UNIVERSITY OF TROMSØ UiT

FACULTY OF BIOSCIENCES, FISHERIES AND ECONOMICS
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Methane emissions from reindeer

Do reindeer fed lichens emit less methane than reindeer on a pelleted feed diet?

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Cover picture: Female reindeer and calf fed lichens at the animal research facilities at the Arctic Biology building, Department of Arctic and Marine Biology, The University of Tromsø.

Photo: Kia Krarup Hansen

PREFACE AND ACKNOWLEDGEMENTS

This Master project is part of the project *New knowledge on methane emission from reindeer and increased local competence in the North* funded by the Reindeer Husbandry Development Fund (Sak 14/10, A5349), and linked to the framework of the International Polar Year as a part of the IPY consortium IPY # 399 EALÁT: *Climate change and reindeer husbandry* and the IPY legacy UArtic EALÀT Institute.

To seek knowledge I participated and presented a poster and a speed-talk at the 16th Nordic Conference on Reindeer and Reindeer Husbandry Research November 16th-18th 2010 (Sundset *et al.* 2010a). I have further participated in the PhD training courses *People in a changing world*, provided by Uarctic Thematic Network held in 22. – 26. March 2011 at Sámi University College, Diehtosiida, Kautokeino, Norway, and the *UArctic EALAT Institute EALLIN Workshop in Sakha Republic (Yakutia)*, Russia, March 2012, both focusing on knowledge, changes and challenges in Arctic societies. At the UArctic course *Adaptation to Globalisation in the Arctic, The Case of Reindeer Husbandry* (University of Oulu, Finland) I studied the *Vulnerability and adaptive capacity of a coastal Reindeer Pasture District in Northern Norway* (Hansen and Mathiesen 2011).

To gain knowledge on different methods for measuring methane emissions from ruminants, I participated in the workshop *Global Research Inventory*, on agricultural greenhouse gas measurement methodologies and techniques, in Reading, Great Britain, October 2011 (Hansen *et al*. 2011). I also visited the Norwegian University of Life Science, Ås, and Aarhus University, Foulum. I thank Prof. Odd-Magne Harstad and Prof. Peter Lund for their contribution and demonstration of techniques during these meetings.

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This master project was conducted under supervision of Prof. Monica A. Sundset (UiT), Prof. Lars P. Folkow (UiT) and Prof. Svein D. Mathiesen (Norwegian School of Veterinary Science, Tromsø and International Centre for Reindeer Husbandry). I thank them for their helpful guidance.

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1. ABSTRACT

Microbial fermentation in the world domesticated ruminant populations is thought to be responsible for as much as 13.5-33% of the global anthropogenic methane (CH_4) emissions (World Resources Institute 2005, EPA 2004, Bodas *et al.*, 2012). However, methane also represents a loss of 2-15% of the gross energy intake in these animals (Blaxter and Clapperton 1965, Holter and Young 1992, Johnson and Ward 1996). The objective of this current project was to study the effect on methane emissions from female reindeer (*Rangifer tarandus tarandus*) fed a mixed lichen diet (dominated by *Cladonia stellaris*) compared to a grass based commercially available pelleted feed (RF from Felleskjøpet, Norway). The pellets contained high concentrations of protein (12.7% of dry matter (DM)) and water-soluble carbohydrates (5.9% of DM) compared to lichens (< 2.6 and 0.1% of DM respectively), while the lichen diet contained as much as 78.7% of DM hemicellulose. Total methane emissions were recorded during 23 hours, twice per animal on the two different diets, using an open-circuit respiration chamber. Feed (~0.440 kg DM) was presented to the animal two hours after initiating the recording in the chamber. The reindeer (n=5) emitted 11.1 ± 1.0 g CH_4 / animal / day when fed pellets, while their mean average methane emission was significant lower (P = 0.0009) when fed the lichen diet (7.3 ± 1.6 g CH_4 / animal / day), given the same amount of DM. The amount of gross energy intake lost in the form of methane from reindeer on a restricted diet was 7.6 ± 1.0% fed pellets and 5.1± 1.6% feeding a lichen diet (P = 0.0002). This study suggests that intake of lichens depress methane emissions in reindeer. The implications of these findings are discussed, and data on methane emissions from reindeer compared to those reported in other ruminants.

2. INTRODUCTION

2.1. Methane emissions

Greenhouse gases in the atmosphere, that absorbs and emits radiation, are essential for life on earth, as they keep the surface of the Earth warm (Moss 1993). The increase in average temperature at the surface of the Earth, known as global warming, is caused by an increase in the concentrations of the greenhouse gases, primary water vapour, carbon dioxide (CO_2), nitrous oxide, ozone and methane. Methane is released from anthropogenic sources induced by

human demands, such as fossil fuel mining and burning, coal mining, oil and gas drilling, rice production, landfills, large scale burning of forest and grassland, waste disposal, as well as from microbial fermentation in domesticated ruminants. But also from natural sources which have always occurred, including wetlands, permafrost, oceans and lakes, termites, wildfires, from hydrates and microbial fermentation in wild ruminants (Solomon *et al.* 2007, IPCC 2007, Kvenvolden 2002). Since pre-industrial times the atmospheric concentrations of methane have doubled (Bolle *et al.* 1986).

Methane absorbs solar radiation in a broad spectrum of light, not competing with other gases in its absorption range. It has 21 times the global warming potential of CO_2 (UNFCCC 2006). Since the atmospheric lifetime of methane is much shorter (12 years) than the lifetime of CO_2, which can stay in the atmosphere for centuries (Solomon *et al.* 2007, IPCC 2007), reducing methane emission is seen as a good option for achieving a short-term solution to global warming (Moss 1993). The United Nations Framework Convention on Climate Change from 1992, and the Kyoto Protocol from 2005, therefore encourage and bind signing countries to reduce their greenhouse gas emissions (UNFCCC 2010), to avoid the Earth being overheated. Increased global temperature will have many implications, especially in the Arctic where the warming is expected to be strongest (ACIA 2005, Solomon *et al.* 2007).

The Norwegian Government has argued to reduce the number of semi-domesticated reindeer in Norway with 30,000 animals due to methane emissions as a contribution to adaptation to global warming (Landbruks- og Matdepartmentet 2009). But apart from a small pilot project published as a conference abstract by Gotaas and Tyler (1994), no data are currently available on methane emissions from reindeer, and little is known about the methanogenic archaea in the reindeer rumen and what factors influence their diversity, density and methanogenesis (Sundset *et al.* 2009a, 2009b).

2.2. Reindeer husbandry

Reindeer are arctic herbivores that have been domesticated mainly in the way of herding for as long as for 2000 years (Federova 2003). In contrast to cattle and sheep, reindeer still free-range all year around on natural pastures. Semi-domesticated reindeer comprise a very small population of ruminants, only ~2 million animals. Including the wild reindeer, the world reindeer population accounts for about 5-6 million animals (Williams and Douglas 1989, Turi 2002). Approximately 40% of the mainland Norway is used as pastures for about 250,000

reindeer (Reindriftsforvaltningen 2009). Due to the great seasonal climate variations found in the Arctic, most reindeer migrate throughout the year. In Norway reindeer are herded in a nomadic system by the Sámi, often between lush coastal summer pastures, where the snow-melt is early, and dry lichen-rich winter pastures at the inland (Skjenneberg and Slagvold 1968, Steen 1968). The metabolic demand and appetite of reindeer varies with photoperiod, being high in summer (August) and reduced to 1/3 in winter (March) (Larsen *et al.* 1985). Reindeer are intermediate adaptable feeders selecting a mixed diet, avoiding fibres as much as possible (Kay *et al.* 1980, Hoffman 1985). In early summer, reindeer feed on young, soft green plants, and selects the emerging growth (Warenberg *et al.* 1997, Staaland and Sæbø 1993, Norberg *et al.* 2001). Through the summer, as the biomass increase, they feed on many different species of grasses, sedges, shrubs, herbs and some deciduous trees. Mushrooms and grasses are part of their early autumn diet (Mathiesen *et al.* 2000, Warenberg *et al.* 1997, Norberg *et al.* 2001). In the long and dark winter, when the ground is covered by snow and the vegetation is of low nutritive quality and biomass (Klein 1990), the reindeer eat a combination of graminoids, shrubs, mosses and lichen (Mathiesen *et al.* 1999, Mathiesen *et al.* 2000, Storeheier 2002a, 2002b). Reindeer are unique because they prefer lichens, high in digestibility and energy content, as part of their winter diet (Norberg *et al.* 2001, Mathiesen *et al.* 2005). Lichens are symbiotic organisms consisting of a fungus, and a photobiont (a cyanobacteria or/and a green algae). The fungus provides protection, minerals and water, while the photobiont is capable of photosynthesis contributing with carbohydrates. Because lichens are poikilohydric, they can tolerate extreme desiccation, and by cryptobiosis (a state were the cells dehydrate to a degree that halts most biochemical activity) they can survive the arctic winter (Brodo *et al.* 2001). But reindeer cannot rely on lichens alone, because the protein and mineral contents in lichens are low (Pulliainen 1971, Nieminen and Heiskari 1989, Storeheier 2002a).

Projected climate change for the Arctic, indicate increasing temperatures and more precipitation (Benestad 2008), with milder and unpredictable winters, greater snowfall and increased frequency of ice-locked pastures (Schuler *et al.* 2010, Putkonen and Roe 2003, Tyler *at al.* 2007). To some extent, the search for food in deep snow cover is strenuous, and therefore represents an energy loss for the animal (Fancy and White 1987, Eira 2012, Eira *et al.* 2012). When looking for adaptation strategies to limit methane emissions from reindeer in relation to e.g. feed and operation, one should bear in mind, apart from scientific findings, the importance of traditional knowledge for a future sustainable reindeer husbandry (Eira *et al.* 2012, Eira

2012). To cope with the consequences of problematic winter condition, which are crucial for the reindeer survival and production, supplementary feeding have increased in the Sámi reindeer husbandry (Hansen and Mathiesen 2011, Tyler *et al.* 2007). In the past lichen has been provided as supplementary feed, but gathering of lichen is time-consuming. The main provided feed today is round bale silage (Nilsen 2011), while the use of commercially produced grass-based pelleted feed (pellets) is increasing (Hamnes 2007). Increased temperatures during spring, summer and autumn would lead to longer growth season (Tømmervik *et al.* 2004, 2005), and changes in vegetation composition (Woodward 1987).

