Oats in the diet of dairy cows

Milk production and enteric methane emissions

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Abstract

The overall aim of this thesis was to investigate whether replacing barley with oats as a grain supplement for dairy cows could reduce enteric methane (CH_4) emissions without compromising milk production. Barley is a more common grain supplement in Sweden, mainly due to higher tabulated feeding values suggesting higher milk production with barley than with oats. In the first paper, different varieties of oats and barley were evaluated *in vitro*. Predicted *in vivo* CH_4 emissions were lower from the oat diets than from the barley diets. In the second paper, barley was replaced by hulled oats as a grain supplement to dairy cows fed a grass silage-based diet. Replacing barley with oats decreased organic matter digestibility and metabolisable energy intake but did not affect milk or energy-corrected milk (ECM) yield. Daily CH_4 emissions (g/d) and CH_4 intensity (g/kg ECM) decreased by 4.7 and 4.8%, respectively. In the third paper, dairy cows were fed one of four grain supplements: barley, hulled oats, dehulled oats, or a mix of hulled and dehulled oats. Organic matter digestibility and metabolisable energy intake were similar between the barley diet and the oat diets, but milk and ECM yield were higher with the oat diets. Replacing hulled oats with dehulled oats did not affect milk or ECM yield. Daily CH_4 emissions were similar between the barley diet and the oat diets. Yet, due to higher ECM yield, CH_4 intensity was 5.7% lower with the oat diets. In the fourth paper, we investigated fatty acid composition of milk. Milk fat from cows fed oats contained lower concentrations of saturated fatty acids and higher concentrations of unsaturated fatty acids. In conclusion, replacing barley with oats in the diet of dairy cows does not compromise milk production and could offer a practical strategy to slightly reduce enteric CH_4 emissions and to change milk quality to be more in line with dietary guidelines.

Keywords: grain supplements, greenhouse gas emissions, sustainability, milk quality, energy utilization

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Sammanfattning

Syftet med denna avhandling var att undersöka huruvida ersättning av korn med havre som kraftfoder i mjölkkors foderstat kunde vara en praktisk strategi för att minska enteriska metanutsläpp utan att mjölkproduktionen påverkas negativt. Det är vanligare att utfodra mjölkkor med korn i Sverige. Detta till följd av att korn har högre angivna energi- och proteinvärden i fodertabeller vilket indikerar högre mjölkproduktion med korn. I den första artikeln utvärderades olika sorter av korn och havre *in vitro*. Estimerade *in vivo* CH_4 utsläpp var lägre från havredieterna än från korndieterna. I den andra artikeln ersattes korn med oskalad havre i foderstaten till mjölkkor utfodrade med gräsensilage. Ersättningen minskade smältbarheten av organiskt material och intaget av omsättbar energi men påverkade inte mängden producerad mjölk eller energi-korrigerad mjölk (EKM). De dagliga CH_4 utsläppen (g/d) och CH_4 intensiteten (g/kg EKM) minskade med 4,7 och 4,8 %. I den tredje artikeln, utfodrades mjölkkor med endera korn, oskalad havre, skalad havre eller en blandning av oskalad och skalad havre. Smältbarhet av organiskt material och intag av omsättbar energi var lika mellan korndieten och havredieterna men mängden mjölk och EKM var högre med havredieterna. Ersättning av oskalad havre med skalad havre påverkade inte mängden mjölk eller EKM. De dagliga CH_4 utsläppen var lika stora med havredieterna som med korndieten. Som en följd av större mängd EKM var CH_4 intensiteten 5,7 % lägre med havredieterna. I den fjärde artikeln undersökte vi mjölkens sammansättning av fettsyror. Mjölkfett från kor utfodrade med havre innehöll lägre koncentration av mättade fettsyror samt högre koncentration av omättade fettsyror. Sammanfattningsvis, ersättning av korn med havre i foderstaten till mjölkkor minskar inte mjölkproduktionen och kan vara en praktisk strategi för att minska de enteriska CH_4 utsläppen något samt ändra mjölkens fettsyrasammansättning mer i linje med internationella kostråd.

Nyckelord: kraftfoder, växthusgasutsläpp, hållbarhet, mjölkkvalitet, energiutnyttjande

Dedication

In memory of my father, Christer Fant (1941-2016)

"*Patience you must have my young Padawan*." — Yoda

"*Remember to look up at the stars and not down at your feet. Try to make sense of what you see and wonder about what makes the universe exist. Be curious. And however difficult life may seem, there is always something you can do and succeed at. It matters that you don't just give up.*"
— Stephen Hawking

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Fant P., M. Ramin, S. Jaakkola, Å. Grimberg, A. S. Carlsson, and P. Huhtanen (2020). Effects of different barley and oat varieties on methane production, digestibility, and fermentation pattern in vitro. *Journal of Dairy Science*, 103 (2), 1404-1415.

II. Ramin M., P. Fant, and P. Huhtanen (2021). The effects of gradual replacement of barley with oats on enteric methane emissions, rumen fermentation, milk production, and energy utilization in dairy cows. *Journal of Dairy Science*, 104 (5), 5617-5630.

III. Fant P., M. Ramin, and P. Huhtanen (2021) Replacement of barley with oats and dehulled oats: Effects on milk production, enteric methane emissions, and energy utilization in dairy cows fed a grass silage-based diet. *Journal of Dairy Science*, 104 (12), 12540-12552.

IV. Fant P., H. Leskinen, M. Ramin, and P. Huhtanen. Effects of barley and oats on milk fatty acid composition in dairy cows fed grass silage-based diets (manuscript).

Papers I-III are reproduced with the permission of the publishers.

The contribution of Petra Fant to the papers included in this thesis was as follows:

I. Collected, prepared, and analysed the data. Wrote the manuscript with regular input from co-authors and main supervisor.

II. Contributed to data management and statistical analysis. Worked jointly with co-authors and main supervisor in writing the manuscript.

III. Collected, prepared, and analysed the data. Wrote the manuscript with regular input from co-author and main supervisor.

IV. Collected, prepared, and analysed the data with regular input from supervisors. Wrote the manuscript with regular input from co-authors and main supervisor.

Abbreviations

CH_4VFA	Stoichiometric methane
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
ECM	Energy-corrected milk
FA	Fatty acids
GHG	Greenhouse gas
iNDF	Indigestible neutral detergent fibre
ME	Metabolisable energy
MP	Metabolisable protein
MUFA	Monounsaturated fatty acids
MUN	Milk urea nitrogen
NDF	Neutral detergent fibre
NE	Net energy
3-NOP	3-nitrooxypropanol
OM	Organic matter
pdNDF	Potentially digestible NDF
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
VFA	Volatile fatty acids

1. Introduction

Oats (*Avena sativa* L.) used to be a popular grain supplement fed to dairy cows in Sweden and the other Nordic countries. Recently, oats have increasingly been replaced by barley (*Hordeum vulgare* L.), much due to the higher tabulated feeding values of barley that are used for ration formulation. For example, according to the Nordic Feed Evaluation System (NorFor, 2022), Swedish oats have a 4-15% lower net energy (NE) value and a 5-15% lower metabolisable protein (MP) value than Swedish barley, depending on the fibre content of oats. However, several studies suggest that production performance of dairy cows fed oat concentrate can be similar to or even better than that of dairy cows fed barley concentrate (Heikkilä *et al*., 1988; Martin and Thomas, 1988; Vanhatalo *et al*., 2006). In addition, replacing barley with oats seems to change the fatty acid (FA) composition of milk to be more in line with international guidelines (FAO, 2010; WHO, 2020) for consumption of saturated FA (SFA) (Heikkilä *et al*., 1988; Martin and Thomas, 1988; Vanhatalo *et al*., 2006).

Greenhouse gas (GHG) emissions from milk- and meat production are of global concern. Mitigation strategies for enteric methane (CH_4) emissions from dairy cows and other ruminants have been thoroughly investigated during the last 30 years. The dietary strategies focus on altering the chemical composition of the diet through improvement of forage quality, ration formulation, and addition of supplements. As oats and barley differ in their chemical composition, replacing barley with oats may impact enteric CH_4 emissions. When barley was replaced by oats in a preliminary *in vitro* study (unpublished data), predicted *in vivo* CH_4 emissions decreased. As both oats and barley grow well in Nordic conditions, replacing barley with oats as a grain supplement in the diet of dairy cows could provide a practical CH_4 mitigation strategy.

1.1 Enteric methane emissions

1.1.1 Methane and climate change

Methane is a GHG, and its emissions have a considerable impact on climate change. Methane is the second most important GHG after carbon dioxide (CO_2), accounting for about 16% of total anthropogenic GHG emissions (Figure 1; Blanco *et al.*, 2014). The atmospheric lifetime of CH_4 is only 12 years compared with up to 200 years for that of CO_2 (Myhre *et al.*, 2013). On the other hand, CH_4 has a higher heat absorption capacity which gives CH_4 a global warming potential of 28 times that of CO_2 (Myhre *et al.*, 2013). After about 12 years in the atmosphere, CH_4 molecules are converted into CO_2 through oxidation with hydroxyl radicals (OH) in the troposphere (Ehhalt and Heidt, 1973). There are both natural and anthropogenic sources of atmospheric CH_4. Natural sources include wetlands, termites, oceans, and geological seepage, whereas anthropogenic sources include leakages during mining, drilling and transport of fossil fuels, agriculture, and waste (Saunois *et al.*, 2020). From pre-industrial times until 2010, the global surface concentrations of CH_4 have increased by 1077 ppb (Table 1), an increase mostly driven by increases in anthropogenic CH_4 emissions (Myhre *et al.*, 2013). From 2010 until 2020, CH_4 concentrations have increased by 80 ppb (Table 1). More than 80% of the change between 2010 and 2019 may be explained by changes in terrestrial emissions of CH_4 in the tropics (Feng *et al.*, 2022).

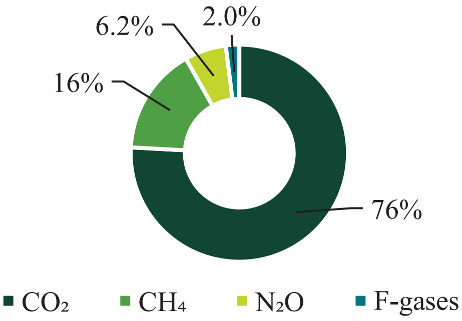

Figure 1. Shares of global anthropogenic GHG emissions in 2010. The global shares are weighted based on the global warming potential of each GHG according to the Kyoto-protocol (Blanco *et al.*, 2014).

Table 1. Global annual mean concentrations of CO_2, CH_4, and N_2O in the atmosphere for year 1750, 2010, and 2020 (Myhre *et al.*, 2013; Dlugokencky and Tans, 2021).

	Concentrations		
Gas	Year 1750	Year 2010	Year 2020
CO_2, ppm	278	389	412
CH_4, ppb	722	1799	1879
N_2O, ppb	270	323	333

1.1.2 Methane emissions from agriculture and ruminants

Figure 2 illustrates sources and shares of anthropogenic CH_4 emissions in Sweden 2020. Out of the total anthropogenic CH_4 emissions (182.6 kt), agriculture was responsible for the largest share accounting for 70% (Naturvårdsverket, 2021). Out of the total CH_4 emissions from agriculture, enteric fermentation in ruminants accounted for 64% and manure management for 6% (Naturvårdsverket, 2021). If we were to look at the emissions on a global scale, rice cultivation would also be part of the emissions from agriculture. It is worth mentioning that a recent study shows that CH_4 emissions from leakages due to use of fossil fuels are greatly underestimated and could be 25-40% higher than current estimates indicate (Hmiel *et al.*, 2020). If that is the case, the emission share from agriculture would be smaller than reported by Naturvårdsverket for 2020.

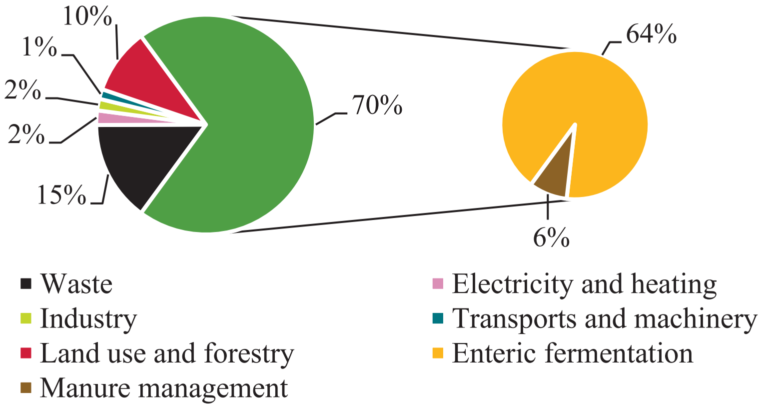

Figure 2. Shares of total anthropogenic CH_4 emissions by source (pie to the left) and shares of total CH_4 emissions from agriculture by source (pie to the right) in Sweden year 2020 (Naturvårdsverket, 2021).

Methane emissions from enteric fermentation arise mainly from anaerobic fermentation of feedstuff in the forestomachs of ruminants such as cattle, sheep, goats, and buffaloes, whereas a minor part arises from hindgut fermentation in monogastric animals such as horses and pigs (FAO, 2021). Out of the global enteric CH_4 emissions, beef cattle, dairy cattle, sheep and goats, and buffaloes are responsible for 54.3, 17.7, 12.2, and 11.1%, respectively (FAO, 2021). In Sweden, dairy and beef cattle are responsible for 37.9 and 48.8%, respectively, of the total enteric CH_4 emissions (Figure 3; Naturvårdsverket, 2021). From 1990 to 2020, the total enteric CH_4 emissions from dairy and beef cattle have decreased slightly, mostly due to decreasing animal populations in Sweden (Naturvårdsverket, 2021).

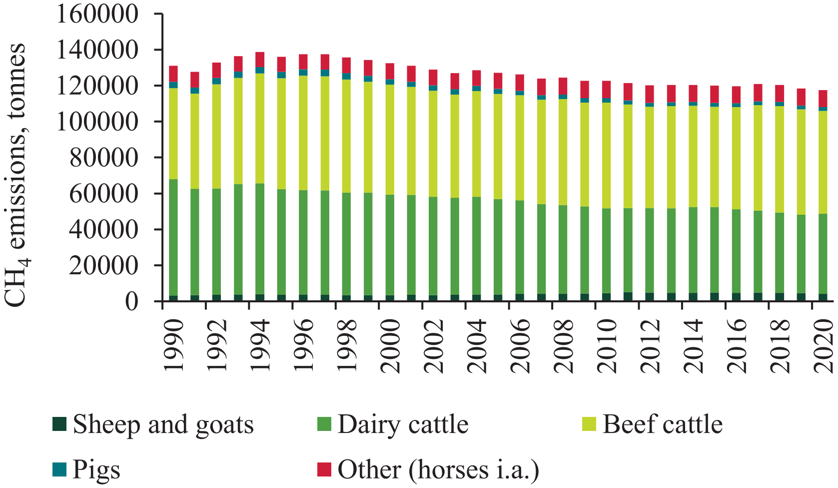

Figure 3. Total CH_4 emissions (tonnes) from enteric fermentation by animal species from 1990 to 2020 in Sweden (Naturvårsverket, 2021).

It is important to not only stare blindly at total GHG emissions, but to also consider emission intensities, i.e., the amount of GHG emitted per kg of product. Beef cattle used for meat production have a higher emission intensity than dairy cattle used for milk production. According to a life-cycle assessment, the global emission intensities for meat from beef cattle, meat from dairy cattle, and milk from dairy cattle are about 50, 17 and 10 kg CO_2-eq/100 g of protein (assuming 32 g protein/L of milk) (Poore and Nemecek, 2018). Differences in emission intensities also exist between different parts of the world. FAO (2021) reports GHG emission intensities including emissions from enteric fermentation (CH_4), manure management (CH_4 and N_2O), and manure application to soils and manure left on pasture (N_2O emissions). According to their report, the GHG emission intensity for milk production (expressed as kg CO_2-eq/kg milk) in Sweden is low compared with the global average and slightly lower than the average for the whole of Europe (Figure 4). There is a decreasing trend in the emission intensities of milk production in most parts of the world, mainly due to increased milk production per cow (Jordbruksverket, 2021).

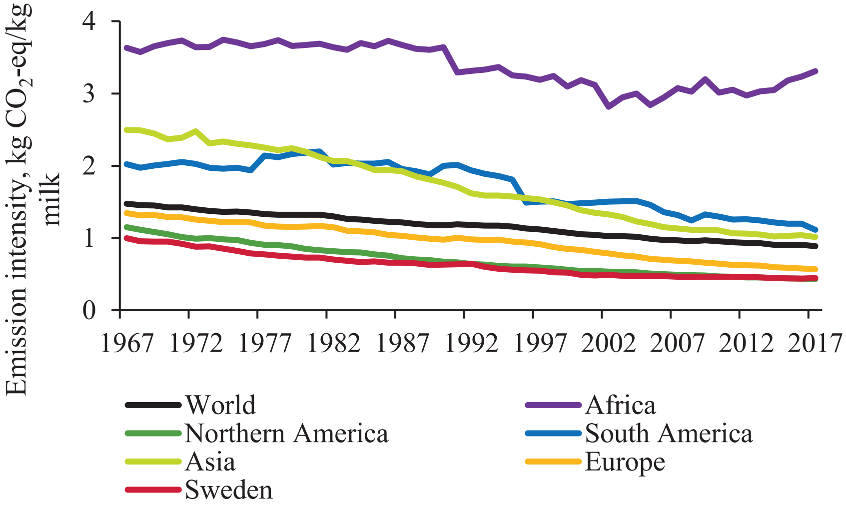

Figure 4. Development of GHG (CH_4 and N_2O) emission intensities (kg CO_2-equivalent/kg milk) between 1967 and 2017 for milk production on a global scale (World), in 5 of the world's continents, and in Sweden (FAO, 2021).

It is worth noting that when shares of CH_4 emissions from different anthropogenic sources are presented (Figure 2), it is assumed that CH_4 emissions from enteric fermentation and CH_4 emissions due to leakages from the use of fossil fuels affect atmospheric CH_4 concentration similarly, which is not exactly the case. As discussed earlier, CH_4 molecules are converted into CO_2 after approximately 12 years in the atmosphere (Ehhalt and Heidt, 1973). The CO_2 introduced to the atmosphere from enteric CH_4 is biogenic carbon originating from carbon stored in plants such as grass and cereals (Harris *et al.*, 2018). When grass and cereals re-grow, they capture atmospheric CO_2 through photosynthesis to build carbohydrates (Nelson, 2011), which in turn are eaten and digested by ruminants. This is called the biogenic carbon cycle (Figure 5). The CO_2 molecules introduced to the atmosphere from fossil fuel leakages originate from long-term storage of carbon in oil and coal and represent new carbon being added to the atmosphere. Even though the contribution of ruminants to increasing atmospheric CH_4 concentrations and climate change may not be as large as has been predicted (Hmiel *et al*., 2020; Feng *et al*., 2022), finding strategies to decrease enteric CH_4 is still important because if enteric CH_4 emissions do not increase, atmospheric concentrations of enteric CH_4 will be in a steady state (Figure 5).

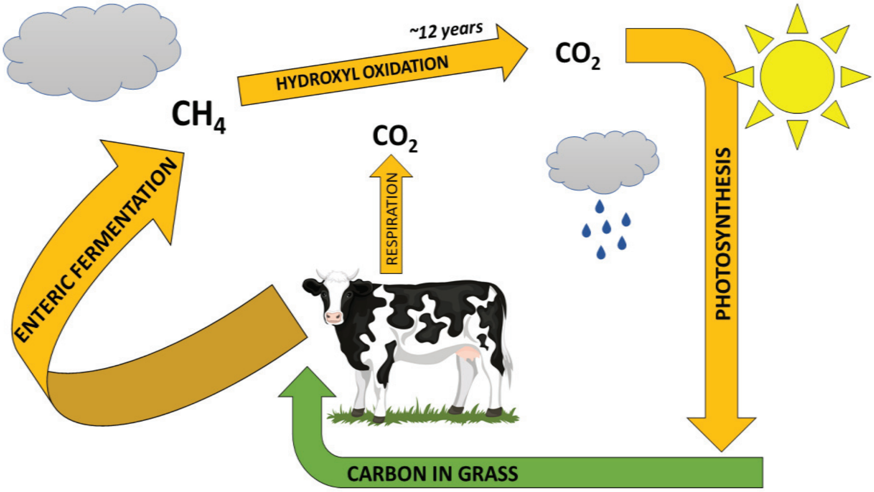

Figure 5. The biogenic carbon cycle.

1.1.3 Enteric fermentation in ruminants

Enteric fermentation of feed in the forestomachs of ruminants contributes to anthropogenic CH_4 emissions. It is, however, important to remember that through the specialized digestion abilities, ruminants can convert fibrous grass and non-protein nitrogen into human edible energy and protein. The price of this extraordinary skill is CH_4 emissions. The stomach of ruminants is divided into four compartments: the rumen, reticulum, omasum (the forestomachs), and the abomasum. The fermentation occurs mainly in the two first compartments, often referred to as the reticulorumen, and to a lesser extent in the omasum (Van Soest, 1994). The forestomachs are inhabited by anaerobic microorganisms from four kingdoms: bacteria, archaea, fungi, and protozoa. Digestion processes and metabolism are carried out by the enzymes of these microorganisms as the ruminant does not possess enzyme excreting cells in the forestomach walls (Van Soest, 1994).

After enzymatic breakdown of polysaccharides such as cellulose and starch into monosaccharides such as glucose, the fermentation process takes place, during which glucose is metabolized via glycolysis to pyruvate (Czerkawski, 1986). Pyruvate is further metabolized through various metabolic pathways to volatile fatty acids (VFA) (Van Soest, 1994). The VFA are absorbed through the rumen wall and used as an energy source by the ruminant. The major VFA are acetic, propionic, and butyric acid, and the minor VFA are isobutyric, valeric, and isovaleric acid (Van Soest, 1994). Glycolysis also releases energy that is captured as adenosine triphosphate (ATP) and used by the microorganisms for maintenance and for microbial growth from uptake of ammonia (NH_3) and amino acids (Czerkawski, 1986).

During glycolysis and oxidative decarboxylation of pyruvate to acetyl-CoA (first step in formation of acetic and butyric acid), hydrogen is released, thereby reducing cofactors such as NAD^+ into NADH (Czerkawski, 1986). For the fermentation process and the energy supply to both microbes and animal to continue, NADH needs to be re-oxidized into NAD^+. Due to the anaerobic conditions in the rumen, oxygen cannot serve as an electron acceptor and instead, CO_2 serves as an electron acceptor, forming CH_4 (Figure 6; Czerkawski, 1986). This process, methanogenesis, is the main hydrogen sink in the rumen under natural conditions. Methanogenesis is carried out by methanogenic archaea to gain energy in the form of ATP (Van Soest, 1994). Methanogenesis requires several enzymatic reactions to occur, and these can be inhibited by specific dietary additives, of which some will be discussed in the next section. Most of the ruminal CH_4 is removed and emitted into the air through eructation (silent belching), but a smaller part can also pass through the rumen wall and be exhaled via the lungs, which can account for up to 30% of the total (Hoernicke *et al.*, 1965).

$$CO_2 + 4\ H_2 \longrightarrow CH_4 + 2\ H_2O$$

Figure 6. Methanogenesis in the rumen (Czerkawski, 1986).

Although methanogenesis is the major contributor to removal of reduced cofactors, it is not the only hydrogen sink in the rumen. The metabolic pathways for production of propionic and valeric acid also serve as hydrogen sinks with a net uptake of hydrogen, whereas production of acetic and butyric acid results in a net release of hydrogen (Van Soest, 1994). Another hydrogen sink is microbial growth, as the microbes utilize reduced co-factors during both amino acid and FA synthesis (Czerkawski, 1986). Biohydrogenation of dietary unsaturated FA also serves as a hydrogen sink (Czerkawski *et al*., 1966), although its contribution is generally considered to be small. Alternative hydrogen sinks may also be introduced in the rumen by dietary addition of nitrate or sulphate (van Zijderveld *et al*., 2010; van Zijderveld *et al*., 2011). The extent of enteric CH_4 production is affected by several dietary factors. As CH_4 is only produced from digested nutrients, increases in dry matter intake (DMI) and diet digestibility increase total enteric CH_4 production (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013). The chemical composition of the diet also affects enteric CH_4 production and will be discussed in more detail in relation to dietary strategies for mitigation of enteric CH_4 emissions in the next section (1.2) and to the discussion in section 5.4.1

1.2 Dietary strategies for mitigation of enteric methane emissions

Enteric CH_4 emissions from ruminants can be mitigated through various dietary strategies. A discussion of the sustainability of a specific strategy should not only consider the magnitude of the CH_4 mitigating effect, but should also consider the strategy's effects on production performance. The United Nations (UN, 2019) has estimated that the world population will grow from 7.7 billion people in 2019 to around 9.7 billion by 2050. Due to the increase in world population size and increased incomes in developing countries (UN, 2019), the demand for livestock products is likely to grow, which will increase total CH_4 emissions. Therefore, mitigation strategies for enteric CH_4 need to be assessed in relation to animal productivity. For a dairy cow, the goal should be to decrease the amount of CH_4 emitted per kg of energy-corrected milk (ECM) produced (CH_4 intensity).

Secondly, one must consider financial and practical aspects of adopting a CH_4 mitigation strategy on commercial farms. For a dairy farmer, the effects of the strategy on milk production as well as the costs of implementing the strategy play vital parts in the financial aspect. Negative effects on milk yield, or neutral effects if implementation costs increase, will not motivate farmers to adopt a strategy no matter how effective it could be for mitigation of CH_4 emissions. Negative effects on animal health will be problematic from an ethical point of view but also financially.

Thirdly, it is important to consider the risk that a specific mitigation strategy for enteric CH_4 emissions could lead to increased GHG emissions from another source within the livestock sector. Figure 7 illustrates the sources and shares of GHG emissions as CO_2 equivalents within the global livestock sector. Enteric fermentation is the largest source accounting for 44% of total GHG emissions from this sector (FAO, 2017). However, production, processing, and transportation of feeds account for up to 42% of total GHG emissions, whereas emissions of CH_4 and N_2O from manure management account for 9% of total GHG emissions (FAO, 2017). For example, mitigation of enteric CH_4 might increase CH_4 emissions from manure management (Hassanat and Benchaar, 2019).

The work in this thesis aimed to investigate the potential of replacing barley grain with oat grain in the diet of dairy cows for mitigation of enteric CH_4 emissions. The focus was on measuring the effects on CH_4 emissions and milk production, but the aspects mentioned above are also considered in the discussion. In the following section, other potential dietary strategies for mitigation of enteric CH_4 will be reviewed, although a comprehensive review will not be provided. The CH_4 intensity is defined as g/kg ECM if not otherwise noted.

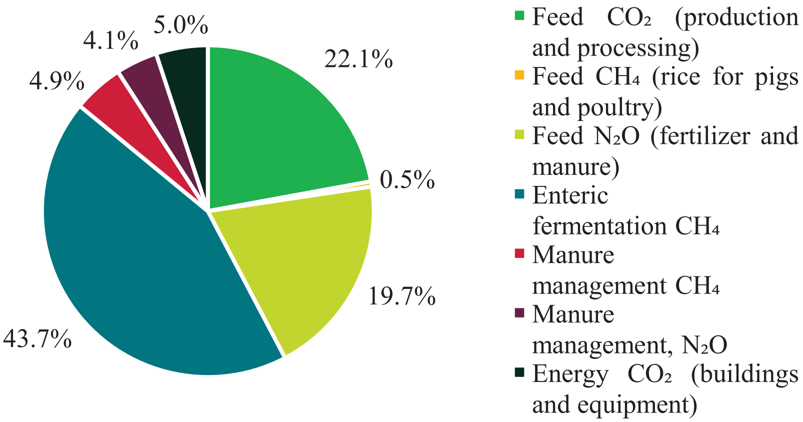

Figure 7. Shares of global greenhouse gas emissions by source within the livestock sector presented as CO_2 equivalents. Livestock include cattle, sheep, goats, buffalo, pigs, and poultry (FAO, 2017).

1.2.1 Forage source and quality

The choice of forage source may impact enteric CH_4 emissions. In a study by Hammond *et al*. (2016), maize silage-based diets led to 13% lower CH_4 intensity compared with grass silage-based diets, due to both lower total CH_4 emissions (g/d) and higher ECM yield. Brask *et al*. (2013) reported lower total CH_4 emissions from maize silage-based diets than from grass silage-based diets, but no effect on ECM yield. In a study by Benchaar *et al*. (2015), replacing red clover silage-based diets with maize silage-based diets decreased total CH_4 emissions slightly, but CH_4 intensity was unaffected despite a slight increase in ECM yield. Gidlund *et al*. (2017) reported no effect of increased ratio of red clover to grass silage on total CH_4 emissions or intensity. Forage quality may also affect enteric CH_4 emissions. Warner *et al*. (2017) reported 22% lower CH_4 intensity from cows fed early-cut grass silage than from cows fed late-cut grass silage, as a result of lower total CH_4 emissions (g/d) and higher ECM.

There is a risk for increased CH_4 emissions from manure management when one forage source is replaced with another. Hassanat and Benchaar (2019) found that manure from cows fed maize silage-based diets had a 54% higher maximum CH_4 production potential than manure from cows fed red clover silage-based diets. Regarding forage quality, there is a risk for increased N_2O emissions as nitrogen losses in manure were higher for early-cut silage than for late-cut silage in the study by Warner *et al*. (2017).

1.2.2 Forage to concentrate ratio

Decreasing the forage to concentrate ratio from 68:32 to 47:53 on an alfalfa and corn silage diet decreased CH_4 intensity by 20% due to decreased total CH_4 emissions and unaffected ECM yield in a study by Aguerre *et al*. (2011). In a study by Bayat *et al*. (2017), decreasing the forage to concentrate ratio from 65:35 to 35:65 on a grass silage diet numerically decreased CH_4 intensity by 25%. The generally considered mechanism behind decreasing CH_4 emissions due to increased concentrate ratio is replacement of neutral detergent fibre (NDF) with starch, which favours production of propionic acid at the expense of acetic acid (Bayat *et al*., 2017), although Aguerre *et al*. (2011) did not observe such a change in molar proportions of VFA.

An important consideration for this CH_4 mitigation strategy is that the utilization of human inedible feed sources for transformation into valuable energy and protein for humans would decrease. Thereby, ruminants would compete with both humans and monogastric animals for the same resources, which is problematic, as the arable land for cultivation of crops is limited (Wilkinson and Lee, 2018).

1.2.3 Macro algae

Supplementing dairy cow diets with red macro algae shows potential for mitigation of enteric CH_4 emissions. Inclusion of *Asparagopsis armata* Harvey at levels of 0.5 and 1.0% on organic matter (OM) basis decreased CH_4 intensity (g/kg milk) by 27 and 60%, respectively, in a study by Roque *et al*. (2019). However, at 1.0% inclusion level, milk yield decreased by 12%. In a study by Stefenoni *et al*. (2021), inclusion of *Asparagopsis taxiformis* Delile at a level of 0.5% on dry matter (DM) basis decreased CH_4 intensity by 26%, but milk yield and ECM yield also decreased. *Asparagopsis* spp. contain several antimicrobial secondary metabolites, of which the halogenated compound bromoform ($CHBr_3$) is most abundant and is thought to inhibit one of the enzymatic reactions required for methanogenesis (Paul *et al*., 2006).

