Methane Production from Dairy Cows

Relations between Enteric Production and Production from Faeces and Urine

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av

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Swedish University of Agricultural Sciences
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Examensarbete 335
30 hp E-nivå

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Supervisors: Jan Bertilsson and Lena Rodhe
Examiner: Peter Udén

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Nr	Titel och författare	År
327	Effects of genotype, age and feed on the fat components of egg yolk 30 hp E-nivå Anna Johansson	2010
328	Värmebehandlat mjölfoder till avelsdjuren för slaktkyckling-produktion Heat treated mash feed for broiler breeders 30 hp E-nivå Malin Karlsson	2010
329	Reduktion av vikt och hull hos överviktiga ponnyer i träning Reduction of weight and body condition in overweight ponies in training 30 hp E-nivå Märta Westlin	2010
330	Effects of different inclusions of oat hulls on performance, carcass yield and gut development in broiler chickens 45 hp E-nivå Ata Mossami	2011
331	Effects of supplemental glutamine and glutamate on growth performance, gastrointestinal development, jejunum morphology and *Clostridium perfringens* count in caecum of broilers 45 hp E-nivå Gholamreza Ebadiasl	2011
332	Automatic registration of dairy cows grazing behaviour on pasture Automatisk registrering av mjölkkors betningsbeteende 30 hp E-nivå Kristina Blomberg	2011
333	Svenska mjölkkor på bete – Värmens påverkan på beteende och produktion hos mjölkkor i en besättning med AMS Swedish dairy cows at pasture – The effect of temperatur and THI on behaviour and production of dairy cows in a Swedish AMS herd 30 hp E-nivå Hanna Alfredius	2011
334	Motivation for eating roughage in sows – as an indication of hunger Suggors motivation att äta ensilage som en indikator på hunger 30 hp E-nivå Tove Bergström	2011

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Table of contents

Figures and tables

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Preface

This study was funded by a project called *Klimatskolan*, a collaboration between the Swedish University of Agriculture Sciences (SLU) and The Federation of Swedish Farmers (LRF) on climate issues. It was conducted as a Master‘s thesis in Animal Science at the Department of Animal Nutrition and Management, SLU from November of 2009 to March of 2010 at Kungsängen Research Centre in Uppsala. The study was part of a larger project lead by Tomas Rondahl where concentrate saving effects of pea/oat silage were investigated.

I want to thank Jan Bertilsson at SLU and Lena Rodhe at Swedish Institute of Agricultural and Environmental Engineering (JTI) for excellent supervision. I would also like to show my gratitude to Rebecca Danielsson (SLU) who helped me with the methane sampling and Johnny Ascue Contreras and Maria del Pilar Castillo (both JTI) for help with the laboratorial analyses at *Genetikcentrum* (GC) as well as to Tomas Rondahl (SLU) for letting me be a part of his project. I would also like to thank Jan Eksvärd at LRF and Ulla Didon at SLU for the great seminar series during the project and for giving me the opportunity to be a part of *Klimatskolan*. I would also like to warmly thank the staff at the laboratory as well as the staff in the cow barn at Kungsängen Research Centre.

Abstract

Methane (CH_4) is a greenhouse gas (GHG) that contributes to the global warming. One of the largest sources of methane is livestock, preferably ruminants which alone counted for 30% of the total agricultural anthropogenic methane emissions in the year of 2000. The reason to why ruminants are such large contributors of methane are that the gas is produced in the rumen by enteric formation and leaves the animals by belching, exhaling or by the excreta.

Diets high in concentrates can result in a lower emission of methane. Also diets with a high content of starch, such as alfalfa-grass, have a methane-decreasing. It is profitable to reduce enteric methane formation since that form of methane is unavoidably lost. Methane emissions from manure, on the other hand, are possible to reduce during storage and manure-derived methane can also be collected and used as biogas fuel. It is earlier shown that it is possible by dietary means to compensate a reduced enteric methane production by a higher or maintained production of methane from manure.

The hypotheses of this study is 1) that the feed regime with high percentage of pea/oat silage results in a lower emission of enteric methane compared to grass silage and 2) that this will result in maintained or higher methane production from the manure.

In this study four rumen fistulated cows of the Swedish Red Breed was included. The cows where held in a separate part of a barn so that the sampling would not be disturbed by the other animals. The study was divided into four periods of one week each and two different treatments were tested; treatment A: 100% grass silage and treatment B: 25% grass silage and 75% pea/oat silage. All individuals were in each treatment twice during the study. Enteric methane was sampled once a day for five days in a row for each period. The gas samples were analyzed for produced methane and sulphur hexafluoride (SF_6) and the amount of methane produced per day was then calculated by the methane:S F_6-ratio. Faeces and urine was sampled during the first period and then analyzed for maximum methane producing capacity (B_o). Analyses were also conducted on dry matter intake, feed nutrient composition, the composition of faeces and urine and on produced milk.

There was, as expected, a significantly higher starch intake in treatment B (25% grass silage, 75% pea/oat silage) than in treatment A (100% grass silage) ($p=0.006$). However, the diet with pea/oat-silage (treatment B) resulted in more methane per kg of ingested starch compared to the diet with grass silage (treatment A), opposite to what was expected. It could neither be shown that a reduction in enteric methane was followed by a higher or maintained production in methane from manure (faeces and urine). Contrary the difference in methane production was similar for enteric production and from manure. Further studies need to be conducted where a larger number of animals are included and the difference in starch content between diets is larger in order to be able to receive significant results.

Sammanfattning

Metan (CH_4) är en växthusgas som bidrar till den globala uppvärmningen. En av de största källorna till metan är boskap, företrädesvis idisslare som stod för 30 % av jordbrukets totala antropogena metanutsläpp år 2000. Anledningen till att idisslare bidrar till så store utsläpp av metan är att gasen produceras i djurens våm genom enterisk bildning och avgår sedan från djuren genom rapningar, utandning eller genom avföringen.

Foderstater med hög koncentratandel kan leda till ett lägre utsläpp av metan. Även foderstater med ett högt innehåll av stärkelse, som alfalfa-gräs, har en metanminskande effekt. Det är lönsamt att minska enterisk metanbildning eftersom denna form av metan oundvikligen går förlorad. Metanemissioner från stallgödsel, å andra sidan, går att minska under lagring och metan från gödsel kan även användas som biogas. Det är tidigare visat att det genom modifieringar i foderstaten går att kompensera en minskad enterisk metanproduktion med en högre eller bibehållen produktion av metan från gödseln.

Studiens hypoteser är 1) att foderstaten med hög procentandel av ärt/havreensilage resulterar i ett lägre utsläpp av enterisk metan jämfört med gräsensliage och 2) att detta resulterar i en högre eller bibehållen produktion av metan från gödseln.

I denna studie ingick fyra våmfistulerade kor i av Svensk rödbrokig boskap (SRB). Korna inhystes i en separat del av ladugården så att provtagningen inte skulle störas av andra djur. Studien var uppdelad i fyra perioder om vardera en vecka och två olika behandlingar testades; behandling A: 100 % gräsensilage och behandling B: 25 % gräsensilage och 75 % ärt/havreensilage. Alla individer fick varje behandling två gånger under studien. Prover på den enteriska metanproduktionen togs en gång per dag fem dagar i rad varje period. Gasproverna analyserades med avseende på producerad metan och svavelhexafluorid (SF_6) och mängden producerad metan beräknades sedan genom att använda förhållandet mellan metan och SF_6. Avförings- och urinprover togs under den första perioden och sedan analyserades dessa för dess maximala metanproducerande kapacitet (B_o). Analyser genomfördes också på torrsubstansintaget, fodersammansättningen, sammansättningen i avföring och urin och på producerad mängd mjölk.

Det var som väntat ett signifikant högre stärkelseintag i behandling B (25 % gräsensilage, 75 % ärt/havreensilage) än i behandling A (100 % gräsensilage) ($p = 0,006$). Foderstaten med ärt/havreensilage (behandling B) resulterade dock i mer metan per kg intagen stärkelse jämfört med foderstaten med gräsensilage (behandling A), tvärtemot vad som förväntats. Det kunde inte heller visas att en minskning av enteriskt metan följdes av en högre eller bibehållen produktion av metan från gödsel (träck och urin). Istället så var skillnaden i metanproduktionen liknande för enterisk produktion och från gödsel. Ytterligare studier där fler djur ingår och där skillnaden i stärkelseinnehåll är större behöver göras för att kunna få signifikanta resultat.

Objectives

Agriculture accounts for about 50% of the global anthropogenic production of methane IPCC Fourth Assessment Report, 2007a), and ruminants are the main contributors (US-EPA, 2006). It is therefore of interest to investigate possible mitigation options for the agricultural sector. The aim of this study is to investigate the amount of methane produced from dairy cows depending on the proportion of pea/oat silage in the diet. Also, the feed influence on the manure properties concerning methane producing potential will be studied. This master thesis is part of a larger project which aim is to provide material for an increased utilization of legumes as forage.

Introduction

Methane (CH_4) is a potent greenhouse gas (GHG) that contributes to the global warming. The main sources of methane from the agricultural sector are rice fields, the burning of biomass, handling of manure and enteric fermentation (IPCC Fourth Assessment Report, 2007a). The production of methane is conducted in an anaerobic environment by means of microorganisms by biological processes such as fermentation (Bertilsson & Börjesson, 2008; US-EPA, 2006).

Approximately 40-50% of the earth's land surface is classified as agricultural land (IPCC Fourth Assessment Report, 2007a). Of the anthropogenic emissions of GHGs, agriculture was in the year of 2005 accounted for 10-12%. To be able to more easily compare the GHGs with each other, the emissions are recalculated as carbon dioxide equivalents (CO_2-eq) where 1 kilogram of methane corresponds to 25 kilograms of carbon dioxide (IPCC Fourth Assessment Report, 2007b). The GHG emissions from the agricultural sector was in the year of 2005 5.1 to 6.1 GtCO_2-eq/yr (IPCC Fourth Assessment Report, 2007a). Of the global anthropogenic emissions of GHGs in 2005, agriculture was accounted for 50% of the methane. This is an increase by 17% from 1990, which gives an average annual increase of about 60 MtCO_2-eq/yr.

At the same time, the agricultural sectors' GHG emissions from the countries in EU decreased from the year of 1995 to the year of 2000 by 20% (European Commission, 2008). This was mainly a consequence of new techniques used in the sector, but also to a large extent due to a reduction in animal numbers. The reduction of GHGs is, in the EU member states, larger in the agricultural sector than in any other sector. Despite this, the agricultural sector is still responsible for the largest part of the anthropogenic emissions of methane and nitrous oxide (N_2O).

Livestock, preferably ruminants, are an important source of methane and accounted for about 30% of the gas' global agricultural anthropogenic emissions in the year of 2000 (US-EPA, 2006). Methane is mainly produced in the rumen by enteric fermentation and it leaves the cows by belching, exhaling or by the excreta. The total anthropogenic emissions in Sweden were in the year of 2008 64 million tCO_2-eq/yr whereof the agricultural sector accounted for around 13% (Naturvårdsverket, 2009). Approximately 1/3 of the GHG emissions from the agriculture are in form of methane and livestock accounts for almost all of these emissions (Bertilsson & Börjesson, 2008; US-EPA, 2006).

Besides being a very potent GHG, production of methane also constitutes a significant loss of energy for the animal (Immig, 1996). Of the gross energy in the diet, about 6-10% is lost in form of methane (Immig, 1996; Johnson & Johnson, 1995). The turn-over of energy in animals is illustrated in figure 1.

Beside the livestock's methane-production from the fermentation of feed, the gas is also produced during storage of manures under anaerobic conditions. In the year of 2004, the global methane emission from manure management was 17.52 million tons of which cattle (dairy and beef) accounted for 7.49 million tons (FAO, 2008). The magnitude of these emissions can be reduced by a range of measures (Clemens and Ahlgrimm, 2001). As an example, it might be possible to reduce emissions from manure by changes in feeding practices (Clemens and Ahlgrimm, 2001; Külling *et al.*, 2003; Hindrichsen *et al.*, 2006; Kreuzer and Hindrichsen, 2006). It is also possible to inversely maximize the production of

methane by anaerobe digestion and the methane can then be used as an energy source (Clemens *et al.*, 2006).