2.3. Rumen methanogenesis

As ruminants, reindeer rely on the symbiotic relationship with microorganisms in their anaerobic rumen and hindgut (Mathiesen *et al.* 2005). The symbiotic microbes, being bacteria, ciliate protozoa, anaerobic fungi and methanogenic archaea, hydrolyse plant polysaccharides to monomers, which are further converted into volatile fatty acids (VFAs), mainly acetic, butyric and propionic acid, and absorbed to meet the animals energy requirements (McDonald *el. al.* 1995, Hobson 1997). Additional end products of the rumen fermentation are hydrogen (H_2) and CO_2, which are combined by methanogens of the domain Archaea to form methane (CH_4) and water (H_2O) (Hungate 1967) in this anaerobic system. Microbial fermentation in the rumen, where 90% of the methane is produced (Johnson *et al.* 2000), is illustrated on Figure 1. The formatted methane cannot be utilised by the animal, and it is hence mostly eructated (up to 98%). Small amounts are emitted as intestinal gas or absorbed in the blood and exhaled through the lungs (Dougherty *et al.* 1965, Johnson *et al.* 2000). The place of emission varies with animals, and digestive systems. In sheep, 80-90% of the formatted methane is excreted via the lungs, whereas all hindgut-methane from the forestomach fermentating kangaroo are lost though anus (Kempton *et al.* 1976). The formation of methane is consequently a loss of energy to the animal, accounting for 2-15% of an animal's gross energy intake (Blaxter and Clapperton 1965, Holter and Young 1992, Johnson and Ward 1996). CO_2 formation in the rumen is simply a release of absorbed CO_2 in the feed, whereas the anaerobic conversion into methane, results in an overall contribution to the greenhouse effect.

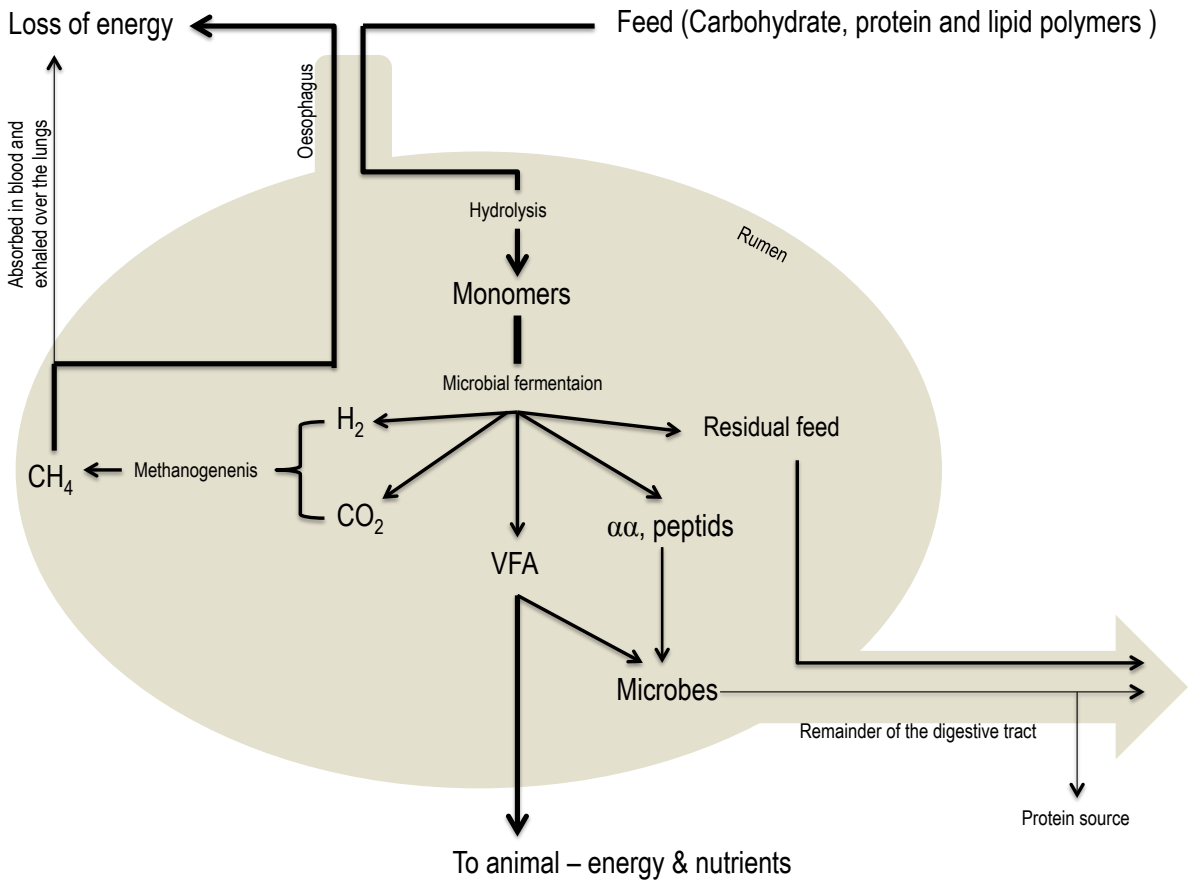

Figure 1. Schematic illustration (modified from Buddle *et al.* 2010; Moss *et al.* 2000) of the hydrolysis and microbial fermentation of carbohydrates, proteins and lipids in the rumen. Ingested polymers hydrolysed to monomers and further though acidogenesis and acetogenesis into amino acids (αα), peptids, volatile fatty acids (VFA) mainly acetic, propionic and butyric acids, releasing hydrogen (H_2) and methane (CH_4). The VFAs are mostly absorbed across the rumen wall and thereby act as a source of energy for the animal. Microbes use energy from VFA for microbial growth from amino acids. Methanogens use CO_2 to reduce the H_2 to form methane ($CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$), which is mostly eructated though the oesophagus, and contribute as a loss of energy for the animal. Methane can also be formed from formate, methanol, methylamines, carbon monoxide or from acetate though acetoclastic methanogenensis (not shown on the figure). Residual feed and rumen microbes enter the remainder of the digestive tract (abomasum, small- and large intestine) for further degradation. The microbial protein forms a significant protein/nitrogen source for the ruminant. The reticulum and omasum are not shown.

Rumen protozoa have also been shown to play a role in methane production (Vogels *et al.* 1980), maybe because the hydrophobic methanogens often live in symbiotic relationship with protozoa (Boadi *et al.* 2004). The methane production is further affected by animal species, age, rumen pH, VFAs, the quantity and quality of diet, feeding strategy, body weight, digestive efficiency, environmental stresses and exercise (Argyle and Baldwin 1988, Beauchemin and McGinn 2005, Boadi and Wittenberg 2002, Grainger *et al.* 2007, Paustian 2006, Steinfeld 2006,

Swainson *et al.* 2007). With-in animal variation in methane production from day-to-day, have also been reported (Blaxter and Clapperton 1965). Studies have shown a direct relationship between the number of methanogens in the rumen and the production of methane (Denman *et al.* 2007). For this reason many attempts including animal-, rumen- or dietary manipulation, have been done to inhibit the growth of methanogens (Eckard *et al.* 2010 and Martin *et al.* 2010).

Previous studies report low ruminal concentrations of methanogens in reindeer compared to domesticated ruminants such as cattle and sheep (Sundset *et al.* 2009a, 2009b, Mathiesen *et al.* 2000). Rumen methanogens can be determined in numbers and diversity through a16S rRNA gene library approach, using primers targeting the *mcrA* gene, only found in methanogenic archaea. Studies of the dominant rumen methanogens in both Svalbard and Norwegian reindeer indicate little variation in the diversity of rumen methanogens between reindeer and other geographically and/or genetically distant ruminants. However, numbers of rumen methanogens are generally lower in Svalbard reindeer ($5.16x10^7$ cells/g in April, $3.02x10^8$ cells/g in October) and Norwegian reindeer ($4.01x10^8$ cells/g in September) (Sunset *et al.* 2009b), than those found in e.g. cattle ($2.9x10^8$-$9.8x10^{10}$ cells/g) (Hook *et al.* 2009, Denman *et al.* 2007, Evans *et al.* 2009). The reindeer rumen microbiota is affected by season and forage/feed chemistry (Mathiesen *et al.* 2000, Aagnes *et al.*, 1995; Olsen and Mathiesen 1998; Sundset *et al.*, 2007), and the amount of methane produced through rumen microbial digestion is consequently also expected to vary with season and dietary changes in these animals. Low densities of rumen methanogens observed in reindeer (Sundset *et al.* 2009b), suggest that reindeer may produce and emit less methane compared to other ruminants such as sheep and cattle. Depressed numbers of rumen methanogens in Svalbard reindeer during winter, when access to food is poor, could be explained as an adaptation to reduce energy loss in favour of survival during harsh arctic winters (Sundset *et al.* 2009b). Lichens are well known for their ability to accumulate secondary metabolites such as the phenolic usnic acid with antimicrobial properties (Sundset *et al.* 2008; 2010b). Previous studies have shown that plant secondary metabolites may have direct toxic effect on the methanogens (e.g. phenolic tannins) or protozoa (e.g. saponins) in other ruminants studied and consequently reduce methane emissions (Bodas *et al.* 2012).

2.4. Aim of the study

The main objective of this MSc project was to study the effect of lichens compared to a grass based pelleted diet on methane emissions from reindeer using the open-circuit respiration chamber technique. The comparative data set from this current study of reindeer are compared with data and findings on methane emissions from other ruminants, to discuss if lichens reduce methane emissions in reindeer. The implications of this study, for the digestive physiology of reindeer and for the environmental ecology of reindeer/reindeer husbandry are discussed.

3. MATERIALS AND METHODS

Measurements were performed January - May 2012, at Department of Arctic and Marine Biology, The University of Tromsø, Norway.

3.1. Experimental animals

Five female semi-domesticated reindeer (*Rangifer tarandus tarandus*) at the age of 1 ½-2 years were used for the experiments. Today, reindeer owners mostly slaughter male calves or bulls, and then consequently male reindeer comprise less than 10% of the herd (Nilsen 1998, Reindriftsforvaltningen 2002). This means that the methane emissions from female reindeer have the greatest impact on the overall emission from reindeer in Norway, and for this reason experiments were conducted on female reindeer. The number of experimental animals chosen were minimalized to a degree that secured valid data, on the background of animal variations.

