McDonald et al., 2002). Many experiments have been performed to try to understand and to decrease this loss of energy. Figure 1 shows the partitioning of food energy in the ruminant.

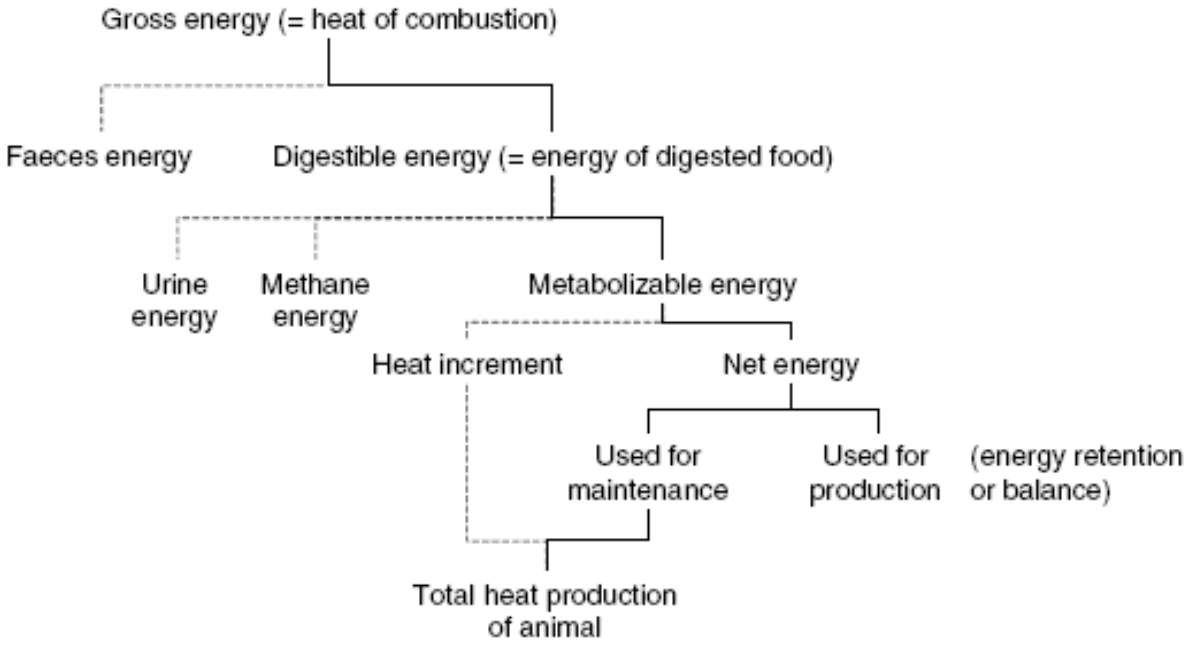

Figure 1. The partitioning of food energy in the ruminant (McDonald et al., 2002) (solid lines indicate energy usage, dashed lines indicate energy wastage).

Carbohydrates

The plants consist mainly of carbohydrates, the carbohydrates are the primary source for microbial growth and thus also the main substrates for ruminal fermentation (Hungate, 1966); Therefore, it is very important how the different carbohydrates affect to the ruminal fermentation. The methane production differs depending on the different types of carbohydrates that are fermented. The carbohydrates influence the ruminal pH and this leads to changes in the micro-flora. The fermentation of cell wall fibre will lead to the production of a higher proportion of acetic acid in the rumen. As a contrast, starch fermentation gives a

higher proportion of propionic acid due to the lowered pH in the rumen which causes changes in the ruminal micro-flora by an increase of amilolitic microbes and decrease of celulolitic microbes.

When the carbohydrates enter the rumen, they come in contact with the bacteria of the rumen and this union causes the hydrolysis of the carbohydrates and the release of glucose and oligosaccharides into the ruminal liquid. Both glucose and oligosaccharides are absorbed by the rumen bacteria (Figure 2), afterwards being incorporated into the glycolisis process which gives as result ATP (energy used by the rumen microorganism), NAD and 2 Pyruvate molecules. There is no oxygen available in the rumen, and for this reason, the pyruvate can be reduced (capturing electrons) producing the end products of the fermentative digestion: the Volatile Fatty Acids (VFA).

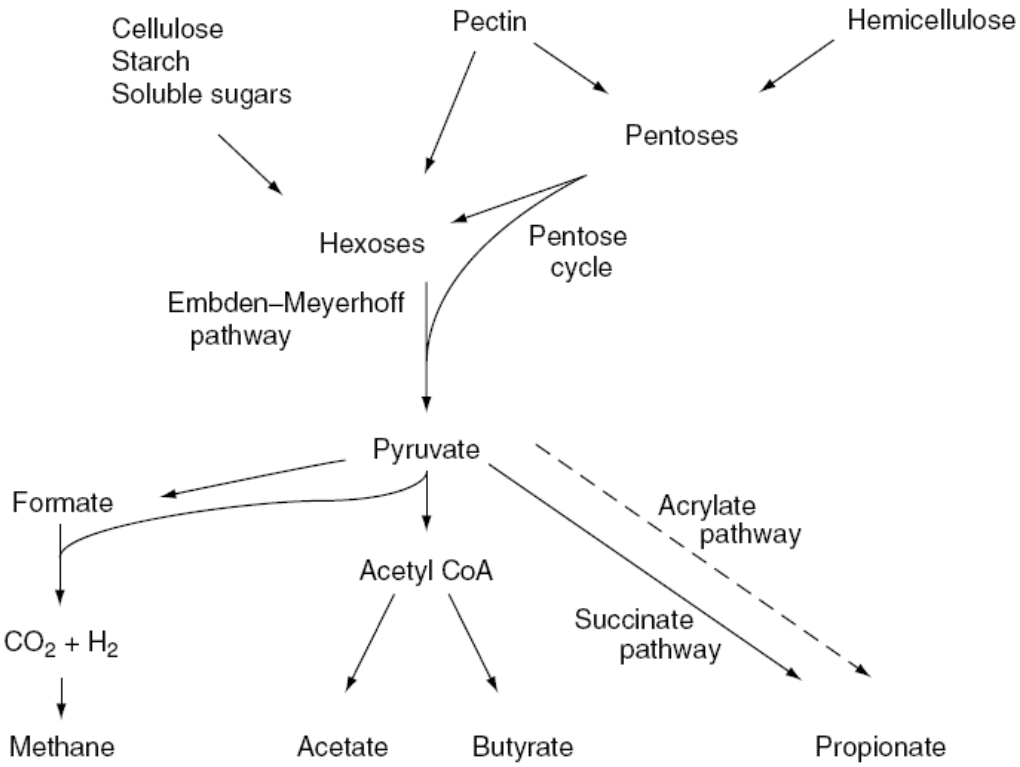

Figure 2. A schematic representation of the major pathways of carbohydrate metabolism in the rumen (Dijkstra et al., 2005).

Volatile Fatty Acids

Volatile Fatty Acids (VFA) are mainly acetate, propionate and butyrate, we can also find valerate, isovalerate, isobutyrate and caproate but in very low proportions (Dijkstra et al., 2005). Volatile fatty acids are end-products of microbial fermentation that takes place in the rumen. They are also the main source of energy for the ruminants, and this energy is used by the lactating cow to produce milk and body fat, but not all VFA have the same degree of efficiency. The propionic acid fermentation is more efficient in the use of the energy than acetic and butyric acids that have a large loss of methane (Wolin, 1960). The type of VFA produced by the animal influences the release of methane and hydrogen, increasing the release of methane when the relation of ruminal VFA [acetic acid+butyric acid]/propionic acid increases (Moss et al., 2000).