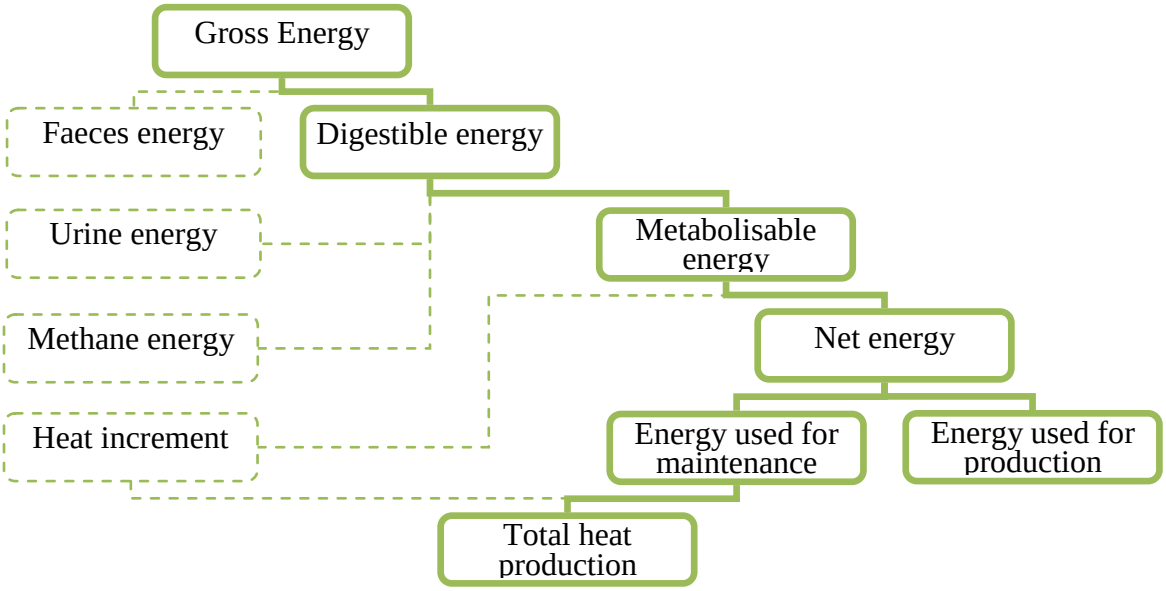

Figure 1. The turn-over of energy in animals. Boxes with dotted outlines represent energy losses. (Modified from McDonald *et al.*, 2002).

For most of the strategies used to minimize the emission of methane, it is still unclear whether the measurements taken also will decrease the manure-derived methane, or if the emission from the manure will be the same or even higher (Kreuzer and Hindrichsen, 2006).

The global consumption of meat is estimated to increase by 85% from 2000 to 2030 to meet the projected demands (The World Bank, 2007). Also the consumption of milk is expected to increase (European Commission, 2008). This is mainly due to the fact that the higher standards of living in the developing countries imply a larger demand for meat and milk, but also partly dependent on the food preferences in the industrial countries. The increase of meat consumption is also a consequence of the worlds' population increase on the whole (US-EPA, 2006).

The production of milk emits 1 kilo of CO_2-eq per kilo produced milk, according to Angervall *et al.* (2008). The production of 1 kilo of cheese emits 10.7 kg CO_2-eq to be compared with the beef production which emits 17 kilo CO_2-eq per kilo product. The largest climate impact in the production of milk occurs at farm level; up to 95% of the total emissions of GHGs take place in the primary production.

Simultaneously with the impact on the climate from livestock's emissions of GHG, the agricultural sector is affected by the climate changes, mostly negatively (COPA-COGECA, 2008). Some areas will however get longer growing seasons and some areas will get more precipitation as a consequence of the changing climate, which contributes to more favourable cultivation conditions (European Commission, 2008).

Literature review

Formation of methane in ruminants

The structure of the digestive system of ruminants enables the conversion of fibrous biomass to energy, allowing the animals to subsist on straw and other products that man cannot use as food (Immig, 1996). In that sense, ruminants are important to humans since they do not eat the same food as man does and therefore there is no need for competition (Moss *et al.*, 2000). However, consumption of fibrous feed also results in the production of methane. When carbohydrates are broken down by micro-organisms into molecules small enough to be absorbed into the bloodstream through the digestive process of enteric fermentation, it brings about the formation of methane as a by-product (IPCC, 2006). In ruminants, formation of methane is the result of fiber fermentation in the rumen (Immig, 1996). Methane is also produced in the hindgut of ruminants and monogastric animals, but in much smaller amounts. Methane emission means, except from being a climate issue, also a loss of energy for the animal corresponding to 6-10% of the gross energy intake (Immig, 1996; Johnson & Johnson, 1995).

Glucose in plant polymers and starch are fermented to puryvate and lactate in an oxidative process under anaerobic conditions (Moss *et al.*, 2000). This gives NADH that, to complete the fermentation of sugars, then are re-oxidized to NAD (figure 2). By transfer of electrons to acceptors other than oxygen, NAD^+ is regenerated. When propionate is formed from succinate the result is the formation of carbon dioxide. This enables carbon dioxide to react with the free H_2 from the re-oxidation of NADH, which results in the formation of methane and water according to following formula: $CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$.

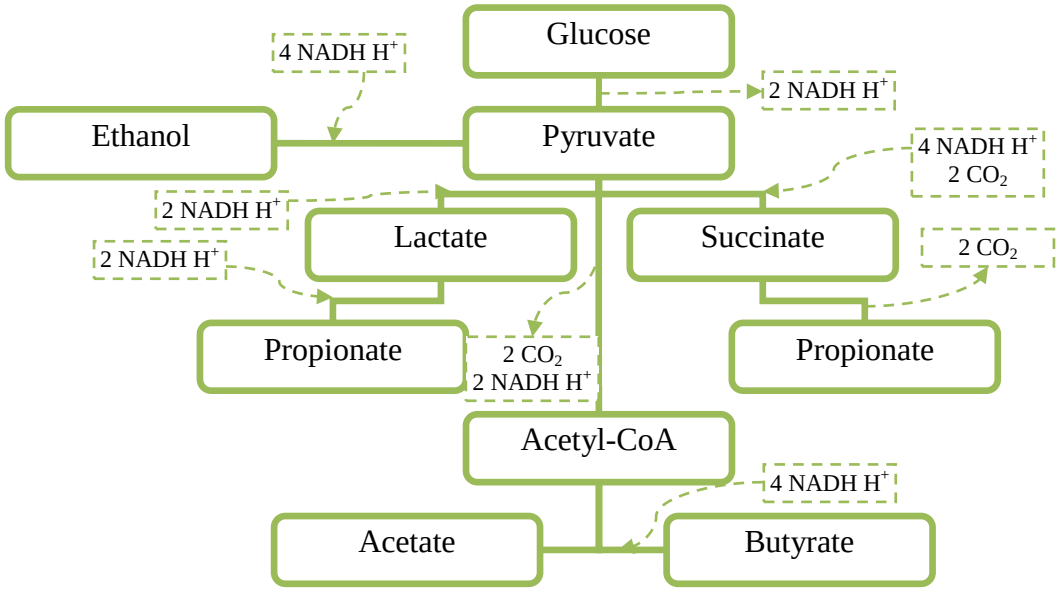

Figure 2. The metabolism of NADH $H^{+\cdot}$ (Modified from Moss *et al.*, 2000).

Manure-derived methane

Manures characteristics

The term ‘manure’ is here jointly describing both the faeces and the urine produced by the animals without additions of bedding material or water. The main basic characteristics of manures which are of importance for the potential of methane production are the content of

volatile solids (VS), which indicates the content of incinerable substances at 550°C, or the amount of carbon. The VS in manure can either be calculated from the dry matter intake (DMI) and digestibility of the feed or by analyzing the manure in laboratory (IPPC, 2006). However, a large part of the VS is not easily degraded by the microbes under anaerobic conditions and does therefore not contribute to the methane production (Sommer *et al.*, 2009). The easily degradable VS have a direct influence on methane production from stored livestock slurry. A reduction in easily degradable VS would therefor reduce methane emissions from stored livestock slurry.

There are different ways of describing the amount of methane produced. The theoretical value describes the methane yield if all carbon is converted to methane whilst the maximum or ultimate methane producing capacity per g VS of the manures (B_o) (IPCC, 2006) is the amount of methane that is formed in a laboratory environment.. The actual methane produced under storage of liquid manure (slurry) on farms depends on the authentic environment and is much lower than both the theoretical and B_o-values. Under Swedish conditions, about 3% of the B_o is produced during storage of cattle slurry (Rodhe *et al*., 2009).

Storage systems for manure

A few different systems of storing manure exist. The most common ways of storing in Sweden is in one fraction which contains urine and faeces mixed with some bedding material and some water during management to give liquid manure or slurry (Ramiran, 2003). It could also be stored in two separated fractions, where the solid manure includes mainly faeces and bedding and the liquid includes urine diluted with water and also some solids, respectively (Külling *et al.*, 2001 and Külling *et al.*, 2003)?. The proportion of dairy farms in Sweden handling their manure as slurry has increased in recent years; in 2003 there were slurry systems on more than 60 percent of the dairy farms (SCB, 2003).

Storage system effects on methane emission

Fresh manure (i.e. manures that is just excreted) does not emit noticeable amounts of methane (Sun *et al.*, 2008). When manure is applied on the field little, if any, methane is produced since the environment is aerobic (Sommer *et al.*, 2009). In Sweden it is required that manure is removed daily from the animal houses with slatted floors, therefore the methane from manure in Sweden mainly originates from storage (Rodhe *et al.*, 2009). There are differences in greenhouse gas potential for manure in terms of the type of storing used. Two studies, differing in the animals' feeding regimes, comparing different manure storing concluded that, independent of feed, the greenhouse gas potential as expressed in CO_2-eq are higher for solid manure systems with urine separately than for slurry systems (Külling *et al.*, 2001; Külling *et al.*, 2003).

The emission of methane is related to storage time and storage temperature (Sommer *et al.*, 2009). The procedure used in Sweden to remove the slurry from the livestock house daily, together with the low storing temperature may imply a 75% reduce of methane emissions compared to scenarios where the slurry is only emptied from the livestock house every fourth month and stored in a higher temperature, as is common in southern parts of Europe. In summary, less storing during the summer and shorter retention times in the livestock houses are two ways of lowering the emission of methane from livestock slurry (Sommer *et al.*, 2009).

Dietary effect on methane from stored slurry

In a study where dairy cows were fed either 175, 150 or 125 g CP/ kg DM, methane emissions from urine-rich slurry were not reduced or rather increased when CP content was reduced (Külling *et al.*, 2001). Grass as a base in the ratio instead of hay decreases the methane emissions from manure during storage, most evidently for slurry and liquid manure storing systems (Külling *et al.*, 2003).

Both the theoretical methane production and the maximal methane yield (B_o) are greater for pig manure than for cattle manure (Møller *et al.*, 2004). According to a study by Møller *et al.* (2004) the theoretical methane production for pig manure is 516 l kg^{-1} VS, for sow manure 530 l kg^{-1} VS and for cattle manure 468 l kg^{-1} VS. The maximal methane yield for pig manure was in the same study 354 l kg^{-1} VS, for sow manure 275 l kg^{-1} VS and for cattle manure 148 l kg^{-1} VS. This is slightly lower than IPCC's estimated B_o-value of manure from dairy cattle in developed countries which is set to 240 l kg^{-1} VS (IPCC, 2006). One reason to the differences between cattle and pigs is that manure from cattle contains larger amounts of slowly degradable carbohydrates and lignin than manure from pigs since cows is given more roughage in their diet. Manure from cows fed on only roughage gives lower yields of methane than manure from cows fed both roughage and concentrate.

Separation of manure is a way to generate manure with a higher methane potential in terms of volume since a fraction without water has a higher proportion of total VS (Møller *et al.*, 2004). Adding straw to the manure enhances the gas potential further. Due to a high content of VS, the methane yield of straw is high and however the use of straw as bedding material increases the methane yield from manure. The methane production of manure increases with 1.8 l methane kg^{-1} or 10% for each 10 g of straw added to 1 kg of manure. However, large differences can be found in the B_o-value for straw depending on pre-treatment of the straw, e.g. cutting length, as well as the digestion conditions.