The reindeer were taken from their herd in Tønsvik outside of Tromsø in northern Norway (69°N, 19°E) at the age of 6 months by the former owner (Mauken/Tromsdalen Reindeer Pasture District), and then transported to the Department of Arctic and Marine Biology, The University of Tromsø, Norway. At arrival the reindeer were investigated by a veterinarian, given parasite treatment (10ml Ivomec Oract) and earmarked with the numbers 9, 10, 11, 12 and 13/10 (the year of arrival to the department). The reason for using animals grown up on natural pastures was to achieve as natural rumen microbiome as possible.

All experiments were approved by the Norwegian Animal Research Authority (permit no. 3524 and 4003), and performed in approved animal research facilities at Department of Arctic and Marine Biology, University of Tromsø. The reindeer were held together under a natural-

like photoperiod, with *ad libitum* access to feed (pellets or lichens) and water, to ensure animal welfare and to achieve as plausible and natural results as possible. Their body mass was frequently measured and their surroundings were cleaned daily. As human handling and measuring devices are a part of the experiment, training and habituation was necessary, to avoid useless measurements due to stress during the methane emission studies.

3.2. Experimental setup

Methane emissions from our animals were measured in an open-circuit respiration chamber, as a comparative evaluation of the different techniques revealed that this standard technique still is the most suitable and accurate method to determine methane emission from individual animals (Blaxter 1962, O´Hare *et al.* 2003, Hansen 2010). This experimental set-up provides the possibility to control variables, in contrast to field experiments, allowing the investigation of the effects of a specific manipulation, namely the diet in this current study.

Alternative methods like a ventilated hood/facemask or a closed chamber, were not chosen because they respectively do not measure hindgut methane emission (Liang *et al.* 1989, Suzuki *et al.* 2007) and can lead to hypoxia (Frappell 1989). Johnson *et al.* (2000) also underlines the importance of determining the emissions from individual animals before large-scale measurements from many animals together.

The experimental setup is shown in Figure 2. The open-circuit respiration chamber setup used in this current project, consisted of an aluminium box (1.3 m^3) with a transparent front. The box was situated inside a climatic chamber, to maintain stable temperature. The climatic chamber was set at -3° C, but due to the metabolism of the animal the temperature inside the box was some degrees higher. This temperature was chosen because it is within the thermo neutral zone of reindeer during winter (Nilssen *et al.* 1984). The air from the inside of the box (from now on referred to as *outlet air*), was withdrawn through a tube, the by a negative pressure air pump providing an airflow of 120-140 l/min. Small openings at the bottom of the box allowed fresh air (*inlet air*) into the box. A fan circulated the air inside the box, preventing the respiration air of the animal to leave through the air inlet openings. The flow of the *outlet air* was measured by a mass flow meter (type G-40, Elster A/G Mainz, Stuttgart, Germany). The flow meter was checked against a Singer DTM-325 volumeter that had been controlled by running air from a spirometer through it. *Inlet- and outlet air* gas samples were analysed by an

oxygen analyser (3-SA Oxygen analyser, Applied Electrochemistry Inc., Sunnyvale, CA, USA) and a Binos-100 methane analyser (Rosemount, Germany). The analysers received air via a small pump (Thomas industries inc., Powerair division, Sheboygan wisc. USA) and drying agents (Calciumchlorid, Merck KGaA, Darmstadt, Germany) that removed water vapour from the gas. The drying agents were changed every second day of experiment. Every hour the gas concentrations (CH_4, O_2) of the *inlet air* were measured, to allow comparison of the composition of *inlet and outlet* gas, for the purpose of quantifying methane production of the animal and to assure that the box was always properly ventilated. Before each experiment, the methane analyser was calibrated, using an certified AGA 99.99% pure nitrogen gas as a zero (baseline) and 997 mol-ppm (0,0997%) CH_4 in nitrogen gas as a standard/reference gas. While the oxygen analyser was calibrated against ambient air (20.95% O_2) before experiments and then checked manometrically at intervals. Gas temperature and water vapour content of the *outlet air* were recorded by a thermo- and hygrometer (Vaisala HMI 32), for use in standard temperature and pressure dry (STPD) corrections. All the analogue signals were digitized by an A/D converter (PowerLab/16sp, ADInstruments Pty ltd, Castle Hill, Australia), and then fed into at a computer using the programme Chart v5 for windows (PowerLab, ADInstruments). A video camera was installed, to permit continuous surveillance of the animal.

The use of the open-circuit chamber required intensive work. The experimental animals all had to be adapted to the equipment and the situation in advance of the experiments. The training included leading the reindeer, letting them get used to new noises, people and seeing/feeling the box. Compared to other domesticated ruminants like cows, which are normally born inside a barn and see people every day, it takes longer time to train semi-domesticated reindeer born in the open landscape not used to seeing people. But with patience and people skilled to handle reindeer, it was possible to get the reindeer totally calm inside the box. About a year of training was invested in the animals prior to the experiments. The equipment and experimental design were tested during the autumn 2011, while training the reindeer for the coming experiments. In that way challenges were overcome, and the best possible design revealed.

Some chamber experiments measure methane emissions from more than one animal at the same time, to minimize stress by separating animals used to live in herds (Pinares-Patiño *et al.* 2008, Storm *et al.* 2012). During training, we initially kept two animals in individual boxes facing each other inside the climate chamber, but this did not seem to calm the reindeer. With

some animals it actually had the opposite effect. Additionally animals inside the chamber would affect the background levels of methane and oxygen, hereby complicating analyses of gas concentrations. For those reasons all experiments were carried out with only one animal inside the chamber at a time.

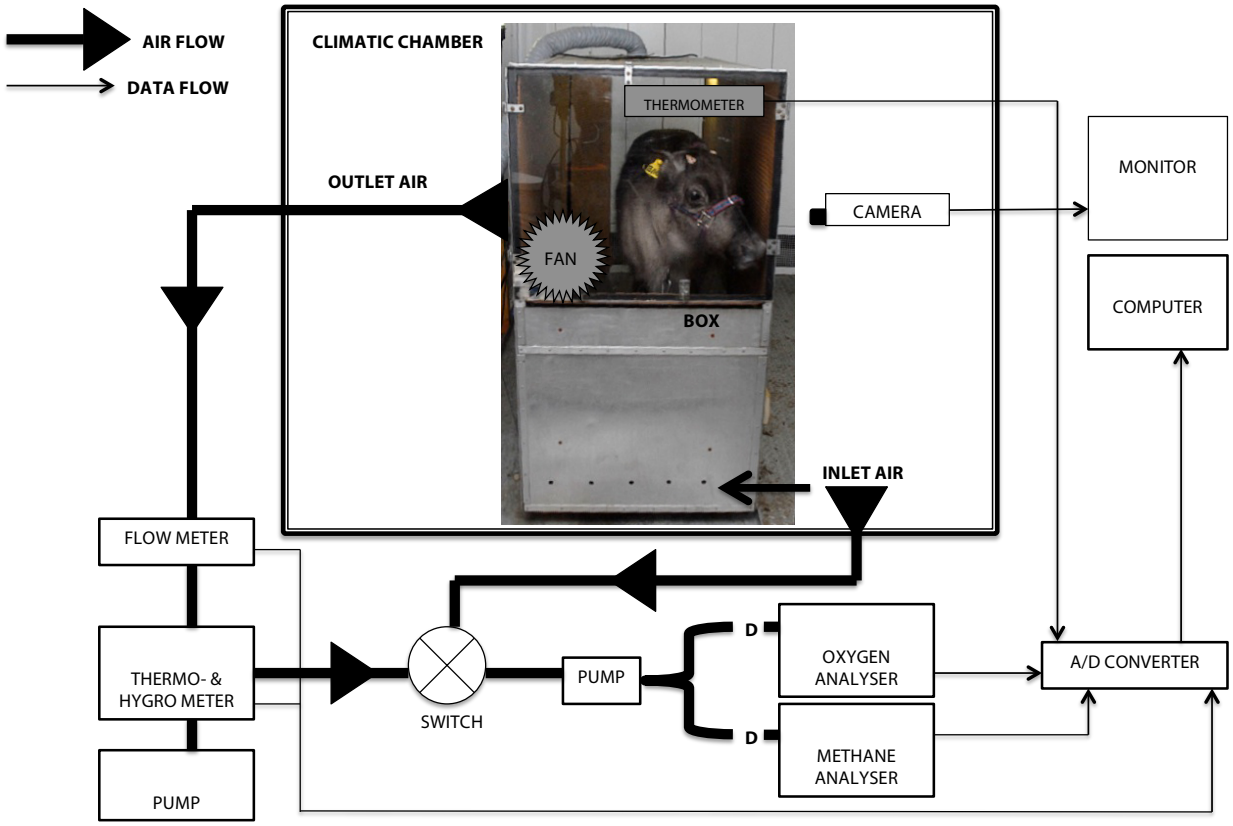

Figure 2. Diagram showing the open-circuit respiration chamber (the box) used to measure methane emissions from reindeer. Concentrations of CH_4, O_2, temperature, humidity and flow rate from the air pulled from the chamber by a pump are monitored continuously, and stored at a computer. "D" is drying agents, which dry the air before it is analysed by the analysers. The "switch" is used to change between analysing the *outlet* and *the inlet air*. Fresh air is let into the box though small holes (arrow). The box holding the reindeer is placed in a climatic chamber to cool the temperature within the thermo neutral zone of a reindeer. A camera was used to monitor the reindeer. Relative sizes of the objects are not to scale.