Methanogenesis

Methanogenesis is a complicated process where many parameters like folic acid and vitamin B_{12} are involved (McDonald et al. 2002). During the fermentation process reduced cofactors like NADH are formed, and these reduced factors have to be reoxidated in order to achieve the chemical reaction by transferring its electrons to acceptors like CO_2.

During the fermentation process H_2 molecules are produced, but the H_2 is used by other ruminal bacteria avoiding the accumulation of these molecules in the rumen (Moss et al. 2000). The major pathway of H_2 elimination is the formation of methane through the reaction: $CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$.

McDonald et al. (2002) summarizes the stoichiometry of the main anaerobic fermentation pathway as follow:

- H producing reactions:

  Glucose $\rightarrow$ 2 pyruvate + 4H
  Pyruvate + H_2O $\rightarrow$ acetate + CO_2 + 2H

- H using reactions:

  Pyruvate + 4H $\rightarrow$ propionate + H_2O
  2 acetate + 4H $\rightarrow$ butyrate + $2H_2O$
  CO_2 + 8H $\rightarrow$ methane (CH_4) + $2H_2O$

Factors which may affect the methane production

Animal parameters

Methane formation depends on the size and the situation of the fermentative zone, and also of the existence of mechanisms that help the feed retention extending the actuation time of the microorganism (DeBlas et al, 2008). Okine et al., (1989) said that mean retention time explains 28% of the methane emissions. It has been estimated that 80% of the organic matter of the feeding is fermented in the rumen (NRC, 2001).

Methane production also varies depending of the age of the animal, the amount is very low in milk fed calves because the milk comes in the abomasum without any fermentation process (DeBlas et al, 2008) and as the animal grows and the rumen develops, the amount of methane gradually increases.

The metabolic live weigh (MLW) also affects methane production, this is more evident in males, (Machmüller et al. 2006) and the following equation for methane production for males was suggested by these authors: CH_4(g/day) = 3.58 MLW ($kg^{0.75}$) – 35.83 (R^2=0.83).

The milk yield also influences the production of methane, Machmüller et al. (2006) have found that when milk yield increases, the methane per kilo milk decreases. This is logical and can be explained by the fact that the energy needed for maintenance is considered approximately the same for the animal irrespective of production level. The methane production that originates from maintenance needs is therefore also estimated to be the same for an individual animal of a specific weight. When the milk yield increases, the DMI also increases but not in the same proportion. With the increased milk yield, there is more milk to carry the "burden" of maintenance needs and methane per kg milk will decrease. Thus, with increased milk yield the methane produced in absolute terms will increase somewhat but the methane per kg milk will decrease.

The methane production is different not only between species, being much more important in ruminants than in monogastrics, and especially in cattle, but also between sexes. In their work, Machmüller et al., (2006) concluded that prediction equations for male cattle are more precise when MLW is used than when estimated DMI (EDMI) is used, whereas for

female, the accuracy of the equations increase when EDMI, daily milk fat and daily milk protein yield are taken into account.

Feeding parameters

The production of methane is directly related to the profile of the VFA in the rumen which in turn is related with the type of feed offered. Johnson et al. (1995) said that there are two main mechanisms that cause fluctuation in methane production; the first one is the amount of dietary carbohydrate fermented in the rumen and the second one is the ratio of VFA produced. Principally, the ratio between propionic acid and acetic acid has a higher impact on methane production. It is expected that methane production decrease when high concentrate diets are given (Leng, 1993). Roughage diets high in cellulose lead to VFA with a very high proportion of acetic acid while diets with a high proportion of concentrates give a large amount of propionic acid. An example of the molar proportion of the VFAs with different rates of concentrate is shown in table 3. However, we have to say that acidosis, laminitis, fertility problems and other problems can occur with rations when diets with a high proportion of concentrate are fed (Moss et al, 2000).

The level of intake can also affect the methane production, when an animal increases its intake, the percentage of gross energy lost in form of methane decreases (Johnson et al., 1993, mentioned in Johnson et al. 1995), but, in absolute terms, the amount produced by the animal is higher.

Table 3. VFA concentration, molar proportions and production rates in the rumen (modified from Dijkstra et al. 2005)

Diet	Intake kg/day	Total VFA concentratio mmol/l	Acetate molar%	Propionate molar%	Butyrate molar%	VFA prod. mol/day
Ryegrass Hay:concentrate(6:4)	12.9[a]	85	68	19	13	79.8
Ryegrass Hay:concentrate(1:9)	12.7[a]	89	52	38	9	90.0

Methane production is also influenced by the digestibility of energy of the diet. Methane losses increase by 0.47 and 0.74 percentage points in roughage and mixed diets respectively when the digestible of energy increases by 10% (Blaxter et al., 1965).

Not all the concentrates have the same effect in methane production, DeBlas et al. (2008), explained that the cereal grains with a high proportion of starchy endosperm like wheat, barley or oats have an easier and faster fermentation giving less methane than those that have a lower proportion like maize, and sorghum. The difference in the fermentation leads to changes in the rumen pH and changes in the proportion of acetic and propionic acids formed, and therefore, in the amount of methane produced.

The quality of the forage and its protein content can also affect the methane production, as is described below.

Researchers have looked for a way of decreasing energetic losses, in particular methane production in ruminants by changes in feed composition. Some additives like ionophores and particularly monensin have been studied. Monensin is a broad spectrum antibiotic obtained from the actinomycete *Streotomyces cinnamonensis* used in some countries. It is not allowed in European Union but it is used in United States. Its main action is to change the fermentation from acetate to propionate (Moss et al. 2000) which leads to the decrease of methane production. However, the widespread use of antibiotics can lead to future problems with bacteria that are resistant to antibiotics and the environmental and economical advantages of using antibiotics to decrease methane production must be weighed against the negative health effects of increased resistance.

Jordan et al. (2006) explained that the addition of fat gives a decrease in methane synthesis because it provokes changes in ruminal flora that decrease the fiber digestion giving a higher formation of propionic acid. However, if the content of fat in the diet is too high, the milk fat content decreases (Palmquist, 1996).

Forage

With the advance of the growing season, the crude fibre content increases in the growing plant, whereas the soluble carbohydrates decrease (Barnett et al. 1961). Therefore forages harvested in an early stage of development usually have a higher digestibility and energy content. Woodward et al. (2004) concluded that methane emissions per mega joule (MJ) decreases when the digestibility of the feed increases.

Methane production can be decreased by grinding and pelleting of forages (Blaxter, 1989, mentioned in Johnson et al. 1995). This is due to the decrease of the retention time compared with forages coarsely chopped. Moss, et al (1994) showed that methane production can be decreased by giving forages in ensiled form and in another paper (1995) they explained that alkali- treatments of poor-quality forage stimulate the rumen degradation of plant cell walls giving an increase in the amount of methane emissions.

Protein supplement

Rumen microorganisms hydrolyse food protein to peptides and amino acids and, some amino acids are later degraded to organic acids, ammonia and carbon dioxide. This deamination leads to the formation of small quantities of VFA such isobutyric or valeric (McDonald, 2002) but there is no evidences that this process affects methane production in the rumen. The degradable protein of the food is used by the ruminal bacteria to synthesise dispensable and indispensable amino acids that are used later by the cow. However, if the feed is deficient in protein, the concentration of ammonia in the rumen will decrease and this will affect to the growth of the ruminal microflora. These changes in the flora can affect the fermentation process of carbohydrates and therefore the methane synthesis. Experiments have been made in order to understand the effect produced by protein in the synthesis of methane. Moss et al. (2002) concluded that when the quantity of soy bean meal given to a group of sheep increased and the proportion of grass silage decreased, the digestibility of organic matter increased and the amount of VFA and the volume of methane per day also increased. This is contradictory with the majority of the papers where it seems that when the proportion of silage decreased, the volume of methane per day decreased.