Reducing enteric methane formation is profitable in all senses since that form of methane is unavoidably lost, while handling of manure offers opportunities to reduce losses during storage (Külling *et al*., 2002). Methane in slurry can also be used as biogas fuel (Külling *et al.*, 2002). If the decomposition of animal manure is subjected mainly to anaerobic digestion, the handling of manure is potentially a larger source of methane than is the gastrointestinal fermentation (Johnson and Ward, 1996).

Factors affecting enteric methane production

Johnson & Johnson (1995) suggested a range of factors that affect enteric methane emissions, such as feed intake, type of carbohydrate fermented, forage processing and lipid addition. These factors have their effects by two different mechanisms. The first mechanism described is the amount of carbohydrate that is fermented in the reticulorumen. The second mechanism is the amount of available hydrogen and the consecutive methane formation through the ratio of VFA produced. The relation between the production of propionic and acetic acids has a relevant impact on methane production. The VFA's regulate the hydrogen supply which controls the production of methane. If carbohydrate would be fermented to acetic acid only, the energy loss from methane formation would be 33% (Wolin & Miller, 1988). If the ratio of acetic and propionic acid was 0.5, the loss of energy as methane would be 0%.

Feed intake

When adding concentrates to the diet of lactating cows, the methane production increased (Hindrichsen *et al.*, 2006). When replacing the hay silage with maize silage, the methane production also increased, both these scenarios were however solely a result of an corresponding increase in DMI and the diets with concentrate supplementation had a lower methane production per kg DMI than did the diets without concentrate. To reduce methane emissions, lauric acid can be used as a supplement to the cows' diet (Külling *et al.*, 2002). This resulted in a decreased enteric emission of methane by 20% but the decrease was also followed by a decreased feed intake.

Type of carbohydrate fermented

Different types of carbohydrates presumably influence methane production through impacts on pH in the rumen as well as on the microbial population (Johnson & Johnson, 1995). A higher proportion of carbohydrates fermented each day (both fiber and starch) imply a lower methane production per DMI. The same is true for feed intake since a higher DMI increases the passage speed which implies that less degradation occurs and consequently less methane is produced (Johnson *et al*,. 2003).

Feed composition

Diets high in concentrates result in a lower emission of methane (Johnson & Johnson, 1995; Hindrichsen *et al.*, 2006). When feeding diets of 90% concentrate the losses of energy as methane might be decreased to half the commonly predicted value of 6% of diet GE (Johnson & Johnson, 1995). A substantial part of the methane emissions from ruminants originate from crude protein, while fat and other ether extract components can decrease the emissions (Clemens and Ahlgrimm, 2001). In a study by McCaughey *et al.* (1999) methane emissions from lactating beef cows grazing on either 100% grass or 22% grass and 78% alfalfa-grass were compared. A significant difference was found and the group that grazed on the alfalfa-grass emitted less methane (373.8 l/day) than the group on grass pasture (411.0 l/day).

Improved production by the cow

When improving the individual cow's production, more milk or meat can be produced per unit. This comes with a higher DMI, but it is at the same time possible to get the same production with fewer units. Every individual animal has a need of energy for maintenance that has to be covered before any production is possible (McDonald *et al.*, 2002). Using fewer animals result in a lower need for energy for maintenance and more can be used for milk or meat production (Moss *et al.*, 2002). This brings about a reduction in produced methane per kg of milk or meat.

Decrease of enteric methane by dietary means –what are the effects on manure-derived methane?

To design dietary strategies with the purpose to decrease methane-emissions are questionable as long as it is not clear whether the manure-derived methane is simultaneously decreased. If the manure is used for production of biogas the scenario is different, in that case it is of interest to maintain the levels of methane in the manure.

Two different scenarios may be of interest in terms of altering the methanogenesis by dietary means. The first is to reduce both enteric and manure-derived methane and the second being to reduce enteric methane but keep or even increase the levels of methane in the manure presupposing that the manure is used as biogas.

Earlier studies have shown that different diets have got an impact not only on enteric methane but also on methane derived from manure. Lodman *et al.* (1993) found a higher methane emission per unit of organic matter in manure from feedlot steers fed with a diet consisting of 11 percent forage and 89 percent concentrate compared to a group that was fed only forage. Hindrichsen *et al.* (2006) found that a group of dairy cows fed hay and grass silages supplemented with concentrates at a ratio of 1:1 had a decreased emission of enteric methane compared to the control group fed maize and grass silage when adjusted to similar DMI. Similarly to the study by Lodman *et al.*, (1993) the manure-derived methane was increased, in this study to a proportion of 30 percent of the enteric methane reduction.

When adding lauric acid, which has a known negative effect on methanogenesis, to the diet of dairy cows, only a small decrease of enteric methane was shown when adjusted to similar DMI (Külling *et al.*, 2002). However, manure-derived methane had an almost nine times higher increase. The lauric acid reduced fiber digestibility in the cow which resulted in more fermentable fiber in the manure. The use of oat hull concentrate, rich in highly lignified fiber, in the diet reduced both enteric methane and manure-derived methane (Hindrichsen *et al.*, 2005).

Pea/oat silage

Studies show that forage-based diets have a higher production of methane in comparison with diets containing more concentrate (Hindrichsen *et al.*, 2006). There are however reasons to why it would be preferable to feed cows large amounts of forage, one being that this is what is natural for them to eat, another being that it is advantageous to feed cows substrates that man cannot eat. Pea/oat silage has earlier shown to have a concentrate-sparing effect (Rondahl *et al.*, 2007) and the high content of starch might have a methane production-decreasing effect because of its high degradability. In a study that used a mechanistic model to investigate methanogenesis in dairy cows, it was shown that replacing sugars from concentrates with starch had a decreasing effect of methane production (Mills *et al.*, 2001).

The voluntary intake of legumes is higher than that of grasses of similar digestibility (Bines, 1985 and Salawu *et al.*, 2002). One reason for this is that legumes seem to have a high degradability of NDF and CP in the rumen (Mustafa *et al.*, 2000). The primary limiting factor of ruminants DMI of silage is the content and digestibility of NDF (Dado & Allen, 1995; Mertens, 1997). There are also other elements that influence the DMI, such as the content of CP (Wright *et al.*, 2000; Broderick, 2003), DM content, levels of ammonia in the rumen (Wright *et al.*, 2000), ammonia N content in the silage (Huhtanen *et al.*, 2002; Wright *et al.*, 2000) and fermentation acids (Huhtanen *et al.*, 2002). The palatability of the feed may also have an impact on the DMI (Huhtanen *et al.*, 2002). The physical capacity of the reticulorumen is also a limiting factor for DMI (Dado & Allen, 1995).

In a study by Salawu *et al.* (2002) Holstein-Friesian cows where fed either grass silage from the second harvest, or pea-wheat bi-crop silage. The study resulted in the conclusion that the pea-wheat bi-crop silage could be fed to dairy cows instead of moderate-quality grass silage. The intake of forage was higher for the bi-crop silage than for the grass silage, however the

DMI was similar for the cows fed bi-crop silage plus 6 kilos of concentrate and grass silage plus 9 kg of concentrates, while the cows fed grass silage plus 6 kilos of concentrate had a lower DMI.

The interest in growing protein crops or protein bi-crops at the own farm has increased in later years, as well in Sweden as in other countries (Wilkins and Jones, 2000; Frank and Swensson, 2002; Salawu *et al.*, 2002). This is most importantly true for organic farming, because of the EU legislation stating that, from the year of 2005, all feedstuff given to animals in organic farming should be organically produced in the largest extent possible (EU regulation no. 1804/1999; Council for the European Union, 1999). In Sweden, organic farms connected to KRAV also have to produce 50% of the feedstuff on the own farm (KRAV, 2010). It is however also of interest for others than organic farmers to find a cheaper replacement for protein sources such as imported soy beans, which often are quite expensive (Salawu *et al.*, 2007).

The challenge for northern Europe, and especially some areas with low temperatures during summer and short growing seasons, is to manage to grow organic crops that give sufficient energy and nutrient requirement for high producing cows. Field peas can be cultivated in almost all of Scandinavia (Rondahl *et al.*, 2006). Field peas are a protein crop that in northern Sweden where the growing season is shorter can be grown as a whole-crop. Mixed pea-cereal crops are preferable, since monocultures of peas are at high risk of lodging (Rondahl *et al.*, 2006).

Criteria that may be used when choosing which cereal to use in the pea-bi crop are the establishments of the crops and the rate of ripening (Rondahl *et al.*, 2006). The establishment of pea is slow during the development of the root nodule which makes it vulnerable to competitors. During this stage of development, barley is more aggressive than oat (Lunnan, 1989), and so oat is a stronger candidate. Besides, oat dries more slowly than barley, which is preferable since field peas mature more slowly than cereals. The pea/oat mixture would also contribute to the crop rotation since barley is more commonly grown as a single crop in Sweden (Rondahl *et al.*, 2006).

Ineffective utilization of nitrogen creates the need of supplementing protein with the diet (Broderick, 2005). The inefficiency in nitrogen use also contributes to environmental impact since, for cows, nitrogen is excreted 2-3 times more in the manure than in the milk. Adding pea in the silage might also have an impact on methane emission as showed by McCaughey *et al.* (1999) where lactating beef cows on alfalfa-grass pasture emitted less methane than did cows on grass pasture.

Methods for measuring enteric methane

There are a range of options to choose between when measuring enteric methane emissions from ruminants (Johnson & Johnson, 1995). Sampling of gas from individuals or from groups of animals can be conducted by enclosure techniques or tracer techniques. Enclosure measurements can either be conducted using a closed circuit or an open circuit (Mc Donald *et al.*, 2002). All techniques have their strengths and weaknesses, so it is important to choose the most suitable method for the current experiment.

By using a respiration chamber, respiratory exchange can be measured (McDonald *et al.*, 2002). In a closed circuit chamber, methane production is measured by sampling and analyzing the air in the chamber. The closed circuit method however demands a large quantity

of soda lime and silica gel to absorb carbon dioxide and water vapour, which is a disadvantage. A more commonly used method of measuring respiratory exchange is the open circuit technique. In an open circuit chamber, air is drawn in and out of the chamber in a controlled flow. The air is sampled for analyses while entering and exiting the chamber and however methane production can be measured using infrared technique. When using the chamber technique, methane-emissions both from the ruminal and post-ruminal processes are measured (McGinn *et al.*, 2006). The chamber method implies that the animals are kept in an enclosed space with controlled ventilation. The flow rate in the chamber is adjusted so that the air pressure in the chamber is positive. A flow of fresh air is fed into the recycling fan to make sure the air in the chamber is moving. The air inside of the chamber is kept at a constant temperature. To measure the methane release from the animals, the methane in the intake and the exhaust air streams is monitored, and the difference between the two flows is calculated.

A head box, or a ventilated hood, can be used to measure methane emissions using the same principles as for the chamber technique (Johnson & Johnson, 1995). In this method, only the head is enclosed in an air tight box. The box allows the animal to move its head inside of the box and a drape is placed around the animal's neck to ensure that no leakage occurs. This is a cheaper method than using a chamber that fits the whole animal. A disadvantage is that it only measures emissions from the mouth and not the rectum. The disadvantage of all enclosure techniques is that they require the animals to be separated from other animals and that it also need some extra training to get them used to the environment.

Methane production can also be measured by tracer techniques. The SF_6 technique measures the methane-emissions from respiration and eructation but do not capture methane released from the rectum (McGinn *et al.*, 2006). This technique involves placing a permeation tube containing SF_6 into the rumen of the animals. This is done some days prior the start of the experiment. The flow of SF_6 from the permeation tube is known and controlled by a Teflon membrane. Air from the nasal cavity is drawn trough tubing into an evacuated canister that is often placed around the animal's neck. The emission of SF_6 is presumed to be identical with the emission of methane; hence the dilution rates for the two gases are the same (Johnson *et al*., 1994).