3.3. Experimental design

Comparative experiments were performed on reindeer fed grass-based pellets, containing 23.3% oats, 18.9% timothy grass, 16.0% wheat bran and 11.2% barley (Felleskjøpet, Norway) and reindeer fed mixed lichens (mainly *Cladonia stellaris*), picked in Østerdalen Southern

Norway. Because experiments on sheep and cows have shown a relationship between methane emission and feed quantity (Murray *et al.* 1999, Gao *et al.* 2011), we wanted to control the amount of the food given to the reindeer in this experiment carefully. During the training of the animals, we observed that some of our reindeer lowered their feed intake while in the respiration chamber. To avoid problems with variable food intake between experiments, we decided to give the animals a limited amount of ~0.440 kg DM feed during each experiment. This amount of feed, kept the reindeer motivated to eat the whole batch during all experiments. It should be kept in mind that the feed intake of reindeer under natural conditions is much reduced in winter (Larsen *et al.* 1985). Prior to the experiments on pellets, the reindeer were given *ad libitum* access to pellets in a 4 weeks habituation period to allow both the digestive tract and the gut microflora time to adapt (Storeheier 2003, Aagnes Utsi 1998). The same procedure was repeated on the lichen diet. The last week before the measurements the reindeer were given the same restricted diet as giving during the experiment, adapting the animals to the experimental feed rations. The water content of the lichens was estimated prior the experiment, to calculate the amount WW corresponding to 0.5 kg wet weight pellets. The feed was kept at minus 20 degrees until 2 hours before feeding, and in plastic bags to avoid evaporation.

In order to obtain the best possible estimate of the daily methane emissions on each specific diet and daily ratio, we tried to conduct measurements over a full 24 h cycle. However, to be able to start a new experiment the day after at the same time, we ran measurements for 23 hours (the 24^{th} hour was estimated, see calculations for further explanation). Test experiments also showed a direct response in methane emission to feeding, and that it took more than 12 hours for methane emissions to return back to baseline (background levels before feed, as illustrated on Figure 3).

Methane emissions were determined for each animal twice on each diet, to compensate for day-to-day variation. The climatic chamber was always cooled for at least 1 hour prior to the experiments, to reach the required temperature. The animal was kept in the box for 1 hour before the registration started, so that the animal, the equipment and the air inside the box were stable. Lights were on during all the experiment, to monitor the animal. The animal body mass (BM) was determined before and after each experiments. All experiments started at 9.10 a.m.

Concentrations of CH_4 and O_2 of the *outlet air* were recorded for 50 minutes/hour, followed by 10 minutes recording of *inlet air* concentrations, to determine background gas levels. The average gas concentration was logged every 30 seconds.

The animal was fed 2 hours into the experiments, to keep their normal routine, and to have a stable measurement on baseline methane levels prior to feeding. Water was given about 6 hours into the experiment, to avoid spill of food due to mixing with water.

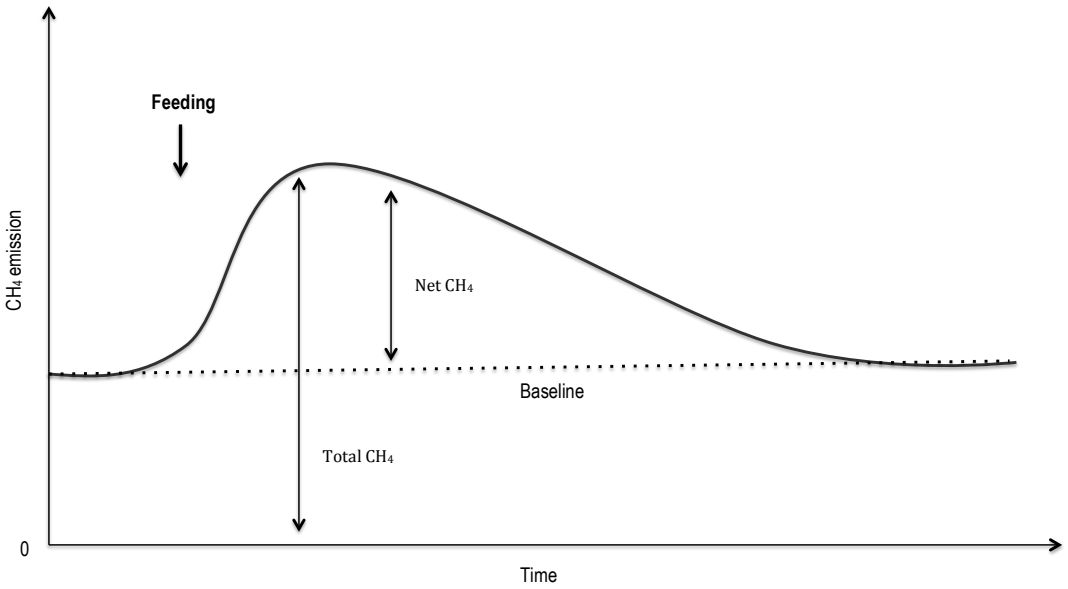

Figure 3. Schematic drawing of total (Total CH_4) and net methane emission (Net CH_4) from a reindeer, showing the baseline methane emission before feeding (dotted line) and the effect of feeding on methane emission. Emissions and time are not scaled in this illustration.

3.4. Calculations

The mean hourly differences in gas concentrations (CH_4, O_2 in %) between *outlet* and *inlet air* were determined, from the respectively 50 minutes of *outlet air* registration, and the 2 x 10 minutes of *inlet air* registration before and after each *outlet air* registration. Between the changeover (by the switch at Figure 2) of the analysers from *outlet air* to *inlet air*, it took some 2-5 minutes before the gas in the tubes were equilibrated. Therefore only the last 45 minutes of *outlet air* registration and the last 5 minutes of *inlet air* registration were used for hourly emission/consumption calculations as shown on Figure 4.

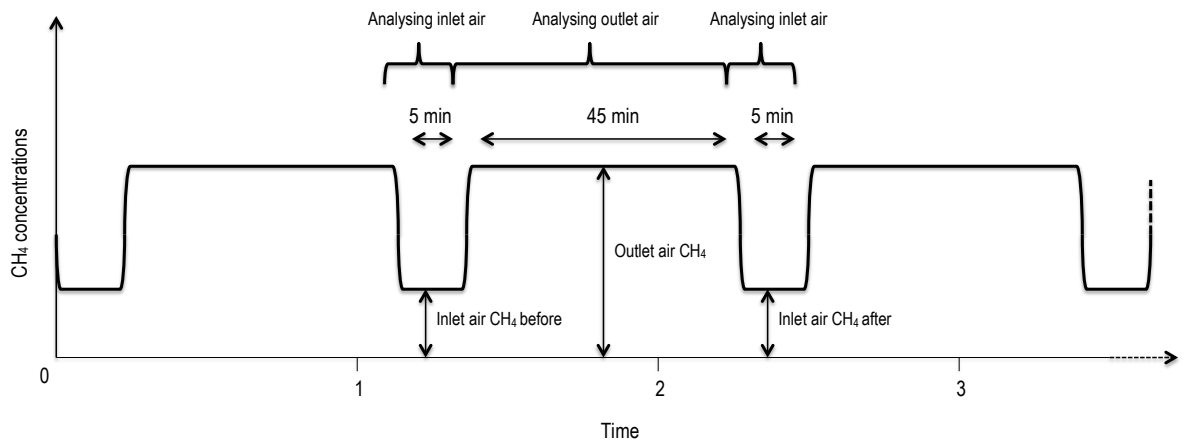

Figure 4. Schematic illustration of registration of methane concentration from an open-circuit respiration chamber experiment, for the calculation of methane emission from reindeer. A methane analyser recorded the methane concentration of the *inlet air* (*inlet air CH4*) for 10 minutes, and then subsequently switched to record the methane concentration of the *outlet air* (*outlet air CH4*) for the following 50 minutes, repetitively for 23 hours. However, to allow equilibration of the methane in the tubes the mean hourly methane emissions were calculated from the last 5 min of the *Inlet air CH4*, and the last 45 minutes of the *Outlet Air CH4* by following question: Δ CH4 % = *Outlet air CH4* % – ((*Inlet air CH4 % before* + *Inlet air CH4 % after*) /2).

The *outlet air CH4* minus the *inlet air CH4* (Figure 4) then represents the percentage methane emitted by the animal (Δ CH4 %). The hourly STPD corrected volume of methane emitted (V_{CH4} (l/hour)) was calculated as following:

$V_{CH4} = \Delta\%\ CH_4 \times 0.01 \times 60\ \text{min/hour} \times \text{Flow}_{STPD}$

The Flow_{STPD} (l/min) is the flow given at standard temperature T (273.15 °K) and pressure P (760 mmHg) registered by the mass flow meter minus the vapour content of the gas, calculated based on the empiric Magnus equation (Koutsoyiannis 2012):

$\text{Flow}_{STPD} = \text{Flow} - (\text{Flow} \times ((RH/100) \times 4.581 \times (10\text{^}((7.5 \times Tgas)/(238+Tgas))))/P)$

Tgas (°C) are the temperature of the measured gas, also registered.

The hourly mean methane emitted in gram (m_{CH4} (g/hour)) was calculated as following equation:

$m_{CH4} = (P \times V_{CH4} \times M) / (R \times T)$

Where M is the molecular weight of CH_4 ($M_H = 1.0079$; $M_C = 12{,}011$) and R = 0.0820574587 L atm K^{-1} mol^{-1} is the gas constant; T and P as above.

Because the animal was kept inside the box 1 hour before each registration, and since the methane level were stable before feeding, we chose to run the experiment for 23 hours, and estimated the last 24th hour as a mean of the 1st and the 23th hour. The total methane emission per day was calculated by summing the 23 mean hourly methane emission (CH_4 (l/hour)), and the 24th estimated methane emission. To see the effect of feeding, this period was divided in before and after feeding.

Gross energy lost as methane was determined by multiplying the total daily methane emission ($V_{CH4, TOT}$; l/day) by the energy content of methane (EC_{CH4} = 39.45 kJ/L, Brouwer 1965, Suzuki *et al.* 2007). The methane emissions in % of gross energy intake (GEI) is then:

CH_4 in % of GEI = (($V_{CH4, TOT} \times EC_{CH4}$) / GEI) x 100

Where GEI is equal to the product of the energy density (Calorimetric heating value in kJ/g; Table 1) of the feed and the daily dry matter intake of the feed (DMI (g/day): that is the ~0.440 kg of pellets or lichen that was offered the reindeer each day. In that way, to be able to compare the emissions from reindeer fed lichen with reindeer fed pellets and to quantify the amount of energy lost in form of methane.