Methods for measuring methane

For the development of strategies that decrease the methane emission from cattle, it is necessary to be able to quantify these emissions under a wide range of circumstances (Johnson et al., 2007). Methane production can be measured with both in vitro and in vivo techniques. In vitro techniques are mainly used to test methane inhibitors because uniform experimental conditions can be maintained in an easier way than in vivo trials and they are cheaper and less time-consuming (Bhatta et al. 2006). In vivo techniques are often preferred as they will show what actually happens in the animal. Each technique has its strengths and weaknesses and the technique must be chosen depending on the question asked (Johnson et al, 1995).

Methods used to measure methane emissions can be divided into direct and indirect measurements (Pinares-Patiño et al. 2008) but also into individual and in group methods. Direct methods for measurement of methane are usually based on putting the animal in a metabolic cage of some sort. The indirect methods are techniques where tracers are used.

In individual systems, the methane emissions can be registered measuring the different concentration in inspired and expired air (Johnson et al. 1995). Most methods include measurements of respiratory gas exchange, they can be divided in different classes according to the use of open or closed circuit, confinement; the open-circuit type being the most common (McLean & Tobin, 1987). Johnson et al. (1995) explained that the disadvantage of these techniques is the expense not only for the construction of the chambers and its equipment but also because of the high labour input for animal training. Another weakness is that the movements of the animals can be restricted and for that reason not all the animals are suited for this type of trial. Other individual techniques use isotopic and non-isotopic tracers. Isotopic methods, as explained by Johnson et al. (1995), involve the use of [^{3}H-] methane or [^{14}C-] methane and cannulated animals and its main weakness is the difficulty of the preparation of the infusion solution because the methane gas has a very low solubility. Within the non-isotopic tracer techniques, one of the most important is the SF_6 tracer technique that is the one that was used in the present experiment and for that reason it is described in more detail below.

Another option to estimate the methane production is to use prediction equations that rely on equations derived from analysis of previously performed animal measurements or in vitro techniques. There are also other prediction methods as the fermentation balance from the conversion of dietary carbohydrates to VFA (Czerkawski, 1986). Blaxter and Clapperton (1965) presented an equation based on feed characteristics where methane is predicted from gross energy digestibility.

The sulphur hexafluoride (SF_6) tracer technique

The SF_6 tracer technique was developed because it has many advantages, for example, it is cheaper than enclosure techniques and it permits the animals to display their normal behaviour.

To carry out this technique, one small permeation tube (Figure 3) must be introduced in the rumen of each cow. Each permeation tube contains the SF_6 tracer gas and the release rate of each one is known before the introduction into the cows. Secondly, a halter fitted with a capillary tube is placed (Johnson et al.2007) and connected to an evacuated sampling canister (Figure 4) where a sample is collected at a constant rate around the mouth and nose of each animal.

Figure 3. SF_6 permeation tube introduced in cow's rumen.

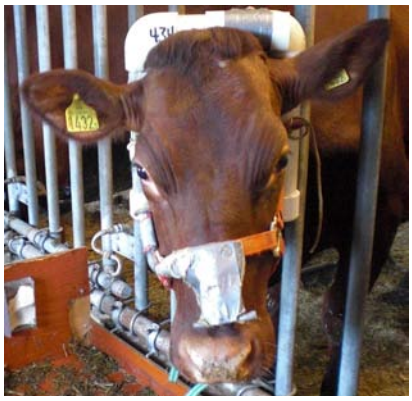

Figure 4. Cow with the halter and the canister for collecting breath samples.

Once the halter and the canister is placed on the cows, they can show their natural behaviour and the breath collection starts. Each 24 hours, the canisters (or yokes) are replaced by new ones and a sample of the collected breath is analysed for methane and SF_6 concentration. The canister is then cleaned with nitrogen infusion.

Moreover, additional halters and canisters are placed near the cows to measure the methane and SF_6 concentration in the background. These measurements are used later to correct those ones obtained from the cows. Pinares-Patiño et al. (2008) explained that the methane emission is calculated using the ratio methane/SF_6 taking into account the background concentrations and the permeation rate of the tube.

The principal disadvantage of this technique is that only the methane from the mouth and nose is measured and it does not include the methane produced in the colon. Even so, Mc Ginn et al. (2006) showed that the methane production estimated with the SF_6 technique was 93 to 95% of that measured using whole-animal chambers. According to Johnson et al. (2007) the difference between these two techniques can be so small mainly because most hindgut methane is absorbed into the blood stream and respired, so it will be measured with the SF6 technique as well.

Material and methods

The experiment was performed at Kungsängens Research Centre in the dairy research barn of the University of Agricultural Sciences, Uppsala, Sweden between 8th of February to 11th of April 2010.

Objective

The objective of this experiment was to measure methane emissions of dairy cows when they were fed different diets (with and without protein supplements combined with two silages differing in protein content) using the SF_6 tracer technique. The experimental time was

divided in three periods of approximately 21 days each, in which the different diets were fed. Each three weeks period had two weeks adaptation to the diet and one week data collection (sampling and measurement).

The three diets were:

Treatment AP: clover/grass silage + cereal + protein supplement

Treatment AC: clover/grass silage + cereal

Treatment BC: grass silage + cereal

Diets where the concentrate consisted of cereals with or without protein supplement were in focus. However, the protein content of the silage may affect the results when cows are fed low amounts of protein in the concentrates (i.e. only cereals). Therefore, two different treatments were included in the experiment where silages with different protein contents were combined with only cereal (treatments AC and BC). The three treatments were also applied to a larger experimental group to study the production response of cows when they are fed only cereals instead of cereals together with a protein supplement. However, the results from the production trial will be published elsewhere and will not be discussed further here.

The study was designed as a double Latin square system with 3 different treatments, 2 orthogonal blocks and 3 cows in each block (table 4). By using the Latin squares design, treatment effects are calculated as differences within each animal and treatment effects can be detected with a lower number of animals. However, the design is more sensitive to missing values and as the animals switch treatments, effects from the previous treatment can sometimes affect the interpretation of the treatment effect.

Table 4. Experimental design with the treatment diets AP[1], AC[2] and BC[3]

	Block 1			Block 2		
Cows	1340	1386	1427	1422	1428	1432
Period 1	BC	AC	AP	BC	AP	AC
Period 2	AP	BC	AC	AC	BC	AP
Period 3	AC	AP	BC	AP	AC	BC

[1]AP = clover/grass silage + cereal + protein supplement; [2]AC = clover/grass silage + cereal; [3]BC = grass silage + cereal

Animals and housing

In the experiment 6 Swedish Red cows (2 were multiparous and 4 primiparous) were randomized to the treatment sequences. At the start of the experiment, the average days in lactation was 194.81 (min. 167 and max. 233) with a milk yield of 24.51 kg milk/day (min. 17.3 and max. 34).

During the adaptation period, the cows were kept in the barn with automatic feeding and milking. The first day of the measurement period the animals were moved to a separate barn with individual cubicles where the cows were tied up during measurement days. The animals were accustomed to changing barns and occasionally being tied up and showed no signs of stress at the change of environment.

During the measurement period, the 6 cows were isolated from other cows in order to avoid the influence of the methane emissions of other cows in the measurements. Around the experimental cows there was a simple construction to minimize the air from other parts of the barn to enter and a special higher ventilation system was used in the section with methane measurements to increase air turnover and decrease the background methane, with the purpose to give a good accuracy of the methane samples taken from each cow.

Feeding and treatments

As previously mentioned, the cows were fed three different diets during the three periods. Two different silages were used in the experiment, silage A and B.