After a sampling period of 24 h, the canister is filled with nitrogen to over-pressure (McGinn *et al.*, 2006). Before taking samples from the canisters, the gases need to be mixed for at least 1 hour. The samples are then taken with a syringe via a septum port on the canisters and the SF_6 and methane are analyzed using gas chromatography (GC). To determine the methane emitted from the animal, the ratio between SF_6 and methane from the canisters are calculated. There may be other sources of methane in the proximity of the equipment since the animals often are close to each other. Therefore additional canisters are also used to measure the background methane. In a study by McGinn *et al.* (2006), the emitted methane was 4% lower when using the SF_6 tracer technique than when using the chamber technique. The differences are most likely due to that the chamber technique measures all methane emitted while the SF_6 tracer technique do not measure methane emitted from the rectum. Similar studies by Boadi *et al*. (2002) and Johnson *et al*. (1994) found no significant differences between enclosure and tracer techniques.

Methods for measuring manure-derived methane

Emissions of methane from manure occur during a much longer period and in much smaller amounts than enteric emission. Therefore measuring methane from manure is even more

demanding than measuring enteric methane. The methods used to determine manure-derived methane stretch from large scale experiments to laboratory scale experiments where bottles are used for storing.

The volume of gas produced by a substrate can be determined by placing the substrate in a bottle and seal it with rubber lids (Møller *et al.*, 2003; Rodhe *et al.*, 2009). The bottles are then kept in a temperature controlled space. The gas production can be calculated either by measuring the pressure in the headspace of the bottle or by connecting the bottle to a gas sampling bag and then use a syringe to measure the volume of sampled gas. Methane can then be analyzed for by chromatography. To mimic the storage of manure on farms, the sampling should go on for a period that resembles farm conditions. Külling *et al.* (2001) used open 10 l buckets for methane production measurements. Gas volume was measured using a closed chamber technique constructed of a second bucket placed upside down on the bucket with substrate. For gases that emits in a slow rate as methane, it is preferable to use the closed chamber technique rather than a dynamic chamber. The emission rate of methane are calculated from the gradually enrichment of methane to the air in the headspace.

Gas production can also be measured in larger scale. Rodhe *et al.* (2009) developed a pilot-scale method to measure gas production from manure using a closed chamber technique. Some conditions were found to be important to resemble farm scale conditions, amongst these where to keep the temperature at similar levels to full-scale storage systems, to expose the manure to changing weather conditions, to fill and empty the storage tank in similar intervals as on a farm and to register the amount and properties of the slurry entering and leaving the storage tank. The tanks where equipped with an airtight and flexible lid so that the head space could be varied.

Hypotheses

The hypothesis of this study is that 1) a starch rich diet with high percentage of pea/oat silage results in a lower emission of enteric methane compared to grass silage and that 2) this will be followed by a higher or maintained production of methane from the manure.

Materials and methods

The study was conducted from November of 2009 to March of 2010 at Kungsängen Research Centre in Uppsala.

Experimental design

The study consisted of four periods, each approximately 30 days long. Prior to the first period there was a transition period during which all the cows were fed grass silage and pea/oat silage at a ratio of 75:25 for two weeks. Each period then started with two weeks when the animals were adapting to the new feed regime and the periods were finished off with 5 days of sampling of enteric methane. Faeces and urine was sampled during the first period only. The study was designed as a changeover experiment, meaning that all cows where in both of the treatments during some stage of the study, see table 1.

Table 1. Experimental periods and the order of treatments per cows

Period / Cow ID	1	2	3	4
1328	A	B	B	A
1379	B	A	B	A
1381	B	A	A	B
1403	A	B	A	B

Animal data

The animals taking part in this study were four rumen fistulated cows of the Swedish Red breed at Kungsängen Research Centre in Uppsala. The cows in the study were in their 8th-25th lactation week and their milk yield was assumed to be between 25-50 ECM/day.

Feed regimes

The cows were fed with two different feed regimes during the four study periods.
Treatment A: 100% grass silage
Treatment B: 25% grass silage, 75% pea/oat silage

In addition to the forage, the cows were also fed 7 kilos of concentrate each day (Solid 120, Lantmännen). Minerals were also fed according to Swedish recommendations. The forage was fed *ad lib* and the forage residues were weighted every day. The amount of forage given to each animal was calculated individually for the cows according to table 8 in appendix 1. During the days of adaptation, the animals were fed from rail hung feeding trucks and during the sampling period they were fed manually. The feed was weighed manually throughout the study.

Housing and milking

The cows in the experiment where housed in a tied stable with other cows during the transitional and the adapting phases of the study. During the sampling period, the four cows where relocated to a separate part of the barn, still in a tied stable, secluded from the rest of the animals by wooden walls and a plastic curtain so that the sampling of methane would not be disturbed by the other animals. This secluded part was also equipped with better ventilation

than the rest of the barn. Milking of the cows took place twice a day at 06.00 and 15.30 o'clock and they were milked in buckets.

Samplings and analyses

Sampling of enteric methane

Each cow was equipped with a specially designed halter for the methane sampling. A hose leading to the nostrils along with a capillary tube were fastened to the halter. The capillary tube regulated the intake rate in order to keep it on a constant level. An evacuated yoke was placed on the neck of the cow. The yoke had a vacuum pressure of -20 - -23 inches of mercury (inHg) and it was connected to the hose at the nostrils via a collecting valve, and air from the nostrils and mouth where collected in the yoke due to the vacuum. The yoke was evacuated using a vacuum pump prior to use. The pressure was measured using a manometer.

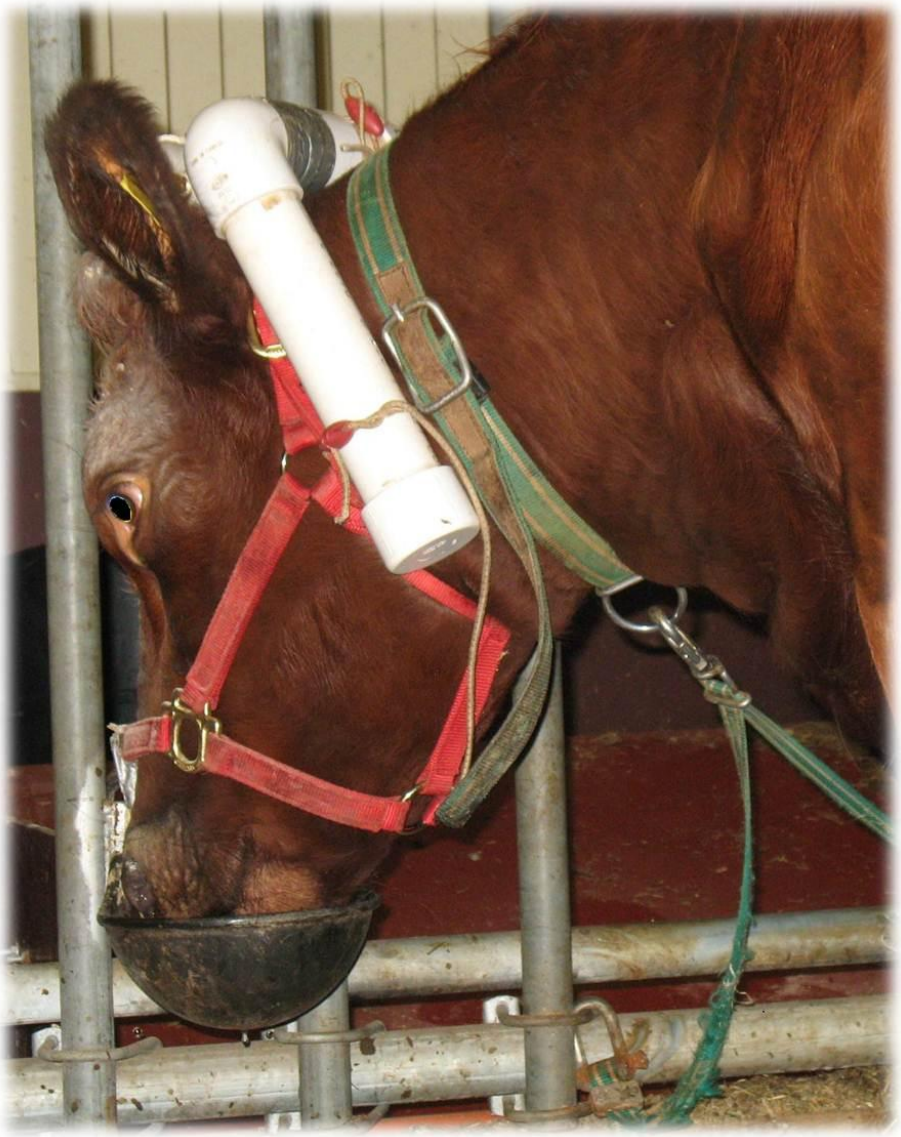

Figure 3. Picture of one of the cows in the experiment equipped with the sampling gear. (Photo by Agnes Willén, 2010).

The method used to measure methane production was SF_6 (sulfur hexafluoride) tracer technique. SF_6 is a highly potent greenhouse gas that has a 23 900 times higher potential for

global warming than carbon dioxide has. The reason to why SF_6 is used is because it is inert and passes through the abdomen of the cow without reacting with other substances therein. A permeation tube containing SF_6 was placed in the rumen of the cows approximately 10 days prior to sampling. SF_6 is released from the capsule and by a Teflon membrane the flow rate is regulated. The flow is constant and was calibrated during the 10 weeks prior to the study, the capsules different flows are shown in table 2. The different capsules had a slightly varying flow and therefore the identification number of each capsule was recorded along with the ID of the corresponding cow. Because of the risk that the gas could affect the equipment, the equipment and the gas was handled in separate rooms.

Table 2. Mean flow of SF_6 from the permeation tube from individual cows (mg/day)

Cow	**1328**	**1379**	**1381**	**1403**
Tube	93	20	17	21
Flow (mg/day)	2.584	3.139	3.254	2.584

An evacuated yoke was placed on the neck of the cow, after which the hose on the halter was connected to the yoke. The time of the connection and the number of the yoke was recorded. The yoke was then tightened to the halter using elastic straps. After approximately 24 hours of sampling, the hose was disconnected from the halter and the time of day was recorded. The yoke was then removed from the cow. Immediately after disconnecting the yoke, the pressure was measured and recorded. When the pressure was in the range of -3 to -12 mbar, the equipment was working. There was also three measuring points in the stable, measuring the background methane. These yokes was also connected to halters and where treated in the same way as the yokes on the cows.

The yokes were then filled up with N_2 at a pressure of 1 bar. This was made to create overpressure and thereby enable gas sampling. After a minimum of one hour of mixing of the gases, the sampling of gases was carried out. This was made by using a 60 ml syringe to sample gas from the yoke. Around 60 ml of gas was then injected in a 22 ml test tub equipped with a rubber lid. A cannula was inserted through the lid trough which redundant gas could escape to make sure that the test tub only contained the gas from the yoke. This procedure was repeated five times during period 1 and six times during period 2. The yokes were then washed with 2 bar N_2 three times in order to be clean before the next sampling.

Sampling of methane from faeces and urine

Faeces and urine was sampled from the four cows during period 1. Urine was sampled twice a day, five days in a row and it was collected in a bucket either while the cow voluntarily evacuated or via stimulation by massaging just under the vulva. Faeces were sampled twice a day, five days in a row and it was collected in a bucket either while the cow voluntarily evacuated or by manually gathering via the cows' rectum. The samples were then weighed and mixed together to one uniform composite sample per cow. Faeces and urine from each cow was mixed by a ratio of 2.4:1 according to earlier studies where urine and faeces was collected (Bertilsson, personal message, 2010). One sample of mixed faeces and urine (FU) from each cow (n=4) was then analyzed along with one pure urine sample (U) from two individual cows (n=2).

Enteric methane analyses

The concentrations of methane, SF_6 and CO_2 in the samples from the yokes were analyzed using a Gas Chromatograph (GC) (Pekin-Elmermodel Claus 530, Shelton, CT, USA), by Gunnar Börjesson (institutionen för mikrobiologi, SLU, Uppsala). Each sample was analyzed in triplicate. The emissions of methane were calculated using following formula:

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

Johnson *et al.*, 1994

QCH_4 is the methane produced per day, QSF_6 is the known flow of SF_6 from the capsule. $[CH_4]$ and $[SF_6]$ are the measured concentrations from the yokes on the cows and $[CH_4b]$ and $[SF_6b]$ are the measured concentrations from the background yokes.