Although the mitigating effect of *Asparagopsis* spp. on enteric CH_4 emissions is large, there are some concerns. Depending on growth conditions, *Asparagopsis* spp. may contain iodine at concentrations that are toxic to the animal (Hillman and Curtis, 1980). Another concern is that large-scale production of algae would require heated pools, which might increase GHG emissions from production. This and the requirement for freeze-drying the algae to maintain proper activity of $CHBr_3$ would also increase feed costs.

1.2.4 3-nitrooxypropanol

A small chemical compound, 3-nitrooxypropanol (3-NOP), has recently been identified as a CH_4 mitigating agent through its inhibition of the enzyme that catalyses the last step of methanogenesis (Duval and Kindermann, 2012). In a study by Melgar *et al*. (2020a), 3-NOP supplemented to dairy cow diets as part of a premix at levels of 60 mg/kg of feed DM decreased CH_4 intensity by 25%, with no negative effect on milk production. In another study by Melgar *et al*. (2020b), inclusion of 3-NOP at levels of 40 and 200 mg/kg of feed DM decreased CH_4 intensity by 25 and 45%, respectively, with no negative effect on milk production.

A report by the European Food Safety Authority concluded that a 3-NOP additive, Bovaer®, can be used as a CH_4 mitigating agent in dairy cows without negative effects on production, animal health, or health of milk consumers (EFSA, 2021) and marketing of the additive was recently approved within the European Union. The cost of 3-NOP supplement or of a premix containing 3-NOP would be lower than the cost of macro algae, although it will still increase feed costs. Supplementation with 3-NOP might decrease DMI (Melgar *et al*., 2020a), but it is questionable whether this decrease would compensate for increased feed costs. Regarding effects of 3-NOP on other GHG, a study by Owens *et al*. (2020) showed that GHG or NH_3 emissions from manure are not affected by dietary 3-NOP supplementation.

1.2.5 Lipid supplements

Lipid supplements have consistently been found to mitigate enteric CH_4 emissions (Beauchemin *et al*., 2008). Bayat *et al*. (2018) supplemented dairy cow diets with either rapeseed oil, safflower oil, or linseed oil at a level of 50 g/kg of diet DM on a grass silage-based diet. Each plant oil supplement decreased CH_4 intensity by 23% without affecting milk yield and ECM yield. In a study by Chagas *et al*. (2020), supplementing a grass silage-based diet with rapeseed oil at 40 g/kg DM decreased CH_4 intensity by 24% without negative effects on milk or ECM yield. In another study, replacement of rapeseed meal with high-oil rapeseed cake on a grass silage-based diet decreased CH_4 intensity by 12% and increased milk and ECM yield (Bayat *et al*., 2021). Fatty acids in lipid supplements are not fermented and so does not contribute to production of enteric CH_4 but do contribute with energy for milk production (Johnson and Johnson, 1995). Lipid supplements may also affect fibrolytic microbes negatively, which increases the relative importance of propionic acid as a hydrogen sink (McAllister *et al*., 1996; Ungerfeld, 2015).

Dietary lipid supplementation might increase CH_4 emissions from manure. The maximum CH_4 production potential from manure increased by 17% when corn silage and red clover silage-based diets were supplemented with linseed oil at 4% of DM in the study by Hassanat and Benchaar (2019). Møller *et al*. (2014) also reported a higher CH_4 yield (mL per gram of volatile solids) from diets supplemented with extra crude fat compared with diets without fat supplementation. However, Ramin *et al*. (2021) reported similar CH_4 emissions from manure with and without rapeseed oil supplementation on a grass silage-based diet.

1.3 Oats and barley

Figure 8. Oats (taller straws) and barley (shorter straws) growing together in the field. Photo: Petra Fant.

1.3.1 Production and usage

In 2020, the top 5 producers of oats were Canada, Russia, Poland, Spain and Finland, whereas Sweden was the 10th largest producer (FAO, 2021). About 60% of the global production of oats is used for animal feed, mostly for horses and ruminants directly on-farm (FAO, 2021). Most feed oats are used as grain, but oats are also used as a whole-crop green oats for grazing, ensiling, or hay making. The use of oats as feed is steadily declining and its recognition as a health food for humans has increased its popularity within the food industry (Rasane *et al*., 2015). For example, studies show that oats are suitable for patients with celiac disease (Holm *et al*., 2006) and that the high content of dietary fibre, especially soluble β-glucan, in oats compared with other cereals may protect against cardiovascular disease (Wu *et al*., 2019). Oats are now commonly used for bread, breakfast cereals, biscuits, porridge, and oat drinks (Rasane *et al*., 2015). Other areas of use for oats are within the industry for production of cosmetics, pharmaceuticals, and plasticizers (Strychar, 2011). The top 5 producers of barley in 2020 were Russia, Spain, Germany, Canada, and France (FAO, 2021). Sweden was the 21st largest producer. About 55% of the global production of barley is used for animal feed (FAO, 2021). The rest is malted and used mostly within the brewing and distilling industry and a smaller part (~2%) within the food industry (Newton *et al*., 2011).

1.3.2 Growing environment and agronomy traits

Both oats and barley grow well in Nordic conditions. Oats require more moisture to produce a given unit of DM than any other cereals, except rice, and therefore, is well suited for moist temperate climates (Forsberg and Reeves, 1995). Oats are also more adaptable to different soil types than barley and grow well even on acidic soils (down to a pH of 4.5), although the highest yields are given between a pH of 5.3 and 5.7 (Forsberg and Reeves, 1995). On the other hand, oats are more sensitive to saline conditions than barley, and slightly more sensitive than wheat or rye (Forsberg and Reeves, 1995). Regarding nutrient requirements, oats and barley are quite similar.

Barley is usually grown in more favourable areas than oats which gives a slightly higher grain yield per hectare for barley (Figure 9; Jordbruksverket, 2022). In 2020, the yields of oats and barley in Sweden were 4530 and 5070 kg/ha, respectively (Jordbruksverket, 2022). Both oats and barley respond quite similarly to yearly weather variations (Figure 9). In 2018, the yields of both cereals were exceptionally low due to an unusually long period of extreme heat and lack of rain in Northern Europe. As oats are taller than barley, they are more susceptible to lodging which might decrease yields depending on the development stage when lodging occurs (Berry *et al*., 2004).

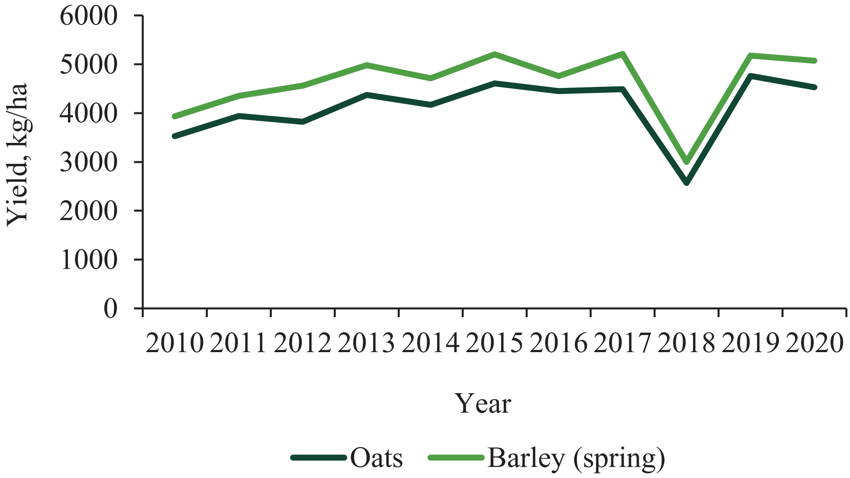

Figure 9. The yield of grain per hectare (kg/ha) for oats and spring barley in Sweden between 2010 and 2020 (Jordbruksverket, 2022).

1.3.3 Chemical composition and feeding value

As mentioned earlier, the chemical composition of the diet impacts emissions of enteric CH_4. Oats and barley display several differences in chemical composition that might affect both enteric CH_4 emissions and dairy cow production performance. Variation also exists within grain species depending on growing environment, weather, and variety. In Paper I, eight different varieties of both oats and barley were assessed for chemical composition and their effects on digestibility and enteric CH_4 emissions *in vitro*.

In oats, the hull constitutes around 25% of the whole grain and in barley only about 13% (Evers and Millar, 2002). The greater proportion of hull in oats is reflected by higher content of the major hull constituents; cellulose, hemicellulose, and lignin (NDF) (NorFor, 2022). Due to the higher lignin content, oats are less digestible than barley. According to Nordic feed tables for Swedish feed grains, the OM digestibility of barley is 80.3% and of oats with the lowest NDF content 74.6% (Table 2; NorFor, 2022). Barley on the other hand, has a greater proportion of endosperm compared with oats (Evers and Millar, 2002), which is reflected by a higher starch content in barley (NorFor, 2022). In the endosperm of both barley and oats, the major cell wall polysaccharide is β-glucan. Although the total content of β-glucan is similar between oats and barley, the content of soluble β-glucan and the ratio of soluble β-glucan to total β-glucan are greater in oats than in barley (Lee *et al.*, 1997). Protein content of oats is generally similar to or slightly higher than that of barley (NorFor, 2022).

Oats have a higher fat content than barley (NorFor, 2022). In barley, fat content may vary between 19 and 41 g/kg DM and in oats between 30 and 110 g/kg DM, depending on variety and growing environment (Welch, 1978; Zhou *et al.*, 1999). The FA composition of the fat also differs. Although the three major FA in both oats and barley are palmitic acid (16:0), oleic acid (18:1), and linoleic acid (18:2), oats tend to have a higher proportion of 18:1 and lower proportions of 18:2 and linolenic acid (18:3) (Welch, 1975; Welch 1978).

Oats also contain avenanthramides, a type of phenolic compound with antioxidant activity that are not present in barley or any other cereal (Peterson, 2001). These compounds could potentially act as inhibitors on enteric CH_4 emissions. The total content of the major avenanthramides in oats varies between 71 and 152 mg/kg and is affected by variety and growing environment and may decrease during heat treatment (Dimberg *et al.*, 1996; Emmons and Peterson, 2001). The groat is the main storage site for the avenanthramides (Dimberg *et al.*, 1996).

Due to the hulls of oats being more loosely connected to the outer layers of the groat in oats than in barley, the hulls of oats can be removed before feeding to increase digestibility. The OM digestibility of dehulled oats is 82.0% according to Finnish national feed tables (Table 2; LUKE, 2022). Since the chemical components are distributed differently between the groats and the hulls, the chemical composition of dehulled oats differ from that of hulled oats. The content of fat, crude protein (CP), and starch is higher, while the content of NDF is lower in dehulled oats than in hulled oats (Biel *et al.*, 2014). Both hulled oats (Paper I, Paper II, and Paper III) and dehulled oats (Paper III) were evaluated in this thesis for their effects on diet digestibility, milk production, enteric CH_4 emissions, and milk FA composition (Paper IV). Table 2 shows, in addition to OM digestibility, the energy- and protein values for barley, hulled oats, and dehulled oats according to Nordic feed tables (NorFor, 2022) and Finnish national feed tables (LUKE, 2022).

Table 2. Energy- and protein values and organic matter digestibility of barley, hulled oats, and dehulled oats for ruminants according to The Nordic Feed Evaluation System (NorFor, 2022: Sweden) and Finnish national feed tables (LUKE, 2022).

	Barley	**Hulled oats[1] (lowNDF)**	**Hulled oats[2] (medNDF)**	**Hulled oats[3] (hiNDF)**	**Dehulled oats**
NorFor[4]					
NEL20, MJ/kg DM	7.21	6.89	6.52	6.16	-
AAT20, g/kg DM	95	90	86	81	-
OMD20, %	80.3	74.6	71.2	67.5	-
LUKE[5]					
ME, MJ/kg DM	13.2	-	12.4	-	14.2
MP, g/kg DM	96	-	93	-	107
OMD, %	84.0	-	76.0	-	82.0

[1] Hulled oats (lowNDF) = low neutral detergent fibre content, 230 g/kg DM.

[2] Hulled oats (medNDF) = medium neutral detergent fibre content, 285 g/kg DM in NorFor, 290 g/kg DM in LUKE.

[3] Hulled oats (hiNDF) = high neutral detergent fibre content, 343 g/kg DM.

[4] NEL20 = net energy for lactation at 20 kg/d dry matter intake, AAT = metabolisable protein at 20 kg/d dry matter intake, OMD20 = organic matter digestibility at 20 kg/d dry matter intake.

[5] ME = metabolisable energy, MP = metabolisable protein, OMD = organic matter digestibility; barley values are for 60-64 kg/hl and hulled oat values are for >58 kg/hl.

2. Objectives

The overall objective of this thesis was to examine whether replacement of barley with oats as a grain supplement to dairy cows fed a grass-silage based diet could provide a mitigation strategy for enteric CH_4 emissions without compromising production performance of dairy cows.
The specific objectives were to:

I. Evaluate different varieties of hulled oats and barley in terms of chemical composition and their effects on ruminal fermentation, digestibility, and CH_4 emissions *in vitro*.

II. Investigate the effects of gradual replacement of barley with hulled oats as a grain supplement in the diet of dairy cows on milk production and enteric CH_4 emissions measured by the GreenFeed system.

III. Investigate the effects of different types of oats, hulled versus dehulled, as grain supplements fed to dairy cows on milk production and enteric CH_4 emissions measured by the GreenFeed system.

IV. Characterize and compare the fatty acid composition of milk from cows fed barley, hulled oats, or dehulled oats as grain supplements on a grass silage-based diet.

3. Materials and Methods

3.1 Paper I

An *in vitro* gas production study was conducted to investigate the effects of different varieties of barley and oats on CH_4 emissions, digestibility, and fermentation characteristics. Eight varieties of each grain (hulled) were incubated with grass silage (forage to concentrate ratio 50:50 on DM basis) in glass bottles containing buffered rumen fluid. To replicate rumen conditions, the bottles were submerged in a continuously agitated water bath at 39°C. The experiment consisted of three runs of 48 h incubations, each run including 16 treatments with two replicates and four blanks containing only buffered rumen fluid. Figure 10 shows the set-up of the *in vitro* gas production experiment in one of three water baths.

To record gas production, we used a fully automated technique as described by Cone *et al*. (1996). To determine CH_4 concentration, head space gas was sampled at 2, 4, 8, 24, 32, and 48 h of incubation and a sample size of 0.2 mL gas was injected to and analysed by a gas chromatograph (Varian Star 3400 CX FID Gas Chromatograph; Varian Inc., Palo Alto, CA). At 48 h of incubation, liquid samples were collected from each incubation bottle to determine VFA concentration by ultra-performance liquid chromatography (Puhakka *et al*., 2016). The pH-value in the liquid residue was also measured at 48 h. The *in vitro* digestibility was determined as true DM digestibility by transferring the incubation residues to nylon bags and boiling in ND-solution.

The gas and CH_4 data collected during the *in vitro* runs were subjected to a set of models to predict total gas and CH_4 emissions *in vivo* by applying the method developed by Ramin and Huhtanen (2012). We also predicted CH_4 end-point values stoichiometrically (CH_4VFA, mL) by using the total amounts of acetic, propionic, and butyric acid produced during incubations according to the equation by Wolin (1960). In addition, we predicted CH_4 emissions by using the mechanistic Nordic dairy cow model Karoline (Danfær *et al*., 2006) revised by Huhtanen *et al*. (2015). Finally, CH_4 emissions were predicted based on feed intake and chemical composition of feeds by an empirical equation developed by Ramin and Huhtanen (2013). Data were analysed by ANOVA using the MIXED procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC). The statistical model included the fixed effects of grain, variety within grain, run, and a random effect of position in water bath. Data based on VFA measurements were pooled within treatment per run and therefore, the random effect of position in water bath was excluded from the model for these variables.

Figure 10. The set-up of the *in vitro* gas production experiment in one of three water baths (Paper I). Bottles containing feed samples and buffered rumen fluid are submerged in a water bath and the bottles are connected with tubes to gas recording boxes.

3.2 Paper II

An *in vivo* study was conducted to investigate the effects of gradual replacement of barley with hulled oats as a grain supplement to dairy cows on ruminal fermentation, digestibility, milk production, CH_4 emissions, and energy utilization. The study was conducted at Röbäcksdalen experimental farm of the Swedish University of Agricultural Sciences, Umeå, Sweden (63° 45’ N; 20° 17’ E). Sixteen Nordic Red dairy cows in early- to mid-lactation were used in a replicated 4 × 4 Latin square design. The cows were blocked based on parity and milk yield, and randomly allocated to treatments. The four periods consisted of 11 days of adaptation and 10 days of sampling. The basal diet comprised grass silage (58% of diet DM) and rapeseed meal (12% of diet DM). The four experimental grain supplements (30% of diet DM) were formulated so that barley would be gradually replaced by oats at levels of 0, 33, 67 and 100% on DM basis. Cows were fed diets as a total mixed ration *ad libitum* and were milked twice daily.

Feed intake and milk yield were recorded daily but only data from the last ten days were used for statistical analysis. Milk samples were collected at four consecutive times at the end of each period. Emissions of CO_2 and CH_4, and consumption of O_2, were measured by the GreenFeed system (C-Lock Inc., Rapid City, SD, USA), as described by Huhtanen *et al*. (2015). Samples of rumen fluid were collected from eight cows (two blocks) after morning milking on the last day and analysed for VFA. The samples were collected with a stomach tube (RUMINATOR), as described by Geishauser (1993). Faecal grab samples were collected from the same eight cows twice a day on the three last days of each period and pooled within cow and period. Diet digestibility was determined by using both indigestible NDF (iNDF) (Huhtanen *et al*., 1994) and acid-insoluble ash (Van Keulen and Young, 1977) as internal markers.

Energy-corrected milk was calculated according to Sjaunja *et al*. (1990). Gross energy intake and gross energy digestibility were predicted according to Ramin and Huhtanen (2013). Urinary energy was calculated according to Guinguina *et al*. (2020) and heat production according to Brouwer (1965). The efficiency of metabolisable energy (ME) use for lactation was calculated according to AFRC (1993) using coefficients derived from Guinguina *et al*. (2020). Data were analysed by ANOVA using the MIXED procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC). The statistical model included the fixed effects of diet, block and period and a random effect of cow within block. For the digestibility data, marker was used in the model as repeated measurements. All treatment effects were investigated by specifying linear and quadratic contrasts.

3.3 Paper III

In paper III, a second *in vivo* study was conducted at Röbäcksdalen experimental farm of the Swedish University of Agricultural Sciences, Umeå, Sweden. The objective was to investigate the effects of replacing barley with hulled oats and dehulled oats as a grain supplement to dairy cows on ruminal fermentation, digestibility, milk production, CH_4 emissions, and energy utilization. Sixteen Nordic Red dairy cows in early- to mid-lactation were included in a 4 × 4 Latin square design replicated over four periods. Cows were blocked based on parity and milk yield, and randomly allocated to treatments. Periods consisted of 18 days of adaptation and ten days of sampling. The basal diet comprised grass silage and the forage to concentrate ratio was 60:40 on DM basis. The four experimental concentrates were barley, hulled oats, a mixture of hulled and dehulled oats 50:50 on DM basis, and dehulled oats. The concentrates were a pelleted mixture of the experimental grain and rapeseed meal (80:20 on weight basis). Cows were fed diets as a total mixed ration *ad libitum* and were milked twice daily.

Measurements were made and samples collected as described in Paper II, except for rumen fluid which was sampled at the start of the sampling period on day 19. Diet digestibility was determined by using iNDF as an internal marker (Huhtanen *et al*., 1994). Energy-corrected milk and energy utilization parameters were calculated and predicted according to the methods described in Paper II. The data were subjected to ANOVA using the MIXED procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC). The model included the fixed effects of diet, block, and period and a random effect of cow within block. Three orthogonal contrasts were specified. The barley diet was compared with the overall mean of the hulled oat, oat mixture, and dehulled oat diet and gradual replacement of hulled oats with dehulled oats was investigated using linear and quadratic contrasts.

3.4 Paper IV

The objective of Paper IV was to investigate the effects of replacing barley with hulled oats and dehulled oats as a grain supplement on FA composition of milk from cows fed a grass silage-based diet. For Paper IV, milk samples for determination of milk FA composition were collected from eight cows (two blocks) during each of the two *in vivo* experiments described in Paper II and Paper III. To analyse milk FA composition, FA methyl esters of lipid in feed and milk samples were prepared according to Shingfield *et al*. (2003) and total FAME profile determined by gas chromatography (6890N, Agilent Technologies, Santa Clara, CA). Milk FA output (g/d) was calculated with the assumption that all milk fat is triacylglycerols. Transfer efficiency of FA was calculated as milk FA output/FA intake (g/d) × 100.

Data were subjected to ANOVA using the MIXED procedure of SAS (Version 9.4, SAS Inst., Inc., Cary, NC). Data for milk FA composition were analysed separately for each study according to the statistical models described in Paper II and Paper III. Relationships between intake and output of FA in milk were examined by using a mixed model linear regression on combined data from Paper II and Paper III, with study, diet within study, and period within study as random effects.

4. Results

4.1 Paper I

The contents of crude fat, NDF and iNDF were higher in the oat varieties than in the barley varieties, whereas content of CP was more variable. For example, crude fat varied between 41.7 and 60.9 g/kg DM in oats and between 25.7 and 30.1 g/kg DM in barley. True DM digestibility was lower ($P < 0.01$) for the oat diets than for the barley diets and differed ($P = 0.04$) between different varieties within the species. Total VFA production was lower ($P < 0.01$) for the oat diets than for the barley diets, but was similar between different varieties within the species. The pH at 48 h of incubation was higher ($P < 0.01$) for the oat diets than for the barley diets and varied between different varieties within the species ($P < 0.01$). Molar proportions of VFA were not affected by dietary treatment, except for a greater ($P = 0.03$) proportion of valerate for the barley diets than for the oat diets.

Predicted *in vivo* total gas and CH_4 emissions were lower ($P < 0.01$) from the oat diets than from the barley diets but were similar between different varieties within the species. Ratio of CH_4 to total gas and predicted *in vivo* CH_4 in relation to true DM digestibility were not affected by species or variety. Predicted CH_4VFA was also lower ($P < 0.01$) from the oat diets than from the barley diets. The CH_4 predictions made by both the mechanistic and the empirical equation agreed well with the predicted *in vivo* CH_4 emissions, with a root mean square error of 0.80 for the mechanistic and 0.78 g/kg DM for the empirical model.

4.2 Paper II

Gradual replacement of barley with hulled oats resulted in increased dietary contents of crude fat, NDF, and iNDF. The intake of crude fat, NDF, and iNDF increased linearly (P at least ≤ 0.02), with increasing dietary inclusion of oats. Intake of DM and CP were not affected by the replacement. The effect of the replacement on digestibility was expressed as the mean for the two markers, as no interaction between diet and marker was observed. Replacing barley with oats decreased (P at least ≤ 0.03) apparent total-tract digestibility of DM, OM, NDF, and potentially digestible NDF (pdNDF) linearly. Milk yield, ECM yield, yield of milk constituents, feed efficiency, and body weight were not affected by the replacement. Concentrations of protein ($P < 0.01$) and fat ($P = 0.05$) in milk decreased linearly with increasing inclusion of oats. Milk urea and milk N efficiency were not affected by the replacement.

Replacing barley with oats decreased (P at least ≤ 0.05) total CO_2 emissions (kg/d), total CH_4 emissions (g/d), CH_4 yield (g/kg DM), CH_4 intensity (g/kg ECM), and ratio of CH_4 to CO_2 linearly. In addition, the respiratory quotient decreased linearly ($P = 0.03$) with increasing inclusion of oats. We observed no effect of the replacement on total VFA concentration or molar proportions of VFA in rumen fluid. Replacing barley with oats increased ($P < 0.01$) predicted dietary gross energy content linearly. Faecal energy increased linearly ($P < 0.01$), whereas gross energy digestibility, digestible energy intake, energy loss as CH_4, and ME intake decreased linearly (P at least ≤ 0.01) with increasing inclusion of oats. Heat production, milk energy, and efficiency of ME utilization for lactation were unaffected by the replacement.

4.3 Paper III

Dietary content of CP and crude fat were greater in the oat diets than in the barley diet, whereas the opposite was true for starch content. Total DM intake was similar between the barley and the oat diets and tended to decrease linearly ($P = 0.09$) when hulled oats were replaced by dehulled oats. Cows fed the oat diets had higher ($P < 0.01$) intakes of CP, crude fat, and iNDF, but lower (P at least ≤ 0.02) intake of starch and pdNDF than cows fed the barley diet. Intakes of CP, crude fat, starch, and neutral detergent solubles increased ($P < 0.01$), whereas intakes of NDF, iNDF, and pdNDF decreased linearly ($P < 0.01$) when hulled oats were replaced by dehulled oats. Total-tract apparent digestibility of the barley diet was similar to the overall mean of the oat diets, but digestibility of DM, OM, CP, NDF, and neutral detergent solubles increased linearly ($P < 0.01$) when hulled oats were gradually replaced by dehulled oats. Cows fed the oat diets produced more (P at least ≤ 0.04) milk, ECM, and milk protein than cows fed the barley diet. Protein concentration was lower ($P < 0.01$) in milk from cows fed the oat diets than that of those fed the barley diet. Gradual replacement of hulled oats with dehulled oats did not affect milk and ECM yield or yield and concentration of milk constituents. Feed efficiency tended to be higher ($P = 0.08$) for cows fed the oat diets compared with the barley diet and increased linearly ($P = 0.01$) when hulled oats were replaced with dehulled oats.

Total CH_4 emissions and CH_4 yield were similar between the oat diets and the barley diet, but CH_4 intensity was lower ($P = 0.01$) for the oat diets than for the barley diet. Gradual replacement of hulled oats with dehulled oats increased (P at least ≤ 0.02) total CH_4 emissions and CH_4 yield linearly but did not affect CH_4 intensity. Total VFA concentrations and molar proportions of VFA in rumen fluid were not affected by dietary treatment. Cows fed the oat diets had similar ME intake as those fed the barley diet, but milk energy was higher ($P = 0.01$) when feeding the oat diets. Efficiency of ME utilization for lactation was similar between the oat diets and the barley diet. Replacement of hulled oats with dehulled oats increased (P at least ≤ 0.01) ME intake and energy balance linearly but did not affect milk energy or efficiency of ME use for lactation.

4.4 Paper IV

In the first *in vivo* experiment (Exp1), gradual replacement of barley with hulled oats decreased ($P < 0.01$) milk fat proportions of 10:0, 12:0, 14:0, 16:0, total SFA, and total SFA + *trans* FA linearly. Milk fat proportions of 18:0, 18:1, total *trans* FA, total monounsaturated FA (MUFA), and total *cis* unsaturated FA increased linearly ($P < 0.01$) with increasing inclusion of hulled oats in the diet. In the second *in vivo* experiment (Exp2), milk fat from cows fed the oat diets had lower ($P < 0.01$) relative proportions of 10:0, 12:0, 14:0, 16:0, total SFA, and total SFA + *trans* FA than milk fat from cows fed the barley diet. Relative proportions of 18:0, 20:0, 18:1, total *trans* FA, total MUFA, and total *cis* unsaturated FA were higher ($P < 0.01$) in milk fat from cows fed the oat diets than from cows fed the barley diet. Replacing hulled oats with dehulled oats increased (*P* at least ≤ 0.04) milk fat proportions of 4:0, total *cis* 18:2, total 18:2, and 18:2n-6 and decreased milk fat proportions of 14:0, *trans*-11, *cis*-15 18:2, and total *cis* 20:1 linearly. The replacement also had or tended to have a quadratic effect on some of the milk FA proportions.

In Exp1, mean transfer efficiency of total C18 FA into milk decreased linearly ($P < 0.01$) with increasing inclusion of hulled oats in the diet. In Exp2, feeding the oat diets led to lower ($P < 0.01$) mean transfer efficiency of total C18 into milk than feeding the barley diet and increasing dietary inclusion of dehulled oats decreased ($P < 0.01$) transfer efficiency linearly. All the investigated FA groups expressed weak positive relationships between intake and output in milk fat. The relationship between intake and output of the C18:1 group was strongest ($R^2 = 0.28$), whereas that of the C18:3 group was weakest ($R^2 = 0.13$).

5. Discussion

5.1 Effects on digestibility and ruminal fermentation

Lower true *in vitro* DM digestibility of the oat diets than of the barley diets in Paper I and the linear decrease in apparent OM digestibility when barley was replaced by hulled oats in Paper II are expected changes, as the proportion of hull to the whole grain is higher in oats compared with barley (Evers and Millar, 2002). Considering that the oat varieties used in Paper I had a mean NDF content of 332 g/kg DM, the 7%-units lower *in vitro* true DM digestibility that we observed corresponds well with the reported difference (12.8%-units) between barley and high-NDF oats by NorFor (2022). As grain inclusion was only 50% of diet DM in our *in vitro* study, the difference was diluted by similar digestibility of the grass silage. In Paper II, the NDF content of hulled oats was 257 g/kg DM and grain inclusion in the diet was 30%. Thus, the linear decrease in apparent OM digestibility of 2.8%-units when barley was replaced by hulled oats in Paper II is also consistent with the difference reported by NorFor (2022) for low- to medium-NDF oats. Moreover, Vanhatalo *et al*. (2006) supplemented dairy cow diets with coarsely ground barley or oats (40% of diet DM) with grass silage or grass-red clover silage as a basal diet and reported 3.6% lower apparent OM digestibility on diets supplemented with oats. In contrast, Tosta *et al*. (2019) reported similar apparent OM digestibility between dairy cow diets supplemented with either rolled barley or rolled oats. In their study however, grain inclusion was only 15% of diet DM and the barley diet had a higher NDF content than the oat diet.

In Paper III, similar OM digestibility between the barley diet and the overall mean for the oat diets (hulled, hulled/dehulled 50:50, dehulled) is explained by numerically lower values for the hulled oat diet, numerically higher values for the dehulled oat diet (compared with the barley diet), and the linear increase in OM digestibility when hulled oats were replaced by dehulled oats. When oats are dehulled, a major part of the indigestible lignin fraction of whole oat grain is removed (Salo and Kotilainen, 1970). Oat hulls may contain lignin up to 76 g/kg DM depending on cultivar and growing location (Crosbie *et al*., 1985). NorFor (2022) does not report OM digestibility values for dehulled or naked oats, but LUKE (2022) reports an 8%-unit higher OM digestibility for dehulled oats compared with hulled oats. The linear increase in OM digestibility of 6%-units in Paper III is higher than would be expected based on the reported values and considering that dietary grain inclusion was only 30% in our study. Although lower DMI increases diet digestibility by increasing the retention time of feed in the rumen, allowing more time for feed digestion (Tyrrell and Moe, 1975), the numerical 0.6 kg decrease in DMI observed with increasing inclusion of dehulled oats is not sufficient to explain the greater difference in OM digestibility in our study. The greater differences could instead be due to the use of different oat varieties and their differences in lignin content. LUKE (2022) reports slightly higher OM digestibility for barley than dehulled oats (Table 2). Although not tested in Paper III, apparent digestibility of DM, OM, and NDF was numerically higher for the dehulled oat diet than for the barley diet. This is in line with the results of Mustafa *et al*. (1998), where ruminal digestibility of DM and NDF were higher for naked oats than for barley.