The grass called silage B, consisted of Meadow Fesccue (*Festuca pratensis L.*), Timothy (*Phleum pretense L.*) and Red Clover (*Trifolium pretense L.*) harvested for silage in early June (Table 5) and stored in a tower silo.

Two thirds of silage A (on dry matter basis) consisted of grass silage harvested from the same ley as silage B but at an earlier date and stored in another tower silo. To this early harvested silage one third (on dry matter basis) red clover round bale silage was added to increase the protein content. The mixture was called silage A. Table 5 summarizes some

information about the two tower silages. The silages analyses were performed before the experiment began, late 2009 in order to decide the mixture and the different rations.

Table 5. Silages composition.

	Silage Type	Harvest date	CP %	ME MJ/kg ts	NDF
Silage A	Mixture grass & clover	28/05/2009	17	11,3	40.0
Silage B	Grass silage	08/06/2009	13,2	11,3	47.9

The cereal given was made of 35,65% of barley, 34% of wheat, 25% oats and 2% molasses with gave a pellet with a crude protein (CP) content of 11,77% of DM. The protein supplement was made of 47,49% of soybean expeller, 15,64% of rapeseed cake, 15% oats, 12% rapeseeds crushed, 4,33% roasted soybean, 2,74% wheat and 1% of molasses that gave a pellet with a CP content of 33,83% of DM. The cereal and protein supplements were offered as pellets.

Each cow was fed concentrates according to the specific treatment at a level that would cover the energy requirements at the production level that the individual animal had at the start of the experiment in February, assuming a silage intake of 15 kg DM. During the adaptation period the animals were offered silage *ad libitum* (following the feeding regimes in the earlier mentioned production experiment). At the start of the measurement period, the amount of silage intake during the two adaptation weeks was calculated for each individual and then kept at the same level throughout the measurement week. The same level of concentrate feeding and the same system for silage feeding was applied throughout the experiment. The animals were fed 4 times per day; 20% of the corresponding ration was given at 6 a.m., 25% at 9 a.m. and 12 p.m., and 30% at 17 p.m. when 100g of minerals were added to the ration. During the two adaptation weeks, the cows were fed concentrates according to the experimental plan using transponders and an automatic concentrate feeder. Silage intake during the pre-period was registered individually though a system with transponders and feed troughs on weight cells that could register consumption at an individual level using automatic registrations. During the measurement week, the feed was weighed daily as well as the leftovers. Leftovers were marginal in amounts.

Feed samples

During the measuring period, registration of feed offered as well as leftovers was made for each cow. Daily, one sample of feed was taken and frozen for later analyses. The samples of silage were pooled to a one sample of feed per week, giving six different samples at the end of the experiment (2 silages and 3 periods). The analyses performed were DM, ADF, CP, NDF, metabolisable energy (ME), following Bertilsson and Murphy methodology (2003), besides, iNDF was also analyses following Norfor (2007) and gross energy content using a isoperibol calorimeter (Parr 6300, Parr Instrument Company, Moline, IL, USA). Ash content was analyze using Van Keulen and Young methodology (1977). Lignin, and quality traits (pH, ammonium-N and lactic and butyric acid) were also analyse. The results of the analysis are presented in table 6. The leftovers were collected daily and pooled into one bag for each animal, at the end of the week DM analysis were made for each sample.

Furthermore, sampling of cereal and protein supplement was performed in a similar manner with a total of 6 samples (one per period for cereals and protein supplements, respectively). These samples where analysed to determine DM, ash, CP, starch, NDF, iNDF, ADF and lignin content.

Table 6. Silages, cereal and protein supplement analysis, mean ± standard deviation

	Silage A	Silage B	Cereal	Prot.Suppl.
DM content %	35.2±1.8	35.1±1.2	87.5±0.2	90.2±0.5
Ash % of DM	8.6±0.1	7.7±0.2	5.9±0.3	7.5±0.03
CP % of DM	16.3±1	13.6±0.6	12.7±0.7	34.5±0.2
NDF % of DM	42.4±0.8	49±0.4	15.4±0.5	12.9±0.2
iNDF g/kgNDF	194±14.4	163±1.9	108±6.8	-26±6.6
ME MJ/kgDM	11.5±0.02	11.7±0.1	18.01±0.1	21.44±0.1
ADF % of DM	28.9±0.4	29.9±0.5	6.6±0.3	9.8±0.1
Lignin % of DM	6.6±0.4	5.6±0.2	1.8±0.1	2.2±0.1
Gross energy MJ/DM	19.12±0.8	19.28±0.2	-	-
pH	3.92±0.04	3.89±0.1	-	-
Ammonium-N %	0.066±0.00	0.047±0.00	-	-
Lactic acid % of DM	9.51±0.6	9.67±0.3	-	-
Butyric acid % of DM	<0.02	<0.02	-	-

Milking

The automatic milking system was used during adaptation weeks and a bucket milking system was used during measurement days with milking at 6 a.m. and 15:30 p.m.

During the experiment, milk yield was registered daily for each cow and milk samples were taken during two consecutive days during each methane measuring period (4 milkings) and during two consecutive milkings during each pre-period. The milk was analysed for fat, protein, lactose and somatic cells by infrared spectroscopy (MilkoScan^{TM} FT120, Foss, Denmark).

Methane sampling: The SF_6 tracer technique

Measurements in methane production were done with the SF_6 tracer technique. This technique needs firstly the introduction of one permeation tube in the rumen of each cow. Approximately 10 days before the experiment begins, the number of each tube is recorded as well as its release rate (Table 7) and one tube is put into the rumen of the cow. The permeation tube body is made from a piece of brass rod and the SF_6 flow is regulated with Teflon membrane. Just after the end of its construction, one tube contains about 2500 mg of SF6 but it is important to know the exact content of each permeation tube and the release rate. As a preparation before the experiment the tubes are calibrated, and once a week during at least ten weeks each tube is weighed to know the release rate of each permeation tube. This release rate will be constant during around 4 months and it will be used later for the calculation of methane production.

Table 7. Permeation tubes introduced in the cows

Cow number	1340	1386	1422	1427	1428	1432
Tube number	24	2	23	1	22	8
mg SF_6/day	3,054	2,331	2,961	3,845	3,373	4,032

Once the SF_6 capsules are introduced in the cows, a halter is placed around the head of the animals, the halter size has to be appropriate because the location of the inlet over the nostrils is very important for good sampling. In the halter a capillary tube is fitted and during the collection time this is connected to a yoke. At the end of the capillary tube (near the nostrils) a filter will be placed in order to avoid the liquid entrance but allow the air to enter. Before connecting a new yoke the yoke must be cleaned in a proper way by flushing it with nitrogen gas, and then the yoke is provided with a vacuum pressure of -25 Hg using a vacuum pump. The connection time of the yoke has to be noted, and then the air sampling begins. Because of the vacuum, with the help of the equipment (capillary tubes), the air near the nostrils of the cow will be sucked in at a low and constant rate throughout the collection period.

Air collection takes place during 24 hours, then the yokes are disconnected from the halter and the time is noted. An over-pressurised yoke is connected with the halter to remove any water that could have entered into the halter's tube during the collection and after one minute the overpressure yoke is removed and a new yoke is placed on the cow´s neck, its number and the connection time is noted. After the yoke is disconnected it is necessary to check that the sampling is good by connecting a manometer and checking if the pressure after sampling is between -3 and -12 Hg, showing that there is still under-pressure in the yoke which shows that the sampling has been going on for the entire 24 hours sampling period (Figure5). If the sampling is not correct the halter is replaced for a new one and repaired for later use. When the sampling is correct, the yoke is filled with nitrogen gas that gives a pressure of 13 Hg and permits the sample collection. Before the sample collection it is necessary to wait at least one hour in order to permit the mixture of the gases inside the yoke. For the collection of the samples, a syringe of 60 ml is used to collect the gas from the yoke and then to introduce the gases into a test tube of 20 ml. Six samples in different test tubes were taken per yoke. After sampling was completed, the yoke was emptied and cleaned three times in a row with nitrogen gas (2 bars).