Analyses of methane from faeces and urine

Nitrogen content in the FU sample was determined using the Kjeldahl method where the organic substances in the substrates are decomposed by oxidation to liberate nitrogen as ammonium sulphate. The solution is then cooled and mixed with water where after it is distilled and filtered with hydrochloric acid to convert ammonium to ammoniac.

The DM content of the FU sample and the U sample was determined by placing substrates in crucibles in an oven at 103°C over night and weighing the crucibles with content before and after heating. Ash content was determined by placing the crucibles in 550°C for three hours and weighing the crucibles with content before and after heating.

The maximum methane production per g VS (B_o) were analyzed for the six substrates (two U and four FU samples) by using a laboratory-scale batch digestion test in 1-litre bottle along with inoculums at 37°C as described by Rodhe *et al.*, (2009). The substrate (either urine or the faeces-urine mixture) was weighed and put in a 1 liter glass bottle. Inoculums and water was then added after weighting and the bottles were closed tightly with a rubber lid. The total volume of substrate, inoculums and water was somewhere around 600 ml. A triplicate digestion test was made for each substrate. The bottles were placed on a shaking table with a rotation speed of 130 rph in a room with a constant temperature of 37°C to ensure soft and even mixing and heating of the samples. The laboratory-scale batch digestion test was run for 100 days.

The pressure in the bottles was tested with a digital pressure meter (GMH 3110) with a pressure sensor (GMSD 2 BR; -1000 tp 2000 mbar) and recorded. The pressure was then recalculated to produce standard gas volume (0°C, 101.3 kPa). Gas was sampled from the bottles using a 5 ml syringe. 2 ml of gas was then injected into a 20 ml test tub with rubber lid. Each substrate was analyzed in triplicates. The bottles with substrate were after that evacuated for air. This was done using a cannula coupled to a plastic bag that sucks out the gas until there was no pressure left in the bottle. The sampling from the bottles took place daily during the first days and then approximately once every 7-10 days. The date and time for sampling was recorded. At the times for sampling, reference samples were also taken from a tube with 99% methane gas in order to see changes during handling of the samples.

The concentration of methane in the FU and U samples were analyzed using a Gas Chromatograph (GC) (Pekin-Elmermodel Claus 530, Shelton, CT, USA), by either Johnny Ascue Contreras or Maria del Pilar Castillo, Swedish Institute of Agricultural and Environmental Engineering, Uppsala.

Feed sampling and analyses

Samples of the grass and the pea/oat silages where taken each day. The samples were put in a freezer with a maximum temperature of -18°C and were analyzed after the study was finished. The samples were pooled to form a composite sample from each animal and measurement period. The feed samples were then analyzed for content of DM, ash, starch, NDF, crude protein and energy. The feed analysis where conducted as a part of the larger study. The VS in the feed was calculated in order to be able to compare methane production from feed with methane production from manure. Formula used: ([DMI]*((100-[ash content in % of DMI])/100).

Milk samples

In each of the four periods, milk where taken from each cow two days in a row and analyzed by the milk laboratory at Kungsängen Research Centre in Uppsala. The method used was infrared technique (Milkoscan FT120, Foss, Denmark) and the analyses performed were for content of fat, protein and lactose. Milk yield, the amount of cells and the yield of energy corrected milk (ECM) were also measured.

Animal weighing

The animals were weighed two consecutive days one time per period and an average weight for each period and animal was calculated.

Statistic analyses

The concentrations of methane and SF_6 samples from the yokes, the production of methane as well as the maximum methane unit producing capacity per g VS of the manures (B_o) were calculated using Excel 2007 (Microsoft Office, 2007). The statistical analyses were carried out using SAS 9.1 (SAS Institute Inc., Cary, NC). The model used in most cases was mixed procedure. The GLM procedure was used for the analyses of FU and U since there were only results from one period.

Ethics

The study set-up was approved by the animal experiment ethical committee in Uppsala according to the permission number C/29/8.

Results

Feed

Energy

The mean daily total intake of Gross Energy (GE) was for treatment A (100% grass silage) 434.6 MJ/day and for treatment B (25% grass silage, 75% pea/oat silage) 419.0 MJ/day, as shown in Table 3. There was no significant difference between MJ intake between either the two different treatments ($p=0.459$) or between the periods ($p=0.744$).

Starch

The mean daily total intake of starch was for treatment A (100% grass silage) 1.7 kg/day and for treatment B (25% grass silage, 75% pea/oat silage) 2.8 kg/day, as shown in Table 3. There was a statistic difference between treatments concerning intake of starch ($p<0.0001$) but not between periods ($p=0.930$).

NDF

The mean daily total intake of NDF was for treatment A (100% grass silage) 7.7 kg/day and for treatment B (25% grass silage, 75% pea/oat silage) 7.1 kg/day, as shown in Table 3. There was no statistic difference between either treatments ($p=0.701$) or periods ($p=0.288$) concerning intake of NDF.

Protein

The mean daily total intake of protein was for treatment A (100% grass silage) 3.7 kg/day and for treatment B (25% grass silage, 75% pea/oat silage) 3.3 kg/day, as shown in Table 3. There was a statistic difference but between treatments ($p=0.048$) but not between periods ($p=0.305$) concerning intake of protein.

Dry matter intake

The average daily intake per cow and period is shown in table 9 appendix 1. The mean daily total intake of DM was for A (100% grass silage) 22.9 kg DMI/day and for treatment B (25% grass silage, 75% pea/oat silage) 22.3 kg DMI/day, as shown in Figure 4.

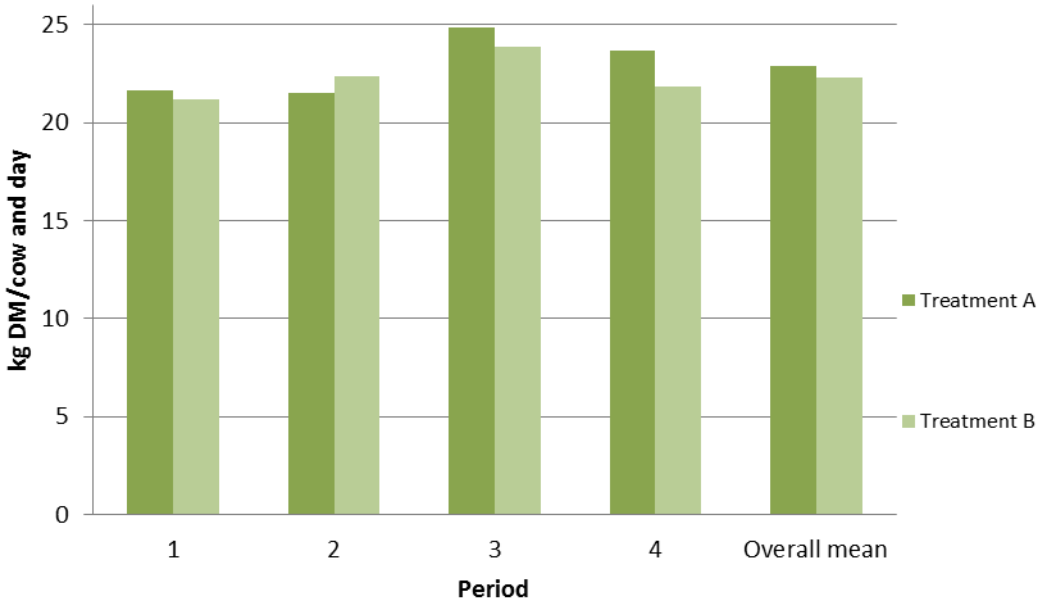

Figure 4. Intake of DM per cow and day for each treatment and period. A =100% grass silage, B=25% grass silage, 75% pea/oat silage.

No significant differences were found between either treatments (p=0.472) or periods (p=0.125).

Table 3. Summary of feed data for the two different treatments (GE = gross energy, NDF = non detergent fiber, DMI = Dry matter intake, MJ = mega joule)

Treatment		GE (MJ/day)	Starch (kg/day)	NDF (kg/day)	Protein (kg/day)	DMI (kg/day)
A	mean	434.6	1.7	7.7	3.7	22.9
	min	367.5	1.7	6.2	3.3	17.2
	max	503.9	1.7	9.4	4.3	28.3
	StDev	39.72	0	0.91	0.31	2.39
B	mean	419.0	2.8	7.1	3.3	22.3
	min	356.8	2.5	5.8	2.9	17.2
	max	480.0	3.0	8.6	3.9	28.3
	StDev	45.50	0.16	1.06	0.35	2.50
P-value treatments		0.459	<0.0001	0.701	0.048	0.472
P-value periods		0.744	0.930	0.288	0.305	0.125

Animal weighing

The weights of the animals are presented in figure 12 in appendix 1. There were no significant differences in animal weights between either periods or treatments.

Milk yield

The cow's individual production of milk are presented in table 10 in appendix 1.The mean milk production per cow in treatment A (100% grass silage) was 27.0 kg/day and in treatment B (25% grass silage, 75% pea/oat silage) 24.2 kg/day see table 6. There were no differences in milk yield between treatments (p=0.126) but between periods concerning milk yield (p = 0.015).

The mean production of ECM per cow was for treatment A 28.2 kg/day and for treatment B 26.9 kg/day. The production of ECM per treatment and period is shown in table 6.

Enteric methane production

The enteric production of methane per cow and period is presented in table 11 in appendix 1 and the enteric production per treatment and period is shown in table 12 in appendix 1. The mean production per cow of enteric methane was for treatment A (100% grass silage) 504 g/day and for treatment B (25% grass silage, 75% pea/oat silage) 657 g/day which is shown in table 4. There was a significant difference treatments (p=0.045) and between periods (p=0.039) concerning enteric methane production, see table G. Figure 5 shows the mean value of enteric methane production per treatment for each of the four periods and the overall mean for all the periods together.

Table 4. Mean, minimum, maximum and standard deviation (StDev) for enteric methane production per cow and day during all four experimental periods

Treatment		**Enteric CH_4 (g/cow and day)**
A	mean	504
	min	214
	max	962
	StDev	194.4
B	mean	657
	min	235
	max	1032
	StDev	186.6
P-value treatments		0.045
P-value periods		0.039

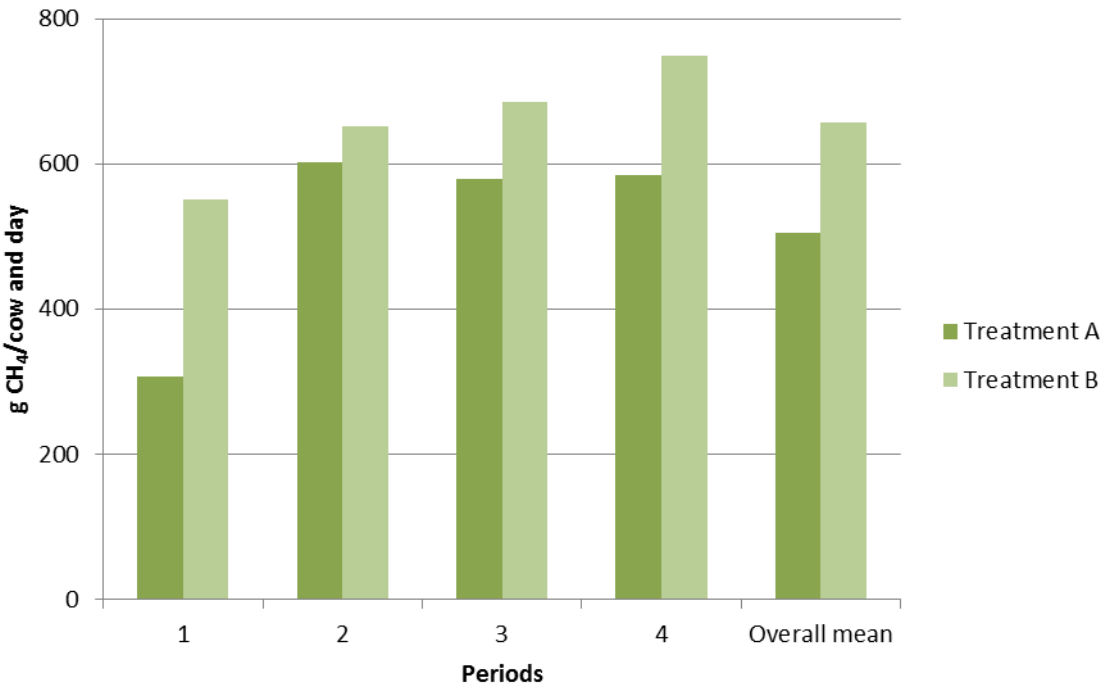

Figure 5. Mean enteric methane production (g/day and cow) for the two treatments. A =100% grass silage, B=25% grass silage, 75% pea/oat silage.