The effects of replacing barley with hulled oats on ruminal fermentation are inconsistent between the papers in this thesis and existing literature. In Paper I, lower diet digestibility and higher amounts of non-fermentable FA with the oat diets than with the barley diets led to lower *in vitro* total VFA production with the oat diets. In contrast, we observed no effect of gradual replacement of barley with hulled oats on total VFA concentrations despite a linear decrease in diet digestibility in Paper II. This inconsistency could be explained by the difference between the *in vitro* and *in vivo* environments, as there is no continuous absorption of VFA *in vitro*. However, an *in vivo* study by Vanhatalo *et al*. (2006) found lower total VFA concentrations in rumen fluid from cows fed hulled oat diets than from cows fed barley diets. The study by Vanhatalo *et al*. (2006) is similar to Paper II, as cows were fed grass silage as basal diet and grain inclusion was 40% of diet DM. Tosta *et al*. (2019) also reported lower total VFA concentration on rolled oat diets than on rolled barley diets when the basal diet consisted of barley silage and alfalfa hay and grain inclusion was 15% of diet DM, whereas Gozho and Mutsvangwa (2008) found no effect of replacing barley with oats on total VFA concentration in rumen fluid.

In both Paper II and III, relatively high standard errors for total VFA concentration indicate that the sample means may not be very accurate due to relatively high random variation. In comparison to the studies by Vanhatalo *et al*. (2006) and Tosta *et al*. (2019), where rumen fluid was sampled using cannulated cows and on several occasions throughout the sampling day, we sampled rumen fluid by using a stomach tube and only once per sampling day. Rumen fluid samples collected by a stomach tube may become contaminated by saliva, and compared with collection through rumen cannula, may not be representative for the total VFA concentration (de Assis Lage *et al*., 2020). In addition, VFA concentrations in rumen fluid show diurnal variation in response to eating behaviour and therefore samples should be collected on several occasions to better represent the 24-h feeding cycle and decrease random variation.

Regarding molar proportions of VFA, the sampling method is less likely to have affected the results (de Assis Lage *et al*., 2020). Lower *in vitro* molar proportion of valerate on the oat diets in Paper I is consistent with the results of Vanhatalo *et al*. (2006). However, Vanhatalo *et al*. (2006) also reported decreased proportion of butyrate, whereas Tosta *et al*. (2019) and Gozho and Mutsvangwa (2008) did not find any effect on molar proportions of VFA. Overall, based on the papers included in this thesis and the previous studies, the effects of replacing barley with hulled oats on ruminal fermentation pattern are most likely small.

5.2 Effects of barley and oats on production performance

5.2.1 Milk and energy-corrected milk yield

Milk yield is an important factor contributing to the economy of the dairy farmer. Lower tabulated energy value of hulled oats compared with barley (NorFor, 2022; LUKE, 2022) suggests that milk and ECM yield would be compromised when barley is replaced by hulled oats on an equal DM basis. However, in Paper II, milk yield and ECM were unaffected by the replacement. In Paper III, although not tested, milk yield was numerically higher (+1.2 kg/d) with the hulled oat diet than with the barley diet and the overall mean for the oat diets was significantly higher (+1.4 kg/d) than for the barley diet. In agreement with this, previous studies consistently show that milk yield is maintained (Gozho and Mutsvangwa, 2008; McKay *et al*., 2019) and in several cases even increases (Heikkilä *et al*., 1988; Martin and Thomas, 1988; Ekern *et al*., 2003; Vanhatalo *et al*., 2006; Tosta *et al*., 2019) when barley is replaced by hulled oats as a concentrate supplement in dairy cow diets. It is difficult to make a direct comparison to and among these studies regarding milk yield response as the basal diet, proportion of experimental grain of diet DM, and the chemical composition of the oats and barley fed vary greatly between studies. A simple description of the previous studies (including Paper II and Paper III) is shown in Table 3. For a study to be included, experimental grain inclusion had to be at least 15% of diet DM. Consequently, the study by McKay *et al*. (2019) with only 4-7% grain inclusion was excluded.

Table 3. Description of previously published studies investigating replacement of barley with hulled oats as grain supplement in the diet of dairy cows, including Paper II and III.

Reference	Country	Basal diet	F:C ratio	Barley of DM	Oats of DM
Heikkilä *et al*., 1988 (five studies)	Finland	Grass silage & Hay (1 kg)	~64:36	36%	37%
Martin & Thomas, 1988	UK	Hay	34:66	54%	54%
Ekern *et al*., 2003	Norway	Grass silage	~45:55	37%	42%
Vanhatalo *et al*., 2006	Finland	Grass & red clover silage	60:40	40%	40%
Gozho and Mutsvangwa, 2008	Canada	Barley silage & alfalfa hay	50:50	31%	31%
Tosta *et al*., 2019	Canada	Barley silage & alfalfa hay	54:46	15%	15%
Paper II	Finland	Grass silage	58:42	30%	30%
Paper III (hulled oats)	Finland	Grass silage	60:40	30%	30%

Based on the studies presented in Table 3, the relationship between milk yield on barley diets and milk yield on oat diets is illustrated in Figure 11, and the corresponding relationship for ECM yield in Figure 12. The mean value for oats used from Paper III is for the hulled oat diet. For studies only reporting yield of fat-corrected milk or if both fat-corrected milk and ECM yield were missing, ECM was calculated according to Sjaunja *et al*. (1990)

based on reported milk yields and milk concentrations of protein, fat, and lactose.

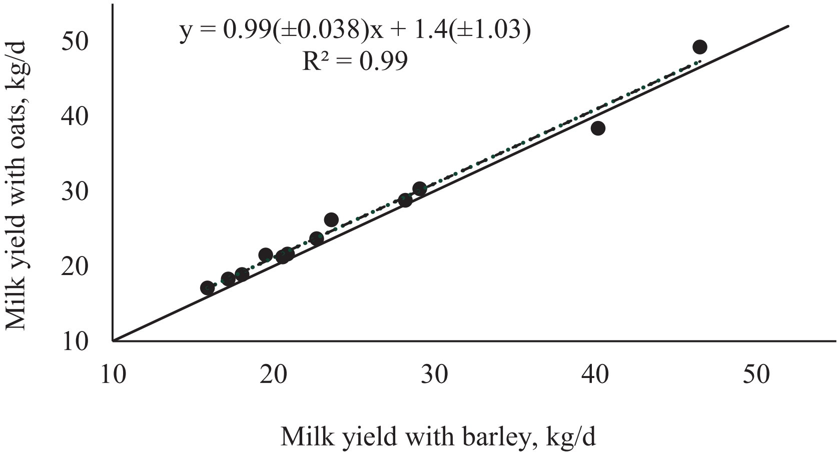

Figure 11. Relationship between milk yield (kg/d) with barley diets (X) and milk yield with oat diets (Y). Based on Heikkilä *et al.*, 1988 (five studies), Martin and Thomas, 1988, Ekern *et al.*, 2003, Vanhatalo *et al.*, 2006, Gozho and Mutsvangwa, 2008, Tosta *et al.*, 2019, Paper II, and Paper III (oat diet = hulled oat diet).

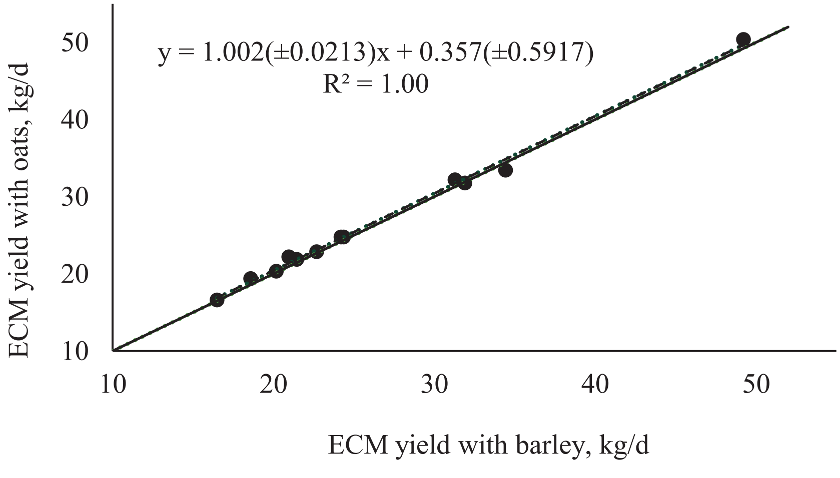

Figure 12. Relationship between ECM yield (kg/d) with barley diets (X) and ECM yield with oat diets (Y). Based on Heikkilä *et al.*, 1988 (five studies), Martin and Thomas, 1988, Ekern *et al.*, 2003, Vanhatalo *et al.*, 2006, Gozho and Mutsvangwa, 2008, Tosta *et al.*, 2019, Paper II, and Paper III (oat diet = hulled oat diet).

Slopes with values very close to one (Figure 11 & Figure 12) indicate that the responses in milk and ECM yield to replacement of barley with hulled oats are not related to production level in the range of 15.9-49.2 kg of milk/d and 16.5-50.4 kg of ECM/d. Based on previous studies, Paper II, and Paper III, cows fed oat concentrate produce on average 1.1 kg/d more milk ($P < 0.01$) and 0.4 kg/d more ECM than cows fed barley concentrate ($P < 0.05$). The lower response in ECM yield than milk yield is due to lower fat and protein concentrations in milk from cows fed oats, which will be discussed later.

The mechanisms behind increased milk yields when barley is replaced by oats in dairy cow diets are still not entirely clear. In Paper II, dietary starch was partly replaced with crude fat with increasing inclusion of hulled oats (Figure 13). In Paper III, dietary crude fat content was higher and starch content lower in the oat diets than in the barley diet (Figure 14).

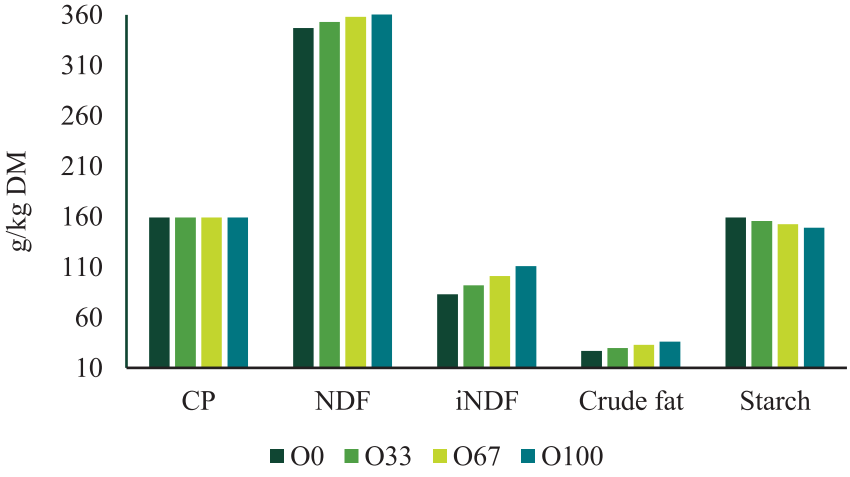

Figure 13. Dietary contents (g/kg DM) of crude protein (CP), neutral detergent fibre (NDF) indigestible NDF (iNDF), crude fat and starch in Paper II. O0 = 0% oats, O33 = 33% oats, O67 = 67% oats, and O100 = 100% oats in grain supplement.

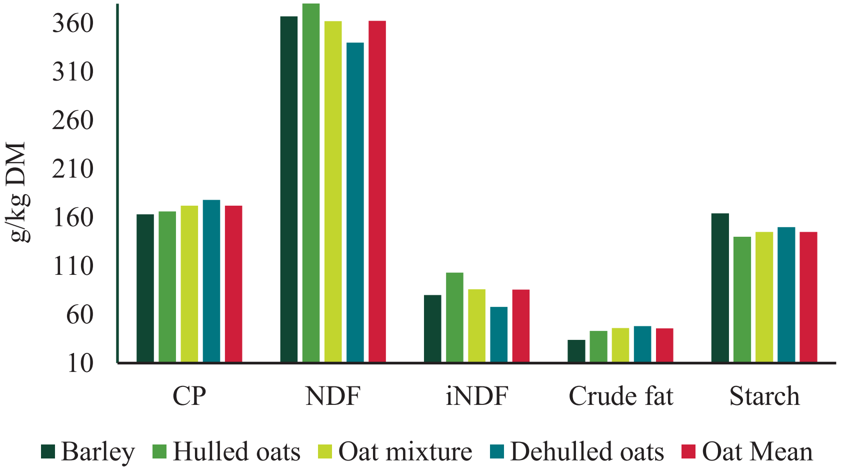

Figure 14. Dietary contents (g/kg DM) of crude protein (CP), neutral detergent fibre (NDF) indigestible NDF (iNDF), crude fat, and starch in Paper III.

Already 80 years ago, Maynard *et al*. (1940) reported slightly higher milk and ECM yield when dietary starch was replaced by fat (isodynamic amounts) through feeding a concentrate with 50 to 70 g/kg DM ether extract instead of a concentrate with 7 to 40 g/kg DM ether extract. A meta-analysis by Rabiee *et al*. (2012) concluded that addition of fat to dairy cow diets consistently increases milk yields, with an overall milk production response of +1.05 kg/cow per day when the estimated average increase in ether extract was 25.9 g/kg of diet DM. However, results were heterogeneous depending on the type of supplemental fat. In paper II, complete replacement of barley with hulled oats increased dietary crude fat content by 9.3 g/kg DM and milk yield numerically by 0.6 kg/day. In paper III, crude fat content was 9.0 g/kg DM higher and milk yield 1.2 kg/d higher (numerically) on the hulled oat diet than on the barley diet. In addition, in Paper III, crude fat content was 11.7 g/kg DM higher and milk yield was 1.4 kg/d higher on the oat diets than on the barley diet. It is important to consider that replacement of barley with hulled oats does not only increase dietary fat content, but it also increases dietary NDF and iNDF content (Figure 13 & 14) and decreases diet digestibility (Paper II, III, Vanhatalo *et al*., 2006). Therefore, the response in milk and ECM yield to incremental levels of fat in oats are offset by a drop in digestibility.

The positive response in milk yield due to incremental dietary fat may be explained by an increased direct incorporation of preformed long-chain FA into milk fat. Milk FA with chain lengths from 4:0 to 12:0, most 14:0 and part of 16:0 are synthesized *de novo* in the mammary gland (Palmquist *et al*., 1969; Palmquist, 2006). Acetic acid (through acetyl-CoA) produced during ruminal fermentation is the main precursor for *de novo* FA, whereas β-hydroxybutyrate derived from butyric acid contributes with a minor part of carbon to *de novo* milk FA (Palmquist *et al*., 1969). *De novo* FA synthesis also requires reducing equivalents in the form of NADPH. Around one half of the NADPH required is obtained from acetic acid through the isocitrate pathway and the other half from glucose metabolism through the pentose phosphate pathway (Bauman *et al*., 1970). Increased supply of especially C18 to the mammary gland has been shown to inhibit *de novo* synthesis of short to medium-chain milk FA (Souza and Williamson, 1993). Decreased milk fat concentrations of 12:0, 14:0, and 16:0 when barley was replaced by hulled oats in Paper II, and when barley was replaced by the oat diets in Paper III (Paper IV), indicates that *de novo* synthesis was inhibited to some extent. As *de novo* synthesis of milk FA is decreased, the need for oxidation of glucose through the pentose phosphate pathway to yield NADPH also decreases. Thus, glucose is spared and may be used for lactose synthesis, which in turn is the driver of milk yield. This way, replacing starch with fat can increase the energetic efficiency of the diet.

In Paper II, the EB of cows tended to change both linearly and quadratically, with the lowest value when barley was completely replaced by oats. This could indicate a change in energy partitioning towards milk production to maintain similar milk energy despite the linear decrease in ME intake. As opposed to Paper II, where diet digestibility and ME supply decreased when barley was replaced by hulled oats, diet digestibility and ME supply were similar between the barley diet and the average of the oat diets in Paper III. Based on predicted ME intake and energy losses due to heat production, the barley diet and the oat diets also supplied similar NE. This together with higher milk energy and numerically higher efficiency of ME use for lactation with a relatively low *P*-value (0.12) when cows were fed the oat diets, suggest an altered energy partitioning towards milk production when barley is replaced by oats. In a study by van Knegsel *et al*. (2007), cows in early lactation were fed a diet high in glucogenic nutrients (mainly starch) or a diet high in lipogenic nutrients (fat and fibre) supplying similar amounts of NE. Cows on the lipogenic diet partitioned more of the NE intake to milk production than cows on the glucogenic diet. Similarly, in a study by Boerman *et al*. (2015), mid-lactation dairy cows were supplied similar NE with a high-starch diet or a high-fat and high-fibre diet. Cows fed the high-fat and high-fibre diet partitioned more energy towards milk production and less energy towards body fat reserves than on the high-starch diet. Interestingly, when hulled oats was replaced by dehulled oats in Paper III, both dietary starch and fat content increased similarly (Figure 14) and similar amounts of ME were partitioned towards milk production.

The fact that body condition scores were not assessed in Paper II or Paper III could be criticized. Assessment of body condition scores before the start of both feeding trials and regular assessment during the trials could have provided valuable information. Moreover, collection of blood samples and analysis of glucose, insulin, non-esterified FA, and triglyceride concentrations could have provided additional information regarding metabolism. Although EB was numerically lower on the hulled oat diets (Paper II, Paper III), it was still positive, and it is unlikely that cows needed to mobilize body fat to maintain milk yields.

Although replacing barley with oats seem to maintain or improve the production performance of dairy cows consistently, the same does not appear to hold for production performance of beef cattle. In a study by Dion and Seoane (1992), where fattening steers were fed a hay-based diet and different cereal grains, average daily gain and feed efficiency were similar between oat and barley diets. In the study by Huuskonen (2009), barley was replaced by oats in the diet of growing and finishing dairy bulls fed a grass silage-based diet. They found that replacing barley with oats decreased live weight gain and impaired feed efficiency. Less consistent results for beef cattle could be expected, as replacing barley with oats seems to favour milk production.

Maintained or increased milk yields when barley is replaced with oats seem to be due to both increased energetic efficiency and repartitioning of energy to favour milk production. In a study by Banks *et al*. (1976), milk yield increased when diets deficient in fat were supplemented with oil. It could be so that dairy cow diets with barley concentrate and without oil supplementation are deficient in fat, making milk production unnecessary inefficient. When cows were fed the barley diet in Paper IV, the output of total C18 in milk was numerically higher than the intake, which could indicate that the barley diet not supply a sufficient amount of C18.

5.2.2 Milk protein concentration and yield

The decrease in milk protein concentration when barley was replaced by hulled oats in Paper II and lower protein concentration in milk from cows fed the oat diets than from cows fed the barley diet in Paper III are consistent with the findings of several previous studies (Heikkilä *et al*., 1988; Martin and Thomas, 1988; Ekern *et al*., 2003; Vanhatalo *et al*., 2006; Tosta *et al*., 2019). In contrast, Gozho and Mutsvangwa (2008) and McKay *et al*. (2019) reported that milk protein concentrations were similar between oat supplemented diets and barley supplemented diets. Figure 15 shows the relationship between milk protein concentrations with barley diets and with oat diets. Based on previous studies, Paper II and Paper III, protein concentration in milk from cows fed oat concentrate is on average 1.2 g/kg lower than in milk from cows fed barley concentrate ($P < 0.01$).

Lower protein concentrations in milk from cows fed oat concentrate may be explained by a dilution effect. The studies reporting lower protein concentrations in milk also reported higher milk yield on oat diets (Heikkilä *et al*., 1988; Martin and Thomas, 1988; Ekern *et al*., 2003; Vanhatalo *et al*., 2006; Tosta *et al*., 2019), whereas studies reporting similar milk protein concentrations reported similar milk yield on oat and barley diets (Gozho and Mutsvangwa, 2008; McKay *et al*., 2019). This is also consistent with the results of Paper III. In addition, although not significant, milk yield was numerically higher when barley was completely replaced by hulled oats in Paper II.

When milk protein yield remains constant (Paper II, Vanhatalo *et al*., 2006), a dilution effect indicates that milk protein synthesis is not affected negatively by replacing barley with hulled oats, despite NorFor (2022) reporting a 5-14 g/kg lower MP value and LUKE (2022) reporting 3 g/kg DM lower MP value for hulled oats than for barley. In Paper II, dietary CP contents were similar between diets, but the NRC (2001) reports that the rapidly degradable fraction A of CP is 65% in oats and 30% in barley, which would lead to greater ammonia nitrogen (N) losses when feeding oats. Although we did not investigate ruminal N digestion, similar milk urea nitrogen (MUN) concentrations when barley was replaced by oats in Paper II does not support greater ammonia N losses with oats. Vanhatalo *et al*.

(2006) did investigate N digestion when barley was replaced by hulled oats and reported similar ruminal ammonia N concentrations, similar MUN concentrations, similar flows of non-ammonia N to the duodenum, and similar milk protein yield when feeding oats as when feeding barley. Based on the results of this thesis and Vanhatalo *et al*. (2006), it seems quite clear that the MP values for hulled oats in comparison with barley are underestimated.

In Paper III, only 42% of the increase in milk yield contributed to dilution of protein concentration, as milk protein yield was 2.8% higher and milk yield was 4.9% higher on the oat diets than on the barley diet. Out of the previous studies, only Ekern *et al*. (2003) reported higher milk protein yield with oat diets. Common to both Paper III and Ekern *et al*. (2003), CP content was higher in the oat concentrate than the barley concentrate. In the studies by Heikkilä *et al*. (1988) and Vanhatalo *et al*. (2006), CP content was similar between barley and oats or slightly higher in barley. Higher milk protein yield from cows fed oats could indicate increased milk protein synthesis. Around 95% of analysed CP in milk is true protein (Davies *et al*., 1983) and the largest single component of milk non-protein N is urea (Wolfschoon and Klostermeyer, 1981). In Paper III, higher MUN concentrations (+1.4 mg/dL) in milk from cows fed oats instead of barley were only a minor contributor to slightly higher milk protein yield with oats. Higher MUN concentrations with oat diets are in harmony with the higher dietary CP content of the oat diets (Nousiainen *et al*., 2004).

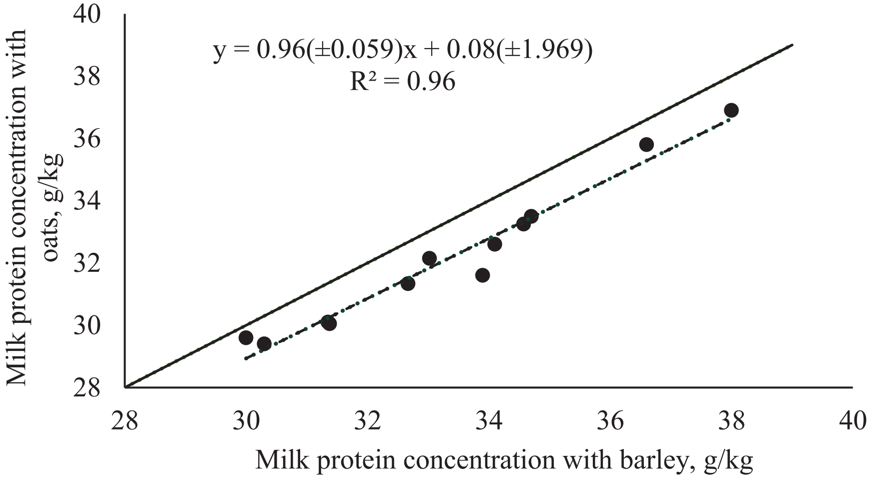

Figure 15. Relationship between milk protein concentration (g/kg milk) with barley diets (X) and milk protein concentration with oat diets (Y). Based on Heikkilä *et al*., 1988 (five studies), Martin and Thomas, 1988, Ekern *et al*., 2003, Vanhatalo *et al*., 2006, Gozho and Mutsvangwa, 2008, Tosta *et al*., 2019, Paper II, and Paper III (oat diet = hulled oat diet).

5.2.3 Milk fat concentration and yield

The effects of replacing barley with oats on milk fat concentration have been less consistent than the effects on milk protein concentration (Figure 16). Decreasing milk fat concentration with increasing inclusion of hulled oats in Paper II agrees with the findings of Heikkilä *et al*. (1988), Martin and Thomas (1988), and Ekern *et al*. (2003). On the contrary, Tosta *et al*. (2019) found higher milk fat concentrations with oat diets than with barley diets, whereas Vanhatalo *et al*. (2006), Gozho and Muswangwa (2008) and McKay *et al*. (2019) did not find any effect of oats on milk fat concentration. Despite larger inconsistencies between studies, fat concentration in milk from cows fed oats is on average 1.7 g/kg lower than in milk from cows fed barley concentrate ($P < 0.05$) based on previous studies, Paper II, and Paper III (hulled oat diet). In general, the fat content between oats and barley varies more than the CP content and it could be that the response in milk fat concentration to replacement of barley with oats is more sensitive to inclusion rate of experimental grain, forage to concentrate ratio, and the basal diet. In Paper II, milk fat yield was unaffected by replacing barley with oats and the decrease in milk fat concentration was mainly due to a dilution effect, similarly to as with milk protein concentration. In Paper III, milk fat concentration was numerically lower (-1.1 g/kg milk), and milk fat yield tended to be higher (+44 g/d) with the oat diets than the barley diet, which suggest that supply of FA to the mammary gland was increased with oats.

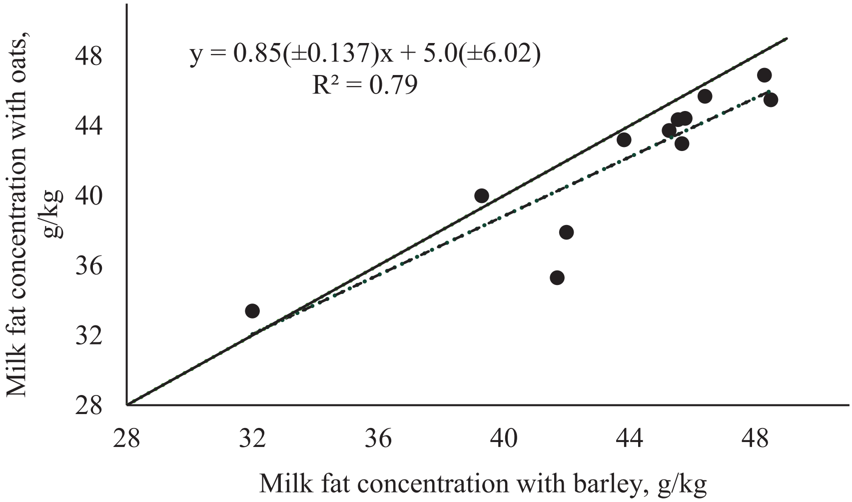

Figure 16. Relationship between milk fat concentration (g/kg milk) with barley diets (X) and milk protein concentration with oat diets (Y). Based on Heikkilä *et al.*, 1988 (five studies), Martin and Thomas, 1988, Ekern *et al.*, 2003, Vanhatalo *et al.*, 2006, Gozho and Mutsvangwa, 2008, Tosta *et al.*, 2019, Paper II and Paper III (oat diet = hulled oat diet).

5.2.4 Milk fatty acid composition

In Paper IV, we evaluated the effect of replacing barley with hulled oats (Exp1) and the effect of replacing barley with both hulled and dehulled oats (Exp2) on milk FA composition. Lower relative proportions of 10:0, 12:0, 14:0, 16:0, and total SFA in milk from cows fed oat grain instead of barley grain (Exp1 and Exp2) are consistent with the findings of previous studies (Heikkilä *et al.*, 1988; Martin and Thomas, 1988; Ekern *et al.*, 2003; Vanhatalo *et al.*, 2006). Also, in agreement with the previous studies, these changes were accompanied by higher relative proportions of 18:0, 18:1, and total MUFA in milk from cows fed oats (Exp1 and Exp2). Replacing barley with oats increased the intake of 16:0 and C18, and the supply of these FA to the mammary gland (Paper IV), which may inhibit *de novo* FA synthesis (Souza and Williamson, 1993; Wright *et al.*, 2002). As *de novo* synthesis in the mammary gland gives rise to all the 12:0, most of the 14:0, and around 50% of the 16:0 found in milk fat (Palmquist, 2006), inhibition of *de novo* synthesis could explain lower milk fat proportions of these FA in milk from cows fed oats. Part of the explanation could also be a dilution effect, which can be especially strong when FA are expressed as proportions. For example, the increase in intake of 18:1 was three-fold compared with the increase in intake of 16:0. When milk fat proportions of 18:0 and 18:1 increase, proportions of some other FA will inevitably decrease. In addition, although not observed in Paper II, replacing barley with hulled oats could be expected to decrease ruminal total VFA production if OM digestibility is reduced (Paper I, Vanhatalo *et al*., 2006). As acetic acid is the main precursor for *de novo* FA (Palmquist *et al*., 1969), the available acetic acid for *de novo* FA synthesis would also be lower with the oat diet in Paper II. However, in Paper III, where OM digestibility was similar between the barley diet and the oat diets, smaller differences in the supply of acetic acid for *de novo* FA synthesis could be expected.

Following dietary recommendations from the World Health Organization (WHO, 2020) and the Food and Agriculture Organization of the United Nations (FAO, 2010), dietary SFA should be replaced with polyunsaturated FA (PUFA) and MUFA due to the link between consumption of SFA and risk of developing cardiovascular disease. In Exp1 and Exp2 (Paper IV), milk fat proportions of total SFA decreased by 2.9 and 2.6 g/100 g FA, respectively, when barley was replaced with oats. As milk proportions of total PUFA also decreased slightly (Exp1), milk SFA were in this case replaced by MUFA, which increased by 3.0 and 2.7 g/100 g FA in Exp1 and Exp2, respectively. These changes in milk FA composition induced by replacing barley with oats are small compared with the changes brought about by some other dietary strategies. For example, supplementing dairy cow diets with 50 g/kg DM rapeseed oil decreased milk SFA by 14.9 and increased milk MUFA by 14.8 g/100 g milk FA in a study by Bayat *et al.* (2018). Milk PUFA were not affected by rapeseed oil supplementation. Nevertheless, feeding oats instead of barley can provide a more financially attractive strategy for modulating milk FA composition, as oil supplements could increase feed costs more than replacing barley with oats. In addition, greater changes in milk FA composition could possibly be brought about if barley was replaced by oat varieties with higher fat content.

5.3 Effects of dehulled oats on production performance

As DMI is a major driver of milk production (Hristov *et al*., 2004) the response in milk and ECM yield to replacement of hulled oats with dehulled oats as a grain supplement might have been partly offset by the linear tendency toward decreased DMI (Paper III). Despite the tendency for decreased DMI with increasing inclusion of dehulled oat, increased OM digestibility led to a linear increase in ME supply. Although calculated ME supply increased by 18 MJ/d, the replacement led only to a small numerical increase in milk yield (+0.5 kg/day) and ECM yield (+0.7 kg/day). In comparison, milk yield and ECM yield were 1.4 and 1.1 kg/d higher on the oat diets than on the barley diet, although calculated ME supply was only 3 MJ/d higher on the oat diets (Paper III). In Paper IV, the linear decrease in transfer efficiency of total C18 FA into milk from 96 to 76% with increasing inclusion of dehulled oats indicates that incremental dietary C18 was partitioned towards body fat reserves rather than the mammary gland and incorporation into milk fat. As opposed to the effect of replacing barley with hulled oats, replacement of hulled oats with dehulled oats did not increase the output of total C18 into milk, although total C18 intake increased linearly. Partitioning of incremental C18 to body fat is further supported by the findings in Paper III, where energy balance increased linearly from 18 to 30 MJ/d with increasing inclusion of dehulled oats in the diet.