The oxygen consumption (V_{O2}) was calculated in the same way as the methane emissions, based on the difference in O_2 concentration of the air let in to the box (*inlet air*) and the O_2 concentration of the air pulled from the animal box (*outlet air*). V_{O2} was not adjusted for possible changes in the respiratory quotient (RQ = $CO_{2\ eliminated}$ / $O_{2\ consumed}$), since the CO_2-analyser was not functioning properly. This was not considered to be critical, since determination of the oxygen uptake rate/metabolism of the animals was not the focus of the present study.

3.5. Chemical analyses

Prior each experiment representative samples of the feed given, were taken, and stored for further analyse. To estimate the feed intake in dry matter weight (DM), a sample of each of the given diets was dried, as following. Blue silica gel (Chemi-Teknik AS, Oslo, Norway) was dried in an incubator at 100°C for one day, and afterwards places in a closed container, to create a dry environment. Beakers to contain the samples was then dried in the oven for one day, and later cooled in the containers holding silica gel. The frozen samples (10 containing pellets and 9 containing lichens) were thawed. Breakers and samples was weight before (wet weight, WW) they was dried initially at 60 °, then at 110 °C, until completely dry (dry weight, DW). This was checked, by weighing the samples several days until they had the same weight as the day before. The DM was calculated as following: DM = (DW/WW) x 100.

Samples á 400g pellets (n=2) and 300g lichens (n=3) from a mixture of the representative samples from each experiment, were taken. The samples were sent, stored frozen in closed bags, to Eurofins Food & Agro Testing Norway AS. Analyses by Eurofins were performed as following:

The *water* content of the samples was determent following the EU Directive 71/393 m (List of Official EU Methods of analysis) About 50 g of the samples was chrushed or divided, and the analyses were performed just after opening of the samples. A container with its lid was weight, and about 5 g of the sample was then weighed into the container. The container without lid was placed in an oven preheated at 103°C. The samples were dried for 4 hours. The lid was placed at the container, and the latter was removed from the oven, to cool in a desiccator for 30-45 min. The samples were then dried again. The water content is based on the water loss. Performed by Eurofins Food & Agro Testing Norway AS (Trondheim).

To determine the *calorimetric heating value*, the feed samples was burned in an oxygen bomb calorimeter, type PARR 6300 (method SS-ISO 1928). The calorific value is the measured amount of energy released per unit weight of fuel. Performed by Eurofins Food & Agro Testing / Eurofins Environment Testing, Sweden AB (Lidköping) (BS-ISO 1928:2009, SS-EN 14918:2010, SS-EN 15400:2011). From the amount of combustible energy (measured by the calometric heating value) the gross energy intake (GEI) of the animal are measured as following: GEI = calometric heating value x DMI.

The determination of the *crude protein* content of the feed is based on the nitrogen (ammonium) content, according to the Kjeldahl method (EU DIR 93/28 m, List of Official EU

Methods of analysis). Sulphuric acid and a catalyst was added to the sample, which was digested by heating. The solution is made alkaline with sodium hydroxide solution, and heated until the ammonia is distilled over. The excess sulphuric acid is titrade in the collection flask with a standard solution of sodium hydroxide, to determent the amount of ammonium. Performed by Eurofins Food & Agro Testing Norway AS (Trondheim).

According to EU Derective 98/64 m, the *fat* content of the samples is determined as following. Five gram of the sample was placed in an exraction trimble, and covered with a fat-free wad of cotton wool. The trimble with the samples were then extracted with light petroleum, for 6 hours in a extractor and dried for 1.5 hours in an oven. The samples were then cooled in a desiccator and weigh, and dried again for 30 minutes to ensure that the weight of the oils and fats remains constant (List of Official EU Methods of analysis). Performed by Eurofins Food & Agro Testing Norway AS (Trondheim).

The *ash* content was determined relative to the EU Directive 71/250 m. Five gram of each sample was weight and placed in a calcined and tared crucible for ashing. The crucible was heated gradually until the substance carbonizes, and thereafter put into a muffle-furnace at 550 °C. It was kept at this temperature until the ash was white, light grey or reddish, since it then appears to be free from carbonaccous particles. The crucible is placed in a desiccator to cool. The residue is weight (List of Official EU Methods of analysis). Performed by Eurofins Food & Agro Testing Norway AS (Trondheim).

Water-soluble carbohydrates were extracted from the sample with boiling water. The amount of glucose and fructose are enzymatically determined, could be determined after acid hydrolysis. In the presence of ATP and hexokinase (HK), glucose and fructose are phosphorylated to respectively glucose-6-phosphate and fructose-6-phosphate. Phosphoglucose isomerase (PGI) was added to transfer fructose-6-phosphate to glucose 6-phosphate. By glucose-6-phosphate dehydrogenase (G6P-DH), gluconate-6-phosphate was formed, and NADP is reduced to NADPH. The absorbance was measured by spectrophotometry at 340 nm before and after the addition of HK/G6PDH (5.9), and the increase in absorbance is proportional to the glucose concentration (Boehringer Mannheim 1984, Larsson and Bengtsson 1983) Analysis were performed by Department of Soil and Environment, Swedish University of Agricultural Sciences (Skara, Sweden)

Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL): The NDF was determined by Eurofins Food & Agro Testing/Eurofins Environment

Testing, Sweden AB, Lidköping, as following: Ground samples were weight and added 100% ND solution and sodium sulphite. Covered with aluminium foil with small holes, the samples were incubated for 16 hours in a 90 °C oven. Amylase was added 1 hour before the end of incubation. After filtering through tarred crucibles with fitted discs and washing with water, the crucible was dried overnight and weight. The NDF is the weight difference before and after treatment (Tecator AN 304, LidNär.0A.21, Chai and Udén 1998). ADL and ADL were determined according to Robertson and Van Soest (1981), using the permanganate method performed by Department of Soil and Environment, Swedish University of Agricultural Sciences (Skara, Sweden). NDF represent the total cell wall content of Hemicellulose, cellulose and lignin. ADF is a subcomponent of the NDF, containing cellulose and lignin. The lignin content of the feed is given by ADL. The content of hemicellulose is therefore calculated as NDF – ADF, and cellulose as ADF – ADL.

The chemical composition of the 5 feed samples varied little, and the means (±SD) from respectively pellets and lichen are presented in Table 1.

3.6. Statistical analyses

The study was conducted as a paired design, with two different treatments/diets, mixed lichens and pelleted reindeer feed. Each reindeer was its own pair, given first pellets and then after 4 weeks the same reindeer was given lichens. The data have been analysed using a paired *t*-test. The data were paired, in order to minimize the variance of the treatments, to detect if the differences between the diets are significant.

The paired *T*-test is based on the *t*-distributed test statistic T ($T = mean\ D\ /\ (S_D / \sqrt{n}$, where D is the difference of the pair values, S_D^2 is the variance and n is the number of samples, the reindeer in this experiment)). By using the *significance level* $\alpha = 0.025$, the null hypothesis H_0 (Saying that reindeer emit more or the same methane emission when feed lichen compared to pellets ($H_0 = \mu_1 \leq \mu_2$)) were tested. If $T > t_\alpha$ (t_α is found in a table of the *t*-distribution, in Kleinbaum *et al.* 1998 and Løvås 2004) the alternative hypothesis that reindeer feed lichen emit less methane than reindeer feed pellets ($H_1 = \mu_1 > \mu_2$) is accepted.

In order to do the test, we must assume that each observed pair is independent of the others (as is the case with 5 individual animals), and that all pairwise difference is approximately normally distributed (this is important during experiments with less than 30 observations). The normal distribution cannot be tested with only 5 observations, this we just most assume.

To quantify how unusual the observed result would be if H_0 was true, the *P*-value is compute. If *P* is smaller than 0.025 the H_0 is rejected, but if the *P* is larger than 0.025 the H_0 is not rejected. (Kleinbaum *et al.* 1998, Løvås 2004).

4. RESULTS

4.1. Feed chemistry

The chemistry of pellets (Felleskjøpet, Norway) and the mixed lichens (mainly *C. stellaris*) are very different. Grass-based pellets produced for reindeer are compared to lichen rich in protein, fat, ash, water-soluble carbohydrates (WSC), lignin and cellulose. While lichen mostly consisted of hemicellulose, comprising 78.7 ± 1.08% of DM (Table 1).

Table 1. Feed chemistry mean (±SD) of pellets and lichen. Analyses performed by Eurofins.

	Pellets (n=2)	**Lichen** (n=3)
Calometric heating vaule (kJ/g DM)	18.5 ± 0.05	17.4 ± 0.85
Dry matter (%)	88.9 ± 0.10	38.2 ± 1.86
(% of dry matter):		
Crude protein	12.7 ± 0.10	< 2.6 (below the detection line)
Fat	4.7 ± 0.16	1.7± 0.16
Ash	7.8 ± 0.10	< 2.6 (below the detection line)
Water-soluble carbohydrates	5.9 ± 0.05	0.1 ± 0.06
Lignin	7.2 ± 0.15	2.0 ± 0.10
Cellulose	5.2 ± 0.16	1.4 ± 0.35
Hemicellulose	32.9 ± 0.22	78.7 ± 1.08

4.2. Body mass and feed intake

The reindeer that were chosen for the experiments had a similar body mass (BM), as shown in Table 2. During the experiments feeding lichen the BM of the reindeer was lower than during the experiments feeding pellets. BM and dry matter intake (DMI) are presented in Table 2.

Table 2. Body mass (BM) and dry matter intake (DMI) during measurements on methane emission from reindeer (n=5) fed pellets and lichen respectively.

Reindeer	Pellets			Lichen		
	Date	BM (kg)	DMI (kg)	Date	BM (kg)	DMI (kg)
9	29.02	62	0.428	30.04	51	0.458
	02.03	60	0.428	03.05	50	0.505
10	26.01	64	0.435			
	09.02	63	0.437	04.05	55	0.487
11	30.01	60	0.436	12.04	57	0.478
	01.02	61	0.437	14.04	55	0.340
12	31.01	65	0.436	13.04	57	0.462
	08.02	64	0.437	19.04	54	0.409
13	07.02	57	0.437	17.04	52	0.398
	13.02	55	0.435	20.04	48	0.512
Mean	Jan.-Mar.	**61**	**0.434**	Apr.-May	**53**	**0.447**

4.3. Methane emissions

Feeding reindeer a pelleted feed, increased methane production above a baseline, right after feeding and in the 8-12 following hours, while the methane emissions from the reindeer fed lichen (green, light green) did not increase in response to feeding compared to baseline values (Figure 5).