Methane from faeces and urine

The results from the analyses of the DM content and ash content of faeces and urine (FU) and pure urine (U) are presented in table 13 in appendix 1 and the results from the N content analysis of (FU) is presented in table 14 in appendix 1. Since there was only one result per cow from the B_o experiment, the GLM procedure with t-test was used as statistical model. The result from the analysis was that no significant difference between the two treatments was found ($p = 0.233$).

The results from the FU-experiment showed a B_o-value of 160 l CH_4 kg^{-1} VS for treatment A (100% grass silage) and 211 l CH_4 kg^{-1} VS for treatment B (25% grass silage, 75% pea/oat silage) after 114 days of incubation. The B_o-value for U was -46 l CH_4 kg^{-1} VS for treatment A and 188 CH_4 kg^{-1} VS for treatment B after 114 days. The results with standard deviation (StDev) are shown in table 5.

Table 5. The maximum methane production and standard deviation (StDev) per g VS (B_o) from faeces and urine (FU) and urine (U)

	FU		U	
Treatment	**l CH_4 kg^{-1} VS**	**StDev**	**l CH_4 kg^{-1} VS**	**StDev**
A	160	37.74	-46	0.26
B	211	11.46	188	8.58

Figure 6 shows the production of methane from FU per treatment over time. The substrate was stored for 114 days. Figure 7 shows the production of methane from FU and U from each cow individually during the time of storage where 1328U and 1379U are U samples. Cow's

with ID 1328 and 1403 is given the diet for treatment A and 1379 and 1381 for treatment B. Each sample was analyzed in triplicates and the figure shows the mean values.

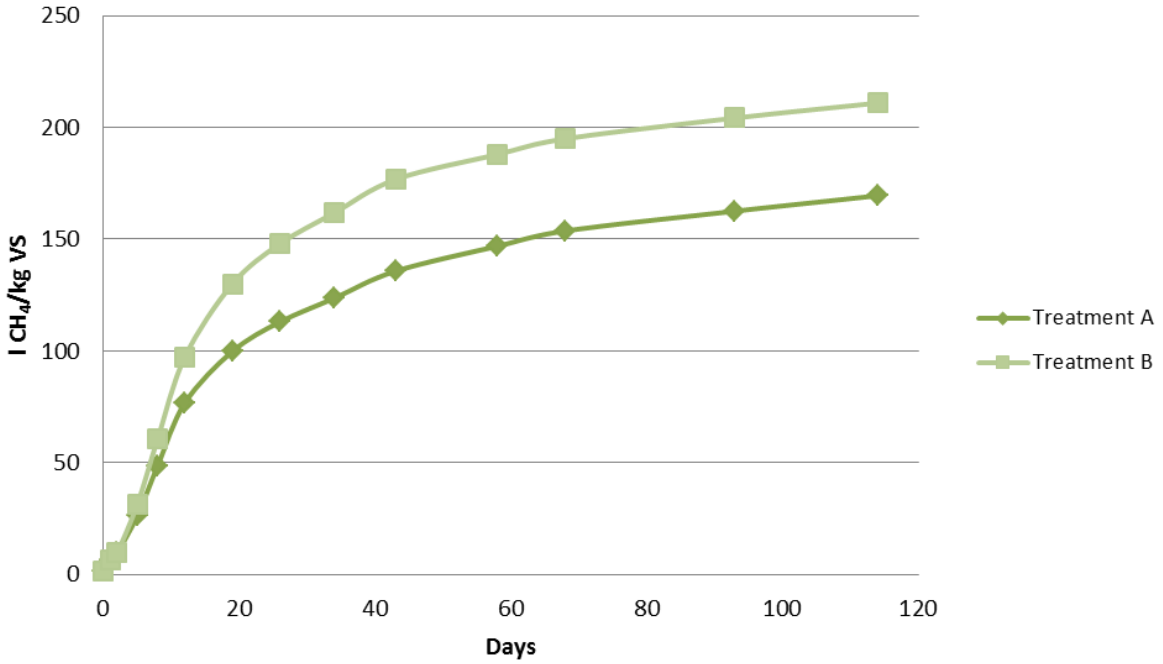

Figure 6. Mean methane producing potential for faeces and urine (FU) for the two treatments from period 1.

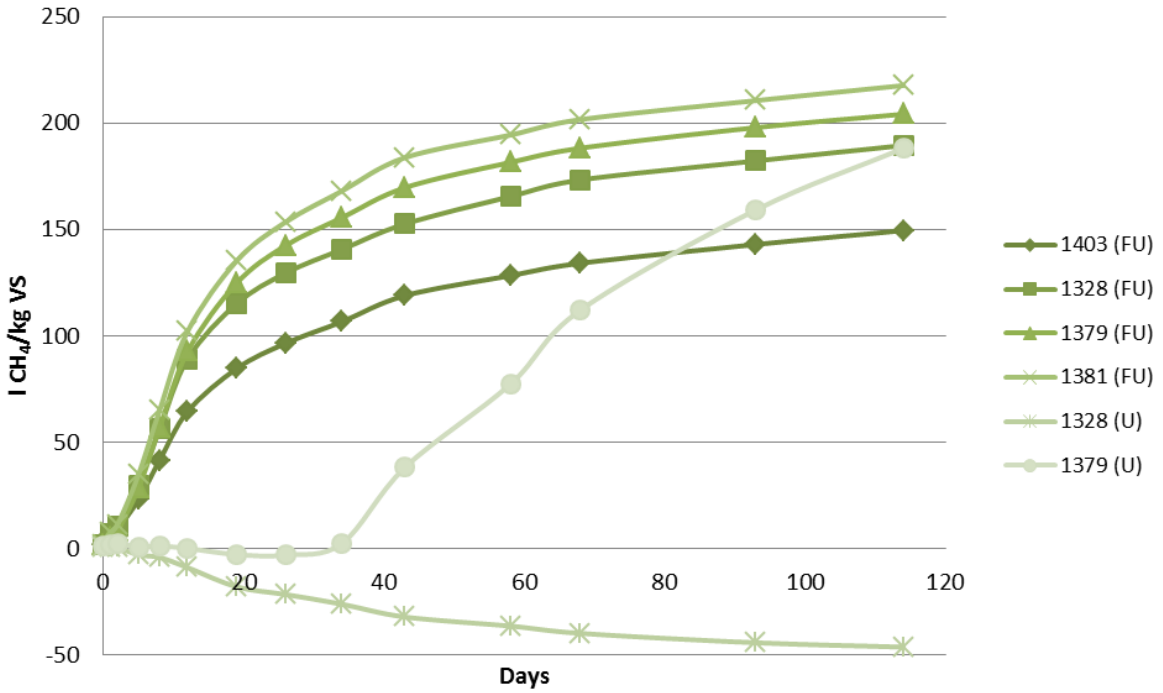

Figure 7. Mean methane producing potential for faeces and urine (FU) and urine (U) for the individual cows from period 1.

Enteric methane relations

Starch

The mean daily value for g produced enteric methane per kg starch in the feed was for treatment A (100% grass silage) 341 and for treatment B (25% grass silage, 75% pea/oat silage) 196. There was not a significant difference in g produced enteric methane per kg starch in the feed between treatments (p=0.063) or between periods (p=0.225). Figure 8 shows a linear trend for the relations between enteric methane production and the intake of starch. However, there was no significance in the linear trend (p<0.0.63).

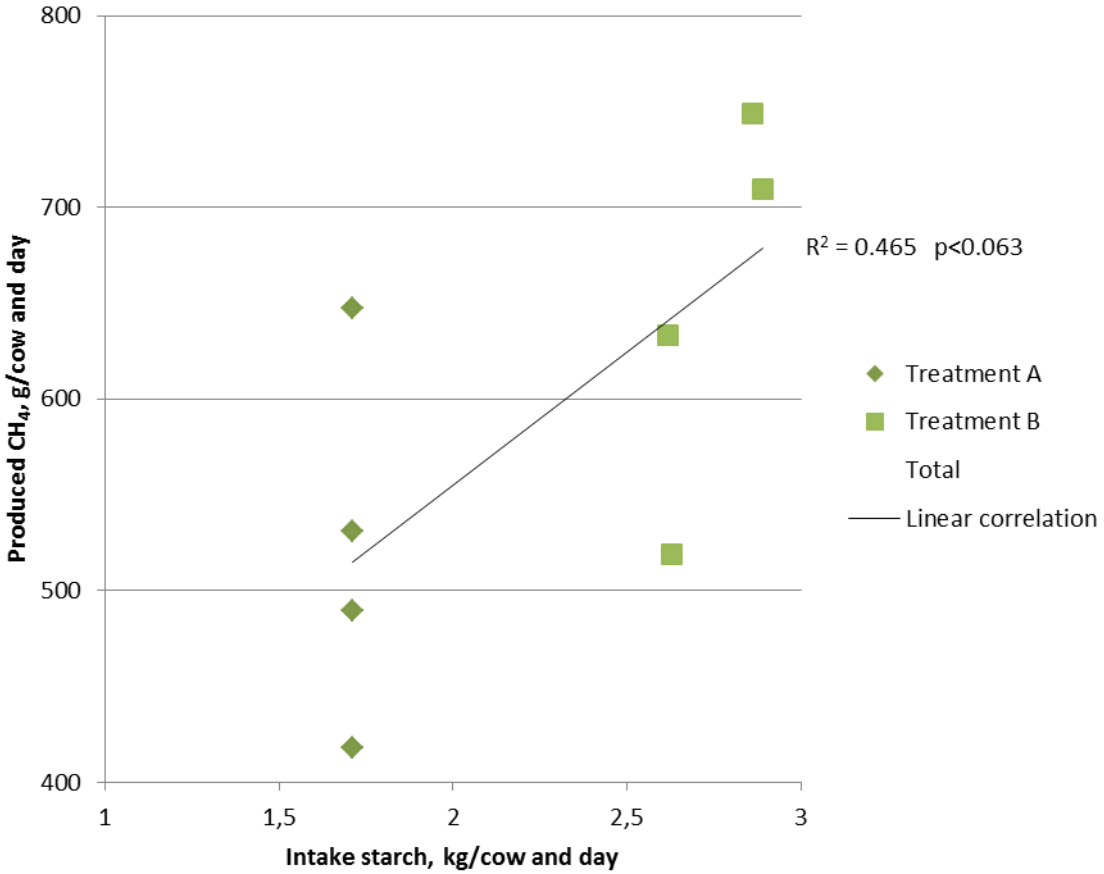

Figure 8. Relations between enteric methane production (g CH_4/day) and starch intake (kg/day).

NDF

The mean daily value for g produced enteric methane per kg NDF in the feed was for treatment A (100% grass silage) 78 and for treatment B (25% grass silage, 75% pea/oat silage) 74. There was no significant difference in g produced enteric methane per kg NDF in the feed between either treatments (p=0.992) or periods (p=0.261).

Protein

The mean daily value for g produced enteric methane per kg protein in the feed was for treatment A (100% grass silage) 160 and for treatment B (25% grass silage, 75% pea/oat silage) 160. There was no significant difference in g produced enteric methane per kg protein in the feed between either treatments (p=0.793) or periods (p=0.275).

DMI

The mean daily value for g produced enteric methane per kg DM was for treatment A (100% grass silage) 22 g methane/kg DM and for treatment B (25% grass silage, 75% pea/oat silage) 29 g methane/kg DM.

There was a significant difference between treatments (p=0.028) and between periods (p=0.034) concerning enteric methane production per DM intake.

Milk yield

The production of g enteric methane per kg ECM was for treatment A 21.9 g/kg ECM and for treatment B 17.6 g/kg ECM, se table 6. There was no significant differences between either treatments (p=0.303) or periods (p=0.193) concerning enteric methane production per kg ECM.