Based on the results of this thesis, the potential of dehulling oats as a means to increase profitability on dairy farms through increased digestibility and milk and ECM yield is questionable. Dehulling is both laborious and costly for the farmer. In practice, the observed increase in feed efficiency when hulled oats was replaced by dehulled oats (Paper III) means that the farmer would need to feed less DM per day of dehulled oats to obtain the same milk yield as with feeding hulled oats, but dehulling would still not be profitable if the hulls go to waste. It is, however, possible that the response in milk and ECM yield to dehulled oats depends on the lactation stage of the dairy cow. In Paper III, cows were in positive energy balance and may therefore not have been very sensitive to increased ME supply when hulled oats was replaced by dehulled oats. Cows in negative energy balance are likely to have a greater response in milk yield to dehulled oats. Potentially, dehulling of oats could be profitable if fed to cows in negative energy balance postpartum.

5.4 Effects on enteric methane emissions

5.4.1 Underlying mechanisms

Several dietary factors are known to affect enteric CH_4 emissions. Dry matter intake and diet digestibility are both positively related to total CH_4 production in the rumen (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013), as CH_4 is produced only from digested DM. Although predicted *in vivo* CH_4 emissions (g/kg DM) were 8.9% lower on the oat diets than on the barley diets in Paper I, the emissions were similar when expressed relative to *in vitro* true DM digestibility. In agreement with Paper I, Paper II found that enteric CH_4 yield (g/kg DMI) decreased by 4.4% when barley was replaced by hulled oats, but when CH_4 emissions were expressed relative to kg of OM digested no difference was observed between the diets. Finally, in Paper III, CH_4 yield increased by 6.6% when hulled oats was replaced by dehulled oats, but CH_4 emissions expressed relative to kg of OM digested were unaffected by the replacement. These results indicate that the major CH_4 mitigating effect of hulled oats is due to lower digestibility compared with barley.

Another dietary factor that affects enteric CH_4 emissions and differs between barley and oats is fat content, which is negatively related to CH_4 production in the rumen (Beauchemin *et al.*, 2009; Grainger and Beauchemin, 2011; Ramin and Huhtanen, 2013). This effect can be mediated through several mechanisms. Replacement of fermentable matter, such as starch, with non-fermentable FA decreases the extent of fermentation and thereby the need for re-oxidation of NADH into NAD^+ and elimination of hydrogen through methanogenesis (Johnson and Johnson, 1995). Also, addition of dietary fat may impair the function of the fibrolytic microbes, which shifts ruminal fermentation pathways towards production of propionic acid, a hydrogen sink (McAllister *et al.*, 1996; Ungerfeld, 2015). Although oats had higher crude fat content than barley, the dietary differences were still relatively small (10 g/kg DM in Paper I if assuming 25 g/kg crude fat in grass silage, 9.3 g/kg DM in Paper II) and it is unlikely that the addition of fat with oats would have been sufficient to negatively affect fibre digestion. This is further supported by that we did not observe a shift in ruminal fermentation pathways towards production of propionic acid (Paper I, Paper II, Paper III). However, replacing barley with oats did replace fermentable matter with non-fermentable FA which, although too small to observe, most likely played a role in the CH_4 mitigating effect of oats. Further elucidation of the CH_4 mitigating mechanisms is complicated by the fact that a change in one chemical component will inevitably lead to a change in another component. This was evident in Paper I, where inclusion of both iNDF content and crude fat content in the regression model caused a multicollinearity problem with a high variance inflation factor (5.9) and insignificance for variables that were significant in the univariate model.

Because a preliminary *in vitro* study (unpublished data) showed a greater decrease in CH_4 emissions than could be accounted for by differences in digestibility and crude fat content when barley was replaced by hulled oats, this thesis also investigated whether oats could contain specific compounds inhibiting methanogenesis in the rumen. In Paper I, the differences between the oat diets and the barley diets in CH_4 end-point values at 48 h of incubation and predicted CH_4VFA values were similar (9.8 and 10%, respectively) indicating that the difference in predicted *in vivo* CH_4 emissions was accounted for by lower digestibility and replacement of fermentable matter with non-fermentable FA with oats. Furthermore, the predictions of CH_4 emissions made by both the empirical and mechanistic model in Paper I agreed well with the observed predicted *in vivo* CH_4 emissions. Based on the results of this thesis, oats do not contain any specific CH_4 mitigating compounds.

In Paper I, the absence of an effect of different varieties of the same grain species on predicted *in vivo* CH_4 emissions may be explained by relatively small variations between the varieties regarding digestibility and fat content. In hindsight, oat varieties with higher fat content could have been included in this thesis. In Paper I, the highest crude fat content observed was 60.9 g/kg DM for the oat variety Akseli and the mean for all oat varieties was 48.7 g of crude fat/kg DM. In Paper II and Paper III, crude fat content of hulled oats was 52.0 and 50.0 g/kg DM, respectively, and of dehulled oats 64.0 g/kg DM. Although these values are within the normal variation of fat content in oats (30-110 g/kg DM; Zhou *et al*., 1999), the higher end of the spectrum (< 60 g/kg DM) was not represented. In addition, breeders have been able to bring forward oat varieties with a fat content up to 180 g/kg DM, called "high-oil oats" (Frey and Holland, 1999). As fat content increases in high-oil oat varieties, CP and β-glucan content also increase while starch content decreases (Peterson and Wood, 1997). It could be expected that replacing barley with “high-oil oats” would have a greater CH_4 mitigating effect than observed with the oat varieties used in this thesis.

5.4.2 Potential of strategy

In Paper II and Paper III, CH_4 intensity decreased by 4.8% and 5.7%, respectively, when barley was replaced by oats. These effects are small in comparison with many other dietary strategies, such as lipid supplements (Bayat *et al*., 2018; Bayat *et al*., 2021), 3-NOP (Melgar *et al*., 2020a; Melgar *et al*., 2020b), and macro algae (Roque *et al*., 2019; Stefenoni *et al*., 2021). Even so, a strategy that is implemented on a commercial farm will have a greater effect than a strategy that is not implemented at all. As observed in Paper II, Paper III, and earlier studies (Heikkilä *et al*., 1988; Vanhatalo *et al*., 2006), production performance of dairy cows is maintained or can even be improved by replacing barley with oats. In comparison, macro algae additives may have negative effects on milk yield (Stefenoni *et al*., 2021) and will increase feed costs, which would affect farmer economy negatively unless farmers receive adequate reimbursement for implementing the strategy. Lipid supplements are promising and depending on the source, milk yield is maintained (Bayat *et al*., 2008) or increases (Bayat *et al*., 2021). However, they might suppress feed intake at high-doses and increase feed costs. If the cereal grains for feed are grown on the dairy farm, feed costs will include costs for seeds, fertilizers, pesticides, machinery use, and processing. Oats are generally known to require low input during crop production and according to a report by Flysjö *et al*. (2008), the use of fertilizers and pesticides are similar for barley and oats in Sweden. This thesis did not include an economic analysis of replacing barley with oats in dairy cow diets, but this should be addressed in the future.

The risk of increasing GHG emissions from other parts of the production chain should also be considered when discussing the potential of a dietary strategy (Figure 7; FAO, 2017). The lower digestibility when feeding oat supplemented diets than when feeding barley supplemented diets (Paper I, Paper II) could increase CH_4 emissions from manure. Emissions from manure were not measured in the studies included in this thesis. However, increasing inclusion of hulled oats in the diet did not increase faecal output of potentially digestible OM in Paper II, which indicates that CH_4 emissions from manure would not be affected by replacing barley with oats. To ensure that CH_4 emissions from manure do not increase, further research should be conducted where emissions from manure are measured directly.

A significant part of the total GHG emissions from the livestock sector originates from production of feeds (Figure 7). Studies suggest that GHG emissions from production of barley and oats are similar, although the emissions may depend on weather conditions and soil type. Rajaniemi *et al.* (2011) conducted a study in Finnish conditions where GHG emissions from production and use of fertilizers and seeds, soil and fuel for machinery were included in the analysis. In a conventional production system, predicted GHG emissions for production of barley were 1930 kg CO_2-eq/ha and 0.57 kg CO_2-eq/kg grain (Rajaniemi *et al.*, 2011). The corresponding emissions for oats were 1800 kg CO_2-eq/ha and 0.57 kg CO_2-eq/kg grain. Total GHG emissions were also similar between the grains when a reduced tillage and a direct drilling production system was used. Moreover, a life cycle assessment study conducted in Norwegian conditions, including a broader range of emission sources, showed similar GHG emissions from crop production (Korsaeth *et al.*, 2012). Production of 1 tonne of barley emitted 966 kg CO_2-eq, whereas 1 tonne of oats emitted 963 kg CO_2-eq.

Another sustainability aspect of crop production is the use of pesticides, since it can have a negative impact on biodiversity (Beketov *et al.*, 2013). According to the report by Flysjö *et al.* (2008), the volumes of herbicides, fungicides, and insecticides applied to oats and barley in Sweden are similar when expressed related to grain yield (grams of active substance/kg grain). In organic cropping systems, the use of pesticides is prohibited and therefore, the weed suppressing abilities of the crop itself are essential for integrated weed management. Oats have increased in popularity within organic cropping systems much due to its higher competitive ability against weeds compared with barley or wheat (Seavers and Wright, 1999). In addition, oats are well known for their suitable properties as a break crop in rotations.

6. Conclusions

Cows fed hulled oats as grain supplement produced similar amounts of milk and ECM as cows fed barley as grain supplement, despite lower tabulated energy- and protein values for oats. When barley was replaced by both hulled and dehulled oats, the yields of milk and ECM increased. Replacing hulled oats with dehulled oats increased diet digestibility but did not increase milk or ECM yield. Milk protein concentrations were lower with oat diets than with barley diets, but milk protein yields were still maintained.

Replacing barley with hulled oats decreased both daily enteric CH_4 emissions and CH_4 intensity. Replacing barley with both hulled and dehulled oats did not affect total enteric CH_4 emissions, as they increased with increasing inclusion of dehulled oats in the diet, but higher ECM yield with the oat diets still led to lower CH_4 intensity. The lower daily enteric CH_4 emissions when feeding hulled oats instead of barley were mainly due to the lower digestibility of oats. The responses in both milk production and CH_4 emissions will be dependent on the differences in chemical composition between barley and oats and the magnitude of the responses will therefore differ depending on which varieties are used and their growing conditions.

Based on the work of this thesis, replacing barley with oats as a grain supplement does not compromise the production performance of dairy cows and could offer a practical strategy to slightly decrease CH_4 intensity of milk production. Moreover, the FA composition of milk from cows fed oats is slightly more in line with international dietary guidelines. Although the individual effects of oats on milk production, enteric CH_4 emissions, and milk quality are relatively small, the combined effects together with several positive agronomy traits makes oats a strong competitor as a grain supplement for dairy cows in temperate climates.

7. Future perspectives

Future research should evaluate oat varieties with higher fat contents (> 60 g/kg DM) than the varieties included in this study. Investigating the effects of replacing barley with “high-oil oats” on dairy cow production performance, enteric CH_4 emissions, and milk FA composition would be of particular interest. As the higher fat content in oats compared with barley seems to play a key role in maintaining or even improving production performance, feeding “high-oil oats” could potentially lead to even greater feed efficiency and lower CH_4 intensity than feeding regular oats. In relation to this, the effects on energy metabolism should be studied more in depth to achieve a greater understanding of the biological mechanisms behind improved production performance with oats.

In a broader perspective, future research should focus on the different sustainability aspects of replacing barley with oats as a grain supplement for dairy cows. For example, additional studies measuring GHG emissions from feed production under different conditions should be conducted and GHG emissions from manure of cows fed barley or oats should be assessed. Moreover, as the potential of any CH_4 mitigation strategy depends on whether it is adopted on commercial farms, the effect of feeding oats instead of barley on dairy farmer economy should be examined. A whole-systems analysis regarding both environmental and economic sustainability would be especially useful.

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Popular science summary

In the past, oats were a popular grain supplement for dairy cows in Sweden. Recently, oats have been replaced by barley mostly due to higher tabulated energy- and protein values compared with oats. Yet, several studies show that the productivity of dairy cows fed oats is similar to or even higher than when they are fed barley. In addition, barley and oats differ in their chemical composition, differences that potentially could affect enteric methane emissions. Methane is a greenhouse gas, and its emissions contribute to climate change. Enteric methane is produced as a byproduct during fermentation of feed in the forestomachs of ruminants to yield energy. As enteric methane emissions are affected by the chemical composition and digestibility of the feed, they can be mitigated by manipulating the diet. Any potential dietary strategy for methane mitigation need to be assessed in relation to how it affects milk production, i.e., the decrease in methane emissions per kg produced milk (methane intensity). The aim of this thesis was to investigate whether barley could be replaced by oats as a means to mitigate enteric methane emissions without negatively affecting production performance of dairy cows.

The first study evaluated eight different varieties of both barley and oats (hulled) in a laboratory environment regarding diet digestibility and methane emissions. Grain and grass silage were incubated in glass bottles containing rumen fluid and total gas production and methane concentrations were measured during the experiment. We observed lower methane emissions from the oat varieties than from the barley varieties but no differences between different varieties of the same grain species. The second study investigated how replacement of barley with hulled oats as a grain supplement in the diet of dairy cows affects diet digestibility, milk production and enteric methane emissions. The study was conducted at

Röbäcksdalen experimental farm in Umeå and included 16 dairy cows fed grass silage as basal diet. When barley was replaced with oats, the digestibility of the diet decreased, but milk yield and energy-corrected milk yield were not affected. In addition, increasing inclusion of oats in the diet decreased enteric methane emissions. When the grain supplement consisted of 100% oats, daily methane emissions (g/d per cow) were 4.7% lower and methane intensity 4.8% lower than when the grain supplement consisted of 100% barley.

The third study was also conducted at Röbäcksdalen experimental farm in Umeå and included 16 dairy cows fed a grass silage-based diet. This study investigated how replacement of barley with hulled oats and dehulled oats, and how replacement of hulled oats with dehulled oats, affects diet digestibility, milk production and enteric methane emissions. We observed higher average milk yield and energy-corrected milk yield when cows were fed the oat diets (hulled and dehulled) than when they were fed the barley diet. Replacing hulled oats with dehulled oats did not affect milk yield or energy-corrected milk although diet digestibility increased. Methane intensity was 5.7% lower with the oat diets than with the barley diet but replacing hulled oats with dehulled oats did not affect methane intensity. In the fourth study, we examined how grain species (barley vs. oats) and type of oats (hulled vs. dehulled) affects the fatty acid composition of milk. For this purpose, we collected milk samples from the cows participating in the second and third study. Replacing barley with oats decreased the concentration of total saturated fatty acids and increased the concentration of total monounsaturated fatty acids in milk. Replacement of hulled oats with dehulled oats did not affect total concentrations of saturated or monounsaturated fatty acids. Based on the results of this thesis, replacing barley with oats as a grain supplement in the diet of dairy cows has potential to mitigate enteric methane emissions without negatively affecting production performance.

Populärvetenskaplig sammanfattning

Havre var förr i tiden ett populärt kraftfoder i mjölkkors foderstat i Sverige. På senare tid har havren ersatts av korn, mycket till följd av att korn har ett högre angivna energi- och proteinvärden jämfört med havre. Dock visar flera studier att mjölkkors produktivitet är den samma oberoende av om kraftfodret består av korn eller havre. Korn och havre uppvisar dessutom flera skillnader i kemisk sammansättning, skillnader som kan påverka utsläppen av enterisk metangas. Metan är en växthusgas vars utsläpp bidrar till klimatförändringen. Enterisk metan bildas i förmagarna hos idisslare som en biprodukt vid fermentering av fodrets näringsämnen för att utvinna energi. Eftersom enteriska metanutsläpp påverkas av fodrets kemiska sammansättning och smältbarhet kan de minskas genom att göra ändringar i foderstaten. Det är viktigt att potentiella strategier utvärderas i relation till mängden erhållen produkt, det vill säga hur mycket metanutsläppen minskar per kg producerad mjölk (metanintensitet). Syftet med denna avhandling var att undersöka huruvida ersättning av korn med havre i mjölkkors foderstat kunde vara en praktisk strategi för att minska metanutsläppen utan att produktiviteten påverkas negativt.

Den första studien utvärderade åtta olika sorter av både korn och havre (oskalade) med avseende på smältbarhet och metanutsläpp i laboratoriemiljö. Spannmål och gräsensilage inkuberades i glasflaskor innehållande våmvätska och total gasproduktion och metankoncentrationer mättes under försöket. I genomsnitt producerades mindre metan från havresorterna än från kornsorterna, men inga skillnader observerades mellan olika sorter av samma spannmålsart. Den andra studien undersökte hur ersättning av korn med havre (oskalad) som kraftfoder i mjölkkors foderstat påverkar smältbarhet, mjölkavkastning och enteriska metanutsläpp. Studien utfördes i Röbäcksdalens forskningsladugård i Umeå och inkluderade 16 mjölkkor vars grundfoderstat bestod av gräsensilage. Studien visade att när korn ersattes med havre sjönk foderstatens smältbarhet men mjölkavkastningen och mängden energi-korrigerad mjölk förblev oförändrade. Därtill ledde en ökad andel havre i foderstaten till lägre metanutsläpp. När spannmålskomponenten bestod av endast havre var metanutsläppen per dag och ko 4,7 % lägre och metanintensiteten 4,8 % lägre än när spannmålskomponenten bestod av endast korn.

Även den tredje studien utfördes i Röbäcksdalens forskningsladugård i Umeå och inkluderade 16 mjölkkor vars grundfoderstat var gräsensilage. Denna studie undersökte hur ersättning av korn med både oskalad och skalad havre samt hur ersättning av oskalad havre med skalad havre påverkar smältbarhet, mjölkavkastning och enteriska metanutsläpp. Studien visade att mjölkavkastningen och mängden energi-korrigerad mjölk var högre när korna utfodrades med havre (både skalad och oskalad) än när de utfodrades med korn. Ersättning av oskalad havre med skalad havre påverkade inte mjölkavkastningen eller mängden energi-korrigerad mjölk trots att smältbarheten ökade. Metanintensiteten var 5,7 % lägre med havre än med korn som kraftfoder men att ersätta oskalad havre med skalad havre påverkade inte metanintensiteten. I den fjärde och sista studien undersökte vi hur valet av spannmål (korn vs. havre) och typen av havre (oskalad vs. skalad) i foderstaten påverkar mjölkens fettsyrasammansättning. För detta ändamål togs mjölkprover från korna som deltog i den andra och tredje studien. Ersättning av korn med havre minskade den totala halten av mättade fettsyror och ökade den totala halten av enkelomättade fettsyror i mjölken. Att ersätta oskalad havre med skalad havre påverkade inte de totala halterna av mättade eller omättade fettsyror. Baserat på resultaten från denna avhandling, skulle ersättning av korn med havre som spannmålskomponent i mjölkkors foderstat kunna leda till lägre metanutsläpp utan negativ påverkan på produktiviteten.

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Effects of different barley and oat varieties on methane production, digestibility, and fermentation pattern in vitro

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ABSTRACT

The objective of this in vitro study was to determine the effects of different barley and oat varieties on CH_4 production, digestibility, and rumen fermentation patterns in dairy cows. Our hypothesis was that oat-based diets would decrease CH_4 production compared with barley-based diets, and that CH_4 production would differ between varieties within grain species. To evaluate this hypothesis, we conducted an in vitro experiment using a fully automated gas production technique, in which the total gas volume was automatically recorded by the system. The experiment consisted of triplicate 48-h incubations with 16 treatments, including 8 different varieties of each grain. The grain varieties were investigated as a mix with an early-cut grass silage (1:1 ratio of grain to silage on a dry matter basis) and mixed with buffered rumen fluid. We estimated predicted in vivo total gas production and CH_4 production by applying a set of models to the gas production data obtained by the in vitro system. We also evaluated in vitro digestibility and fermentation characteristics. The variety of grain species did not affect total gas production, CH_4 production, or fermentation patterns in vitro. However, in vitro-determined digestibility and pH were affected by variety of grain species. Grain species affected total gas and CH_4 production: compared with barley-based diets, oat-based diets decreased total gas production and CH_4 production by 8.2 and 8.9%, respectively, relative to dry matter intake. Grain species did not affect CH_4 production relative to in vitro true dry matter digestibility. Oat-based diets decreased digestibility and total volatile fatty acid production, and maintained a higher pH at 48 h of incubation compared with barley-based diets. Grain species did not affect fermentation patterns, except for decreased molar proportions of valerate with oat-based diets. These results suggest that replacing barley with oats in dairy cow diets could decrease enteric CH_4 production.

Key words: oats, barley, methane, in vitro

INTRODUCTION

After water vapor and CO_2, methane (CH_4) is the third most important greenhouse gas contributing to climate change. Compared with CO_2, CH_4 is characterized by a greater efficiency in absorbing heat energy and a shorter atmospheric lifetime (12–13 yr), resulting in a global warming potential 28 times greater than CO_2 (IPCC, 2014). Ruminant livestock account for approximately 37% of the global anthropogenic CH_4 emissions; a greater part of that originates from enteric fermentation, and a lesser part from manure (FAO, 2006). In addition, enteric CH_4 production represents an energy loss to the animal, ranging from 2 to 12%, depending on diet composition and feeding level (Johnson and Johnson, 1995). For these 2 reasons, research aimed at mitigating enteric CH_4 emissions from ruminants is very important.

Enteric CH_4 production in ruminants is affected by DMI (Johnson and Johnson, 1995), diet composition, chemical composition of feed components, diet digestibility, and individual animal characteristics (Yan et al., 2000). Dietary strategies to decrease CH_4 emissions from ruminants have been widely investigated. Many of these involve dietary supplements, such as ionophores (Guan et al., 2006), nitrate (van Zijderveld et al., 2011), secondary plant compounds (Bhatta, 2015), 3-nitroxypropanol (Hristov et al., 2015), and oil supplements (Martin et al., 2008; Beauchemin et al., 2009), which have all been found effective to various degrees. However, for farmers to adopt a CH_4-mitigating dietary strategy, the strategy has to be safe, practically applicable at a farm scale, and economically beneficial to the farmer (Hristov et al., 2013), posing problems for the practical application of the strategies above.

Oats (*Avena sativa*) and barley (*Hordeum vulgare*) are common grains in the diet of dairy cows, especially

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in the Nordic countries, because yields are relatively high and moderate input is required. Many studies suggest that replacing barley with oats in the diet of dairy cows increases milk yield, decreases milk protein and fat concentrations, and improves the composition of milk fat by increasing the concentration of unsaturated fatty acids (Martin and Thomas, 1988; Ekern et al., 2003; Vanhatalo et al., 2006). In terms of chemical composition, oats express a greater concentration of oil and fiber, with a greater proportion of soluble β-glucans than barley; barley expresses a higher concentration of starch (Lee et al., 1997). In addition, oats contain several types of antioxidants, such as phenolic compounds and avenanthramides, which are not present in barley (Peterson, 2001). All of these differences in chemical composition between oats and barley could influence enteric CH_4 production.

The first objective of this study was to compare oats and barley (and different varieties of each grain) in a grass-silage-based diet in terms of CH_4 production, digestibility, and fermentation patterns using an in vitro gas production system. Our hypothesis was that replacing barley with oats would decrease CH_4 production, and that CH_4 production would differ between varieties of grain species. The second objective was to conduct a regression analysis to explain possible mechanisms of the differences in CH_4 production and compare observed responses to the model-predicted responses as a means of evaluating whether oats have a specific CH_4 mitigation effect.

MATERIALS AND METHODS

Grain Samples

The experimental feed material consisted of 8 varieties of oats and 8 varieties of barley. All grains were hulled and obtained as seed samples from Boreal Plant Breeding Ltd. (Jokioinen, Finland). The grain samples (including hull) were dried at 60°C in a forced-air oven for 48 h and milled through a 1.0-mm screen using a laboratory mill (Retsch SM2000; Rheinische, Haan, Germany).

Experimental Design and Treatments

The in vitro gas production experiment consisted of triplicate runs of 48 h in vitro. Each run included 36 samples: 16 treatments × 2 replicates and 4 blank samples containing only buffered rumen fluid. Feed samples and buffered rumen fluid were mixed in serum bottles (250 mL; Schott, Mainz, Germany) and incubated in a water bath. Before each run, serum bottles were randomized to the 36 positions in the water bath so that each treatment had a different position during each run. The ratio of incubated grain to silage was 1:1 on a DM basis; each treatment contained 500 mg of barley or oats, and 500 mg of silage. The silage was early-cut silage harvested from primary growth of a third-year lay dominated by timothy (*Phleum pratense*). The fields were fertilized with 70 kg of N/ha in the spring. The silage samples were treated in the same way as the grain samples.

In Vitro Incubations

All experimental procedures were approved by the Umeå Ethics Committee for Animal Research, Sweden, and by the National Animal Ethics Committee, Finland. Rumen fluid was collected approximately 2 h after morning feeding from 2 fistulated Nordic Red dairy cows in late lactation that were fed a TMR of grass silage and concentrate (600:400 g/kg on a DM basis) ad libitum. The concentrate consisted of rolled barley and rape seed meal (800:200 g/kg on a DM basis). The rumen fluid was kept in 2 steel thermoses that had been prewarmed and flushed with CO_2 to ensure an anaerobic environment. The pH value of the rumen fluid (mean 6.27, standard deviation 0.12) was recorded (744 pH Meter; Metrohm Ltd., Herisau, Switzerland) before it was filtered through 4 layers of cheesecloth into a measuring cylinder continuously flushed with CO_2. A total of 483 mL of rumen fluid was transferred through a funnel into another measuring cylinder containing 483 mL of buffer solution mixed with micro- and macrominerals as described by Menke (1988), at 39°C under constant stirring and continuous flushing with CO_2. The buffered rumen fluid was supplemented with peptone (pancreatic digested casein; Merck, Darmstadt, Germany). Feed samples were incubated in 60 mL of buffered rumen fluid and placed in a water bath at 39°C with continuous agitation for 48 h. The in vitro gas production experiment was conducted using a fully automated gas production technique described by Cone et al. (1996), in which the total gas volume is automatically recorded at 0.2-h intervals and corrected for the normal atmospheric pressure (101.3 kPa).

Gas Sampling

To predict CH_4 production in vivo based on the data from the in vitro system, we used the method of Ramin and Huhtanen (2012). Gas sampling was performed at 2, 4, 8, 24, 32, and 48 h of incubation by drawing a gas sample from each bottle in a gas-tight syringe (Hamilton, Bonaduz, Switzerland) through the rubber suba seal. A sample size of 0.2 mL of gas was injected into a gas chromatograph (Varian Star 3400 CX FID Gas Chromatograph; Varian Inc., Palo Alto, CA), equipped with a thermal conductivity detector. A standard mixture of CO_2 (900 mmol/mol) and CH_4 (100 mmol/mol) was used as a calibration gas (AGA Gas AB, Sundbyberg, Sweden), and gas sample peaks were recognized by comparison with the standard gas. The duration of the gas sampling process was approximately the same as that of the incubation process (35–45 min), ensuring an equal fermentation time in all bottles.

VFA, pH Measurements, and Digestibility Analysis

Each bottle was sampled for VFA analysis at 48 h of incubation by drawing a sample of 0.3 mL fluid residue using a liquid syringe. The fluid samples were pooled within treatment and run and stored in Eppendorf tubes at −18°C until further analysis. Concentration of VFA was determined by liquid chromatographic analysis using a Waters Acquity ultra-performance liquid chromatography apparatus (Waters, Milford, MA), as described in detail by Puhakka et al. (2016). The concentration of total VFA (mmol/L) was calculated as the difference between total VFA concentration in the sample and average total VFA concentration in the blanks. Total VFA production (mmol) was calculated by multiplying the difference in VFA concentration (sample − blank) by the sample volume (60 mL). The pH of each bottle at 48 h of incubation was also determined (744 pH Meter; Metrohm Ltd.). The in vitro digestibility was determined as true DM digestibility (**TDMD**). Sampling for TDMD was performed at 48 h of incubation by transferring the feed sample residue from each bottle into a preweighed polyester bag with a pore size of 11 μm. All excess liquid was carefully pressed out through the pores of the bag. The nylon bags were sealed and stored at −18°C until further analysis. On the day of analysis, the sample bags were thawed and boiled in an NDF solution for 1 h with added heat-stable α-amylase and sodium sulfite. Sample bags were dried at 60°C for 48 h before weighing.

Chemical Analysis

Dry matter concentration was determined by drying the grain (including the hull) and silage samples at 105°C for 16 h. Ash concentration was determined by incinerating the samples at 500°C for 4 h (AOAC International, 2012) and OM concentration was calculated as the difference between 1,000 and the ash concentration. Nitrogen concentration was determined using the Kjeldahl method (AOAC International, 2012) and a Tecator digestion system (Tecator Digestion Auto and Tecator Scrubber; Foss Analytical, Hillerød, Denmark) and a distillation and titration apparatus (Kjeltec Auto 2300; Foss Analytical). We calculated CP concentration by multiplying the N concentration by 6.25. We determined crude fat concentration by ether extraction and HCl hydrolysis (Foss Soxtec 8000 extraction unit, SoxCap 2047 hydrolysis unit; Foss Analytical). The NDF concentration was determined using the method described by Van Soest et al. (1991), using a 1020 hot and 1021 cold extractor (Tecator Fibertec System; Foss Analytical) with addition of heat-stable α-amylase and sodium sulfite. The NDF concentration was expressed free of residual ash (**aNDF**). Indigestible NDF (**iNDF**) concentration was determined by applying a 288-h in situ incubation as described by Krizsan et al. (2015), using polyester bags with a pore size of 6 μm and a pore area equal to 5% of the total surface area. Sample bags were incubated in 2 lactating Ayrshire dairy cows fitted with a rumen fistula at the research farm at the Department of Agricultural Sciences at the University of Helsinki, Finland. The cows were fed a diet based on grass silage and concentrate (60:40 on a DM basis). Indigestible NDF was expressed inclusive of residual ash. Frozen silage samples were thawed and pressed before analysis. Ammonia-N was analyzed by direct distillation after adding MgO using a Kjeltec 2100 Distillation Unit (Foss Analytical). We analyzed VFA and lactic acid according to the method of Ericson and André (2010).

Predicted In Vivo Methane Production

We calculated the cumulative CH_4 production (mL) at each time point (0.2 h) according to the following equation:

$$V_{CH4}\ (\text{mL}) = V_{HS}\ (\text{mL}) \times CH_4\ (\text{mL/mL}) + V_{GP}\ (\text{mL}) \times A \times CH_4\ (\text{mL/mL}),$$

where V_{CH4} was the total CH_4 production at each time point; V_{HS} was the headspace volume; CH_4 was the CH_4 concentration in the headspace; V_{GP} was the gas production volume; and coefficient A was the ratio of CH_4 concentration in the outflow gas to the headspace. Coefficient A (0.55) was predicted using a mechanistic model described by Ramin and Huhtanen (2012).

Methane concentration at 0.2-h time intervals was estimated by fitting a logarithmic regression of measured CH_4 at 6 time points, as described by Ramin and Huhtanen (2012). Methane production (mL) at each time point (0.2) was calculated as total gas production × calculated CH_4 concentration. The kinetic parameters of total gas production and CH_4 production at each time point (0.2) were predicted separately by fitting the data to the 2-pool Gompertz model described by Schofield et al. (1994), using the NLIN procedure in SAS version 9.4 (SAS Institute Inc., Cary, NC) according to the following equation:

$$V_t = V_1 \times \text{Exp}\{-\text{Exp}[1 - k_1 \times (t - L_1)]\} + V_2 \times \text{Exp}\{-\text{Exp}[1 - k_2 \times (t - L_2)]\},$$

where V_t is measured total gas or CH_4 volume at time t; V_1, k_1, and L_1 are asymptotic cumulative gas production (mL/g of DM), rate (1/h), and lag (h) parameters, respectively, for the first pool (rapid); V_2, k_2, and L_2 are the corresponding parameters for the second pool (slow); and t is incubation time.