One measurement on methane emissions from animal no. 10 (not shown) was unusable because analysed gas was not pulled from the box during the first 3 hour of the measurement. During experiment on reindeer no. 13 (date 13.02) flow data were not recorded. The methane emission from this experiment has been estimated using the mean flow of the 9 other experiments on reindeer fed pellets (the pump effect was not adjusted between experiments).

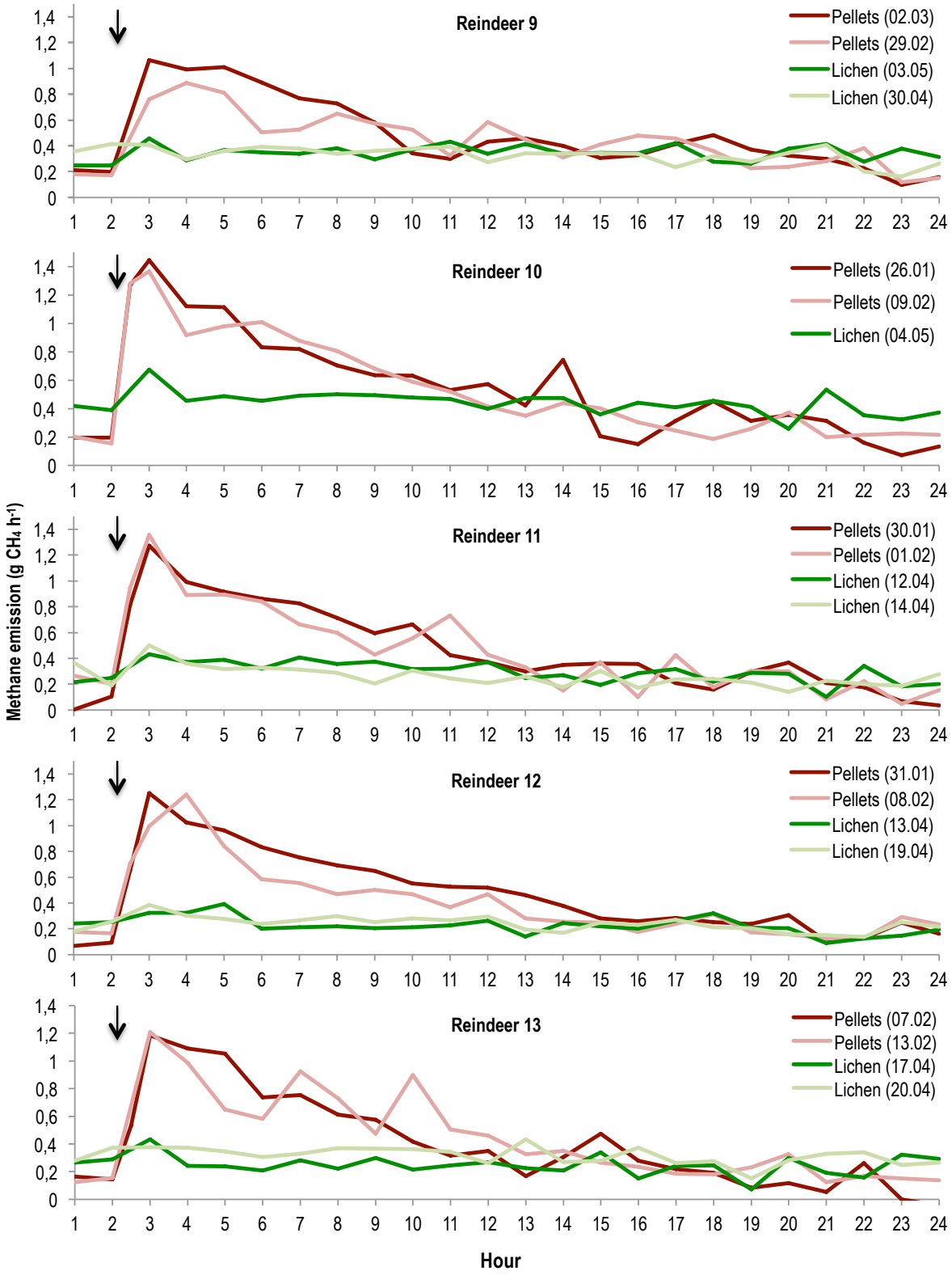

Figure 5. Methane (CH_4) emissions from reindeer (n=5) per hour, during 23-hours experiments using an open-circuit respiration chamber. The 24th hour is estimated. The reindeer were fed 2 hours into the experiment (arrows), with respectively a grass-based pelleted feed (red, light red) and a mixture of lichens (green, light green). Dates (dd.mm) of the experiments are indicated.

The methane emissions from reindeer fed lichens was on average 7.3 ± 1.6 g/day compared to 11.1 ± 1.0 g/day from reindeer fed the same amount of DM pellets. Since T = 7.3005 > t_α = 2.776, H_0 at significance level 0.025 were rejected. H_0 could actually have been rejected at significance level 0.005. Further the *P*-value is small (0.0009), which strengthens the rejection of the H_0, at the chosen significance level. There was also a significant difference between the methane energy loss in % of GEI (*P* = 0.0002) from the two diets. Thereby we can conclude that the methane emissions from reindeer fed lichen is significantly lower than methane emissions from reindeer fed pellets.

Table 3. Methane emissions per animal (CH_4 g/day; CH_4 in % of gross energy intake (GEI)) from experiments on reindeer fed pellets and lichen respectively.

Reindeer	Pellets			Lichen		
	Date	CH_4 g	CH_4 in % of GEI	Date	CH_4 g	CH_4 in % of GEI
9	29.02	10.7	7.4	30.04	8.0	5.5
	02.03	11.8	8.2	03.05	8.3	5.2
10	26.01	13.0	8.9			
	09.02	12.4	8.4	04.05	10.7	6.9
11	30.01	11.0	7.5	12.04	7.2	4.7
	01.02	10.9	7.4	14.04	6.4	5.9
12	31.01	11.3	7.7	13.04	5.5	3.8
	08.02	9.7	6.6	19.04	5.8	4.5
13	07.02	9.6	6.5	17.04	6.0	4.8
	13.02	10.9	7.4	20.04	7.7	4.7
Mean ± SD	Jan.-Mar.	**11.1** ± 1.0	**7.6** ± 0.7	Apr.-May	**7.3** ± 1.6	**5.1** ± 0.9

4.4. Oxygen

The oxygen concentration of the *outlet air* never dropped below 20.48 %, showing that the box was well ventilated at all times. The estimated mean oxygen consumptions by the reindeer fed pellets were higher when the reindeer were fed pellets (481.7 l/day) than fed lichen (361.8 l/day). This difference is significant (*P* = 0.0001) also in relation to BM (*P* = 0.0070), using a paired t-test.

5. DISCUSSION

5.1. Lichens depress methane emissions from reindeer

This study has demonstrated that reindeer fed mixed lichens and pelleted feed in controlled experiment produce methane, despite that early studies have found that number of methanogens in reindeer rumen was low (Sundset *et al.* 2009a, 2009b, Mathiesen *et al.* 2000). Methane emissions from reindeer fed lichens was significantly lower ($P = 0.0009$) compared to methane emissions from pelleted fed reindeer (Table 3, Figure 5). In fact, reindeer fed lichens emit 33% less methane compared reindeer fed pellets even when methane emissions was expressed in % of GEI.

Why is it so that reindeer fed lichens emit less methane compared to when fed pellets? Lichens synthesize and accumulate a wide variety of phenolic secondary compounds, such as usnic acid. Phenolic compounds, e.g. tannins, have been shown to reduce methane production by 13-16% (Waghorn *et al.* 2002, Woodward *et al.* 2004, Carulla *et al.* 2005, Grainger *et al.* 2009, Puchala *et al.* 2005), while other studies indicate that tannins have no effect on methane production (Beauchemin *et al.* 2007). Phenolic compounds are diverse, and can effect methane production both through effecting ruminal microbes and their fermentation. Previous studies have also suggest that the efficiency of rumen fermentation, and hence methane emissions, depends on the amount of dietary carbohydrate and the percentages of the different VFAs regulating the available H_2 (Johnson and Johnson 1995). Feed chemistry, including secondary compounds, rumen pH, digestibility, passage rate and microbial rumen diversity, size and activity affect both these factors (Beauchemin and McGinn 2005, Moe and Tyrell 1979, Moss *et al.* 2000). Acetate and butyrate promote methane production by producing CO_2 and H_2, while propionate is a competitive pathway to methane formation by utilizing H_2 (Figure 1). Therefore if all energy were fermented to propionate, the potential loss of substrate energy as methane would be zero (Moss *et al.* 2000, Wolin and Miller 1988). Furthermore, fermentation of carbohydrates to VFAs depends on microbial protein synthesis, and *visa versa*, the production of microbial cells depends on energy from carbohydrate degradation, as illustrated by Figure 6 (Ørskov 1992). This means that both nitrogenous compounds and carbohydrates are needed to produce microbial cells as well as VFAs and methane. Different effects of the secondary compounds, feed content of carbohydrates and proteins, as well as digestibility on methane production are discussed in the following.

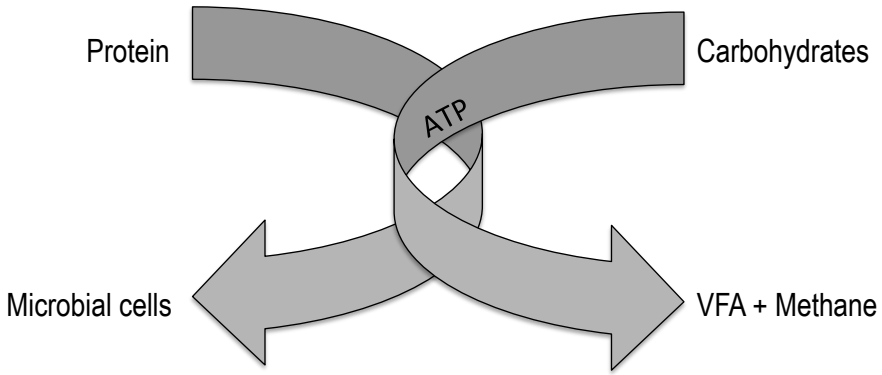

Figure 6. Illustration (modified from Ørskov 1992) of the interdependence of carbohydrate fermentation and microbial protein production. Carbohydrates are fermented to volatile fatty acids (VFA) and methane, producing ATP, being the driving force for microbial growth from amino acids and non-protein nitrogen (e.g. ammonia).