Table 6. Milk production in kg and ECM, mean for treatment and period, and mean produced methane (g) per kg ECM

Period / Treatm.		1	2	3	4	Overall mean	n
A	g CH_4/kg ECM	13.2 (10.2)[1]	24.9 (5.7)	27.8 (3.7)	21.5 (.)[2]	21.9 (8.0)	7
	kg ECM	30.2 (8.3)[1]	29.7 (5.1)	24.8 (8.1)	27.9 (6.8)	28.2 (5.9)	8
	kg milk	31.3 (10.5)[1]	27.6 (4.3)	23.3 (9.3)	25.8 (7.1)	27.0 (6.9)	8
B	g CH_4/kg ECM	13.4 (8.6)[1]	20.0 (.)[2]	18.5 (0.2)	22.0 (.)[2]	17.6 (5.2)	6
	kg ECM	32.2 (0.2)[1]	29.4 (9.4)	27.6 (4.3)	18.6 (17.7)	26.9 (9.5)	8
	kg milk	30.0 (0.7)[1]	25.9 (8.8)	25.2 (5.6)	15.7 (15.5)	24.2 (9.0)	8

[1]Standard deviation (StDev) within brackets
[2]n=1, therefore no value for StDev

Methane from faeces and urine

Because there were only data from one period concerning methane from FU and U, no statistics where done on comparisons with methane from FU and U. However the relationship between methane from FU and enteric methane was investigated by plotting the values in a graph, see figure 9. There was no significant linear trend between the two forms of produced methane.

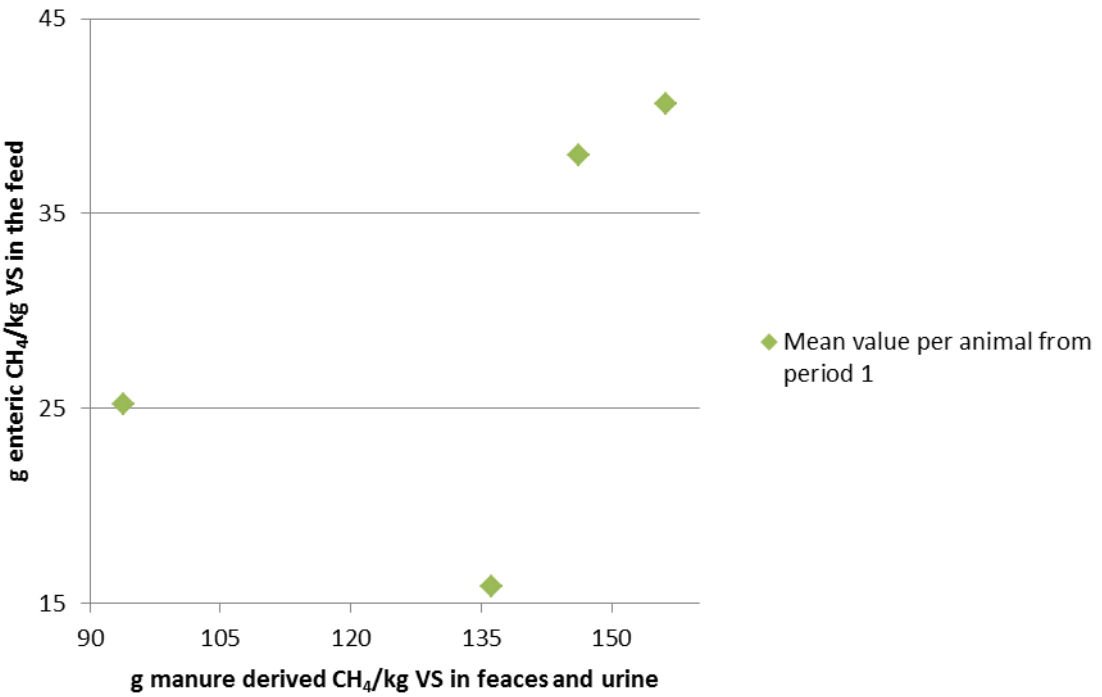

Figure 9. Relations between enteric methane production (g CH_4/VS in feed) and methane from faeces and urine (FU) production (g CH_4/kg VS in faeces and urine).

Summary of methane results

Table 7. Means, standard deviation (StDev) and significance levels for some of the variables studied, significant differences are shown in italic

Variable	Treatment						Significance levels	
	A	n	StDev	B	n	StDev	Treatment	Period
Enteric methane								
CH_4 (g/day)	504	30	194.44	657	35	186.61	*0.045*	*0.039*
CH_4/ kg starch (g/kg)	340	7	136.78	196	6	60.92	0.063	0.225
CH_4/ kg NDF (g/kg)	78	7	32.39	74	6	22.24	0.992	0.261
CH_4/ kg protein (g/kg)	160	7	62.91	160	6	50.02	0.293	0.275
CH_4/DMI (g/kg)	22	30	8.92	29	35	7.10	*0.028*	*0.034*
CH_4/ kg ECM (g/kg)	22	7	8.04	18	6	5.20	0.303	0.193
Methane from faeces and urine								
FU CH_4 (l/kg VS)	160	2	41.14	211	2	9.53	0.233	-

Discussion

The first hypothesis of the study was that using a large amount of legumes in the cow's feed (i.e. treatment B) would have a decreasing effect on the enteric methane production from the cows. After analyzing the results from the study, this did not seem to be the case. The difference between treatments proved to be significant, but the cows that were fed pea/oat silage emitted more methane (657 g/day) than did the cows that was fed grass silage (504 g/day). This is opposite to previous studies where lactating beef cows on alfalfa-grass pasture emitted less methane (373.8 l/day) than cows on grass pasture (411.0 l/day) (McCaughey *et al.*, 1999). The result is neither in line with the idea that a decrease in methane production can be obtained by replacing sugars from concentrates with starch (Mills *et al.*, 2001). There where however not as much starch in the pea/oat silage as expected due to a pea aphid infestation that caused losses of pea pods (Rondahl, 2010). This might explain the default expected effect.

The second hypothesis of this study was that a low enteric methane production was somewhat followed by a high or maintained production of methane from the faeces and urine. This is interesting since methane from manures is easier to utilize as energy than enteric methane. This turned however out not to be the case. There were no significant differences between the two treatments, but the results indicated that treatment A (grass silage) gave less produced methane than did treatment B (pea/oat silage), which was the same as for the enteric methane produced.

There was a significant difference between period 1 and all other period concerning enteric methane production. This difference might be due to that one of the cows had significantly lower methane production this period compared to the later three periods, and thereby reduced the total production during this period. It might also be a result of DMI intake, since the intake of DM was slightly lower as a total during period 1 compared to the other periods. A lower DMI has in previous studies proved to decrease methane production (Külling *et al.*, 2002; Hindrichsen *et al.*, 2006).

Rodhe *et al*. (2009) showed that about 3% of the maximum methane production for cattle slurry is produced during storage when considering Swedish conditions, and that is how the amount of methane from the faeces and urine mixture is calculated in figure 10 and 11. As visible in figure 10, the trend is that treatment B generated more methane both from enteric production and from the faeces and urine. It is however important to note here that the VS in the two different methane sources are different. When considering enteric methane it is the VS content of the feed that is implied and considering methane from faeces and urine it is the VS content of the faeces and urine mixture that are referred to. If the faeces and urine will be used for biogas production it is preferable to choose to give the cows treatment B (pea/oat silage), but this will unfortunately also bring about a higher enteric methane production.

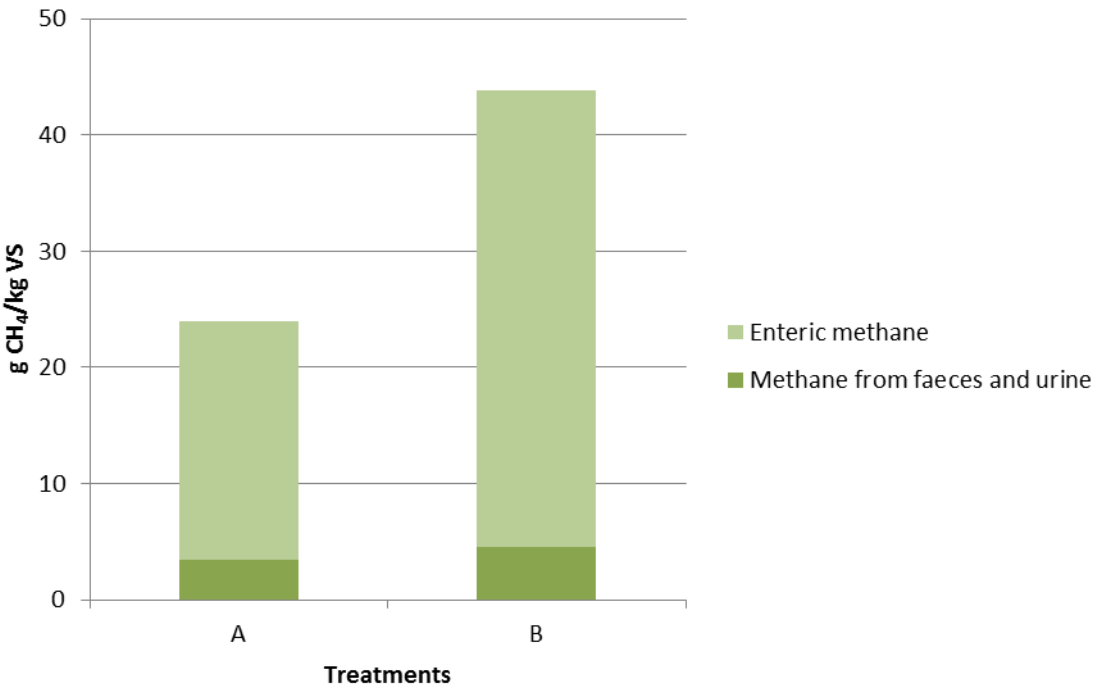

Figure 10. Distribution of enteric methane and methane from faeces and urine per treatment from period 1 (g CH_4/kg VS).

When presenting the results on individual level (Figure 11), the trend is the same as on treatment level. When emitting higher amounts of enteric methane, as for cow 1379 and 1381, the amount of methane from faeces and urine also seems to be higher. When a linear trend was created to compare methane derived from FU and enteric methane fermentation no significant difference were found. It did however seem that a higher value of enteric methane gave a higher value of methane derived from FU. These findings are not in line with a study by Hindrichsen *et al.* (2006) who found that when enteric methane production was decreased by dietary means, the methane production from the manure was simultaneously increased. It is however difficult to draw any conclusions from the results from the present study since there are not that many earlier studies that have compared enteric produced methane and methane production from manure.

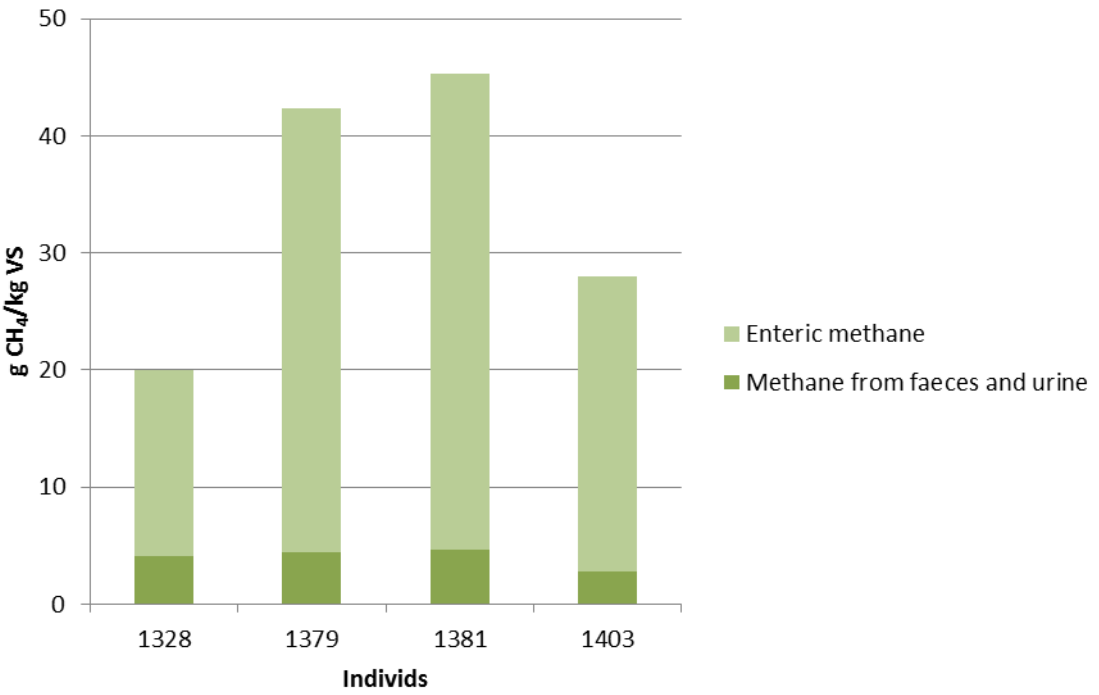

Figure 11. Distribution of enteric methane and methane from faeces and urine per individual from period 1 (g CH_4/kg VS).