Figure 5. Checking of the sampling method.

Apart from the cow's samples, the collection of the background air is needed in order to be able to measure the amount of SF_6 and methane that is present in the air and correct the values for each cow for the background content of methane. Three measurement units (halter + yoke) were placed around the cows, and the air was collected and the samples were taken in the same way as for the cow's ones. The background content of methane in the barn air around the cows was considered as the mean of the methane and SF_6 content in the three background units.

Once the samples were ready, they were sent to the laboratory for analysis of SF_6, methane and CO_2, the analysis are done with gas-liquid chromatography method, model name: Pekin-Elmermodel Claus 530, Shelton, CT, USA).

Methane emission was calculated using the equation:

$$QCH4 = QSF6 \times \frac{([CH4] - [CH4b])}{([SF6] - [SF6b])}$$

(Johnson et al., 1994)

Where QCH4 is the methane production in g/day, QSF6 is the release rate of the capsule, [CH4] and [SF6] are the concentration of the gases measured in the air exhaled by the cows and [CH4b] and [SF6b] are the gases concentration of the background.

Live weight

The animals were weighed at the beginning and at the end of each measurement period.

Statistical analysis

The data was analysed using the SAS software system (SAS Institute Inc., Cary, NC). The mixed procedure of SAS was used. The first analysis included the effect of pre-period and interactions but as none of these factors were significant the final model was the following:

Y (response variable i.e. methane) = block period treatment with

Random factors: cow block*period*treatment*cow

Results

During the experiment some problems occurred with the technique: the cow 1386 lost her SF_6 permeation tube during the first week of the trial, the capsule was not found and loss was not detected until the first laboratory results arrived. Therefore, the data set contains one missing value. The capsule was replaced with another one during the second pre-period and no more problems were observed for this cow.

Due to a writing error, during the first period the cow 1422 was fed too little cereal, and the 1427 too much but, as no interactions between periods and treatments were found, it seems that this has not affected to the general treatment results of the experiment.

Methane production

The average of the estimated methane production was 503 g/day in treatment AC, 437 g/day in treatment AP and 489 g/day in treatment BC. There were no significant differences between treatments ($p=0.28$). Figure 6 shows the methane production for each treatment.

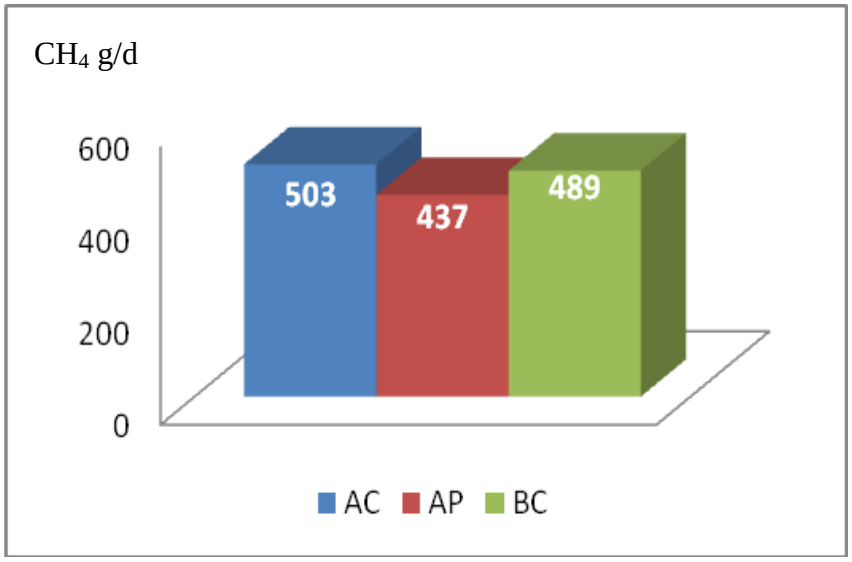

Figure 6. Methane production (g/d) per treatments AP (silage A + cereal and protein supplement); AC (silage A + cereal) and BC (silage B + cereal), least squares means.

Feed consumption

A summary of the main consumption data is shown in table 8.

The overall average intake was 19.7 kgDM/day and 20.4, 20.0 and 18.6 kgDM/day on treatments AC, AP and BC respectively. The differences in DMI were no significant.

Methane production per kg of DMI when animals were on treatment AC was 25.1 g/kgDMI, 22.3 g/kgDMI on AP and 27.2 g/kgDMI on BC. The differences between treatments were not significant but a tendency was found between treatment AP and BC (*p=0.055*). Figure 7 shows the DMI per treatment and the methane per kg of DMI.

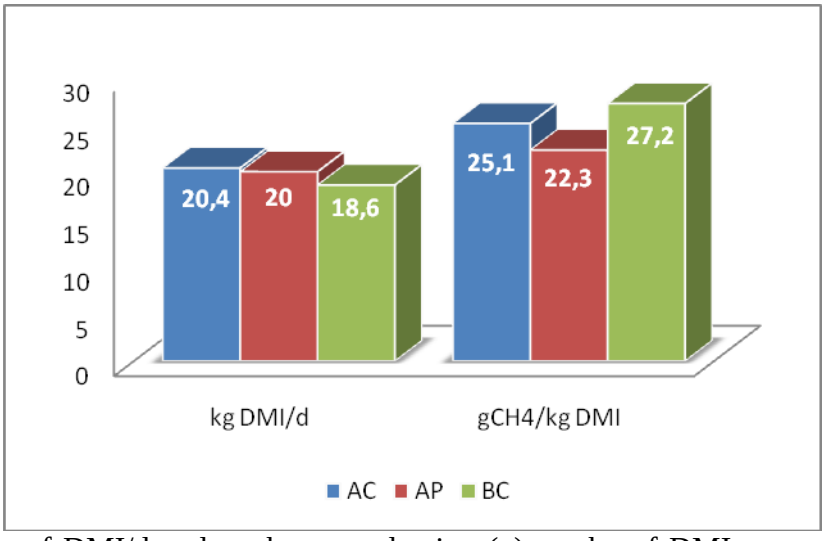

Figure 7. kg of DMI/d and methane production (g) per kg of DMI per treatments AP (silage A + cereal and protein supplement); AC (silage A + cereal) and BC (silage B + cereal), least squares means.

The average of CP intake (Figure 8) was 3.1 kg/d in treatment AC, 4.9 in AP and 2.5 in BC.

Methane production per kg of CP intake (Figure 9) showed significant differences between treatment AC and AP ($p<0.05$) and between AP and BC ($p<0.01$). Between the treatments AC and BC the results showed a tendency ($p=0.092$). The methane per kg CP intake was 162.3, 104.7 and 203.3 g/kg CP intake on treatment AC, AP and BC respectively.

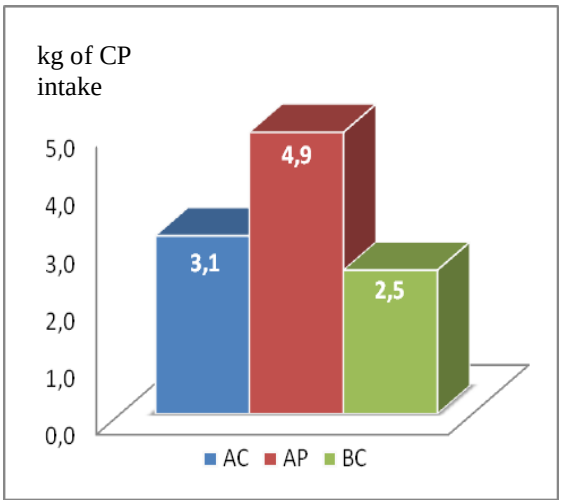

Figure 8. kg CP intake per treatments AP (silage A + cereal and protein supplement); AC (silage A + cereal) and BC (silage B + cereal), least squares means.