5.1.1. *Effect of phenolic plant secondary compounds on methane emission*

More than 600 secondary compounds have been reported in lichens, most of them chemical by-products of the fungi. From the many different lichens found, reindeer prefer lichens of the genus *Cladonia* (Inga 2007, Holleman and Luick 1977, Skogland 1975). Several species of lichens, including the *C. stellaris*, contain large amount of antibiotic usnic acid, 21.5 mg/g DM in *C. stellaris* (Sundset *et al.* 2010b). Apart from its antimicrobial, antiprotozoal and antiviral properties, usnic acid has been shown to prevent infection of wounds, it has been used as a dietary supplement for weight loss (despite of its toxic effects) and it is used an ingredient in cosmetics and perfumery (Guo *et al.* 2008, Han *et al.* 2004, Ingolfsdottir 2002). Usnic acid functions as a protection against harmful UV-B radiation from the sun, and as a defence against herbivores and microorganisms (Cocchietto *et al.* 202, Dailey et al 2008). Sundset *et al.* (2008, 2010b) suggest that microbes in the reindeer rumen though evolution have developed mechanism to cope with high concentrations of these acids, so that they can exploit the energy in the lichens (Palo 1993). Earlier studies have shown that the usnic acid is degraded in the reindeer rumen, and consequently not absorbed by the animal host (Sundset *et al.* 2010b). The novel bacteria *Eubacterium rangiferina* is resistant to usnic acid, but the mechanism by which this feat is accomplished is unknown (Sundset *et al.* 2008). This current study reveals that lichens decrease methane emissions from reindeer, whether it is due to usnic acid directly inhibiting methanogens or protozoa or due to other components in the lichens, still remains unresolved. Methane studies on muskoxen have shown similar reduction in methane production, feeding higher levels of woody browse, which similarly produce a variety of plant

secondary compounds. *In vitro* dry matter digestibility experiments revealed, that energy intake, relative proportions of VFAs and secondary phenolic compounds all have the potential to influence methane production (Lawler 2001).

5.1.2. *How does different carbohydrates effect the acetate/propionate ratio?*

Methane production is influenced by the type of carbohydrate fermented, most likely through impacts on ruminal pH and the microbial population (Johnson and Johnson 1995). Cultivation based studies (Aagnes *et al.* 1995) indicate low concentrations of VFAs and low acetate/propionate ratios, associated with a 85% reduction in the bacterial populations linked to plant particles in the rumen of reindeer fed pure lichens compared to reindeer feeding a mixed winter diet. Rumen VFA and acetate concentrations in reindeer fed lichens, may explain the low methane production from reindeer fed lichens.

According to Moe and Tyrell (1979) the fermentation of soluble carbohydrates (e.g. water-soluble carbohydrates) by amylolytic bacteria is less methanogenic than cellulolytic bacteria degradation of cell wall carbohydrates, while lignin prevent fibre degradation (Van Soest 1994), and in that way reduce methanogenesis. The low content of WSC in lichens (0.1 in relation to 5.9% of DM of pellets), and lignin (2.0 in relation to 7.2% of DM of pellets), indicates that these components do not explain the reduced methane emission from reindeer fed lichens compared to pellets. Cellulolytic bacteria such as *Fibrobacter succinogenes*, *Ruminococcus flavefaciens, Ruminococcus albus* and *Butyrivibrio fibrosolvens*, which normally also ferment hemicellulose, were not isolated from the rumen fluid of reindeer feed solely lichens in a cultivation study by Aagnes *et al.* (1995). From other domesticated ruminants it is known that hemicellulose from vascular plants are resistant to degradation in the rumen, and therefore become more available for microbial fermentation after enzyme fermentation in the abomasum (Van Soest 1994). But little is known about lichen hemicellulose fermentation in the digestive tract of reindeer (Aagnes *et al.* 1994), which are different from vascular plants both chemically and structurally (Storeheier 2003). According to Johnson and Johnson (1995), greater amounts of any carbohydrate fraction will lead to decreased methane production. This is in agreement with data reported in this current study, where low methane emissions are observed from reindeer fed lichens containing about 40% more carbohydrates (cellulose, hemicellulose and water-soluble carbohydrates) compared to pellets (Table 1).

5.1.3. *Does the low protein contents in lichens depress methane emission?*

The lichen diet had a high energy content (17.4 kJ/g DM), but was low in protein < 2.6% of DM (Table 1). According to Sveinbjörnsson *et al.* (2006) the fermentation of protein give rise to less VFA compared to carbohydrates. Considering this, one would expect the opposite of what we observed, that the methane emission from reindeer feed pellets high in protein (12.7% of DMI) would produce less methane compared to when fed lichens. Rumen microorganisms require nitrogen to synthesis protein. The low protein content in lichens may therefore lead to low stimulation on microbial growth but also low fermentation, and therefore low methane production as illustrated by Figure 6. The low protein content in lichens (Table 1) could therefore explain the low methane production from reindeer feed only lichens, despite nitrogen-reabsorption of urea and mobilisation of body mass (Table 2). Hove and Jacobsen (1975) have showed that reindeer fed a low protein diet of lichens are able to increase the reabsorption of urea nitrogen in their kidneys to as much as 93% of the filtered urea. Despite this efficient renal mechanism, and a possible reduced nitrogen demand in winter, this is not enough protein for the reindeer to maintain a net balance of protein. The animal consequently has to mobilise body protein and thereby lose body mass, as normally seen in late spring in reindeer (McEwan and Whitehead 1970, Jacobsen and Skjenneberg 1977, Bøe and Jacobsen 1981, Staaland *et al.* 1984, Aagnes and Mathiesen 1994), and also observed in this current experiment. The reindeer had a lower body mass after 4 weeks on a pure lichen diet (in April/May), compared to when they were fed pellets in January/March (Table 2). Statistical analyses showed no significant effect of weight on methane emission (personal communication with Elinor Ytterstad, University of Tromsø), and can therefore not explain the whole difference in methane emission between the two diets used.

In conclusion, reindeer require a combination of lichens and vascular plant, to maintain a stable body mass in winter (Tyler *et al.* 1999). Wintergreen parts of graminoids contain 10% protein, approximately the same amount as pellets feed (Storeheier *et al.* 2002c). This additional nitrogen may prevent mobilization of muscle protein as a source of nitrogen, but consequently lead to an increased methane emission from reindeer on natural pastures, compared to the emission measured here on a pure lichen diet.

The oxygen consumption was higher when the reindeer were fed pellets compared to lichens (section 4.4). The metabolism is higher when digesting proteins than carbohydrates (Brody 1945), and may explain the higher oxygen consumption from reindeer fed pellets high

in protein (Table 1). This is also supported by observed higher oxygen consumption the hours just after feeding pellets during the experiments.

5.1.4. *Effect of digestibility and passage rate on methane emissions*

Different digestibility's of feed pellets and the mixed lichen diet provided, may partly explain the difference in methane emissions from reindeer fed pellets compared to lichens. When the digestibility of energy increases, energy lost as methane seems to increase (Blaxter and Clapperton 1965). Digestibility of a similar grass-based pelleted feed (RF-80) to the pellets used in this study was 78.3 % DM (Sletten & Hove 1990). In vitro digestibility study of lichens in inoculum from slaughtered reindeer at winter pasture has shown large inter-specific differences, ranging from 32%-71%. The digestibility of *C. stellaris* is dependent of adaptation to the diet, and have both shown intermediate digestibility, 56-58% (Storeheier 2003), and digestibility as high as 75 % (Jacobsen and Skjenneberg 1975).

When the residence time in the rumen decrease due to increasing passage rate, time available for microbial fermentation decrease and so does methane production. Rapid passage rate also favours propionate production (Moss *et el.* 2000). Since the rumen turnover time in reindeer fed lichen is long (23-69 hours according to Aagnes and Mathiesen 1994), the passage time does not seem to explain the low methane production in reindeer fed lichens. The soft structure of lichens could though have little stimulation on the rumen walls, and by that affect the digestion. Furthermore Johnson *et al.* (1996) found that pelleting of forages decrease the methane production, due to an increase in passage time (Le-Liboux and Peyraud 1999). But these affects are not apparent when the intakes of the diets are restricted, as it was in these experiments.

5.1.5. *Other factors effecting the methane emission from reindeer*

At low ruminal pH, methanogens reduce their ability to utilize H_2. This favours growth of lactic acid bacteria, which are able to use the free H_2. But in general, all interventions which directly inhibit methanogens lead to accumulation of H_2, and therefore contribute to ruminal acidosis, diarrhoea and reduced growth of cellulolytic bacteria and fermentation (Stewart 1977, Hiltner and Dehority 1983, Hoover 1986), and is therefore not a favourable alternative for the animal. Alternative sink for the H_2 cold be used when methanogens are inhibited. Through e.g. reductive acetogenesis H_2 is used to reduce CO_2 to acetic acid by acetogenic bacteria (especially found in termites and the hindgut of mammals) (Demeyer and de Graeve, 1991,

Demeyer et al. 1996). Acetate has the benefit that it can be used as energy for the animal, but acetogenic bacteria normally lose the competition with methanogens in the adult rumen (Breznak and Kane 1990). Also the reduction of CO_2 to acetate is thermodynamically less favourable than the reduction of CO_2 to methane (McAllister and Newbold 2008). Therefore, and because acetoclastic methanogens capability of forming methane from acetate, reductive acetogenesis would only be rewarding if methanogens are already inhibited (Bodas et *et al.* 2012). Significant alternative H_2 sinks are oxygen, nitrates, sulphates, fumarate, malate and acrylate and microbes (Johnson and Johnson 1995, Ungerfeld *et al.* 2007, Ungerfeld and Forster 2011). Rumen function and methane emissions have additionally been tried manipulated by natural additives like, plant extracts, ionophores (e.g. monensin), buffers and minerals (Jouany 1994, Weimer 1998; Santra and Karim 2003, Hart *et al.* 2008, McGinn *et al.* 2004).