The parameters were subjected to a dynamic, mechanistic 2-compartment rumen model as described by Huhtanen et al. (2008), with modifications as described by Ramin and Huhtanen (2012). The model was used to predict the proportion of asymptotic CH_4 production at infinitive time that would be produced during the residence of feed in the rumen and the simulations were performed using POWERSIM version 2.5 (Powersim Software, Bergen, Norway) with a retention time of 50 h, which represented the maintenance level of intake. Predicted in vivo CH_4 production (mL/g of DM) was calculated as CH_4 = proportion × asymptotic CH_4 production (mL/g of DM) and expressed it as grams per kilogram of DM, calculated as follows:

$$CH_4 \text{ (g/kg of DM)} = CH_4 \text{ (mL/g of DM)}/22.4 \text{ (L/mol)} \times 16.04 \text{ (g/mol)},$$

where 22.4 is the gas volume and 16.04 is the molar mass of CH_4.

Stoichiometrically Predicted Methane Production

Stoichiometrically predicted CH_4 (**CH_4VFA**) production was calculated using the amounts of acetate, propionate, and butyrate produced in vitro according to the following equation (Wolin, 1960):

$$CH_4VFA \text{ (mL)} = 22.4 \times (0.50 \times AA - 0.25 \times PA + 0.50 \times BA),$$

where AA, PA, and BA are the total amounts (mmol) of acetate, propionate, and butyrate, respectively, in the feed samples at 48 h of incubation and 22.4 is gas volume (L/mol of gas). The VFA data were corrected for blanks.

Predicted Methane Production Using Mechanistic and Empirical Equations

The Nordic dairy cow model Karoline is a mechanistic dairy cow model described by Danfær et al. (2006). In this study, we used the sub-model predicting CH_4 production, recently revised by Huhtanen et al. (2015), to predict methane. The Karoline model requires a set of input variables, such as the chemical composition parameters of the feedstuff and the kinetic digestion rates for specific feed fractions. If required input variables were not analyzed, they were estimated or derived from feed tables. For grains and silage, potentially digestible NDF was calculated as the difference between aNDF and iNDF. The sugar concentration of oats (18 g/kg of DM) and barley (20 g/kg of DM) was derived from national feed tables (LUKE, 2019). Starch concentration for each individual grain variety was calculated as OM − CP − crude fat − aNDF − sugar. The grain protein fractions of amino nitrogen, peptide nitrogen, soluble protein nitrogen, insoluble protein, and indigestible protein for grains were derived from the Cornell Net Carbohydrate and Protein System feed table (Tylutki et al., 2008). Values for the digestion rate of NDF were set at 0.050 and 0.066/h for oats and barley, respectively. For the digestion rate of starch, we used a value of 0.2/h for both grains. The digestion rate of insoluble protein was set at 0.09 and 0.11/h for oats and barley, respectively. Silage protein fractions and digestion rates were based on the Cornell Net Carbohydrate and Protein System feed table (Tylutki et al., 2008). The Karoline simulations were based on a 60-kg sheep fed at maintenance level of intake (1 kg of DMI/d).

We also predicted CH_4 production using an empirical equation developed and described by Ramin and Huhtanen (2013), which is based on feed intake and the chemical composition of the feed, as follows:

$$CH_4 \text{ (L/kg of DMI)} = 5 - 0.35 \times DMIBW + 0.031 \times OMD_m - 0.043 \times EE + 0.018 \times NDF + 0.018 \times NFC,$$

where DMIBW is DMI per unit of BW (g/kg); OMD_m is OM digestibility at a maintenance level of feed intake (g/kg); EE is ether extract (g/kg of DM); NDF is given in grams per kilogram of DM; and NFC (g/kg of DM) was calculated as OM − CP − NDF − crude fat (NRC, 2001). Empirical CH_4 production (g/kg of DM) was calculated as

$$CH_4 \text{ (g/kg of DM)} = CH_4 \text{ (L/kg of DMI)} \times 1 \text{ (L)}/22.4 \text{ (L/mol)} \times 16.04 \text{ (g/mol)},$$

where 22.4 is the gas volume and 16.04 is the molar mass of CH_4.

Statistical Analysis

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc.). Data for total gas and CH_4 production parameters (g/kg of DM), TDMD (g/kg of DM), and pH values were subjected to ANOVA using the MIXED procedure in SAS according to the following model:

$$Y_{ijkl} = \mu + G_i + V(G)_{ij} + R_k + b_l + \varepsilon_{ijkl},$$

where Y_{ijkl} is the observation; μ is the overall mean; G_i is the effect of grain (i = 2); $V(G)_{ij}$ is the effect of variety within grain (j = 16); R_k is the effect of the run (k = 3); b_l is the random effect of bottle (position in bath); and ε_{ijkl} is the random error term. Because the samples for CH_4VFA (g/kg of DM), total VFA production (mmol), and molar proportions of VFA (mmol/mol) for each run were pooled within treatment, these data were subjected to ANOVA by a different model using the MIXED procedure in SAS:

$$Y_{ijk} = \mu + G_i + V(G)_{ij} + R_k + \varepsilon_{ijk},$$

where Y_{ijk} is the observation; μ is the overall mean; G_i is the effect of grain; $V(G)_{ij}$ is the effect of variety within grain; R_k is the random effect of the run; and ε_{ijk} is the random error term. We tested the data used in both models using the Shapiro-Wilk test for normality and homoscedasticity of residuals. The results were considered statistically significant when $P < 0.05$, and highly significant when $P < 0.01$. We adjusted P-values for multiple comparisons of variety within grain using Tukey's test. To investigate possible relationships between predicted in vivo CH_4 and grain composition (TDMD, OM, CP, aNDF, iNDF, and crude fat) a regression analysis was performed by using the REG procedure in SAS. To investigate possible interactions between grain species and grain composition on predicted in vivo CH_4 production, we performed a regression analysis using the GLM procedure in SAS.

RESULTS

Chemical Composition of Feeds

The chemical composition of the oat and barley varieties and the means and standard deviations within grain species are presented in Table 1. The most pronounced differences between the oat and barley varieties were higher concentration of aNDF, iNDF, and crude fat in the oat varieties compared with the barley varieties. The silage had a DM concentration of 285 g/kg of fresh weight. The concentrations of OM, CP, aNDF, and iNDF were 938, 157, 611, and 102 g/kg of DM, respectively. The fermentation quality of the silage was good, in that it had low concentrations of ammonia N (45.0 g/kg of N). Concentrations of lactic acid, acetic acid, propionic acid, and butyric acid were 99.6, 21.6, 1.06, and 0.27 g/kg of DM, respectively.

Total Gas and Methane Production

The findings for total gas and predicted in vivo CH_4 production measurements are presented in Table 2. Total gas production and predicted in vivo CH_4 production were 8.2 and 8.9% lower ($P < 0.01$), respectively, in oat-based diets than in barley-based diets. The variety of grain species did not affect total gas or predicted in vivo CH_4 production. The mechanistic and empirical CH_4 predictions are illustrated in Figure 1. Both models expressed a good relationship to predicted in vivo CH_4; root mean square errors were 0.80 and 0.78 for the mechanistic and empirical models, respectively. Although R^2 values were not very high (0.67 and 0.68 for the mechanistic and empirical models, respectively), relative prediction errors were 3.6 and 3.5%, respectively, of the observed mean.

Relationships Between Predicted In Vivo Methane Production and Grain Composition Parameters

The results from simple and multiple regression analysis of predicted in vivo CH_4 production and grain parameters are presented in Table 3. The best predictor of CH_4 production was TDMD. Among grain composition parameters, iNDF concentration was the best predictor of CH_4 production. The mean response in CH_4 production to increased iNDF concentration was −0.020 g/kg of DM per 1 g/kg of DM in iNDF. Grain aNDF concentration was only slightly less associated with CH_4 production than iNDF concentration. Using crude fat concentration as a predictor instead of iNDF or aNDF increased the prediction error slightly. However, the mean response in CH_4 production to crude fat (−0.087 g/kg of DM per 1 g/kg of DM crude fat) was greater than that to iNDF or aNDF. Grain CP concentration did not affect CH_4 production. When the data were classified according to grain species (n = 8), we found no interactions between grain type and grain parameters ($P > 0.54$) with respect to predicted in

Table 1. Chemical composition of oat and barley varieties incubated in the in vitro gas production experiment

			Composition, g/kg of DM[1]					
Grain	Variety	DM, g/kg	OM	CP	aNDF	iNDF	Crude fat	NFC[2]
Oats	Riina	911	970	132	311	144	53.5	474
	Nike	902	971	112	347	128	37.1	475
	Niklas	910	969	136	304	143	48.9	480
	Kerstin	908	974	124	391	158	41.7	417
	Meeri	912	970	139	308	147	52.9	470
	Akseli	911	968	134	320	148	60.9	453
	Cilla	910	969	123	369	150	49.6	427
	Haga	917	976	94	310	135	45.0	527
	Mean	910	971	124	332	144	48.7	466
	SD	3.9	2.8	14.9	32.7	9.4	7.40	34.0
Barley	6-row Justus	894	976	152	204	43.8	26.8	593
	6-row Kaarle	898	978	115	190	27.2	25.7	647
	2-row RGT Planet	897	982	105	170	42.9	29.1	678
	2-row Wootan	894	978	144	201	52.2	26.6	606
	6-row Elmeri	897	979	111	214	49.4	29.0	625
	6-row Severi	895	978	137	221	35.9	29.3	591
	2-row Selena	895	979	101	187	38.8	26.3	665
	2-row Tamtam	894	980	91	185	40.5	30.1	674
	Mean	896	979	119	197	41.3	27.9	635
	SD	1.6	1.8	22.2	16.7	7.80	1.70	35.9
Silage[3]		285	938	157	611	102	—	—

[1] aNDF = NDF free of residual ash; iNDF = indigestible NDF.

[2] NFC not analyzed; calculated as OM – CP – aNDF – crude fat (NRC, 2001).

[3] Concentration of ammonia N was 45.0 g/kg of N, lactic acid 99.6 g/kg of DM, acetic acid 21.6 g/kg of DM, propionic acid 1.06 g/kg of DM, and butyric acid 0.27 g/kg of DM.

vivo CH_4 production. Bivariate models did not improve predictions because of collinearity between variables (models not shown).

Digestibility and Fermentation Pattern In Vitro

Results for digestibility expressed as TDMD, pH at 48 h of incubation, and VFA production parameters are presented in Table 4. True DMD decreased by 7.0% in oat-based diets compared with barley-based diets ($P < 0.01$) and variety of grain species also affected TDMD ($P = 0.04$). We observed a negative relationship between iNDF concentration and TDMD among the varieties of oats and barley (Figure 2). Oat-based diets increased the pH value at 48 h of incubation compared with barley-based diets ($P < 0.01$), whereas barley-based diets expressed a higher production of total VFA than oat-based diets ($P < 0.01$). In addition, variety of grain affected pH at 48 h of incubation ($P < 0.01$), but when P-values were adjusted for multiple testing, we observed differences only between some of the barley varieties, ranging from 0.06 to 0.10 pH units. Grain and variety of grain did not affect the molar proportions of VFA, except for a significantly greater proportion of

Table 2. Effects of grain species and variety within grain species on predicted in vivo total gas and methane production

			SEM		P-value	
Item	Oats	Barley	Oats	Barley	Grain	Variety
Total gas, g/kg of DM						
Asymptotic gas	160	173	3.4	3.2	<0.01	0.23
Predicted gas[1]	145	158	2.9	2.7	<0.01	0.30
CH_4						
Asymptotic CH_4, g/kg of DM	25.6	28.2	0.41	0.39	<0.01	0.21
Rate, 1/h	0.063	0.060	0.0011	0.0011	0.03	0.16
Predicted in vivo CH_4, g/kg of DM	21.4	23.5	0.30	0.29	<0.01	0.17
Predicted in vivo CH_4, g/kg of TDMD[2]	27.0	27.5	0.37	0.35	0.26	0.36
CH_4/total gas,[3] %	14.9	15.1	0.22	0.21	0.50	0.67

[1] Predicted in vivo total gas and CH_4 production based on observed values corrected for a mean retention time of 50 h.

[2] TDMD = true DM digestibility determined at 48 h of incubation in vitro.

[3] Predicted in vivo CH_4 (g/kg of DM) divided by predicted total gas (g/kg of DM).

Table 3. Relationships between grain variables (g/kg of DM) and predicted in vivo CH_4 production (g/kg of DM; n = 16)

Item[1]	Intercept	SE	*P*-value	Slope	SE	*P*-value	RMSE[2]	Adjusted R^2
TDMD	−6.9	4.97	0.19	0.036	0.006	<0.01	0.74	0.694
aNDF	26.2	0.81	<0.01	−0.014	0.003	<0.01	0.85	0.589
iNDF	24.3	0.43	<0.01	−0.020	0.004	<0.01	0.84	0.602
Crude fat	25.8	0.75	<0.01	−0.087	0.019	<0.01	0.87	0.574

[1] aNDF = NDF free of residual ash; iNDF = indigestible NDF; TDMD = true DM digestibility.

[2] RMSE = root mean square error.

valerate in barley-based diets compared with oat-based diets.

DISCUSSION

Chemical Composition of Feeds

Oat grain displays a greater proportion of hull than barley grain, accounting for approximately 25% in oats and 13% in barley, whereas barley displays a greater proportion of endosperm than oats, accounting for approximately 76% in barley and 63% in oats (Evers and Millar, 2002). The greater proportion of fibrous hull in oats is reflected in the higher aNDF concentration compared with barley. The greatest difference in aNDF concentration observed in this study was 221 g/kg of DM between the oat variety Kerstin and the barley variety RGT Planet. The endosperm serves as a storage compartment for most of the starch in cereal grains, and the difference in endosperm proportion is reflected in the greater concentration of starch in barley than in oats (Evers and Millar, 2002). We did not analyze starch concentration in this study, but the calculated values (OM − CP − crude fat − aNDF − sugar) of 630 and 450 g/kg of DM in barley and oats, respectively, were within the normal ranges of 510 to 640 g/kg of DM (barley) and 400 to 500 g/kg of DM (oats) reported by Holtekjølen et al. (2006) and Sayer and White (2011).

Oats are known to have a higher oil concentration than barley; most of the oil (96%) is found in the groat, and the rest is found in the hull (Bryngelsson et al., 2002). The groat oil is largely found in the endosperm

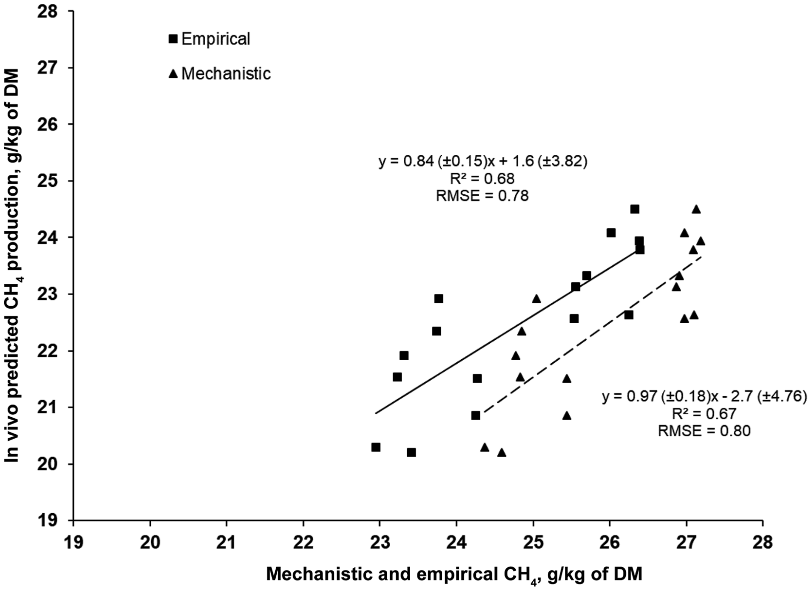

Figure 1. The relationship between predicted in vivo CH_4 production and CH_4 production predicted by the mechanistic Nordic dairy cow model Karoline and the empirical equation developed by Ramin and Huhtanen (2013). RMSE = root mean square error.

Table 4. Effects of grain species and variety within grain species on in vitro TDMD, pH, total VFA production, VFA molar proportions, and stoichiometrically predicted CH_4 production at 48 h of incubation in vitro

			SEM		*P*-value	
Item	Oats	Barley	Oats	Barley	Grain	Variety
TDMD,[1] g/kg of DM	795	855	1.9	1.8	<0.01	0.04
pH	6.26	6.11	0.005	0.005	<0.01	<0.01
Total VFA production, mmol/g of DM	3.30	3.68	0.11	0.11	<0.01	0.55
VFA molar proportions, mmol/mol						
Acetate	630	628	4.4	4.4	0.65	0.51
Propionate	216	214	1.8	1.8	0.63	0.27
Butyrate	130	129	5.0	5.0	0.95	0.88
Isobutyrate	3.9	5.0	0.49	0.49	0.12	0.59
Valerate	12.4	14.3	0.74	0.74	0.03	0.34
Isovalerate	1.1	2.7	0.57	0.57	0.08	0.56
CH_4VFA,[2] mL	24.6	27.3	0.79	0.79	<0.01	0.44

[1] TDMD = true DM digestibility determined at 48 h of incubation in vitro.

[2] CH_4VFA = stoichiometrically predicted CH_4 production using the amount of acetate, propionate, and butyrate produced in vitro (Wolin, 1960).

(86–90%), and smaller proportions are found in the scutellum and embryo (Banaś et al., 2007). Compared with the reported total variation in oil concentration among hulled oat varieties (30 to 110 g/kg of DM; Zhou et al., 1999), the varieties evaluated in this study were in the lower range (37–61 g/kg of DM); high-oil oat varieties in the upper range were not represented. The variations in crude fat concentration among the barley varieties were smaller (26–30 g/kg of DM) than among the oat varieties, but well in line with the reported variations (19–41 g/kg of DM; Welch, 1978).

The variations in CP concentration among the barley varieties found in this study (91–152 g/kg of DM) were well in line with reported variations (99–187 g/kg of DM; Welch, 1978). However, the variations in CP concentration among the oat varieties were smaller than among barley varieties (94–139 g/kg of DM) and compared with reported variations (112–160 g/kg of DM; Welch and McConnell, 2001).

Method

It is difficult to create an in vitro environment exactly similar to the in vivo environment, and our in vitro gas production system had some limitations. There is no absorption of VFA and no continuous intake of feedstuff during incubation. However, the prediction error of CH_4 production was relatively small (9.5% of observed mean) in the study by Danielsson et al. (2017), in which predicted in vivo CH_4 values obtained from the in vitro gas production system were compared with observed values from in vivo studies that measured CH_4 using respiration chambers or the GreenFeed system (C-Lock Inc., Rapid City, SD). The estimated proportion of gross energy (Volden, 2011) lost as CH_4 ranged between 6.1 and 7.4%, close to the predicted values of Ramin and Huhtanen (2012) at a maintenance level of feed intake using the same in vitro gas production approach as used in the present study. In addition, these values were also well in line with values obtained in vivo on grass silage-based diets (Yan et al., 2000).

Because the proportion of grain was only 50% (on a DM basis) of the incubated feed mixture in this study, the observed decrease in TDMD of 7 percentage units for the oat-based diets compared with the barley-based diets fell well in line with the values in national feed tables, which reported a 10- to 12-percentage-unit difference in digestibility between oats and barley (LUKE, 2019). In addition, the pH levels observed in this experiment (6.11 and 6.26 for barley and oats, respectively) at 48 h of incubation may be regarded as in the normal range, indicating that the buffered mineral solution used in vitro was able to imitate the effect of saliva excreted in vivo. Contrary to the findings of this study, Vanhatalo et al. (2006) reported no differences in pH between oats and barley when compared in vivo on a grass-silage-based diet. This inconsistency may be explained by the differences between the in vitro and in vivo environments. The variation in pH at 48 h of incubation expressed a close relationship ($R^2 = 0.90$) with the variation in TDMD among the different grain varieties, whereas total VFA production was less associated ($R^2 = 0.38$) with the variation in pH.

The observed decrease in total VFA production for oat-based diets compared with barley-based diets was consistent with the results of Vanhatalo et al. (2006) and can be explained by the lower digestibility of oats compared with barley. However, the decrease in total VFA production was 10%, whereas the decrease in TDMD was only 7%, indicating that additional factors lowered total VFA production. Because dietary fatty acids are not fermented in the rumen and do not con-

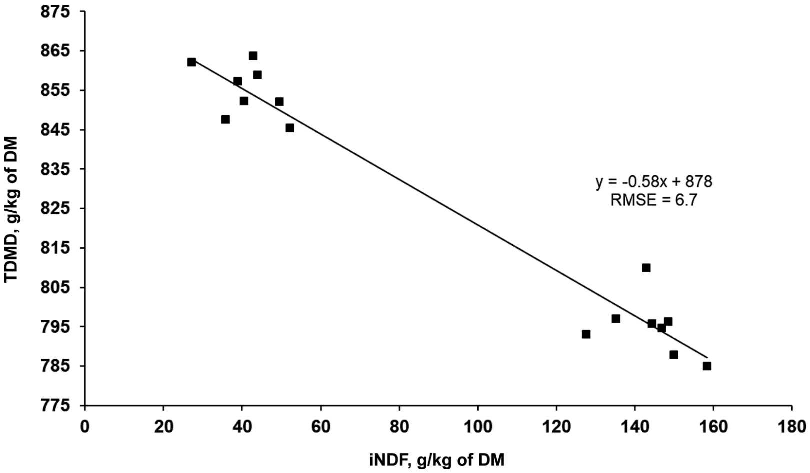

Figure 2. The relationship between true DM digestibility (TDMD) determined in vitro and indigestible NDF (iNDF) concentration of 16 varieties of oats and barley incubated in vitro over 48 h. Observations grouped to the left represent barley varieties, and observations grouped to the right represent oat varieties. RMSE = root mean square error.

tribute to the VFA pool, the higher fat concentration of oats may be an additional factor explaining the lower total VFA production observed for oat-based diets.

Total Gas and Methane Production

To our knowledge, no study has compared CH_4 production between oat- and barley-based diets or between different varieties of the grains in question. The decrease in total gas and predicted in vivo CH_4 production we observed for oat- compared with barley-based diets was most likely related to decreased digestibility, because grain species did not affect CH_4 production relative to TDMD. In the present study, the silage and percentage of grain inclusion were the same for all treatments, and the differences in total gas and CH_4 production between oat- and barley-based diets were most likely related to differences in chemical composition between the grains.

The strong positive relationship ($R^2 = 0.69$) observed between diet TDMD and CH_4 production in the present study was also confirmed by other studies (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013), and by the fact that CH_4 production arises only from the fermentation of digestible matter in the rumen. A better fit between digestible crude nutrients and CH_4 production compared with using crude nutrients in the equation was reported by Jentsch et al. (2007). The negative relationship between crude fat concentration in the grains and CH_4 production we observed in the present study confirmed the findings of several earlier studies (Czerkawski et al., 1966; Beauchemin et al., 2009; Grainger and Beauchemin, 2011). In the present study, CH_4 production decreased by 0.087 g/kg of DM when dietary crude fat concentration increased by 1 g/kg of DM. However, this response in CH_4 production was also influenced by the lower digestibility of oats; as a result, a smaller response in CH_4 production to increased dietary fat concentration would be expected. Ramin and Huhtanen (2013) reported a 0.043 L/kg of DM decrease (equal to 0.031 g/kg of DM) in CH_4 production when ether extract was used in the equation and a 0.096 L/kg of DM decrease (equal to 0.069 g/kg of DM) when ether extract was replaced by fatty acids in the same study.

The CH_4-mitigating effect of increased dietary fat concentration may be explained by 3 underlying mechanisms. First, fermentable substrate is replaced with non-fermentable fatty acids (Johnson and Johnson, 1995), decreasing the extent of fermentation and leading to a lower amount of H_2 available for methanogenesis. Second, dietary fat suppresses the function of fiber-digesting microbes in the rumen, inducing a shift in fermentation pattern toward propionate production at the expense of acetate and butyrate (McAllister et al., 1996). The propionate-producing pathway captures H_2 and acts as an H_2 sink in the rumen. However, this was not the case in the present study, because the fermentation pattern was not affected by grain species. In comparison, Vanhatalo et al. (2006) reported a slightly lower proportion of butyrate when barley was replaced by oats in an in vivo study on a grass-silage-based diet. Third, the biohydrogenation of dietary UFA captures H_2 and thus acts as another H_2 sink in the rumen (Czerkawski et al., 1966). However, the biohydrogenation pathway plays only a minor role in the CH_4-mitigating effect of increased dietary fat, because the complete biohydrogenation of 1 mol of linoleic acid would decreases CH_4 production by only 1 mol. Assuming that the fatty acids in oats contain 1.5 double bonds, complete biohydrogenation could decrease CH_4 by approximately 0.4 g/kg of DM. Therefore, the CH_4-mitigating mechanism of increased crude fat concentration in oats is mostly due to the replacement of fermentable substrate by nonfermentable substrate (fatty acids) in the diet, an explanation that is also supported by the decrease in total VFA production in oat- compared with barley-based diets.

In this study, the nonsignificant effects of iNDF and crude fat on CH_4 production when they were included in the same model is related to the high correlation between these composition parameters, indicated by a high variance inflation factor (5.9). In addition, the variance inflation factor was relatively high (4.0) between TDMD and crude fat concentration, resulting in a nonsignificant effect of crude fat when included with TDMD in the bivariate model. Due to the collinearity problem between grain composition parameters and digestibility, it was difficult to draw any further conclusions about the relative importance of the CH_4-mitigating mechanisms of oats from this study.

Although oats and barley have additional differences in chemical composition, such as the concentrations of soluble β-glucans, phenolic compounds, and avenanthramides, these factors did not seem to play a role in the CH_4-mitigating effect of oats, because the decrease in CH_4 production when replacing barley with oats was as expected based on differences between the grains in terms of digestibility and oil concentration. In addition, the difference between oats and barley in predicted CH_4VFA values (10%) was close to the observed difference (9.8%) when the CH_4VFA was compared with observed CH_4 endpoint values at 48 h of incubation. Because the CH_4VFA equation by Wolin (1960) is based on the amount of digestible carbohydrates to predict CH_4 production through major VFA molar proportions, the CH_4-mitigating effect of oats was accounted for by the decreased digestibility and replacement of fermentable carbohydrates by fat. The mechanistic and empirical CH_4 values expressed close relationships to predicted in vivo CH_4 production values. The relationships were similar between mechanistic CH_4 predictions and predicted in vivo CH_4, as well as between empirical CH_4 predictions and predicted in vivo CH_4. In contrast to our second hypothesis, grain variety did not significantly affect CH_4 production, because of relatively small variations in digestibility and oil concentration between different varieties of the 2 grains.

CONCLUSIONS

Production of CH_4 was 8.9% lower for oat-based diets than for barley-based diets, and in vitro TDMD and total VFA production were also lower for oat-based diets than barley-based diets. The CH_4-mitigating effect of oats observed in the present study was most likely related to the lower digestibility and higher oil concentration of oats compared with barley. Based on the results of this experiment, we conclude that replacing barley with oats in a grass-silage-based diet could decrease enteric CH_4 production in dairy cows. Differences between grain varieties did not reach statistical significance. The relationships between composition and CH_4 production suggest that selecting grain varieties with low iNDF and high fat concentration can reduce CH_4 emissions.

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The effects of gradual replacement of barley with oats on enteric methane emissions, rumen fermentation, milk production, and energy utilization in dairy cows

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ABSTRACT

This study evaluated the effects of gradual replacement of barley with oats on enteric CH_4 emissions, rumen fermentation, diet digestibility, milk production, and energy utilization in dairy cows fed a grass silage-based diet. Sixteen lactating Nordic Red dairy cows received a total mixed ration [58:42 forage:concentrate on dry matter (DM) basis]. Grass silage (*Phleum pratense*) was the sole forage with canola meal (10% of diet DM) as a protein supplement. The effects of gradual replacement of barley with oats on DM basis were evaluated using a replicated 4 × 4 Latin square design with 21 d periods. The grain supplements (30% of diet DM) consisted of 100% barley, 67% barley and 33% oats, 33% barley and 67% oats, and 100% oats. In addition to intake, milk production, and digestibility measurements, CH_4 emissions were measured by the GreenFeed system (C-Lock Inc.). The energy metabolism was estimated from the gas exchange measurements recorded by the GreenFeed unit. The last 10 d of each period were used for recordings of gas exchanges, feed intake and milk production. Dry matter intake, body weight, milk yield, and energy-corrected milk yield were not affected by gradual replacement of barley with oats in the diet. Increased inclusion of oats linearly decreased CH_4 emissions from 467 to 445 g/d, and CH_4 intensity from 14.7 to 14.0 g/kg energy-corrected milk. In addition, the ratio of CH_4 to CO_2 decreased with increasing inclusion of oats in the diet. Digestibility of organic matter, neutral detergent fiber, and potentially digestible neutral detergent fiber decreased linearly with increasing inclusion of oats. Increased inclusion of oats linearly increased fecal energy from 121 to 133 MJ/d, whereas urinary energy and heat production were not affected by dietary treatment. This resulted in a linear decrease in metabolizable energy intake. However, increased levels of oat in the diet did not significantly affect energy balance or efficiency of metabolizable energy utilization for lactation. This study concludes that barley could be replaced with oats in the diet of dairy cows fed a grass silage-based diet to mitigate CH_4 emissions without having any adverse effects on productivity or energy balance. However, the effect of replacing barley with oats on CH_4 emissions is dependent on the differences between barley and oats in the concentrations of indigestible neutral detergent fiber and fat.

Key words: digestibility, energy balance, feed efficiency, grass silage, greenhouse gases

INTRODUCTION

Within the agricultural sector, CH_4 is a major contributor to total global greenhouse gas emissions (Wuebbles and Hayhoe, 2002). Up to 39% of total global anthropogenic CH_4 emissions are arising from ruminants in which a greater part originates from enteric fermentation (Gerber et al., 2013). Methane is 28 times more effective in trapping heat in the atmosphere than CO_2, but has a shorter turnover time (about 10 years) in the atmosphere than CO_2 (IPCC, 2014). In the Kyoto Protocol in 1997, mitigation of CH_4 emissions have been of interest, in addition to mitigation of CO_2 emissions. Moreover, production of CH_4 from cows represents an energy loss ranging from 2 to 12% depending on the diet (Johnson and Johnson, 1995).

Despite intensive research during the last decade, practical innovations on-farm to lower CH_4 emissions are limited. Feed additives such as ionophores may be effective for lowering CH_4 emissions, but the use of these additives is prohibited in the European Union. Plant extracts have been demonstrated effective in vitro (Calsamiglia et al., 2007), but the amounts required for cows make these supplements too costly and their availability may also constrain widescale use. Nitrate supplementation is an effective means for lowering CH_4 emissions (Van Zijderveld et al., 2011), but unless care-

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fully monitored may pose a health risk to the animal and increase N emissions when included in diets supplying adequate RDP. Replacement of starch sources with plant oils or oilseeds have consistently lowered CH_4 emissions (Beauchemin et al., 2008), but at high levels typically lower feed intake, can depress milk yield, and increase feed costs. The mitigation effect of some other feed additives such as 3-nitrooxypropanol (Hristov et al., 2015) or *Asparagopsis taxiformis* (Roque et al., 2019) has showed potential to decrease CH_4 emissions in dairy cows. However, the literature is scarce on whether the use of these additives would be economically sustainable for the farmer or safe for the animal in the long run.