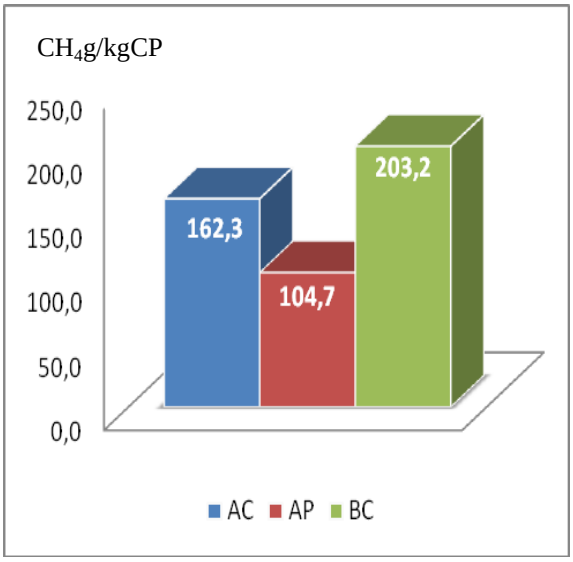

Figure 9. Methane production (g) per kg of CP intake per treatments AP (silage A + cereal and protein supplement); AC (silage A + cereal) and BC (silage B + cereal), least squares means.

The ME intake, treatment AC gave 267.9, AP 270 and BC 244.4 MJ/d. Concerning methane production per ME, the differences due to the treatment showed a tendency ($p=0.090$), and the differences were significant between treatments AP and BC ($p<0.05$). The results were 1.9 g of methane/MJ on treatment AC and 1.6 and 2.0 per AP and BC respectively.

The amount of NDF was 7.4, 7.3 and 7.6 kg/d for treatments AC, AP and BC respectevely. Methane production per kg NDF did not differ significantly between treatments, the values were 71 g/kg NDF per treatment AC, 61 per treatment AP and 68 per BC.

The starch intake was 2.5 kg/d in treatment AC, 1.2 in AP and 2.2 in BC. The methane emissions per kg of starch intake differed significantly for the different treatments ($p=0.019$) and the results were 185 g of methane per kg of starch intake on treatmente AC, 492 on AP and 229 on BC. Significant differences were found between AC and AP ($p<0.05$) and BC and AP ($p<0.05$).

The silage intake had no significant differences between treatments. The intakes of treatments AC, AP and BC were 15.4 kgDM, 15.6 and 14.3 respectively.

Table 8. Means, standard deviation, minimums and maximums values for feed consumption data

Tr.		DMI kg/d	Silage kgDM/d	Cereal kgDM/d	Prot.Sup. kgDM/d	sil.of DMI	ME MJ/d	CP kg/d	NDF kg/d	Starch kg/d
AC	Mean	20.4	15.6	4.8	0	0.6	267.9	3.1	7.4	2.5
	Stand.Dev.	2.9	2.7	1.3	0	0.07	38.5	0.4	1.1	0.7
	Min.	16.8	10.3	3.4	0	0.61	233.9	2.6	5.4	1.7
	Max.	29.7	23.6	6.6	0	0.83	379.8	4.3	10.7	3.4
AP	Mean	20.0	15.6	1.7	2.7	0.78	270	4.9	7.3	1.2
	Stand.Dev.	3.1	2.9	1.8	1.0	0.09	45.3	2.5	1.1	0.9
	Min.	15.1	8.6	0.3	1.6	0.50	196.9	2.8	5.0	0.5
	Max.	26.5	22.2	6.0	4.1	0.85	347.4	10.9	10.1	3.3
BC	Mean	18.6	14.2	4.4	0	0.76	244.4	2.5	7.6	2.2
	Stand.Dev.	3.3	3.2	1.2	0	0.08	41.1	0.4	1.5	0.6
	Min.	9.4	4.9	3.2	0	0.53	138.3	1.2	3.1	1.7
	Max.	23.5	17.8	6.6	0	0.84	316.1	3.1	9.3	3.3

Milk yield

The milk production data from the measurement periods showed extremely high fat contents in milk, much higher than values obtained during the pre-period and much higher than the values generally obtained for the breed. Therefore, it was concluded that the difference between milking systems or sampling had given some type of sampling error. Therefore, milk production data obtained during the second part of the pre-period were used for calculations of milk production data and methane per kg milk. Milk production per cow in treatment AC was 27.8 kg/day, in treatment AP 29.4 kg/day and 27.5 kg/day in treatment BC, the differences between treatments were not significant.

The energy corrected milk (ECM) production was 28.6 kg/day for treatment AC, 30.9 kg/day for treatment AP and 30.1 kg/day for treatment BC which means that there were no differences between treatments.

The milk fat content did not differ significantly between treatments and was 4.2%, 4,3% and 4.7% for the treatments AC, AP and BC respectively. No differences were found in protein and lactose in the milk, the amount of protein was 3.5% for the three treatments and the amount of lactose was 4.8 for all of them.

Concerning the methane production per kg milk, the differences between treatments were not significant for overall treatments and the values were 18g/kg milk, 15.4 and 18.2 for treatments AC, AP and BC respectively.

The production of methane per kg ECM was 17.5 g/kg ECM on treatment AC, 14.6 on AP and 16.9 on BC. These results were no significantly different.

Table 9 summarize the milk data presented above together with means and variance. Figure 10 shows the difference between treatments in methane emission per kg milk.

Table 9. Milk yield (kg/day.cow), ECM (kg/day.cow) and methane production (g/kg milk) and (g/kg ECM) for the three different treatments, least squares means ± standard dev.

	Treatments		
	AC	AP	BC
Milk (kg/d.cow)	28.6 ± 1.6	29.0 ± 5.7	28.2 ± 7.1
ECM (kg/d.cow)	29.4 ± 2.6	30.5 ± 6.4	31.3 ± 10.2
gCH_4 /kg milk	17.6 ± 9.7	15.4 ± 4.1	18.1 ± 4.7
gCH_4/kgECM	16.9 ± 8.3	14.7 ± 3.8	16.8 ± 4.6

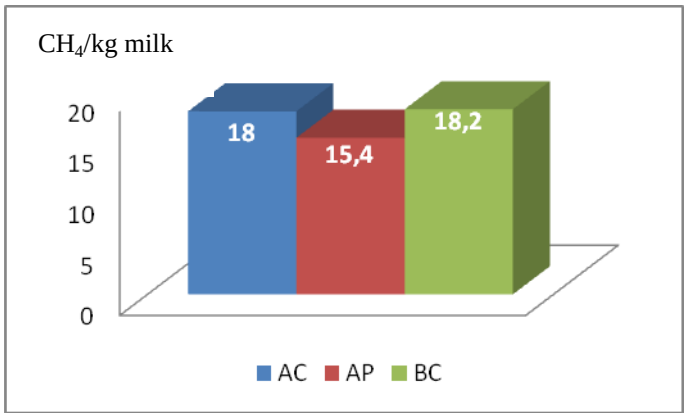

Figure 10. Methane production per kg milk per treatments AP (silage A + cereal and protein supplement); AC (silage A + cereal) and BC (silage B + cereal), least squares means.