Increased dietary fat content have also shown to reduce methane emissions in domestic ruminants (Eugene *et al.* 2008, Martin *et al.* 2010). Chain length and degree of unsaturated fatty acids, reduction of the amount of organic matter and an inhibition of rumen protozoa are suggested explanations. Individual fatty acids have further shown to decrease methane production by enhance the production of propionic acid and by providing an alternative to CO_2 in biohydrogenation (Czerkawski *et al.* 1966, Johnson and Johnson 1995).

Differences in rumen methane production from animal to animal may be due to individual inherent differences leading to ecological changes in the ruminal microbial ecosystems (Nkrumah *et al.* 2006). As presented in Table 3 there are only smaller variations between the methane emissions from the reindeer fed the same diet, which could also be explained by small differences in DMI (Table 2).

In the last half-century, reindeer pastures have suffered substantial encroachments, due to e.g. expansion of cities, road network and industry (Hansen and Mathiesen 2011, Ressursregnskap for reindriftsnæringen 2008/09). Locked pastures due to difficult snow conditions, together with encroachments, means less lichens available for the reindeer. Svalbard reindeer (*Rangifer tarandus platyrhynchus*) and the reindeer (*Rangifer tarandus tarandus*) brought to South Georgia by Norwegian whalers are examples of reindeer living without access to lichens (Staaland and Punsvik 1980, Staaland 1986, Mathiesen *et al.* 1999). Locked pastures and encroachments, have forced especially coastal reindeer pasture districts to supplementary feed the reindeer with e.g. pellets during the winter. Furthermore climate projections indicate that pastures in Finnmark, were most of the reindeer in Norway are distributed, would

experience increased ratio of locked pastures and encroachments (Hansen and Mathiesen 2011, Tyler *et al.* 2007). The use of supplementary pellets could therefore increase in future. The consequence of changed winter diet for reindeer on mainland Norway, could as revealed from this study, lead to higher emissions of methane from reindeer.

5.2. Methane emissions from reindeer compared to other ruminants

The recorded finding from this current study, disclosing the effect of lichens on methanogenesis in the reindeer rumen, is the first actually measured data on methane emissions from reindeer. This project reveals that earlier guesstimating, trying to use other ruminant species (sheep and goats), to predict the methane emissions from reindeer, to be is not possible. Yearly enteric methane emissions from reindeer were suggested to be 11 kg CH_4 /animal/year (SSB 2009), equal to a daily emission of about 30.1 g CH_4 /reindeer/day.

Studies on methane emissions from cattle shows vide variations (Hindrichsen *et al.* 2005, Nkrumah *et al.* 2006, Beauchemin and McGinn 2005), but are generally much higher than what measured from reindeer in this current study (Table 5). Methane emissions from sheep have also shown variable values in relation to diet and intake (Pinares-Patiño *et al.* 2003, Table 5). Methane energy losses relative to GEI from other domesticated ruminants mainly range from 5-6.5%, varying from 2-15% (Blaxter and Clapperton 1965, Holter and Young 1992, Johnson and Ward 1996).

Various methane emissions from different ruminants (Table 5) may, except for being different species and BM, be explained by different levels/chemistry of feed and different gastro-intestinal systems, and microbial environment (Blaxter and Clapperton 1965, Moe and Tyrell 1979, Johnson and Ward 1996). Together with many different experimental setup and methods to quantify methane emission from ruminants, this makes it problematic to compare data on methane emissions from different ruminants.

Table 5. List of methane emissions from different ruminants, feeding different diets and levels of fed intake, measured by different methods respectively open-circuit respiration chamber (C), SF_6 tracer technique (SF_6), SF_6 tracer technique in open-circuit respiration chamber (SF_6 in C), and the SF_6 tracer technique used on pasturing animals (SF_6 on pastures).

Animal	Diet	DMI (kg)	CH_4 (g/animal/day)
Dairy cow[1]	Ryegrass + grain	13.8 + 5	322.0 (C); 331.0 (SF_6 in C)
Beef cattle[2]	Barley silage/grain	7.4	166.2 (C)
Sheep[3]	Ryegrass/write clove pastures	Ad libitum	23.0 – 37.3 (SF_6 on pastures)
Sheep[4]	Lucerne Silage	0.765	13.9 (C) 14.8 (SF_6) 16.1 (SF_6 in C)
Goat[5]	Crabgrass/tall fescue	0.67	10.6 (C)
	Tannin-containing legume	1.11	7.4 (C)
Reindeer[6]	Pellets	0.439	11.1 (C)
	Lichen	0.447	7.3 (C)

[1]Grainger *et al.* 2007, [2] McGinn *et al.* 2004, [3]Pinares-Patiño *et al.* 2003, [4]Pinares-Patiño *et al.* 2011, [5]Puchala *et al.* 2005, [6]Current study.

If trying to extrapolate data on methane emissions from reindeer revealed in experimental studies to free-ranging reindeer on natural pastures, one must be careful, because of different feeding conditions. Studies of other ruminants have shown that rumen methane production increases with increasing feed intake (Blaxter and Clapperton 1965), and that percentage of GEI lost as methane decreases by an average of 1.6% per level of intake, due to increased passage of feed out of the rumen (Johnson *et al.* 1993, Johnson and ward 1996). Studies on cows (Storm *et al.* 2012) have been performed with the climatic chamber placed in the barns where the animals have their daily routines. Reindeer are usually free-living and are thus more difficult to study. Field experiments with reindeer on pastures would thus give more information of their actual methane emission, also in relation to seasonal variations in plant species eaten. In order to be able to compare methane emission from reindeer with methane emissions from other animal, comparable studies with equal experimental setup and design as this experiment, are performed on sheep (by Marte Nielsen, master student, The University of Tromsø).

The controlled chamber experiment used, gave us the opportunity to measure the effect of one specific feed type, namely lichens. This study reveals that reindeer loses less GEI in form of methane fed only lichens (5.1 ± 0.9%) compared to when fed pellets (7.6 ± 0.7%). Possible due to low feed intake in this study, these values are high in relation to cattle. GEI is not a measure on the actual energy available for the animal, because of differences in feed

digestibility. Other differences between the two diets, as well as reindeer compared to cattle, can therefore be concealed.

Methane emissions registered before feeding the reindeer (Figure 5) are possible due to remaining feed in the gastro-intestinal system or microbes eating each other or endogenous nitrogen from the host. On Figure 5 the methane emissions from reindeer rise (from the baseline exemplified at Figure 3) just after given pellets. Feeding lichens do however not rise methane emissions from above this baseline in reindeer (Figure 5). This means that Net CH_4 (Figure 3) is zero. This indicates that the effect of lichens on methanogenesis, whether it is due to usnic acids, protein/carbohydrate composition or other factors, is significant. Therefore it is also uncertain that the methane emission would rise, if the amount of lichen were increased. This is supported by measurements on methane emission from goats (Table 5), which showed similar reduction in methane emissions feeding a tannin-containing legume (*Lespedeza cuneata*), even though feed intake was increased with about 40% compared to a crabgrass/tall fescue (Puchala *et al.* 2005). This study indicates that by eating lichens, reindeer could potentially maximize the conversion of dietary energy into metabolic energy, and save energy by emitting less methane.

5.3. Global methane emissions

Worldwide, 13.5-33% of the greenhouse gas emission comes from agriculture (World Resources Institute 2005, EPA 2004, Bodas *et al.* 2012. In New Zealand, where the comprehensive export is based on ruminant livestock, 50% of the greenhouse gas emission is related to agriculture (Leslie 2007). In comparison agriculture in Norway only accounts for 9% of the total greenhouse gas emissions within Norway, most of it being methane emission. About half of this methane is emitted from livestock and manure, and furthermore 85% of this is from microbial fermentation in ruminants. This means that only 3-4% of the greenhouse effect projected, could potentially come from methane from ruminants in Norway (NIR 2003, SSB and SFT 2007, Gundersen *et al.* 2009). Though from 1993 there has been a plateau of the atmospheric methane concentrations, as the methane emission has become equal to the removal. Since the world population of large ruminants have continued to rise, this questions the relationship between the changes in atmospheric methane concentrations and the ruminant production (Solomon *et al.* 2007).

The number of reindeer in Norway is about 250,000 (Reindriftsforvaltningen 2009), compared to 875,000 cattle, and 920,000 adult sheep (SSB 2011, numbers from 2010).

Worldwide there are 5-6 million reindeer (Wiliams and Douglas 1989, Turi 2002), compared to 1.4 billion cattle and 1 billion sheep worldwide (FAOSTAT 2003), see Table 6.

Table 6. World ruminant populations.

Animal type	World population
Cattle	1,371,100,000[1]
Sheep	1,024,000,000[1]
Pigs	956,000,000[1]
Goats	767,900,000[1]
Buffaloes	170,700,000[1]
Horses	55,500,000[1]
Camels	19,100,000[1]
Wild Reindeer	3,300,000-3,900,000[2]
Domesticated Reindeer	2,000,000[3]

[1]FAOSTAT (2003) [2]Williams and Douglas 1989 [3]Turi 2002

The size of the circumpolar reindeer population is small in relation to the size of the other domesticated ruminants in the world (Table 6) and makes methane emissions from reindeer small regardless of being fed lichens or pellets. The most obvious solution on reducing methane emissions from ruminants would be to reduce the number of ruminant animals in the agriculture world wide, as argued by the Norwegian Government, (Landbruks- og Matdepartmentet 2009). But because of increased demand of human protein in future, such reduction is difficult, and has to be seen in relationship with other greenhouse gas emissions globally. Reduction of reindeer husbandry, a unique livelihood for Arctic indigenous peoples for more than thousand years, based on the assumption of methane emission should therefore be adjusted.

6. CONCLUSION

Methane emissions from ruminants represent both an environmental problem and a potential loss of dietary energy. Our studies indicate that reindeer fed lichens emit less methane compared to when fed a pelleted fed. This might instead saves energy in favour of survival. The increasing use of pellets as supplementary feed in the Sámi reindeer husbandry due to climate change and encroachment, may threaten this unique contribution to reduced methane emissions.

The low protein content in lichens or actions of secondary phenolic compounds such as usnic acid, could explain low methane emission from reindeer fed lichens in this current project. Further studies are needed to better understand what factors influences rumen methanogenesis in reindeer.

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