In temperate climates, oats (*Avena sativa*) and barley (*Hordeum vulgare*) are commonly used as concentrate supplements in the diet of dairy cows. Recently, oats have mostly been replaced by barley, partly due to the higher tabulated ME value (13.2 vs. 11.5 MJ/kg of DM; LUKE, 2020) and MP value (96 vs. 93 g/kg of DM; LUKE, 2020) of barley compared with oats, which favors barley in ration-formulating programs. However, previous production studies have found that replacement of barley grain with oat grain in the diet of dairy cows maintains milk yields on the same level (McKay et al., 2019), or even increases milk yields (Martin and Thomas, 1988; Ekern et al., 2003; Vanhatalo et al., 2006), whereas milk protein and fat concentrations might decrease. Barley and oats differ in their chemical composition and digestibility, and these factors could potentially affect enteric CH_4 production in dairy cows. Oat grain is less digestible and provides the diet with a higher oil and fiber concentration, but lower starch concentration, compared with barley (Lee et al., 1997). Increasing the oil concentration in the diet of dairy cows mitigates enteric CH_4 emissions (Beauchemin et al., 2009; Grainger and Beauchemin, 2011). In addition, enteric CH_4 emissions decreased with decreasing diet digestibility (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013), because CH_4 is produced only from digested matter. A recent in vitro study by Fant et al. (2020), where gas production kinetics were used to predict CH_4 emission in vivo, reported 8.9% lower CH_4 emissions from incubated oat-based diets compared with barley-based diets.

The objective of this study was to investigate the effect of gradual replacement of barley with oats in the diet of lactating dairy cows on enteric CH_4 emissions, rumen fermentation, milk production, and energy utilization. We hypothesized that replacement of barley with oats will decrease enteric CH_4 emissions without compromising animal performance in terms of milk yield and ECM yield.

MATERIALS AND METHODS

Experimental Design

All animals were cared for according to the rules and guidelines proposed by the Swedish University of Agricultural Sciences Animal Care and Use Committee and the National Animal Research Authority. The production trial was conducted at Röbäcksdalen experimental farm of the Swedish University of Agricultural Sciences, Umeå, Sweden (63°45′N; 20°17′E) in fall 2016. Sixteen lactating Nordic Red dairy cows, with mean DIM 82 ± 29.6, BW of 618 ± 82 kg, and milk production 31.8 ± 3.50 kg/d at the start of the experiment, were used in a replicated 4 × 4 Latin square design with 21-d periods and balanced for the carry-over effect. The cows were divided into 4 blocks according to parity and milk yield and allocated randomly to treatments. Grass silage was the sole forage with heat-treated solvent-extracted canola meal containing low levels of glucosinolates and erucic acid (ExPro; 10% of diet DM) as a protein supplement. The oats and barley were purchased from a local feed company (Lantmännen). Grass silage was prepared from a primary growth perennial leys dominated by timothy (*Phleum pratense*), with approximately 5 to 10% of red clover (*Trifolium pratense*) on DM basis. The fields were fertilized with 70 kg of N/ha in spring. The ratio of forage to concentrate for the experimental diets was 58:42 on a DM basis (Table 1). The dietary treatments comprised gradual replacement of barley with oats in grain supplement (30% of diet DM) at levels of 0, 33, 67 and 100% on a DM basis. The resulting 4 diets (Table 1) were defined as: 100% barley (**O0**), 67% barley and 33% oats (**O33**), 33% barley and 67% oats (**O67**) and 100% oats (**O100**). The O0 diet was formulated according to Lypsikki (Huhtanen and Nousiainen, 2012) and in other diets barley was gradually replaced with oats on weight basis. The cows were housed in an insulated freestall barn equipped with an automatic feed intake recording system and free access to fresh water. The cows were fed a TMR ad libitum, and the diets were mixed using a TMR mixer (Nolan A/S) and delivered in the feed troughs 4 times/d (0300, 0800, 1400, and 1800 h) by an automatic feeding wagon. The ingredient composition of the different mixtures is shown in Table 1.

Sample and Data Collection

Feed intake was recorded individually on daily basis throughout the trial in Roughage Intake Control feeders (Insentec B.V.), but only the data collected from the last 10 d of each period were used for the statisti-

Table 1. Ingredient composition of formulated diets (g/kg of DM)

Item	Diet[1]			
	O0	O33	O67	O100
Grass silage	580	580	580	580
Heat-treated canola meal expeller	100	100	100	100
Oats	0	100	200	300
Barley	300	200	100	0
Minerals[2]	20	20	20	20

[1] In addition to the TMR, the cows received a concentrate mixture during visits to the GreenFeed system (C-Lock Inc.); O0 = 100% barley; O33 = 67% barley and 33% oats; O67 = 33% barley and 67% oats; O100 = 100% oats.

[2] NaCl and $CaCO_3$.

cal analysis. Cows were weighed on 3 consecutive days after morning milking at the end of each period.

Milk yield was recorded throughout the trial with gravimetric milk recorders (SAC; S.A. Christenses and Co Ltd.), but only data collected from the last 10 d of each period were used for the statistical analysis. Cows were milked twice daily at 0600 and 1600 h in a 2 × 8 milking parlor. Milk samples were collected at 4 consecutive milkings during the last 3 d of each period, starting from d 19 in the afternoon. Milk samples were kept in special plastic bottles, preserved with 2-bromo-2-nitropropane-1,3-diol (Bronopol, Valio Ltd.), stored at 4°C, and sent for analysis within 1 wk from sampling.

Samples of grass silage were collected twice a week during the last 2 wk of each period. A subsample was frozen at −20°C for later determination of fermentation quality. Concentrate samples were taken once a week during the last 2 wk of each period. Grass silage and concentrate samples were dried at 60°C for 48 h for later determination of chemical composition and digestibility trial. The dried silage and concentrate samples were ground to pass a 1-mm (for chemical composition analysis) or 2-mm (for digestibility trial) sieve using a cutter mill (SM 300, Retsch Ltd.).

Fecal grab samples (300 mL) were collected from the rectum of 8 cows (from 2 blocks) at 0600 and 1400 h on the last 3 consecutive days of each period. Fecal samples were pooled within cow and period, and dried at 60°C in a forced-air oven for 48 h. A subsample of the dried fecal sample was ground using a cutter mill (SM 300, Retsch Ltd.) to pass through a 1-mm sieve for chemical composition analysis. For the digestibility trial, another subsample was ground with pestle and mortar to pass through a 2-mm sieve for indigestible NDF determination (**iNDF**).

Methane emissions, CO_2 emissions, and O_2 consumption were measured by the GreenFeed system (C-Lock Inc.) as described by Huhtanen et al. (2015), but only data from the last 10 d of each period were used for statistical analysis. For determination of O_2 consumption, the GreenFeed uses a paramagnetic O_2 sensor. Moisture is measured in real time to mathematically dry the air on a second basis as is best practice in chamber measurements. The calculations are then on a dry-gas basis with humidity taken into account, the rest of the calculations are the same as described by Huhtanen et al. (2015) for CH_4 and CO_2 emissions. The device was programmed to allow each cow in the experiment to visit at minimum 5-h intervals. During each visit, the cows were given 8 drops of 50 g concentrate every 40 s. The span gas (a mixture of CO_2, CH_4, and O_2) and zero gas (N_2) calibrations were performed once a week and CO_2 recovery tests were conducted every other week. The average recovery was 104 ± 2.7. The airflow rates and gas concentrations were measured continually and by using the gas sensor information, a volumetric flux (L/min) of gases emitted/consumed by the animal could be calculated. A concentrate was given to the cows to attract them for regular visits to the GreenFeed. The concentrate was a commercial concentrate, of which intake was taken into account in the total DMI calculations (Table 2). The head position was also recorded by the system during the visits and data with inappropriate head position was filtered out by the system.

For determination of total VFA concentration and molar proportions of VFA, ruminal fluid samples were collected from 8 cows (2 blocks) after morning milking during the last day of the sampling week of each period. The samples were collected using a stomach tube (RUMINATOR) as described by Geishauser (1993) and filtered through a 2-layer cheesecloth. The first sample (approximately 500 mL) of ruminal fluid collected directly after insertion of the stomach tube was discarded to avoid saliva contamination. A subsample of 2.5 mL was then taken from the glass jar filled with new ruminal fluid (500 mL) and was mixed with 0.5 mL of 25% metaphosphoric acid and then kept in −18°C until further analyses.

Chemical Analysis

The DM concentration of feed and feces was determined by oven drying at 105°C for 16 h, followed by ash determination by combustion of the dried samples at 500°C for 4 h (AOAC International, 2000). Total N concentration was determined by the Kjeldahl method using a Block Digestor (SEAL Analytical) followed by a continuous flow analysis using an Auto Analyzer 3 Unit (SEAL Analytical). Crude protein concentration was calculated as total N concentration × 6.25. Crude fat concentration was determined by ether extraction and HCl-hydrolysis according to AOAC method 954.02 (AOAC International, 2000), and starch concentration in concentrates was determined with an YSI Analyzer (YSI 2950D-1 Biochemistry Analysers) at the Dairy One Forage Laboratory (Ithaca, NY). The NDF concentration was determined using heat stable α-amylase and sodium sulfite (Mertens, 2002) in Ankom^{200} Fiber Analyzer (Ankom Technology Corp.).

Indigestible NDF concentration was determined for feed and fecal samples as described by Huhtanen et al. (1994). A sample size of 2 g was weighed into polyester bags of 11.5-μm pore size and incubated for 288 h in triplicates in the rumen of 3 cannulated cows fed a grass silage-based diet (60:40 forage-to-concentrate ratio). The iNDF was expressed exclusive of residual ash. Acid-insoluble ash (**AIA**) was determined for feed and fecal samples according to Van Keulen and Young (1977) with some modifications; samples were boiled in acid for 10 min, filtrated, and combusted in 600°C for 1 h.

The frozen silage samples were thawed and pressed, the silage juice was diluted 1:1 with distilled water, and the liquid was used for determination of ammonia N using a Kjeltec 2100 Distillation Unit (Foss Analytical Ltd.) and pH. In addition, lactic acid, and VFA concentrations were analyzed as described by Ericson and André (2010). Silage DM concentration was corrected for volatile losses as described by Huida et al. (1986). The milk samples were analyzed for concentration of lactose, protein, fat, and urea by near-infrared reflectance spectroscopy (CombiFoss 6000, Foss Analytical Ltd.). Individual VFA concentration in ruminal fluid samples was determined using a Waters Alliance 2795 UPLC system as described by Puhakka et al. (2016).

Calculations

Dietary chemical composition was calculated based on the ingredient composition and proportions in each

Table 2. Chemical composition of dietary ingredients (g/kg of DM unless otherwise stated)

Item	Grass silage	Canola meal	Barley	Oats	GreenFeed bait[1]
DM, g/kg	280	908	868	895	896
In DM, g/kg					
Ash	76.0	79.0	25	28.0	66.0
CP	147	356	102	105	194
NDF	434	292	202	257	248
iNDF[2]	89.6	147	43.4	137	82.0
NDS[3]	490	629	773	715	686
Starch	NA	6.0	528	494	NA
Crude fat	25.0	49.0	21.0	52.0	NA
pdNDF[4]	344	145	159	120	166
Fermentation quality					
pH	3.72				
In DM, g/kg					
Lactic acid	57.9				
Acetic acid	15.0				
Propionic acid	0.79				
Butyric acid	0.35				
Formic acid	5.06				
Ethanol	2.36				
2,3-Butandiol	0.35				
Ammonium N, g/kg of N	33.0				
Feeding values[5]					
MP, g/kg of DM	84	210	96	93	112
ME, MJ/kg of DM	11.0	12.4	13.2	11.5	13.3

[1] GreenFeed system (C-Lock Inc.).

[2] iNDF = indigestible NDF.

[3] NDS = neutral detergent solubles, calculated as OM − NDF.

[4] pdNDF = potentially digestible NDF, calculated as NDF − iNDF.

[5] Based on coefficients from feed tables (LUKE, 2020).

diet. Potentially digestible NDF (**pdNDF**, g/kg of DM) was calculated as the difference between NDF and iNDF. Neutral detergent solubles (**NDS**) was calculated as the difference between OM and NDF. Metabolizable energy (MJ/kg of DM) and MP (g/kg of DM) in feed ingredients and diets were calculated based on the coefficients in Finnish national feed tables (LUKE, 2020). Energy-corrected milk was calculated as described by Sjaunja et al. (1990) according to Equation [1]:

$$\begin{aligned}\text{ECM} &= \text{milk yield (kg/d)} \times [38.3 \times \text{fat (g/kg)} \\ &+ 24.2 \times \text{protein (g/kg)} + 16.54 \times \text{lactose (g/kg)} \\ &+ 20.7]/3{,}140, \qquad [1]\end{aligned}$$

where fat, protein, and lactose are the concentrations of these constituents in milk. Feed efficiency was calculated as the ratio of ECM yield (kg/d) to DMI (kg/d). Milk N efficiency was calculated as: Milk N (protein yield/6.38, g/d) divided by N intake (kg/d).

Digestibility of DM, OM, CP, NDF, pdNDF, and NDS were estimated using 2 different markers (iNDF and AIA) in feeds and feces. The formula for estimation of OM digestibility (**OMD**) using iNDF as a marker is presented in Equation [2]. The same equation was used for the other parameters. For AIA-marker-based estimation, iNDF concentration was replaced by AIA concentration in the formula.

$$\begin{aligned}\text{OMD} &= 1{,}000 - 1{,}000 \times \{[\text{iNDF in diet DM (g/kg)}]/ \\ &[\text{iNDF in fecal DM (g/kg)}]\} \times [\text{OM in fecal DM} \\ &\text{(g/kg)}]/[\text{OM in diet DM (g/kg)}]. \qquad [2]\end{aligned}$$

The daily fecal DM output was calculated as the quotient of daily iNDF intake and iNDF concentration in feces. The respiratory quotient (**RQ**) was calculated as the ratio of CO_2 eliminated to O_2 consumed on a volume basis. Gross energy (**GE**) intake (MJ/d) was estimated from DMI (kg/d) according to Ramin and Huhtanen (2013), and GE concentration (MJ/kg DM) calculated from chemical composition based on Equation [3] of Beyer et al. (2003):

$$\begin{aligned}\text{GE intake} &= \text{DMI} \times [(23.6 \times \text{CP} + 39.8 \times \text{EE} \\ &+ 17.3 \times \text{NFC} + 18.9 \times \text{NDF})/1{,}000], \qquad [3]\end{aligned}$$

where EE is ether extract, and the dietary concentrations are expressed as kg/kg of DM, and the coefficients as MJ/kg of DM. Gross energy digestibility (**GED**) was estimated from OMD as GED, g/kg = −11.3 + 0.977 × OMD, as described by Ramin and Huhtanen (2013). Energy losses in feces were calculated based on estimated GED and GE intake. Digestible energy (**DE**) intake (MJ/d) was calculated by subtracting fecal energy from GE intake. Methane energy (**CH_4E**, MJ/d) was calculated considering the molecular weight (16.04 g/mol) and energy combustion (55.5 MJ/kg) of CH_4 as:

$$\begin{aligned}CH_4\text{E (MJ/d)} &= 55.5 \times 16.04 \times CH_4\text{ (L/d)}/ \\ &[1{,}000 \times 22.414\text{ (L)}],\end{aligned}$$

where 22.414 is the standard volume of 1 mol of an ideal gas at 1 atmosphere.

Urinary energy (**UE**) was calculated as described by Guinguina et al. (2020), according to Equation [4]:

$$\begin{aligned}\text{UE} &= -3.6 + 0.37 \times \text{DMI (kg/d)} + 0.006 \\ &\times \text{Forage proportion (g/kg of DM)} + 0.03 \\ &\times \text{CP (g/kg of DM) (adjusted root} \\ &\text{mean square error} = 3.34;\ n = 624). \qquad [4]\end{aligned}$$

Metabolizable energy intake (MJ/d) was calculated as: ME intake (MJ/d) = DE intake − CH_4E − UE. Milk energy (MJ/d) was calculated as 3.14 (MJ/kg) × ECM according to Sjaunja et al. (1990). Heat production (**HP**, MJ/d) was calculated according to Brouwer (1965) in Equation [5]:

$$\begin{aligned}\text{HP} &= 0.01618 \times O_2 + 0.00502 \times CO_2 - 0.00599 \\ &\times \text{UN} - 0.00217 \times CH_4, \qquad [5]\end{aligned}$$

using volumes of O_2 consumption (L/d), CO_2 production (L/d), CH production (L/d), and estimated urinary nitrogen excretion (**UN**, g/d). Urinary nitrogen excretion was calculated as total N intake (g/d) − Milk N (g/d) − Fecal N (g/d) and assuming zero N balance. Energy balance (**EB**, MJ/d) was calculated as

$$\text{EB} = \text{ME intake} - \text{HP} - \text{Milk energy}. \qquad [6]$$

The efficiency of ME use for lactation (***kl***) was calculated as described by AFRC (1993):

$$kl = E_{1(0)}/(\text{ME intake} - \text{ME}_{\text{m}}), \qquad [7]$$

where $E_{1(0)}$ is milk energy output (**E_1**) adjusted to zero energy balance (MJ/d), as calculated from Equations [8] and [9]. The ME_{m} is the ME requirement for maintenance (MJ/d).

$$\text{If EB} > 0,\ E_{1(0)} = E_1 + 0.7277 \times \text{EB}; \qquad [8]$$

$$\text{if EB} < 0,\ E_{1(0)} = E_1 - 0.6943 \times \text{EB}. \qquad [9]$$

The coefficients were derived from a large (n = 841) respiration chamber data set (Guinguina et al., 2020).

Statistical Analysis

The experimental data were analyzed by ANOVA for a replicated 4 × 4 Latin square design using the MIXED procedure of SAS. All data were pooled per cow/period. The statistical model was:

$$Y_{ijkl} = \mu + S_i + P_j + C_k(S_i) + D_l + E_{ijkl},$$

where Y_{ijkl} is a dependent variable and μ is the mean for all observations, S_i is the fixed effect of square i, P_j is the fixed effect of period j, $C_k(S_i)$ is the random effect of cow k within square i, D_l is the fixed effect of diet l, and $E_{ijkl} \sim N\left(0, \sigma_e^2\right)$ represents the residual error. To compare the effects of gradual replacement of barley with oats in the diet, linear and quadratic contrasts were used. Differences were declared significant at $P \leq 0.05$, and a tendency toward significant at $0.05 < P \leq 0.10$.

For digestibility data, we used a repeated measurement statistical model, because 2 methods were used for digestibility determinations (iNDF and AIA). The following model was used:

$$Y_{ijkl} = \mu + C_i + P_j + D_k + M_l + M_l \times D_k + C_i \times P_j \times D_k + E_{ijkl},$$

where Y_{ijkl} is a dependent variable, μ is the mean for all observations, C_i is the fixed effect of cow, P_j is the fixed effect of period, D_k is the fixed effect of diet, M_l is the fixed effect of marker, $M_l \times D_k$ is the interaction between marker l and diet k, $C_i \times P_j \times D_k$ is the interaction between cow i and period j and diet k, and E_{ijkl} is the random residual error $\sim N\left(0, \sigma_e^2\right)$. The model included a REPEATED statement, with type of marker considered as a repeated measures factor. To compare the effects of gradual replacement of barley with oats in the diet, linear and quadratic contrasts were used. Differences were declared significant at $P \leq 0.05$, and a tendency toward significant at $0.05 < P \leq 0.10$.

RESULTS

Diet Composition and Feed Intake

The silage used in the current study was of good quality, as indicated by the low pH-value (3.72) and the moderate concentration of lactic acid (57.9 g/kg of DM, Table 2). Low levels of NH_3-N (mean 33.0 g/kg of N; Table 2) indicated small proteolysis. The CP concentration was similar between oats and barley (105 vs. 102 g/kg of DM; Table 2), and the CP level of the diets was similar, indicating that the experimental diets were isonitrogenous. The concentrations of iNDF, NDF, and crude fat were greater in oats compared with barley, which resulted in increased dietary concentrations of these constituents with increasing inclusion of oats in the diet (Table 3). Total DMI was not affected by gradual replacement of barley with oats (average 22.7 kg/d; Table 4). The intake of NDF ($P = 0.02$), crude fat, and iNDF ($P < 0.01$) increased linearly with increasing inclusion of oats in the diet, whereas the intake of NDS and pdNDF ($P < 0.01$) decreased linearly.

Milk Production, Milk Composition, and Feed Efficiency

Milk yield, ECM yield, and yield of milk constituents were not affected by gradual replacement of barley with oats in the diet (Table 5). The concentrations of lactose and urea remained unchanged, whereas the concentration of milk protein ($P < 0.01$) and milk fat ($P = 0.05$) decreased linearly from 38.0 to 36.9 g/kg and from 48.3 to 46.9 g/kg, respectively, with increasing inclusion of oats. Milk N efficiency was not affected by dietary treatment, with an overall mean of 283 g/kg. Feed efficiency, defined as ECM/DMI, was not affected by replacement of barley with oats (Table 5).

Table 3. Chemical composition of experimental diets (g/kg of DM unless otherwise stated)

	Diet[1]			
Item	O0	O33	O67	O100
In DM, g/kg				
OM	939	938	938	938
CP	159	159	159	159
NDF	347	353	358	364
iNDF[2]	83.0	92.0	101	111
NDS[3]	592	586	580	574
Crude fat	26.7	29.8	32.9	36.0
pdNDF[4]	264	261	257	253
Feeding values[5]				
ME, MJ/kg of DM	11.8	11.6	11.5	11.3
MP, g/kg of DM	103	102	101	101

[1] O0 = 100% barley; O33 = 67% barley and 33% oats; O67 = 33% barley and 67% oats; O100 = 100% oats.

[2] iNDF = indigestible NDF.

[3] NDS = neutral detergent solubles, calculated as OM − NDF.

[4] pdNDF = potentially digestible NDF, calculated as NDF − iNDF.

[5] Based on coefficients from feed tables (LUKE, 2020).

Table 4. Effect of gradual replacement of barley with oats on nutrient intakes

	Diet[1]					*P*-value	
Intake, kg/d	O0	O33	O67	O100	SEM	Linear	Quadratic
Total DM	22.8	22.8	22.6	22.6	0.24	0.44	0.96
Silage DM	12.5	12.5	12.4	12.4	0.13	0.42	0.82
Concentrate DM	10.3	10.3	10.2	10.2	0.11	0.48	0.87
OM	21.4	21.4	21.2	21.2	0.22	0.40	0.95
CP	3.67	3.67	3.64	3.65	0.038	0.70	0.99
NDF	7.82	7.92	7.97	8.09	0.083	0.02	0.91
Crude fat	0.58	0.64	0.70	0.77	0.007	<0.01	0.86
iNDF[2]	1.88	2.09	2.27	2.47	0.022	<0.01	0.98
NDS[3]	13.6	13.5	13.2	13.1	0.14	<0.01	0.98
pdNDF[4]	5.93	5.83	5.70	5.62	0.062	<0.01	0.89

[1] O0 = 100% barley; O33 = 67% barley and 33% oats; O67 = 33% barley and 67% oats; O100 = 100% oats.

[2] iNDF = indigestible NDF.

[3] NDS = neutral detergent solubles, calculated as OM − NDF.

[4] pdNDF = potentially digestible NDF, calculated as NDF − iNDF.

Gas Emissions and Rumen Fermentation

Total VFA concentration and fermentation pattern were not affected by increased inclusion of oats in the diet (Table 6). Total CH_4 emissions decreased linearly ($P = 0.01$) from 467 to 445 g/d by gradual replacement of barley with oats in the diet (Table 6). In addition, increased inclusion of oats linearly decreased CH_4 yield ($P = 0.03$), defined as grams of CH_4/kg of DMI, CH_4 intensity ($P = 0.05$), defined as grams of CH_4/kg of ECM, and the ratio of CH_4 to CO_2 ($P < 0.01$). Total CO_2 emissions decreased linearly by gradual replacement of barley with oats in the diet from 13.6 to 13.4 kg/d (Table 6). The RQ, calculated as the ratio of CO_2 eliminated to O_2 consumed on a volume basis, decreased linearly ($P = 0.03$) from 1.125 to 1.109 by replacing barley with oats. The diurnal pattern in CH_4 emissions were consistent among the 4 different treatments with a similar pattern among diets (Figure 1).

Digestibility, Energy Utilization, and Efficiency Traits

Because there was no interaction between diet and marker (e.g., for OMD: $P = 0.20$) the diet effects were expressed as the mean of 2 markers. The type of marker (iNDF vs. AIA) used in the digestibility analysis had a significant effect only on DMD and OMD ($P < 0.01$), with higher values with AIA for DMD (mean 713 vs. 705 g/kg) and OMD (mean 729 vs. 722 g/kg), compared with the iNDF marker (Table 7). Apparent total-tract digestibility of DM, OM, NDF ($P < 0.01$), and pdNDF ($P = 0.03$) decreased linearly with gradual replacement of barely with oats. Crude protein digestibility tended to change quadratically ($P = 0.06$) since the lowest

Table 5. Effect of gradual replacement of barley with oats on milk production

	Diet[1]					*P*-value	
Item	O0	O33	O67	O100	SEM	Linear	Quadratic
Yield							
Milk yield, kg/d	28.2	28.3	28.0	28.8	0.72	0.28	0.39
ECM, kg/d	31.9	31.5	31.1	31.8	0.77	0.76	0.15
Protein, g/d	1,067	1,066	1,032	1,057	22.3	0.32	0.39
Fat, g/d	1,357	1,332	1,311	1,345	43.3	0.51	0.14
Lactose, g/d	1,311	1,303	1,313	1,349	35.5	0.13	0.22
Concentration							
Milk fat, g/kg	48.3	47.3	46.9	46.9	1.27	0.05	0.31
Milk protein, g/kg	38.0	37.9	37.0	36.9	0.55	<0.01	0.98
Milk lactose, g/kg	46.6	46.0	46.8	46.9	0.33	0.12	0.12
Milk urea, mmol/L	4.18	4.14	4.12	4.11	0.148	0.55	0.85
Milk N efficiency, g/kg	286	285	277	284	2.80	0.30	0.22
ECM/DMI	1.40	1.39	1.37	1.41	0.030	0.69	0.12
BW, kg	622	628	623	626	12.6	0.66	0.64

[1] O0 = 100% barley; O33 = 67% barley and 33% oats; O67 = 33% barley and 67% oats; O100 = 100% oats.

value was observed for the O0 diet (661 g/kg), but minimal differences between the other diets.

Predicted GE concentrations slightly increased with increasing proportion of oats in the diet due to higher fat concentration, but GED decreased linearly ($P <$ 0.01, Table 8). Increased level of oats in the diet linearly increased fecal energy ($P < 0.01$), which led to a linear decrease in DE intake ($P < 0.01$). Methane energy ($P = 0.01$), and the ratio of CH_4 to GE intake ($P = 0.03$), decreased linearly by gradual replacement of barley with oats. Predicted UE was not affected by dietary treatment, however, ME intake decreased linearly ($P < 0.01$) with increasing inclusion of oats in the diet, mostly as a consequence of the lower DE intake. No significant effect of dietary treatment was observed on HP, milk energy, or efficiency of ME utilization for lactation. Energy balance was positive and varied quadratically ($P = 0.06$), because the lowest value (23.7 MJ/d) was observed for diet O100 (Table 8).

DISCUSSION

The main objective of this study was to investigate the effects of gradual replacement of barley with oats, as a concentrate supplement, in the diet of dairy cows on enteric CH_4 emissions and milk production. We hypothesized that replacement of barley with oats will decrease enteric CH_4 emissions without compromising productivity. Methane intensity (expressed as g/kg of ECM) decreased by 4.8% when barley was completely replaced by oats in the diet, and although this is not a large decrease, we accept our hypothesis. Furthermore, we investigated ruminal fermentation, digestibility, energy utilization, and energy balance to find possible explanations for our results. The CH_4 mitigating effect of oats compared with barley has been confirmed in vitro (Fant et al., 2020), but there is a lack of in vivo studies comparing oats and barley in terms of enteric CH_4 emissions. Regarding the chemical composition of the oats and barley used in this study, the CP concentrations were exceptionally low. However, because both grains expressed similarly low CP concentrations, it is unlikely that this influenced our results.

Feed Intake and Rumen Fermentation

Feed intake was not affected by increased inclusion of oats in the diet, which is consistent with the findings of McKay et al. (2019) and Tosta et al. (2019) where cows were fed ad libitum. Because OMD decreased with increasing inclusion of oats, the absence of an effect of replacement of barley with oats on total VFA concentration in ruminal fluid was unexpected. Vanhatalo et al. (2006) and Tosta et al. (2019) both reported a 7% lower total VFA concentration in the ruminal fluid of cows fed oat diets compared with barley diets. However, by judging from the relatively high standard error of the mean in the current study, it is likely that the means for total VFA concentration are not very accurate. Once-a-day sampling method with a stomach

Table 6. Effect of gradual replacement of barley with oats on gases and volatile fatty acids production

	Diet[1]					*P*-value	
Item	O0	O33	O67	O100	SEM	Linear	Quadratic
Concentrate intake by GreenFeed,[2] kg/d	1.20	1.25	1.24	1.21	0.046	0.90	0.23
CH_4, g/d	467	454	449	445	9.54	0.01	0.37
CH_4, g/kg of DMI	20.5	19.9	19.8	19.6	0.25	0.03	0.41
CH_4, g/kg of OM digested	29.6	28.5	29.0	29.2	0.572	0.79	0.30
CH_4, g/kg of ECM	14.7	14.6	14.6	14.0	0.24	0.05	0.41
CO_2, kg/d	13.6	13.5	13.2	13.4	0.09	0.05	0.13
CO_2, g/kg of DMI	600	594	588	596	6.3	0.50	0.28
CO_2/ECM, g/ kg	433	436	433	427	6.2	0.40	0.41
CH_4/CO_2, g/kg	34.0	33.4	33.6	32.9	0.25	<0.01	0.92
O_2, kg/d	8.85	8.87	8.75	8.85	0.056	0.59	0.45
RQ[3]	1.125	1.112	1.105	1.109	0.0072	0.03	0.15
Total VFA, mmol/L	75.4	92.3	83.6	87.5	8.27	0.29	0.26
Molar proportion, mmol/mol							
Acetic acid	695	680	685	693	7.4	0.97	0.14
Propionic acid	150	167	157	161	4.6	0.30	0.17
Butyric acid	123	123	128	117	5.5	0.62	0.33
Isovaleric acid	4.4	3.5	4.1	3.7	0.31	0.36	0.43
Isobutyric acid	10.4	9.2	9.9	9.4	0.44	0.22	0.38

[1] O0 = 100% barley; O33 = 67% barley and 33% oats; O67 = 33% barley and 67% oats; O100 = 100% oats.

[2] GreenFeed system (C-Lock Inc.).

[3] RQ = respiratory quotient, calculated as CO_2 eliminated/O_2 consumed on a volume basis.