Weight

There were no significant differences in the weight of the cows between the beginning and the end of the experiment.

Feed intake and feeding level

Table 10 shows the percentage of the needs (% of MJ and % of AAT) covered by the diet according to their production level. The values are based on intake and milk yield data from each cow on each treatment and then an average over treatment has been calculated. The table shows that without protein supplement, it was impossible to reach the protein needs for the animals. However the energy needs were completely covered, thanks to the cereals supply.

Table10. Needs covered by the treatments (%). CP, forage, NDF and starch content (%). ECM and silage intake per cow (kg/d).

		Treatment AC	Treatment AP	Treatment BC
% of MJ covered	Mean	106	108	103
	Max.	137	116	139
	Min.	83	97	78
% of AAT covered	Mean	89	100	88
	Max.	115	111	119
	Min.	70	92	66
% of CP in diet	Mean	16	19	13
	Max.	16	20	13
	Min.	16	18	13
% of forage in diet	Mean	76	78	77
	Max.	83	85	84
	Min.	66	57	68
% of NDF	Mean	34	34	40
	Max.	36	36	43
	Min.	32	29	37
% of starch	Mean	12	6	11
	Max.	17	16	16
	Min.	8	2	8
ECM (kg/d)	Mean	28.3	30.5	29.7
	Max.	33.6	43.3	47.9
	Min.	24.6	22.5	18.9
Silage intake(kg/d)	Mean	14.7	15.5	14.3
	Max.	19.2	18.9	17.6
	Min.	10.4	11.5	9.2

Discussion

Methane production

In the literature there is no evidence that the protein content in the ration offered to the cows can affect or modify the rate of VFA and therefore the amount of methane produced. For this reason, the hypothesis in this experiment was that there will be no significant differences between the three treatments studied.

The three treatments gave a similar methane production, in average 473 g/d. This amount of methane was higher than others studies where a similar proportion of silage was given (Grainger et al., 2007) were the average of methane production was around 340 g/d. On the other hand, it is also interesting to compare the obtained results with some obtained in the same experimental barn (for the similarity of the experimental conditions with management, etc.). Danielsson, R., (2009) found that the average methane production of diets with 70% forage was 300.5 g/d and 317 g/d with 90% forage. In this case, the average % silage was around 77% for the three treatments, so it seems that the methane produced by the cows in the experiment presented here was higher than the values obtained in the earlier experiment performed under similar conditions. However, these differences can be explained by the higher average level of milk yield and feed intake that had the cow of the present compared with the earlier experiment (28.6 kg milk/d vs. 20.1 kg milk/d and 19.7 kg DMI/d vs. 15.5 kg DMI/d, respectively). If the methane per kg milk of the two experiments is compared, the average is 16.5 g/kg milk vs. 15.1 so, the values seem to be comparable with previous values.

Nevertheless, the influence of the protein level in the diet in methane production has, to our knowledge, not been extensively studied yet, and for that reason it is difficult to compare the obtained results with previous findings where production from cows on similar diets (with different protein levels) have been studied.

Feed parameters

The feed composition can largely affect the ruminal methane production, but in this case, as said in the results, only significant differences were found in methane per CP intake, per ME and per starch. Concerning the methane production per kg protein intake the differences could perhaps be explained by the fact that the cows on the AC and BC treatments were underfed with regard to protein. That means that the emissions of methane were higher when less protein content was offered to the cows. The differences found in methane per MJ intake were due to the higher ME content on the AP diet.

Another significant difference was the methane per kg of starch intake. Treatments AC and BC were fed only cereals and therefore much more starch compared to treatment AP where a considerable amount of the concentrate ration consisted of the protein supplement. So, it is normal that higher levels of starch were found in the diets AC and BC and therefore less methane production per kg of starch intake.

Another point is that there was no differences between the treatments AC and BC, which only differs in the quality of the silage (harvest date and protein content). Some authors like DeBlas et al. (2008) explained that young and high quality forage give rise to a more intensive fermentation process and therefore a higher amount of methane than more mature forages. On the other hand, Blaxter et al., (1965) described in their study that the apparent digestibility of the forage is positively related with the amount of methane produced. Nevertheless, in the present experiment no differences were found between the two silage qualities.

Even if significant differences were found only in some cases, the results showed always the same ranking order between treatments: with the lowest methane production during treatment AP, followed by AC and the highest when cows were on treatment BC. Thus, it is logical to think that the energy utilisation was more efficient on the AP diet when the cows were fed a balanced diet where both energy and protein requirements were met (table 10). Underfeeding with protein (AC and BC) may have led to a situation where the higher level of energy compared with the level of protein could have led to a higher energy loss giving higher methane production.

Milk yield

A higher production in treatment AP could be expected because this ration covered requirements for both energy and protein while animals on the AC and BC diets were fed below AAT requirements when estimated according to the Swedish feed tables (Spörndly, 2003) as shown in table 10 above. However, the results were not different between treatment either in milk yield or in ECM. Besides, the composition did not differ between treatments either. Due to earlier mentioned problems with the milk yield data from third week of each treatment the milk yield during the measurement period could not be used. Instead data from the second adaptation week was used in the calculations. It is probable that the time period between the start of feeding a diet and the milk yield measurement occasion (approximately 10 days) was too short for the differences in production to be manifested. During the second week of feeding, the diets may not have given any clear milk yield responses.

Methane production per kg milk and per kg ECM was similar for all the treatments, because the milk yield did not vary. If one of the treatment would have lead to higher milk production, a decrease of methane per kg milk would be expected because the methane production that originates from maintenance needs is the same even when the milk yield is higher because the energy needed for maintenance does not vary. Therefore, with increased milk yield the methane produced in absolute terms will increase somewhat but the methane per kg milk will decrease.

SF_6 method

As mentioned before, the SF_6 tracer technique does not measure the methane formed in the colon, and for that reason the measurements are 4-7% lower than measurements performed using the chamber technique (Ginn et al., 2006). However, it has been shown in many trials mentioned in the literature that the SF_6 tracer technique is reliable for methane measurements giving 93-95% of the values obtained in chamber measurements. The method is also suitable for comparing the methane production between treatments.

During the experimental time, some cows contaminated the tubes of the halter with water. This was probably due to their behaviour when they drink, or because of the quantity of saliva was higher than in other cows. These problems were solved by extending measurements during one or two days into the adaptation period that was shortened slightly for these cows. However, the adaptation period was never less than ten days for any cow. The blockage problems seem less frequent or even nonexistent when the cows are on pasture but this option was no possible in our case (Danielsson R., personal communication, 2010).

Another problem in indoor trials is the difficulty to know if the backgrounds are reliable because more factors can affect the results obtained. This problem does not appear in outdoor trials because methane measurements for a specific cow is not affected by methane in the ambiance released by the others cows, so backgrounds measurements are not needed.

Environmental impact

Methane production from cattle, and from ruminants in general, have to be studied in a general way. Therefore, it does not matter if an individual cow produces less methane than another one if the first one produces much less milk than the second one. The methane emissions must be related to the level of production. The same can be applied for meat production. The aim is to quantify the environmental effect (residuals products that can be emitted to water, soils, atmosphere, etc.) that one kg of milk has throughout the life cycle.

Conclusion

The hypothesis made at the beginning of the experiment seems to be in accordance with the obtained results because no significant differences were found either in absolute methane production or methane per kg DMI when diets with or without protein supplement in the concentrate ration were fed. However, the results may indicate that unbalanced diets where AAT needs are not covered may increases the methane production compared with balanced diets. As experiments studiyng the protein influence of dietary protein level on methane production are very few, it would be interesting to investigate this question further. In general

terms, more research is needed to finally find methods that decrease the energy loss and therefore the methane production in dairy cows and that are in accordance with each management system (organic, extensive, etc.). It seems that the most effective and efficient method is to increase the milk production of each cow to decrease the methane per kg of milk produced.