The faeces and urine (FU) showed a lower B_o-value for both treatments (A=160 l CH_4 kg^{-1} VS, B=211 l CH_4 kg^{-1} VS) compared to the default value of 240 l CH_4 kg^{-1} VS for cattle slurry from developed countries that are given from IPCC (2006). The B_o for faeces and urine in the present study were also lower than for cattle slurry taken from a dairy farm in Sweden measured in the same laboratory using the same techniques (294 l CH_4 kg^{-1} VS) (Rodhe *et al.*, 2009). One difference between the present study and the comparing studies is that the substrate in this study was sampled directly at excretion from the cows and therefore there were no addition of residues of feed or bedding material present. This might explain the relatively low B_o from faeces and urine. The reason to why the faeces and urine in the study are sampled directly from the animals is that the aim was to investigate the feeds impact on production of methane from faeces and urine and therefore it had to be free from bedding materials and feed residues. This is different from what is common when doing methane production analyses from manure, because most studies are interested in investigating all of the material that normally ends up in the manure storing system, including water and bedding material.

When measuring maximal methane production (B_o) from manure, it is common to incubate the manure for 100 days (Rodhe *et al.*, 2009). However, in this study, the substrate was stored for another two weeks, in total 114 days. This was done to make sure that the production of methane was stabilized which was not the case after 100 days. However, this does not seem to have influenced the results since a longer incubation should imply a higher level of produced methane, if any difference at all, and the result was the opposite.

The result from the storing of pure urine shows a partitioned result. The urine from the cow in treatment A had a negative B_o-value, whilst the urine from the cow in treatment B started off with a negative B_o-value but after a month of storing it steadily increased to a B_o of 188 l CH_4 kg^{-1} VS. High content of nitrogen (N) in the substrate can have an inhibiting effect on

methane production (Hansen *et al.*, 1998). In a study where swine manure was used, the authors found that a concentration of free ammonia of 1.1 g-N/litre or more had an inhibiting effect on the methane production. The N-content might be the reason to why B_o for the urine showed these results.

One reason to why the methane production did not show the expected results could be the ratio between faeces and urine used. The faeces to urine ratio were 2.4:1, which was calculated from earlier studies (Personal message, Bertilsson, 2009). However, there are not common in this kind of studies to mention the ratio between faeces and urine, probably because it is common to sample the materials when it is already mixed, and therefore it is not sure that the ratio used corresponds to the normal conditions.

As suspected, there were significant differences between the treatments concerning intake of starch. There was however not a significant difference between the treatments concerning produced enteric methane per kg ingested starch. This is not in line with the result of an earlier study where it was shown that methane production can be reduced by including feeds rich in starch that act to enhance propionic acid (Johnson & Johnson, 1995). Also a study by Russel & Jeraci (1984) showed that using starch as the energy source inhibited methane production.

When creating a linear trend for both treatments together to investigate the relations between enteric methane production and the intake of starch, no significance was obtained. There was however a trend that as the amount of starch in the diet raises so does the enteric methane production. This is not quite in line with the result discussed above and might be an effect of the fact that the individuals in treatment A all ate the same amount of starch.

The enteric methane production per kg DMI was higher for treatment B (pea/oat silage) than for treatment A (grass silage). This is not a result of higher DMI for treatment B, as could be suspected, since there were no significant differences between treatments concerning DMI. When only comparing numbers but not significance, the DMI was even slightly lower for treatment B then for treatment A which makes it even clearer that the DMI is not the reason for differences in enteric methane production.

The amount enteric methane produced per kg of ECM is lower for period 1 then the rest of the periods. This is probably an effect of the low enteric methane production from one individual cow along with the slightly higher production of ECM during this period.

Another interesting property of pea/oat silage that earlier studies have shown is a concentrate saving effect. In this study, the cows were fed with the same amounts of concentrate but with different ratios of grass silage versus pea/oat silage. According to earlier studies, the cows fed with the higher amount of pea/oat silage should produce more milk. This was however not the case for this study. There were no significant differences between the treatments at all concerning milk yield.

The sampling of enteric methane was conducted during five days in each period. Though, during period 2, one extra day of sampling was added and the first day was removed from the analysis since there were some problems with the gear that first day. During the other periods, some of the values were not used due to them being deviate and sometimes some values were even missing. Missing and deviating values was most often due to equipment problems such as water in the yokes or clogging of the hose.

When missing values from one of the three backgrounds occurred, it was dealt with by using the average value from that specific background the remaining four days of the period. There was one of the four cows that sometimes got suspiciously low values on enteric methane production. One conceivable reason to the low values is leaking from the fistula, this specific cow did show more leakage from her fistula than did any of the other cows in the study. No previous studies have been found on the correlation of leaking fistulas and enteric methane production, so this is only my guessing.

Conclusions

It could not be concluded, as was the hypothesis, that a diet with high content of pea/oat silage decreases the amount of produced enteric methane compared to a diet with grass silage. Neither were there any signs that a lower enteric methane production is followed by a high or maintained production of methane from the faeces and urine as suspected. Diets with pea/oat silage contains as expected significant higher amounts of starch than diets with grass silage. It was however not shown that a diet with high content of pea/oat silage generates less methane per kg of ingested starch than a diet with grass silage. Further studies need to be conducted and to be able to receive results of a higher significance a larger number of animals need to be included in the study and the difference in starch content has to be larger.

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Appendix 1

Table 8. Adjustment of the silage ratio depending on residues

Residues	**Ratio**
No residues	Increase ratio with 3.0 kg/day
0.1-3.0 kg residues	Unchanged ratio
3.1-6.0 kg residues	Decrease ratio with 2.0 kg/day
More than 6.0 kg residues	Decrease ratio with 4.0 kg/day

Table 9. Average daily DMI in kg per cow and period

Cow ID \ **Period**		**1**	**2**	**3**	**4**
1328	Mean	22.0	24.5	26.2	25.0
	StDev	0.42	0.42	2.01	1.38
	Min	21.3	24.0	23.3	23.4
	Max	22.4	25.0	28.3	26.7
1379	Mean	20.7	20.1	21.5	22.3
	StDev	1.04	1.69	0.90	2.12
	Min	19.9	17.2	20.3	19.3
	Max	22.2	21.4	22.5	24.3
1381	Mean	21.6	22.8	23.3	23.8
	StDev	1.51	1.91	1.56	1.62
	Min	19.2	19.6	22.0	21.3
	Max	23.3	24.5	25.9	25.6
1403	Mean	21.3	20.2	26.6	19.9
	StDev	1.57	1.46	1.63	1.70
	Min	19.3	18.3	24.3	17.2
	Max	23.3	21.9	28.3	21.8

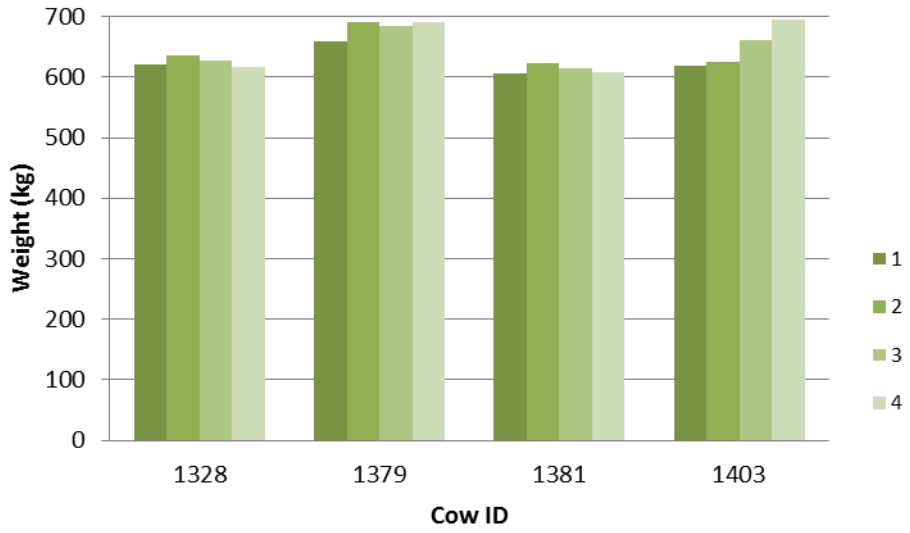

Figure 12. Individual animal weights in kg per period.

Table 10. Milk yield and energy corrected milk (ECM) per cow and period

Cow ID \ Period		1	2	3	4
1328	kg milk	38.7	32.1	29.1	30.8
	kg ECM	36.1	36.0	30.6	32.7
1379	kg milk	30.5	24.5	21.2	20.7
	kg ECM	32.3	26.1	24.5	23.1
1381	kg milk	29.5	30.6	29.9	26.6
	kg ECM	32.0	33.3	30.5	31.1
1403	kg milk	23.8	19.7	16.7	4.7
	kg ECM	24.3	22.7	19.1	6.1

Table 11. Enteric CH_4 (g/day), mean for cow and period

Cow ID \ Period	1	2	3	4	Overall mean
1328	240	681	817	714	613
1379	517	567	521	486	523
1381	591	687	608	804	673
1403	391	614	569	658	558

Table 12. Enteric CH_4 (g/day), mean for treatment and period

Period	Treatment	CH_4 (g/day)	StDev	n
1	A	307	122.0	9
	B	550	174.4	9
2	A	601	180.8	7
	B	652	160.8	9
3	A	580	113.4	7
	B	685	199.7	9
4	A	584	184.3	7
	B	750	183.9	8
Overall mean	A	504	194.4	30
	B	657	186.6	35

Tabell 13. Results from DM and ash analyses for FU and U

ID	Crucible	Tare (g)	Weigh-in (g)	After drying (g)	After burning (g)	DM %	Mean DM %	Ash %	Mean Ash %
1403 (FU)	2f	17.876	7.020	18.782	18.048	12.91	12.94	2.45	2.46
	580	22.444	6.999	23.351	22.616	12.96		2.46	
1328 (FU)	3f	18.543	8.651	19.381	18.697	9.69	9.70	1.78	1.76
	553	21.260	6.013	21.843	21.364	9.70		1.73	
1379 (FU)	526	21.894	7.691	22.768	22.030	11.36	11.16	1.77	1.76
	444	17.997	8.815	18.962	18.151	10.95		1.75	
1381 (FU)	508	21.934	6.283	22.547	22.009	9.76	9.81	.	1.67
	561	21.809	6.414	22.441	21.916	9.85		1.67	
1328 (U)	8	43.072	20.230	42.980	43.592	4.49	4.52	2.57	2.58
	10	47.858	20.368	48.782	48.384	4.54		2.58	
1379 (U)	13	44.296	21.048	45.618	44.861	6.28	6.28	2.68	2.64
	14	43.494	20.244	44.766	44.019	6.28		2.59	

Tabell 14. Results from N content analyses of FU

ID	Tube	Results (%)	Mean (%)
1403	8	0.71	0.69
	9	0.68	
	10	0.68	
1328	11	0.42	0.42
	12	0.42	
	13	0.42	
1379	14	0.52	0.52
	15	0.52	
	16	0.52	
1381	17	0.48	0.48
	18	0.48	
	19	0.48	