Table 7. Effect of gradual replacement of barley with oats on digestibility using 2 different marker methods (iNDF and AIA)[1]

Item	Marker	Diet[2]				SEM	*P*-value[3]	
		O0	O33	O67	O100		Linear	Quadratic
Digestibility, g/kg								
OM		738	732	722	710	3.4	<0.01	0.46
iNDF	722					2.4		
AIA	729							
DM		721	715	705	693	3.6	<0.01	0.40
iNDF	705					2.6		
AIA	713							
NDF		631	606	587	566	7.4	<0.01	0.77
iNDF	592					5.2	0.18	
AIA	602							
CP		661	679	684	674	7.4	0.19	0.06
iNDF	670					5.2	0.20	
AIA	679							
pdNDF[4]		827	822	820	800	8.5	0.03	0.42
iNDF	815					6.0	0.57	
AIA	820							
NDS[5]		806	812	810	806	3.6	0.86	0.16
iNDF	806					2.6	0.14	
AIA	811							
Fecal pdOM output,[6] kg/d		3.53	3.54	3.49	3.59	0.106	0.83	0.70

[1] iNDF = indigestible NDF; AIA = acid-insoluble ash.

[2] O0 = 100% barley; O33 = 67% barley and 33% oats; O67 = 33% barley and 67% oats; O100 = 100% oats.

[3] *P*-value for marker if not reported < 0.01.

[4] pdNDF = potentially digestible NDF.

[5] NDS = neutral detergent solubles.

[6] pdOM = potentially digestible OM.

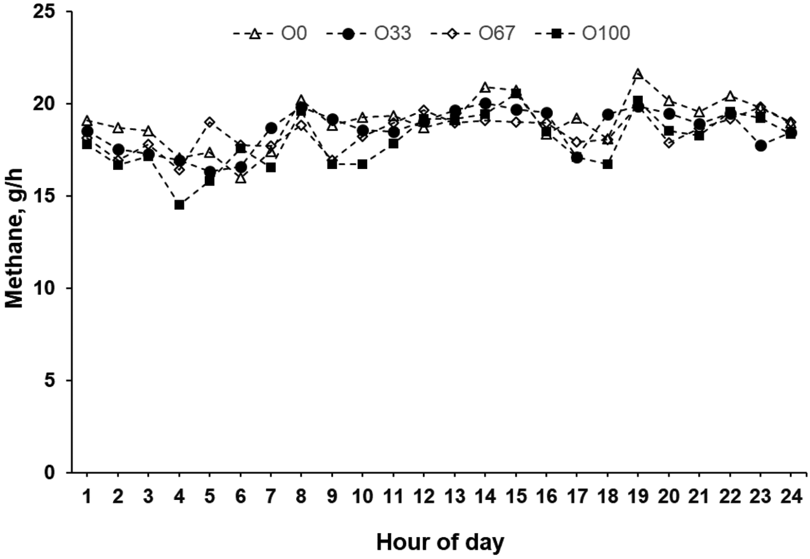

Figure 1. Diurnal pattern of CH_4 emissions in dairy cows fed 4 different diets. Diets were 100% barley (O0), 67% barley and 33% oats (O33), 33% barley and 67% oats (O67), and 100% oats (O100). The standard deviation for all the visits ranged from 1.37 g/h (at 0500 h) to 4.33 g/h (at 1200 h).

tube can increase random variation in the data due to salivary contamination and time elapsed after the last meal.

Regarding the effect of replacement of barley with oats on molar proportions of VFA in ruminal fluid, the sampling method is less likely to have affected the results. However, there are somewhat conflicting findings reported in the literature. Vanhatalo et al. (2006) reported decreased proportions of butyrate and valerate when barley was replaced by oats in the diet of dairy cows, whereas, in agreement with our findings, McKay et al. (2019) and Tosta et al. (2019) found no effect on molar proportions of VFA when barley was replaced by oats.

Milk Production, Milk Composition, and Feed Efficiency

In our study, gradual replacement of barley with oats in the diet of dairy cows had no effect on milk yield or ECM yield. On the contrary, Martin and Thomas (1988), Ekern et al. (2003), Vanhatalo et al. (2006), and Tosta et al. (2019) reported increased milk yields when oat diets were compared with barley diets. In the study by Vanhatalo et al. (2006), milk yield and ECM increased by 0.7 and 0.3 kg/d, respectively, when coarsely ground barley was replaced by coarsely ground oats on a grass silage-based diet where silage to concentrate ratio was 60:40 and the concentrate consisted of oat or barley grain with a mineral supplement of 53 g/kg concentrate. In agreement with our results, McKay et al. (2019) found no difference in milk yield when oats were compared with barley on a grass silage-based diet or on pasture. However, in the study by McKay et al. (2019), the grass silage-to-concentrate ratio was approximately 90:10, only 42% of the concentrate consisted of barley or oat grain, and the cows were in late lactation (275–289 DIM).

The previously reported increases in milk yield could partly be due to the increase in dietary fat concentration when barley is replaced by oats in the diet. Supplementation of dairy cow diets with different sources of fat can increase milk yields by providing additional DE for the animal (Rabiee et al., 2012). However, the amount of fat supplementation when barley is replaced by oats is relatively small. In the study by Vanhatalo et al. (2006), the dietary increase in fat content was 12 g/kg of DM, whereas it was 9.3 g/kg of DM in our study. The increase in dietary fat concentration is also confounded with other changes in diet composition, such as an increase in iNDF concentration and hence, lower digestibility. The composition of oats and barley also differs between different varieties of the same grain species, making it difficult to elucidate the mechanisms involved. Compared with barley-based diets, the increase or maintenance of milk yield by dairy cows on

Table 8. Effect of gradual replacement of barley with oats on energy utilization

	Diet[1]					*P*-value	
Energy utilization	O0	O33	O67	O100	SEM	Linear	Quadratic
GE content,[2] MJ/ kg of DM	18.40	18.50	18.50	18.60			
GE digestibility,[3] g/kg	711	705	695	683	3.5	<0.01	0.46
GE intake,[3] MJ/d	421	422	420	422	4.4	0.94	0.95
Fecal energy,[4] MJ/d	121	124	128	133	1.6	<0.01	0.44
DE intake,[5] MJ/d	299	298	292	288	3.0	<0.01	0.74
CH_4-E,[6] MJ/d	26.0	25.2	25.0	25.0	0.30	0.01	0.39
CH_4-E/GE intake	0.060	0.059	0.059	0.058	0.0007	0.03	0.42
CH_4-E/DE intake	0.086	0.084	0.085	0.085	0.001	0.59	0.24
Urinary energy,[7] MJ/d	13.1	13.0	13.0	13.0	0.08	0.44	0.95
ME intake, MJ/d	260	259	254	250	2.8	<0.01	0.65
Milk energy, MJ/d	100.0	99.0	97.5	100.0	1.20	0.77	0.15
Heat production,[8] MJ/d	126	129	125	127	1.1	0.66	0.60
Energy balance, MJ/d	30.0	34.4	30.4	23.7	2.91	0.10	0.06
k_l[9]	0.625	0.625	0.630	0.627	0.0040	0.74	0.71

[1] O0 = 100% barley; O33 = 67% barley and 33% oats; O67 = 33% barley and 67% oats; O100 = 100% oats.

[2] GE = gross energy; GE content predicted according to Beyer et al. (2003): (23.6 × CP + 39.8 × Ether extract + 17.3 × NFC + 18.9 × NDF)/1,000.

[3] Predicted according to Ramin and Huhtanen (2013).

[4] Predicted as GE intake − DE intake.

[5] DE = digestible energy, DE intake predicted as GE intake − Fecal energy.

[6] CH_4-E = methane energy.

[7] Predicted from Guinguina et al. (2020).

[8] Calculated according to Brouwer (1965).

[9] k_l = efficiency of ME use for lactation, calculated according to AFRC (1993) with coefficients derived from Guinguina et al. (2020).

oat-based diets (despite the lower DE supply) could be the result of differences in energy partitioning. In this case, less energy is partitioned into body fat reserves, as suggested by our study (Table 8).

The observed decrease in milk protein concentration when barley was replaced by oats is well in line with several other studies. Compared with the 1.1 g/kg of milk decrease in our study, Ekern et al. (2003), Vanhatalo et al. (2006), and Tosta et al. (2019) reported that milk protein concentration decreased by 1.2, 1.2, and 0.9 g/kg milk, respectively. In the study by Vanhatalo et al. (2006), the lower milk protein concentration was suggested to be caused by the dilution of same amount of protein to slightly greater volume of milk, because no effect on nitrogen (N) utilization was observed. As discussed earlier, Ekern et al. (2003) and Tosta et al. (2019) also found increases in milk yield, whereas in the study by McKay et al. (2019), milk yield and milk protein concentration were unaffected by treatment, thereby supporting the theory of a dilution effect on milk protein concentration when barley is replaced by oats. In this study, however, we observed no significant effect on milk yield, only a small numerical increase of 0.6 kg/d with increasing inclusion of oats. In addition, no effect of diet was observed on milk N efficiency, and the total average for this variable was 283 g/kg milk which is close to the average value of 277 g/kg for milk N efficiency found in a meta-analysis by Huhtanen et al. (2008). This suggests that the effect on milk protein concentration observed in this study is due to numerical variation in milk yield (dilution effect).

Considering lower tabulated MP concentration of oats compared with barley (93 vs. 96 g/kg DM; e.g., LUKE, 2020) similar milk protein yield in cows fed oats and barley is unexpected. This suggests that MP value of oats is underestimated compared with barley. Rapidly degradable fraction A of CP is much greater in oats than in barley (65 vs. 30%; NRC, 2001). In contrast, NAN flow to the small intestine was higher for oats diet compared with barley in cows fed silage-based diets (Vanhatalo et al., 2006). Similarly, in sheep NAN flow was greater with naked oats compared with barley (Martin, 1990). These reported NAN flows with barley and oat diets are more consistent with milk protein yield responses than tabulated MP values (e.g., Volden and Nielsen, 2011) or degradation parameters (NRC, 2001). The concentrations of MUN between barley and oat diets (Vanhatalo et al., 2006; current study) were similar. This together with numerically lower ruminal ammonia N concentration (Vanhatalo et al., 2006) does not support greater ammonia N losses from the greater immediately in situ degradable N fractions in oats compared with barley.

There are conflicting results in the literature regarding the effect of grain source on milk fat concentration. In our study, milk fat concentration decreased by 1.4 g/kg milk, when the O0 diet was replaced by the O100 diet and that no effect was observed on the dietary effect on total milk yield. This indicates that the effect on milk fat concentration is mainly due to the dilution effect. Martin and Thomas (1988) and Ekern et al. (2003) reported that milk fat concentration decreased by 4.1 and 6.4 g/kg milk, respectively, when oats was replaced by barley. On the other hand, Vanhatalo et al. (2006) and McKay et al. reported no difference in milk fat concentration, whereas Tosta et al. (2019) reported a small increase (0.7 g/kg milk) in milk fat concentration when rolled barley was replaced by rolled oats in the diet. In addition, when the diet was based on pasture in the study by McKay et al. (2019), the oat diet increased milk fat concentration and milk fat yield compared with the barley diet. These conflicting results may be explained by the differences between studies discussed earlier, regarding silage to concentrate ratio, level of inclusion of oats and barley in concentrate fraction, and silage type and quality

In line with the results of McKay et al. (2019), gradual replacement of barley with oats on a grass silage-based diet had no effect on feed efficiency, since neither feed intake nor ECM yield were affected by diet. On the contrary, Tosta et al. (2019) found a slightly higher feed efficiency (ECM/DMI) for rolled oats compared with rolled barley.

Gas Emissions

Studies comparing the effects on CH_4 emissions between diets with oats and barley as the main concentrate ingredients are scarce. A recent in vitro study by Fant et al. (2020), where predicted in vivo CH_4 emissions from incubated oat and barley diets were evaluated, found that oat diets decreased CH_4 emissions by 8.9% compared with barley diets, when CH_4 emissions were expressed as g/kg of DM. Smaller effect of oats in this study is partly due to the smaller proportion of oats in the diet compared with the study by Fant et al. (2020) (30 vs. 50% on DM basis). In this study, the corresponding decrease was smaller, 4.4% (g/kg DMI), when comparing the O100 diet with the O0 diet. In addition, the intensity of CH_4 emissions decreased by 4.8% (CH_4/ECM), indicating that CH_4 emissions were mitigated without compromising animal productivity. The average proportion of CH_4-E to GE intake (0.058) is in line with the findings of Yan et al. (2000), for dairy cows fed a grass silage-based diet, considering the higher DMI in the current study.

The linear decrease in CH_4 emissions when replacing barley with oats in the diet may be partly explained by the linear decrease in OMD, because enteric CH_4 arises only from digested matter. It is well known that a positive relationship exists between diet digestibility and CH_4 emissions (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013), as total CH_4 emissions decrease due to decreased digestibility and vice versa. The amount of digestible fiber in the feed is commonly the main factor contributing to the extent of methanogenesis in the rumen (Moss et al., 2000). Oat grain contains a higher concentration of indigestible fiber compared with barley grain, as shown by the linear increase in dietary iNDF concentration and intake with increasing inclusion of oats in the current study. In addition, the evaluation of 8 different varieties of oats and barley by Fant et al. (2020) reported a greater mean value of iNDF in oats compared with barley (144 vs. 41.3 g/kg DM), and a strong negative correlation between dietary iNDF concentration and digestibility in vitro.

Another possible explanation for the linear decrease in CH_4 emissions with increased inclusion of oats in the diet, is the higher fat concentration in oats compared with barley. Gradual replacement of barley with oats resulted in a linear increase in the concentration and intake of dietary crude fat. Compared with the O0 diet, the O100 diet displayed a 9.3 g/kg DM higher crude fat concentration. Dietary fat concentration is known to display a negative relationship with enteric CH_4 emissions (Beauchemin et al., 2009; Grainger and Beauchemin, 2011; Ramin and Huhtanen 2013). However, changes in rumen fermentation patterns does not explain the differences in CH_4 emissions in this study, because the molar proportions of VFA were not significantly affected by gradual replacement of barley with oats in the diet.

Digestibility, Energy Utilization, and Efficiency Traits

In this study, apparent OMD was 3.8% lower when cows were fed the O100 diet compared with the O0 diet. These results fall well in line with the digestibility values in national feed tables, reporting a 10 to 11% lower digestibility of oats compared with barley (LUKE, 2020). The tabulated difference in digestibility (LUKE, 2020) was diluted in our study, because the inclusion of oat or barley concentrate was only 30% on a DM basis. Well in line with our results, Vanhatalo et al. (2006) reported a 2.8% lower apparent OMD with oat diets compared with barley diets, when the level of concentrate inclusion was 40% and the diet was based on grass silage of good quality. The same authors reported a 6.5% lower NDF digestibility in oat diets compared with barley diets, compared with the 10% difference in our study.

One main reason for the decline in OMD relates to the higher iNDF concentration of oats compared with barley (almost 3 times more iNDF in oats compared with barley in this study). Only minor differences were observed in the digestibility of NDS and pdNDF indicating that the higher iNDF concentration was the main reason for reduced digestibility with increased proportion of oats. Reduced diet digestibility could lead to a larger amount of potentially digestible OM in feces, which would increase the CH_4 emissions from the manure of cows being fed oats compared with barley. However, in this study, the fecal output of potentially digestible OM was similar among diets, indicating that replacing barley with oats does not increase CH_4 emissions from manure.

The current study evaluated the energy utilization in dairy cows when barley was replaced by oats. Fecal energy linearly increased by replacing barley with oats (12 MJ/d more with the O100 diet), reflecting the linear increase in dietary iNDF concentration with increased inclusion of oats in the diet. Metabolizable energy intake declined by replacing barley with oats. The decline in dietary ME concentration (1.2 MJ/kg grain DM) with increased proportion of oats corresponds well to tabulated differences in ME concentration (LUKE, 2020). Similar production despite of lower ME intake suggests that incremental energy supply from barley favored body fat production instead of milk production. Another explanation could be that cows mobilize body fat to support milk production when oat was included in the diet.

However, the HP was not changed by replacing barley with oats that could be due to the fact that in the O0 diet the increased ME intake was mainly recovered as retained energy. The RQ values decreased by replacing barley with oats, indicating direct incorporation of preformed fatty acids to milk fat. However, the value of averaged RQ (1.112) was close to the values reported for cows using the respiration chamber technique (Aubry and Yan, 2015).

The k_l values obtained in the current study (average 0.63) are similar those calculated according to the NRC (2001) system from observed dietary ME concentration.

CONCLUSIONS

In the present study, replacing barley with oats in the diet of dairy cows decreased total daily CH_4 emissions by 4.6%, and CH_4 intensity by 4.8%. Although the CH_4 mitigating effect of oats is small compared with several other strategies, replacing barley grain with oat grain can be easily adopted by the farmers, especially in areas where oat grain has previously been fed to dairy cows before it was replaced by barley. The CH_4 mitigating effect of oats is most likely due to the higher iNDF concentration and lower digestibility, and higher fat concentration, compared with barley. Increased inclusion of oats in the diet did not affect DMI, milk yield, ECM yield, feed efficiency, or energy balance, but decreased OMD. Caution must be exercised when interpreting energy metabolism values as they were calculated indirectly using the GreenFeed system. Finally, based on the findings from this study, we conclude that replacement of barley with oats in the diet of dairy cows fed a grass silage-based diet could be used as a sustainable strategy to mitigate enteric CH_4 emissions without compromising animal performance.

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Replacement of barley with oats and dehulled oats: Effects on milk production, enteric methane emissions, and energy utilization in dairy cows fed a grass silage-based diet

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ABSTRACT

Sixteen Nordic Red dairy cows, at 80 ± 4.6 d in milk and with an average body weight of 624 ± 91.8 kg, were used in a replicated 4 × 4 Latin square design to investigate the effects of different concentrate supplements on milk production, enteric CH_4 emissions, ruminal fermentation, digestibility, and energy utilization. The cows were blocked into 4 groups based on parity and milk yield and randomly assigned to 1 of 4 experimental concentrates: (1) barley, (2) hulled oats, (3) an oat mixture consisting of hulled and dehulled oats, 50:50 on dry matter basis, and (4) dehulled oats; canola meal was a protein supplement in all 4 concentrates. The cows were fed grass silage and experimental concentrate (forage-to-concentrate ratio 60:40 on dry matter basis) ad libitum. To compare the effects of barley and oats, the barley diet was compared with the overall mean of the hulled oat, oat mixture, and dehulled oat diets. To investigate the effects of gradual replacement of hulled oats with dehulled oats, linear and quadratic contrasts were specified. Milk and energy-corrected milk (ECM) yield were higher on the oat diets compared with the barley diet but were not affected by the type of oats. Concentrations of milk constituents were not affected by grain species or type of oats, except for protein concentration, which was lower on the oat diets than on the barley diet. Feeding the oat diets led to higher milk protein yield and higher milk urea N concentrations. Feed efficiency tended to be higher on the oat diets, and linearly increased with increased inclusion of dehulled oats. Methane emissions (g/d) and CH_4 yield (g/kg of dry matter intake) were unaffected by grain species but increased linearly with increasing inclusion of dehulled oats in the diet. Because of higher ECM yield, CH_4 intensity (g/kg of ECM) was on average 5.7% lower from cows on the oat diets than on the barley diet. Ruminal fermentation was not affected by dietary treatment. Total-tract apparent digestibility of organic matter, crude protein, and neutral detergent fiber was unaffected by grain species but linearly increased with increasing inclusion of dehulled oats. Gross energy content was higher on the oat diets and linearly increased with increasing inclusion of dehulled oats. Feeding the oat diets led to a lower ratio of CH_4 energy to gross energy intake, greater milk energy and heat production but no change in energy balance. Gradual replacement of hulled oats with dehulled oats linearly increased gross energy digestibility, CH_4 energy, metabolizable energy intake, heat production, and energy balance. We observed no effect of dietary treatment on efficiency of metabolizable energy use for lactation. In conclusion, replacing barley with any type of oats increased milk and ECM yield, which led to a 5.7% decrease in CH_4 intensity. In addition, dehulling of oats before feeding is unnecessary because it did not significantly improve production performance of dairy cows in positive energy balance.

Key words: sustainability, agriculture, concentrate supplement

INTRODUCTION

Within the livestock sector, dairy production is the second largest contributor to anthropogenic CH_4 emissions after beef production, with most of the emissions originating from enteric fermentation in the digestive tract (FAO, 2020). As the world population is estimated to grow from 7.7 billion people in 2019 to around 9.7 billion by 2050 (UN, 2019), total CH_4 emissions will increase because of growing demand for livestock products. Therefore, the potential of enteric CH_4 mitigation strategies need to be evaluated in relation to the amount of product (milk or meat) obtained.

Oats (*Avena sativa*) are a common cereal in the Northern Hemisphere and were a popular concentrate supplement for dairy cows in the past. Today, however, barley (*Hordeum vulgare*) is the main concentrate in-

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gredient in the Nordic countries, much due to the fact that ration formulation programs tend to recommend barley before oats, because feed tables state higher ME and MP values for barley (Volden and Nielsen, 2011; LUKE, 2020). However, several studies suggest that barley could be replaced by oats without lowering yields of milk, ECM, and milk constituents, although milk protein concentration is typically lowered (Martin and Thomas, 1988; Ekern et al., 2003; Vanhatalo et al., 2006). Literature is scarce regarding the effect of dehulling of oat grain on milk yield and composition.

Barley, hulled oats, and dehulled oats show several differences in chemical composition that might affect CH_4 emissions from the rumen. Hulled oat grain contains more fat (Liu, 2011) and fiber (Evers and Millar, 2002) compared with hulled barley grain, and because of the higher fiber concentration, digestibility is lower. On the other hand, hulled barley grain contains more starch than hulled oat grain (Holtekjølen et al., 2006; Sayer and White, 2011). Oat grain that has been dehulled contains less fiber, more fat and starch, and has a higher digestibility compared with hulled oats (Biel et al., 2014). Overall, in vivo studies comparing enteric CH_4 emissions between oats- and barley-based diets are scarce, in particular the effect of dehulled oat grain. An in vitro study by Fant et al. (2020) reported that replacement of different varieties of hulled barley grain with hulled oat grain predicted an in vivo decrease of enteric CH_4 yield by 8.9%. A recent in vivo study by Ramin et al. (2021) reported a 4.4% decrease in CH_4 yield when hulled barley was replaced by hulled oats in the diet of dairy cows fed a grass silage-based diet. By evaluating both hulled and dehulled oats in the same experiment, it could be possible to further elucidate the mechanisms involved in the CH_4 mitigating effect of oats.

The objective of this study was to investigate the effects of replacing barley with hulled and dehulled oats on milk production, enteric CH_4 emissions, and energy utilization in lactating dairy cows fed a grass silage-based diet. We hypothesized that replacing hulled oats with dehulled oats would improve production performance in terms of milk and ECM yield. We also hypothesized that total CH_4 emissions and CH_4 emission intensity would decrease when barley is replaced by all types of oats, but that the size of the effect would be dependent on the type of oats.

MATERIALS AND METHODS

The experiment was conducted at Röbäcksdalen experimental farm of the Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences in Umeå, Sweden (63°45′N; 20°17′E) in spring 2018. All experimental procedures were approved by the Swedish Ethics Committee on Animal Research (Umeå, Sweden) and in accordance with Swedish laws and regulations regarding EU Directive 2010/63/EU on animal research.

Experimental Design, Animals, and Diets

Sixteen lactating Nordic Red dairy cows were used in a replicated 4 × 4 Latin square design including 4 dietary treatments, 4 blocks, and 4 periods. Each experimental period lasted 28 d, including 18 d of adaptation to the experimental diet and 10 d of sampling and data collection. The cows were assigned to 4 blocks based on parity and milk yield. At the beginning of the experiment, the cows were on average at 80 ± 4.6 DIM, producing 33.4 ± 1.18 kg of milk/d, and weighing 624 ± 91.8 kg. The cows were kept in an insulated freestall barn, milked twice daily at 0600 and 1600 h, and had free access to water and salt blocks throughout the experiment. The salt block was composed of sodium chloride (39% sodium) without additives (SP Natural, Salinity Ab, Göteborg, Sweden). The cows were fed grass silage and 1 of 4 experimental concentrates (forage-to-concentrate ratio 60:40 on DM basis) as a TMR offered ad libitum. The TMR was prepared using a TMR mixer (Nolan A/S, Viborg, Denmark) and delivered in feed troughs 4 times per day by automatic feeding wagons.

The grass silage was made from primary growth perennial leys of timothy (*Phleum pretense*). The fields were fertilized with 70 kg of N/ha in spring 2017. The grass was wilted and ensiled in bunker silos, and a commercial additive (ProMyrTM XR 630, Perstorp) containing a mixture of formic acid and propionic acid was applied at a rate of 3.5 L/t to preserve the silage. Within each block, cows were randomly assigned to 1 of 4 dietary treatments differing in the type of concentrate (Table 1). The dietary treatments comprised barley, hulled oats, an oat mixture consisting of hulled oats and dehulled oats 50:50 on DM basis, and dehulled oats. The experimental concentrate feeds were a pelleted mixture of the experimental grain component (78.8%), canola meal (18.0%), $CaCO_3$ (1.6%), NaCl (1.0%), MgO (0.4%), and a premix (0.2%), and were obtained from Raisioagro Oy (Ylivieska, Finland).

Sample and Data Collection

Individual daily feed intake was recorded throughout the trial in Roughage Intake Control feeders (Hokofarm Group), and individual daily milk yield with gravimetric milk recorders (SAC, S.A. Christensen and Co. Ltd.). Only data collected from the last 10 d of each period were used for the statistical analysis. The BW of the cows was recorded after morning milking on the last 3 d of each period with a walk-through system (Hokofarm Group) when exiting the milking parlor.

Emissions of CH_4 and CO_2, and consumption of O_2, were recorded throughout the experiment by the GreenFeed system (C-Lock Inc.) described by Huhtanen et al. (2015), and the data were handled in the same way as for feed intake and milk yield. The calibrations, gas recordings, and calculations were performed as described by Ramin et al. (2021). The animals had free access to the GreenFeed, but the minimum visit interval allowed was set to 5 h. To attract the cows to the GreenFeed, they received a maximum of 8 drops of concentrate every 40 s during each visit. Each drop contained 50 g of a commercial concentrate (Komplett Norm 180, Lantmännen Lantbruk AB). In the total DMI calculations, the intake of commercial concentrate from the GreenFeed was taken into account based on the number of drops registered by the system.

To determine milk composition, milk samples (~20 mL) were collected during 4 consecutive milking times, starting from evening milking on d 26 until morning milking on d 28, during each period. Samples were preserved with 2-bromo-2-nitropropane-1,3-diol (Bronopol, Valio Ltd.), stored at 4°C, and sent refrigerated for analysis within 1 wk from sampling. To determine ruminal fermentation parameters, ruminal fluid samples were collected before morning feeding at 0800 on d 21 of each period from 8 cows belonging to 2 blocks. A sample size of approximately 500 mL was collected by stomach tubing (RUMINATOR, Profs Products) as described by Geishauser (1993). After discarding the first sample, the second sample was filtered through 2 layers of cheesecloth, and a subsample of 2.5 mL was mixed with 0.5 mL 25% metaphosphoric acid and stored at −20°C until further analysis.

Fecal grab samples (300 mL) were collected twice a day, at 0600 and 1700 h, on the last 3 d of each period from the same 8 cows that were sampled for ruminal fluid. The 6 fecal samples from each cow were pooled within cow after each period, dried at 60°C for 48 h in a forced-air oven, and ground in 2 different sizes. For chemical composition analyses, the samples were ground with a cutter mill (SM300, Retsch Ltd.) and passed through a 1-mm sieve, and for determination of indigestible NDF (**iNDF**), the samples were ground with pestle and mortar and passed through a 2-mm sieve.

Samples of grass silage were collected 3 times during each sampling period, on d 23, 26, and 28. To determine fermentation quality, a subsample was frozen and stored at −20°C until day of analysis. To determine chemical composition, another subsample was dried in a forced-air oven at 60°C for 48 h. Concentrate samples were collected once during each sampling period, on d 26, and treated in the same way as the dried silage samples. The dried grass silage and concentrate samples were ground and passed through a 1-mm sieve for chemical composition analysis and passed through a 2-mm sieve for determination of iNDF.

Chemical Analyses

Silage, concentrate, and fecal samples were determined for concentrations of DM, ash, CP, NDF, and iNDF. In addition, silage samples were determined for crude fat, and concentrate samples for crude fat and starch concentration. The chemical analyses were performed according to Ramin et al. (2021), except for CP concentration. Concentration of N was determined by the Kjeldahl method with a 2020 Digestor and Kjeltec 2460 Analyzer Unit (Foss Analytical A/S), and the CP concentration was calculated by multiplying N concentration by a factor of 6.25. The NDF and iNDF concentrations were expressed exclusive of residual ash.

The frozen silage samples were determined for pH, ammonia-N, lactic acid, and VFA concentrations. Samples were thawed and pressed, and the undiluted juice used for analysis. Ammonia-N was determined using a Kjeltec 2100 Distillation Unit (Foss Analytical Ltd.). Lactic acid and VFA concentrations were deter-

Table 1. Ingredient composition of formulated diets (g/kg of DM)

	Diet[1]			
Item	Barley	Hulled oats	Oat mixture	Dehulled oats
Grass silage	600	600	600	600
Barley[2]	400			
Oats[2]		400	200	
Dehulled oats[2]			200	400

[1] Oat mixture = mix of hulled oats and dehulled oats 50:50 on DM basis. In addition to TMR, the cows received a concentrate mixture during visits to the GreenFeed system.

[2] Fed as a mineral mixture: barley or oats or dehulled oats (788 g/kg of DM), canola meal (180 g/kg of DM), $CaCO_3$ (16 g/kg of DM), NaCl (10 g/kg of DM), MgO (4 g/kg of DM), and a premix (2 g/kg of DM).

mined as described by Ericson and André (2010). Silage DM concentration was corrected for volatile losses as described by Huida et al. (1986). Milk samples were analyzed for lactose, CP, fat, and urea concentrations, and ruminal fluid samples for VFA concentration as described by Ramin et al. (2021).