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References

Barnett, A. J. G. and R. L. Reid.1961. Reactions in the Rumen. 1st edition.Edward Arnold LTD. Chapter 3.

Bertilsson, J. and M. Murphy.2003. Effects of feeding clover silages on feed intake, milk production and digestion in dairy cows. Grass and Forage Science, 58, 309-322.

Bhatta, H., K. Tajima, N. Takusari, K. Higuchi, O. Enishi, M. Kurihara. 2006. Comparison of sulphur hexafluoride tracer technique, rumen simulation technique and in vitro gas production techniques for methane production from ruminant feeds. International Congress Series 1293: 58-61.

Blaxter, K. L. And J. L. Clapperton. 1965. Prediction of the amount of methane produced by ruminants. British Journal of Nutrition, 19:511-522.

Czerkawski, J. W.1986. An introduction of rumen studies. Pergamon Press, New York.

Danielsson, R. 2009. Metanproduktion hos mjölkkor utfodrade med hög andel grovfoder. Swedish University of Agricultural Sciences. Department of Animal Nutrition and Management. Examensarbete 282.

Danielsson, R. 2010. Personal communication.

De Blas, C., P. García-Rebollar, M. Cambra-López and A.G. Torres. 2008. Contribución de los rumiantes a las emisiones de gases con efecto invernadero. XXIV Curso de Especialización FEDNA.

Dijkstra, J., J. M. Forbes and J. France. 2005. Quantitative Aspects of Ruminants Digestion and Metabolism. 2nd edition. CABI Publishing. Pg 157-176.

Grainger, C., T. Clarke, S. M. McGinn, M. J. Auldist, K. A. Beauchemin, M. C. Hannah, G. C. Waghorn, H. Clark, and R. J. Eckard. 2007. Methane emissions from dairy cows measured using the sulfur hexafluoride (SF_6) tracer and chamber techniques. J. Dairy Sci. 90:2755-2766.

Harper, L.A., O. T. Denmead, J. R. Freney, and F. M. Byers. 1999. Direct measurements of methane emissions from grazing and feedlot cattle. J. Anim. Sci. 77:1392-1401.

Hungate, R.E. The rumen and its microbes. 1966. Academic Press, New York.

Johnson, K. A., M.T. Huyler, H.H. Westberg, B.K. Lamb and P. Zimmerman.1994. Measurement of methane emissions from ruminant livestock using a SF_6 tracer technique. Environ. Science and technology. 28:239.

Johnson, K. A. and D. E. Johnson. 1995. Methane emissions from cattle. J. Anim. Sci. 73:2483-2492.

Johnson, K. A., H. H. Westberg, J. J. Michal and M. W. Cossalman. 2007. The SF_6 tracer technique: Methane measurement from ruminants. In measuring methane production from ruminants. Ed. H. P. S. Makkar and P. E. Vercoe. Chap.3 pg 33-67. Austria, Vienna. IAEA.

Jordan, E., D. K. Lovett, F. J. Monahan, J. Callan, B. Flynn and F. P. O'Mara. 2006. Effect of refined coconut oil or copra meal on methane output and on intake and performance of beef heifers. J. Anim. Sci. 84: 162-170.

KRAV.2009. Standards for KRAV-certified production January 2009. http://www.krav.se/Documents/Regler/englisghEditions/Standards_for_krav-certified_production_january_2009.pdf

Lassey, K. R., M. J. Ulyatt, R. J. Martin, C. F. Walker and I. D. Shelton. 1997. Methane emissions measured directly from grazing livestock in New Zealand. Atmospheric Environment Vol. 31, No. 18, pp. 2905-2914.

Leng, R.A. 1993. Quantitative ruminant nutrition – A green house science. Australian Journal of Agricultural Research 44:363-80.

Machmüller, A. And H. Clark. 2006. First results of a meta-analysis of the methane emission data of New Zealand ruminants. International Congress Series 1293 (2006) 54-57.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan. 2002. Animal nutrition. 4th edition. Longman Group Ltd. Essex, England. Pg, 149, 225.

McDonald, P., R.A. Edwards, J.F.D. Greenhalgh and C.A. Morgan. 2002. Animal nutrition. 6th edition. Pearson Education Limited. Harlow. Essex, UK. Pg, 266-277.

McGinn, S. M., K. A. Beauchemin, A. D. Iwaasa and T. A. McAllister. 2006. Assessnebt of the sulphur hexafluoride (SF6) tracer technique for measuring enteric methane emissions from cattle. J. Environ. Qual. 35:1686-1691.

McLean, J. A. and G. Tobin. 1987. Animal and Human calorimetry. Cambridge University Press. New York. pp, 50-51, 93-96.

Moss, A. R. 1994. Methane production by ruminants- Literature review, Nutr. Abstr. Rev. Ser. B64: 786-806.

Moss, A. R., D. I. Givens, P. C. Garnsworthy. 1995. The effect of supplementing grass silage with barley on digestibility, in sacco degradability, rumen fermentation and methane production in sheep at two levels of intake. Anim. Feed. Sci. Technol. 55: 9-33.

Moss, A. R., J. Jouany and J. Newbold. 2000. Methane production by ruminants: Its contribution to global warming. Ann. Zootech. 49:231 – 253.

Moss, A. R. and D. I. Givens.2002. The effect of supplementing grass silage with soya bean meal on digestibility, in sacco degradability, rumen fermentation and methane production in sheep. Animal Feed Science and Technology, 97 (2002) 127-143.

Norfor. 2007. Norfor in sacco standard. September 10, 2007. Downloaded 15 december 2009 from http://www.Norfor.Info/files/pdf-dokumenter/pdf_lab/analyses/norfor_in_sacco_standard_070910.pdf.

NRC 2001. Nutrient requirement of dairy cattle. National Academy Press. Washigton, D.C. pg 381.

Okine, E. K., G. W. Mathison, R. T. Hardin. 1989. Effects of changes in frequency of reticular xontractions on fluid and particulate passage rates in cattle. J. Anim. Sci. 67:3388-3396.

Palmquist, D. L. 1996. Utilización de lípidos en dietas de rumiantes. XII Curso de especialización FEDNA.

Pinares-Patiño, C. S. and H. Clark. 2008. Reliability of the sulfur hexafluoride tracer technique for methane emission measurement from individual animals: an overview. Australian Journal of Expermental Agriculture, 48: 223-229.

Rohner-Thielen, E. 2010. Area under organic farming increased by 7.4% between 2007 and 2008 in the EU-27. Eusrostat. http://epp.eurostat.ec.europa.eu/cache/ITY_OFFPUB/KS-SF-10-010-EN.PDF

Swedish Dairy Association (Svensk Mjölk). 2010. Milk – key figures milk in Sweden. http://www.svenskmjolk.se/In-English/Statistics/.

Van Keulen J., and B. A. Young.1977. Evaluation of acid insoluble ash as a natural marker in ruminant digestibility tudies. Journal of Animal Science, 44, 282-287.

Watson, R. T., L. G. Meira Filho, E. Sanhueza, T. Janetos. 1992. Climate change, Cambridge University Press, Cambridge, Pg 25-46.

Wolin, M.J. 1960. A theoretical rumen fermentation balance. J. Dairy Sci. 43: 1452.

Woodward, S.L., G. C. Waghorn and P. G. Laboyrie. 2004. Condensed tannins in birdsfoot trefoil (Lotus corniculatus) reduce methane emissions from dairy cows. Proceeding of the New Zealand Society of Animal Production. 64.

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