Calculations

Potentially digestible NDF (**pdNDF**) concentration was calculated as a difference between NDF (analyzed using amylase and expressed exclusive of residual ash) and iNDF concentration, and neutral detergent solubles (**NDS**) concentration was calculated as a difference between OM and NDF. The ME (MJ/kg of DM) and MP (g/kg of DM) values in feeds were calculated using weighted coefficients from Finnish national feed tables (LUKE, 2020). Chemical composition of each diet was calculated based on the chemical composition and inclusion proportion of each feed ingredient. Starch concentration in grass silage was assumed to be 0 g/kg of DM (LUKE, 2020). Energy-corrected milk was calculated according to Sjaunja et al. (1990):

$$\begin{aligned}\text{ECM (kg/d)} &= \text{Milk yield (kg/d)}\\ &\times [38.3 \times \text{Fat (g/kg)} + 24.2 \times \text{Protein (g/kg)}\\ &+ 16.54 \times \text{Lactose (g/kg)} + 20.7] \,/\, 3{,}140. \qquad [1]\end{aligned}$$

where Fat, Protein, and Lactose are the concentrations of these constituents in milk. Milk N efficiency was calculated as milk N (g/d) divided by N intake (g/d), and milk N was calculated as milk protein yield divided by a factor of 6.38. Feed efficiency (**FE**) was defined and calculated as the ratio of ECM (kg/d) to DMI (kg/d). The respiratory quotient (**RQ**) was calculated as the ratio of CO_2 produced (L/d) to O_2 consumed (L/d). Digestibility of dietary components was estimated using iNDF as an internal marker according to the following equation with OM digestibility as an example:

$$\begin{aligned}\text{OM digestibility} &= 1 - [\text{iNDF in diet DM (g/kg)}/\\ &(\text{iNDF in fecal DM (g/kg)}]\\ &\times [\text{OM in fecal DM (g/kg)}/\text{OM in diet DM (g/kg)}]. \qquad [2]\end{aligned}$$

Gross energy (**GE**) intake (MJ/d) was calculated according to Beyer et al. (2003) as follows:

$$\begin{aligned}\text{GE intake (MJ/d)} &= \text{DMI (kg/d)}\\ &\times [(23.6 \times \text{CP} + 39.8 \times \text{EE} + 17.3 \times \text{NFC}\\ &+ 18.9 \times \text{NDF})/1{,}000], \qquad [3]\end{aligned}$$

where EE is ether extract, and concentrations are expressed as kg/kg DM. Gross energy digestibility was predicted according to Ramin and Huhtanen (2013):

$$\begin{aligned}&\text{GE digestibility (kJ/MJ)} =\\ &-11.3 + 977 \times \text{OM digestibility}. \qquad [4]\end{aligned}$$

Gross energy digestibility was predicted by using the observed OM digestibility values for the 8 cows in the digestibility trial. Whereas, for the other 8 cows, OM digestibility was predicted from dietary iNDF concentration using the relationship between iNDF and OM digestibility determined in the digestibility trial. Fecal energy was calculated as:

$$\begin{aligned}\text{Fecal energy (MJ/d)} &= \text{GE intake} - \text{GE intake}\\ &\times (\text{GE digestibility}/1{,}000). \qquad [5]\end{aligned}$$

Digestible energy (**DE**) intake (MJ/d) was calculated as a difference between GE intake and fecal energy. Gross energy in CH_4 (**CH_4E**, MJ/d) was calculated as $0.05524 \times CH_4$ (g/d) according to Kriss (1930). Urinary energy was calculated according to Guinguina et al. (2020) as follows:

$$\begin{aligned}\text{Urinary energy (MJ/d)} &= -3.6 + 0.37 \times \text{DMI (kg/d)}\\ &+ 0.006 \times \text{Forage proportion (g/kg DM)} + 0.03\\ &\times \text{CP (g/kg DM)}, \qquad [6]\end{aligned}$$

Metabolizable energy intake (MJ/d) was calculated as DE intake − CH_4E − Urinary energy. Milk energy (MJ/d) was calculated as ECM (kg/d) × 3.14 (MJ/kg) according to Sjaunja et al. (1990). Heat production was calculated according to Brouwer (1965) as follows:

$$\begin{aligned}\text{Heat production (MJ/d)} &= 0.01618 \times O_2\ \text{(L/d)}\\ &+ 0.00502 \times CO_2\ \text{(L/d)} - 0.00599 \times \text{UN (g/d)}\\ &- 0.00217 \times CH_4\ \text{(L/d)}, \qquad [7]\end{aligned}$$

where UN is urinary N calculated as N intake − Fecal N − Milk N assuming zero N balance. Energy balance (**EB**, MJ/d) was calculated as ME intake − Heat production − Milk energy. The efficiency of ME use for lactation (**k_l**) was calculated according to AFRC (1993):

$$k_l = E_{1(0)}/(\text{ME intake} - \text{ME}_m), \qquad [8]$$

where $E_{1(0)}$ is milk energy output (**E_1**) adjusted to 0 EB (MJ/d) according to Equations [9] and [10], and ME_m is the ME requirement for maintenance (MJ/d).

$$\text{If EB} > 0,\ E_{1(0)} = E_1 + 0.7277 \times \text{EB}; \quad [9]$$

$$\text{if EB} < 0,\ E_{1(0)} = E_1 - 0.6943 \times \text{EB}. \quad [10]$$

The coefficients in Equations [9] and [10] were derived from a large (n = 841) respiration chamber data set (Guinguina et al., 2020).

Statistical Analysis

The experimental data were subjected to ANOVA using the MIXED procedure of SAS version 9.4 (SAS Institute Inc.) and analyzed as a replicated 4 × 4 Latin square according to the following statistical model:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + C_l(S_k) + \varepsilon_{ijkl},$$

where Y_{ijkl} is the dependent variable and μ is the population mean, T_i is the fixed effect of diet i, P_j is the fixed effect of period j, S_k is the fixed effect of square k, $C_l(S_k)$ is the random effect of cow l within square k, and ε_{ijkl} is the random residual error. Interactions $T_i \times S_k$ and $P_j \times S_k$ were excluded from the final model because they were nonsignificant ($P > 0.10$). Due to an unexplained drop in milk production for 1 cow during the last period, the observation of this cow was removed from period 4, resulting in an unbalanced data set. To account for the unbalance, *P*-values were derived by applying the Kenward-Roger approximation for estimating degrees of freedom, and mean values presented in the text and tables are least squares means obtained by the LSMEANS statement in SAS. To compare treatment effects, 3 orthogonal contrasts were specified. To compare the effects of barley and oats, the barley diet was compared with the overall mean of the hulled oat, oat mixture, and dehulled oat diets. To investigate the effects of gradual replacement of hulled oats with dehulled oats, linear and quadratic contrasts were specified. Differences were declared significant if $P \leq 0.05$, highly significant if $P \leq 0.01$, and a tendency toward significant was declared if $0.05 < P \leq 0.10$.

RESULTS

Chemical Composition, Feed Intake, and Digestibility

The grass silage displayed a low concentration of ammonium N (65.3 g/kg N) and a low pH (3.73), indicating a good quality grass silage (Table 2). Hulled oats and dehulled oats both had a higher concentration of ash, CP, and crude fat than barley, with dehulled oats having an even higher concentration of CP and crude fat than hulled oats. The concentrations of NDF and iNDF were greatest in hulled oats, whereas they were lowest in dehulled oats. Barley showed the greatest concentration of starch, whereas hulled oats showed the lowest concentration. As a result of these differences in chemical composition, the concentrations of CP and crude fat were greater in the oat diets than in the barley diet (Table 3). The concentrations of NDF and iNDF were greatest in the hulled oat diet and lowest in the dehulled oat diet.

Table 2. Chemical composition of dietary ingredients

Item	Grass silage[1]	Barley	Oats	Dehulled oats	GreenFeed concentrate[2]
DM, g/kg of fresh matter	259	870	873	886	878
In DM, g/kg					
Ash	65	66	67	66	71
CP	160	168	174	204	206
NDF	489	182	227	116	205
iNDF[3]	90	64	123	35	74
pdNDF[4]	399	118	104	81	131
NDS[5]	446	751	706	818	724
Starch	NA[6]	410	350	376	NA
Crude fat	38	29	50	64	NA
Feeding values					
ME, MJ/kg of DM	10.8	12.7	11.6	12.9	12.1
MP, g/kg of DM	87.0	106	103	116	106

[1] Concentrations were as follows: ammonia-N (65.3 g/kg of N), lactic acid (91.7 g/kg of DM), acetic acid (18.8 g/kg of DM), propionic acid (1.74 g/kg of DM), butyric acid (0.35 g/kg of DM). pH = 3.73.

[2] GreenFeed concentrate was a commercial concentrate (Komplett Norm 180) obtained from Lantmännen Lantbruk AB (Malmö, Sweden).

[3] iNDF = indigestible NDF.

[4] pdNDF = potentially digestible NDF, calculated as NDF − iNDF.

[5] NDS = neutral detergent solubles, calculated as OM − NDF.

[6] NA = not analyzed.

Table 3. Chemical composition of experimental diets

Item	Diet[1] Barley	Hulled oats	Oat mixture	Dehulled oats
In DM, g/kg				
OM	935	934	934	935
CP	163	166	172	178
NDF	367	385	362	340
iNDF[2]	80	103	86	68
pdNDF[3]	287	281	276	272
NDS[4]	568	550	572	595
Crude fat	34	43	46	48
Starch[5]	164	140	145	150
Feed values				
ME, MJ/kg DM	11.5	11.1	11.3	11.6
MP, g/kg DM	94.5	93.4	96.0	98.7

[1] Oat mixture = mix of hulled oats and dehulled oats 50:50 on DM basis.

[2] iNDF = indigestible NDF.

[3] pdNDF = potentially digestible NDF, calculated as NDF − iNDF.

[4] NDS = neutral detergent solubles, calculated as OM − NDF.

[5] Starch concentration in grass silage assumed to be 0 g/kg of DM (LUKE, 2020).

Total DMI (kg/d) was not affected by grain species but tended to decrease linearly ($P = 0.09$) with gradual replacement of hulled oats with dehulled oats (Table 4). The mean intake of CP, iNDF, and crude fat was higher ($P < 0.01$), whereas the intake of starch ($P < 0.01$) and pdNDF was lower ($P = 0.02$) for cows fed the oats diets compared with the barley diet. Gradual replacement of hulled oats with dehulled oats resulted in a linear

Table 4. Effects of barley and oat grain and gradual replacement of hulled oats with dehulled oats on feed intake (n = 63) and apparent total-tract digestibility (n = 31) in lactating cows

	Diet[1]					*P*-value[2]		
Item	Barley	Hulled oats	Oat mixture	Dehulled oats	SEM	Barley vs. Oats	Linear	Quadratic
Intake, kg/d								
Total DM	23.3	23.8	23.4	23.2	0.44	0.60	0.09	0.82
Silage DM	13.2	13.5	13.3	13.1	0.26	0.54	0.08	0.91
Concentrate DM	10.1	10.3	10.1	10.1	0.18	0.50	0.08	0.69
OM	21.8	22.2	21.9	21.7	0.41	0.57	0.09	0.82
CP	3.86	3.99	4.06	4.15	0.073	<0.01	<0.01	0.82
NDF	8.33	8.89	8.28	7.71	0.159	0.71	<0.01	0.84
iNDF[3]	1.85	2.41	1.99	1.58	0.038	<0.01	<0.01	0.71
pdNDF[4]	6.48	6.47	6.28	6.12	0.122	0.02	<0.01	0.89
NDS[5]	13.4	13.3	13.6	13.9	0.25	0.22	<0.01	0.81
Crude fat	0.76	0.96	1.01	1.06	0.018	<0.01	<0.01	0.88
Starch[6]	3.60	3.14	3.21	3.29	0.065	<0.01	0.01	0.94
Digestibility								
DM	0.716	0.682	0.697	0.742	0.0117	0.43	<0.01	0.25
OM	0.734	0.701	0.717	0.761	0.0110	0.45	<0.01	0.25
CP	0.689	0.686	0.691	0.734	0.0130	0.25	<0.01	0.18
NDF	0.598	0.543	0.563	0.620	0.0178	0.19	<0.01	0.32
pdNDF	0.768	0.745	0.741	0.781	0.0230	0.57	0.18	0.38
NDS	0.819	0.807	0.811	0.838	0.0081	0.95	<0.01	0.17

[1] Oat mixture = mix of hulled oats and dehulled oats 50:50 on DM basis.

[2] Probability of significant orthogonal contrasts. Effects tested using orthogonal contrasts were: Barley vs. Oats = barley vs. hulled oats, oat mixture, and dehulled oats; Linear = linear effect of replacement of hulled oats with dehulled oats; Quadratic = quadratic effect of replacement of hulled oats with dehulled oats.

[3] iNDF = indigestible NDF.

[4] pdNDF = potentially digestible NDF, calculated as NDF − iNDF.

[5] NDS = neutral detergent solubles, calculated as OM − NDF.

[6] Starch concentration in grass silage assumed to be 0 g/kg of DM (LUKE, 2020).

Table 5. Effects of barley and oat grain and gradual replacement of hulled oats with dehulled oats on milk production and milk composition in lactating cows (n = 63)

	Diet[1]					P-value[2]		
Item	Barley	Hulled oats	Oat mixture	Dehulled oats	SEM	Barley vs. Oats	Linear	Quadratic
Yield								
Milk yield, kg/d	29.1	30.3	30.5	30.8	1.00	<0.01	0.22	0.97
ECM, kg/d	31.3	32.2	32.2	32.9	1.00	0.01	0.14	0.41
Protein, g/d	1,055	1,076	1,081	1,098	34.2	0.04	0.21	0.67
Fat, g/d	1,336	1,370	1,366	1,404	44.0	0.06	0.21	0.38
Lactose, g/d	1,273	1,320	1,325	1,349	47.7	<0.01	0.16	0.61
Concentration								
Milk fat, g/kg	46.4	45.7	44.9	46.0	0.84	0.18	0.67	0.19
Milk protein, g/kg	36.6	35.8	35.5	35.8	0.54	<0.01	0.91	0.34
Milk lactose, g/kg	43.7	43.5	43.4	43.7	0.36	0.49	0.56	0.43
MUN, mg/dL	9.9	11.3	10.9	11.7	0.45	<0.01	0.25	0.10
Milk N efficiency, g/kg	269	265	261	260	6.2	0.10	0.37	0.68
FE[3]	1.35	1.36	1.38	1.43	0.034	0.08	0.01	0.49
BW, kg	631	641	641	636	19.5	0.08	0.32	0.58

[1] Oat mixture = mix of hulled oats and dehulled oats 50:50 on DM basis.

[2] Probability of significant orthogonal contrasts. Effects tested using orthogonal contrasts were: Barley vs. Oats = barley vs. hulled oats, oat mixture, and dehulled oats; Linear = linear effect of replacement of hulled oats with dehulled oats; Quadratic = quadratic effect of replacement of hulled oats with dehulled oats.

[3] FE = feed efficiency calculated as the ratio of ECM (kg/d) to total DMI (kg/d).

increase ($P < 0.01$) in the intake of CP, NDS, starch, and crude fat, and a linear decrease ($P < 0.01$) in the intake of NDF, iNDF, and pdNDF.

Total-tract apparent digestibility of all chemical constituents for cows fed the barley diet were similar to that of the overall mean for the oat diets (Table 4). Gradual replacement of hulled oats with dehulled oats linearly increased ($P < 0.01$) digestibility of DM, OM, CP, NDF, and NDS, whereas no effect was observed on digestibility of pdNDF.

Milk Production, Milk Composition, and Feed Efficiency

Milk yield was on average 1.4 kg/d higher ($P < 0.01$) for cows receiving the oat diets compared with the barley diet but was unaffected by the type of oats (Table 5). In addition, ECM yield was 1.1 kg/d higher ($P = 0.01$) for cows receiving the oat diets compared with the barley diet and was not affected by the type of oats in the diet. Dietary treatment did not affect concentrations of milk constituents, except for protein concentration, for which the average was lower ($P < 0.01$) for cows receiving the oat diets than for the barley diet. Despite the lower protein concentration, milk protein yield was higher ($P = 0.04$) for cows fed the oat diets, and milk fat yield tended to be higher ($P = 0.06$) as well. In addition, MUN concentrations were higher ($P < 0.01$) and milk N efficiency tended to be lower ($P = 0.10$) in cows receiving the oat diets compared with the barley diet. The FE, defined as the ratio of ECM (kg/d) to total DMI (kg/d), tended to be higher ($P = 0.08$) for cows receiving the oat diets compared with the barley diet, and linearly increased ($P = 0.01$) when hulled oats was gradually replaced by dehulled oats in the diet.

Gas Emissions and Ruminal Fermentation

Methane emissions, expressed as g/d and g/kg of DMI, were unaffected by grain species in the diet, but linearly increased ($P = 0.02$ and $P < 0.01$, respectively) when hulled oats was replaced by dehulled oats (Table 6). On the other hand, CH_4 intensity expressed as g/kg of ECM, was on average 5.7% lower ($P = 0.01$) for cows receiving the oat diets than for those receiving the barley diet but was unaffected by replacement of hulled oats with dehulled oats. When CH_4 emissions were expressed in relation to amount of OM digested, no differences were observed between dietary treatments. The CO_2 emissions followed the same pattern as the CH_4 emissions (Table 6). Dietary treatment did not affect ruminal fermentation in terms of total VFA concentration and molar proportions of VFA (Table 6).

Energy Utilization

Regarding energy utilization parameters, the oat diets displayed a higher ($P < 0.01$) overall mean GE concentration than the barley diet, and among the oat diets, GE concentration linearly increased ($P < 0.01$)

Table 6. Effects of barley and oat grain and gradual replacement of hulled oats with dehulled oats on gas emissions and rumen fermentation in lactating cows (n = 63)

Item	Diet[1]				SEM	P-value[2]		
	Barley	Hulled oats	Oat mixture	Dehulled oats		Barley vs. Oats	Linear	Quadratic
Concentrate intake from GreenFeed, kg/d	1.29	1.34	1.29	1.32	0.040	0.24	0.44	0.13
CH_4, g/d	479	470	474	488	14.1	0.81	0.02	0.49
CH_4, g/kg of DMI	20.7	19.8	20.3	21.1	0.67	0.27	<0.01	0.60
CH_4, g/kg of OM digested[3]	30.4	29.9	29.9	29.2	1.50	0.26	0.37	0.63
CH_4, g/kg of ECM	15.8	14.8	14.9	15.0	0.70	0.01	0.71	0.98
CO_2, kg/d	13.4	13.3	13.5	13.8	0.31	0.19	0.01	0.65
CO_2, g/kg of DMI	578	563	577	597	16.9	0.91	<0.01	0.76
CO_2, g/kg of ECM	441	422	424	423	20.2	0.04	0.86	0.86
O_2, kg/d	9.60	9.68	9.76	9.95	0.206	0.02	0.01	0.52
RQ[4]	1.013	1.002	1.006	1.010	0.0047	0.10	0.15	0.98
Total VFA, mmol/L	79.3	80.5	84.9	75.2	6.60	0.91	0.57	0.41
Molar proportion, mmol/mol								
Acetic acid	667	658	672	663	10.7	0.78	0.70	0.31
Propionic acid	178	183	178	175	6.7	0.88	0.26	0.86
Butyric acid	115	117	111	121	5.4	0.77	0.54	0.10
Isovaleric acid	4.13	4.38	4.20	4.46	0.198	0.35	0.76	0.39
Isobutyric acid	8.14	8.06	8.02	8.05	0.266	0.73	0.97	0.92

[1] Oat mixture = mix of hulled oats and dehulled oats 50:50 on DM basis.

[2] Probability of significant orthogonal contrasts. Effects tested using orthogonal contrasts were: Barley vs. Oats = barley vs. hulled oats, oat mixture, and dehulled oats; Linear = linear effect of replacement of hulled oats with dehulled oats; Quadratic = quadratic effect of replacement of hulled oats with dehulled oats.

[3] CH_4, g/kg of OM digested, is based on 8 observations per diet.

[4] RQ = respiratory quotient calculated as the ratio of CO_2 produced (L/d) to O_2 consumed (L/d).

with increasing inclusion of dehulled oats (Table 7). We observed a tendency for higher ($P = 0.07$) GE intake but lower ($P = 0.08$) GE digestibility on the oat diets than on the barley diet. Gradual replacement of hulled oats with dehulled oats did not affect GE intake but linearly increased ($P < 0.01$) GE digestibility, which led to a linear increase ($P < 0.01$) in DE intake. Grain species did not affect CH_4E, and as a result, CH_4E as a ratio of GE intake was lower ($P = 0.03$) on the oat diets than on the barley diet (Table 7). Increased inclusion of dehulled oats linearly increased CH_4E ($P = 0.02$) and CH_4E as a ratio of GE intake ($P < 0.01$). Calculated urinary energy was higher ($P < 0.01$) on the oat diets than on the barley diet but was not affected by oat type. Intake of ME was not affected by grain species but linearly increased ($P < 0.01$) with increasing inclusion of dehulled oats. Milk energy was higher ($P = 0.01$) on the oat diets than on the barley diet but was not affected by the type of oats. Heat production was higher ($P = 0.04$) on the oat diets than on the barley diet and linearly increased ($P = 0.01$) with increasing inclusion of dehulled oats. The ratio of heat production to ME intake decreased with increasing inclusion of dehulled oats ($P = 0.03$). Grain species did not affect EB but gradual replacement of hulled oats with dehulled oats linearly increased ($P = 0.01$) EB. We observed no effect of dietary treatment on k_l (Table 7).

DISCUSSION

The present study aimed to investigate the effects of replacing barley with hulled and dehulled oats on milk production, enteric CH_4 emissions, and energy utilization in dairy cows. Our hypothesis regarding milk production was that dehulling of oats would improve dairy cow production performance. As milk yield and ECM yield were not significantly affected by increasing inclusion of dehulled oats in the diet, we reject this hypothesis. Our hypothesis regarding CH_4 emissions was that replacing barley with oats would decrease (1) total CH_4 emissions (g/d) and (2) CH_4 intensity (g/kg of ECM) irrespectively of oat type, but that the size of the effect would be dependent on the type of oats. As total CH_4 emissions did not decrease, the first part of the hypothesis is rejected. However, CH_4 intensity decreased on the oat diets as a result of increased ECM yield, and therefore, we accept the second part of the hypothesis, although the size of the effect on CH_4 intensity was not dependent on the type of oats.

The results of this study on the effects of grain species and oat type on energy utilization in dairy cows should be interpreted with caution as most of the variables were estimated by using equations and not directly measured. However, most of the equations are based on large data sets.

Table 7. Effects of barley and oat grain and gradual replacement of hulled oats with dehulled oats on energy utilization in lactating cows (n = 31)

	Diet[1]					*P*-value[2]		
Item	Barley	Hulled oats	Oat mixture	Dehulled oats	SEM	Barley vs. Oats	Linear	Quadratic
GE content,[3] MJ/ kg of DM	18.6	18.8	18.8	18.9	0.002	<0.01	<0.01	<0.01
GE intake,[4] MJ/d	432	447	441	438	8.2	0.07	0.20	0.80
GE digestibility,[5] kJ/MJ	706	671	694	729	5.2	0.08	<0.01	0.25
Fecal energy,[6] MJ/d	127	147	135	119	4.2	0.01	<0.01	0.49
DE intake,[7] MJ/d	305	299	306	319	5.1	0.52	<0.01	0.51
CH_4E,[8] MJ/d	26.4	25.9	26.2	27.0	0.78	0.81	0.02	0.49
CH_4E/GE intake	0.062	0.058	0.059	0.062	0.0020	0.03	<0.01	0.57
Urinary energy,[9] MJ/d	13.5	13.8	13.8	13.9	0.16	<0.01	0.25	0.82
ME intake, MJ/d	265	260	266	278	4.9	0.53	<0.01	0.53
Milk energy, MJ/d	98.4	101	101	103	3.15	0.01	0.14	0.41
Heat production,[10] MJ/d	140	141	142	145	3.1	0.04	0.01	0.58
Heat production/ME intake	0.533	0.544	0.534	0.521	0.0163	0.94	0.03	0.83
Energy balance, MJ/d	27.2	18.0	23.2	30.4	5.15	0.40	0.01	0.81
kl[11]	0.596	0.602	0.601	0.606	0.0113	0.12	0.53	0.47

[1] Oat mixture = mix of hulled oats and dehulled oats 50:50 on DM basis.

[2] Probability of significant orthogonal contrasts. Effects tested using orthogonal contrasts were: Barley vs. Oats = barley vs. hulled oats, oat mixture, and dehulled oats; Linear = linear effect of replacement of hulled oats with dehulled oats; Quadratic = quadratic effect of replacement of hulled oats with dehulled oats.

[3] GE = gross energy, GE content predicted as (23.6 × CP + 39.8 × ether extract + 17.3 × NFC + 18.9 × NDF)/1,000.

[4] Predicted as DMI (kg/d) × GE content.

[5] Predicted according to Ramin and Huhtanen (2013).

[6] Predicted as GE intake – GE intake × (GE digestibility/1,000).

[7] DE = digestible energy, DE intake predicted as GE intake – Fecal energy.

[8] CH_4E = gross energy in CH_4, calculated according to Kriss (1930).

[9] Predicted according to Guinguina et al. (2020).

[10] Predicted according to Brouwer (1965).

[11] k_l = efficiency of ME use for lactation, predicted according to AFRC (1993) with coefficients derived from Guinguina et al. (2020).

Milk Production, Milk Composition, and Feed Efficiency

Higher milk yield (1.4 kg/d) from cows receiving the oat diets than from cows receiving the barley diet is in agreement with several previous studies that have compared the effects of barley and hulled oats on milk production. Martin and Thomas (1988), Ekern et al. (2003), Vanhatalo et al. (2006), and Tosta et al. (2019) found that milk yields increased by 1.2, 2.6, 0.7, and 2.7 kg/d, respectively, when barley was replaced by oats as a concentrate supplement to dairy cows. In contrast, milk yields remained unchanged in the study by Ramin et al. (2021) when barley was replaced by hulled oats on a grass silage-based diet (forage to concentrate 60:40).

The higher tabulated ME and MP values for barley compared with oats (Volden and Nielsen, 2011; LUKE, 2020) suggests lower milk production on oat-based diets, and yet, milk production remains unchanged or even increases when barley is replaced by oats. One explanation for this could be an altered partitioning of glucose. Additional dietary fat could increase direct incorporation of preformed fatty acids into milk fat, thereby decreasing de novo synthesis of fatty acids, as indicated by the observed tendency toward lower RQ values on the oat diets in this study. As de novo synthesis is decreased, the need for oxidation of glucose through the pentose phosphate pathway to yield NADPH decreases (Bauman and Davis, 1975), resulting in partitioning of glucose toward lactose synthesis instead.

If milk yield increases due to higher fat concentration in oats, it could be expected that milk yields would increase with increased inclusion of dehulled oats in the diet, due to the higher concentration of fat in dehulled oats (Biel et al., 2014). In our study, increased inclusion of dehulled oats resulted only in a small (0.5 kg of milk/d) numerical increase in milk yield. Although not significant, this numerical increase is, however, as expected based on the difference in dietary fat content between the hulled oat diet and the dehulled oat diet and the significant increase in milk yield and the difference in dietary fat content between the barley diet and the oat diets.

In our study, the lower concentrations of protein in milk (0.9 g/kg of milk) from cows receiving the oat diets than from cows receiving the barley diet is in line with previous studies (Ekern et al., 2003; Vanhatalo et al., 2006; Tosta et al., 2019; Ramin et al., 2021). This effect may be explained by a dilution of protein concentration when milk yields increase, because the percent changes in milk yield (+4.9%) and milk protein concentrations (−2.5%) are consistent. In agreement with Vanhatalo et al. (2006), milk fat concentration remained unchanged by replacement of barley with oats in our study. On the contrary to these results, milk fat concentration decreased by 4.1, 6.4, and 1.4 g/kg of milk in the studies by Martin and Thomas (1988), Ekern et al. (2003), and Ramin et al. (2021), respectively. Although numerical decrease in milk fat concentration was similar (0.9 g/kg of milk) to the decrease in milk protein concentration, this difference did not reach statistical significance in our study.

Mean MUN concentrations for each diet were all within the recommended range of 10–16 mg/dL depending on level of milk production (Jonker et al., 1998). By analyzing data from 50 production trials (n = 306), Nousiainen et al. (2004) found dietary concentration of CP to be the major dietary determinant of MUN concentration. Higher mean MUN concentration for cows receiving the oat diets (11.3 mg/dL) compared with the barley diet (9.9 mg/dL) is therefore expected based on higher overall mean for dietary CP concentration in the oat diets (172 g/kg of DM) than in the barley diet (163 g/kg of DM). Previous studies have not found any differences between the effects of hulled barley and hulled oats on MUN concentrations (Vanhatalo et al., 2006; Tosta et al., 2019; Ramin et al., 2021). In these studies, however, barley-based diets had CP concentrations that were slightly higher than, or similar to, the oat-based diets. The tendency for lower mean milk N efficiency observed for cows receiving the oat diets is also in harmony with the higher dietary CP concentrations, as an increase in dietary CP concentration is strongly associated with a decrease in milk N efficiency (Nousiainen et al., 2004; Huhtanen and Hristov, 2009). The linear increase in FE with increasing inclusion of dehulled oats could partly be explained by the linear increase in apparent total-tract OM digestibility with increased inclusion of dehulled oats and partly by the linear increase in dietary fat intake with increased inclusion of dehulled oats.

Gas Emissions and Ruminal Fermentation

Ramin et al. (2021) reported that total daily CH_4 emissions decreased by 4.6%, CH_4 yield by 4.4%, and CH_4 intensity by 4.8% when hulled barley was replaced by hulled oats. However, the inclusion of dehulled oats in our study and the observed linear increase in total daily CH_4 emissions and CH_4 yield when hulled oats was replaced by dehulled oats, explains the lack of an effect of grain species on CH_4 emissions in this study. The linear increase in total daily CH_4 emissions (18 g/d) and in CH_4 yield (1.3 g/kg DMI) when hulled oats was replaced by dehulled oats may be explained by the linear increase (0.06) in apparent OM digestibility with increased inclusion of dehulled oats. Diet digestibility is one of the major dietary factors affecting CH_4 emissions from the rumen and is positively related to enteric CH_4 emissions (Blaxter and Clapperton, 1965; Ramin and Huhtanen, 2013). This explanation is also supported by similar CH_4 emissions per kilogram of digested OM observed in our study.

Because dehulling of oat grain decreases the fiber and lignin content and increases both digestibility and fat concentration (Biel et al., 2014), it could be possible to further investigate the CH_4 mitigating mechanisms of oats reported in previous studies (Fant et al., 2020; Ramin et al., 2021). The results of our study indicate that the CH_4 mitigating effect of oats reported in the study by Ramin et al. (2021) diminishes when oats are dehulled to increase digestibility, and so the CH_4 mitigating effect of oats is mostly due to lower digestibility compared with barley. Dietary fat content is negatively related to enteric CH_4 emissions (Giger-Reverdin et al., 2003; Beauchemin et al., 2008; Grainger and Beauchemin, 2011). In this study, dietary fat concentration increased from 43 to 48 g/kg of DM and dietary fat intake from 0.96 kg to 1.06 kg/d when hulled oats was replaced by dehulled oats. In the meta-analysis by Grainger and Beauchemin (2011), a 10 g/kg increase in dietary fat concentration decreased CH_4 yield by 1 g/kg of DM intake, and an increase from 30 to 40 g/kg of DM would result in a 4.7% decrease in CH_4 yield. It is apparent that the difference in fat concentration between hulled and dehulled oats is not sufficient to counteract the positive effect of increased OM digestibility on CH_4 yield with increased inclusion of dehulled oats. For future perspectives, it could be interesting to investigate the effect of high-fat oat varieties on enteric CH_4 emissions.

Considering increased apparent total-tract digestibility with increasing inclusion of dehulled oats in the diet, the lack of an effect of oat type on total VFA concentration in ruminal fluid was unexpected but could partly be due to relatively high random variation caused by the sampling method. Although precaution was taken to avoid salivary contamination, sampling was only performed once a day without considering the diurnal variation in rumen VFA concentrations. To our knowledge, no study has investigated the effects of oat type on total VFA or molar proportions.

Digestibility and Energy Utilization

The lack of an effect of grain species on apparent diet digestibility is expected based on the linear increase in digestibility with increasing inclusion of dehulled oats in the diet. The numerically lower OM digestibility (−0.033) of the hulled oat diet than of the barley diet is in line with Finnish national feed tables (LUKE, 2020) and previous studies (Vanhatalo et al., 2006; Ramin et al., 2021) considering the level of grain inclusion. Although differences between the barley diet and the hulled oat, oat mixture, and dehulled oat diets were not tested separately in this study, the ranking of the grains in terms of digestibility agree with Mustafa et al. (1998), where effective degradability of DM and NDF in barley was lower than that of naked oats but higher than that of hulled oats. In the same study, effective CP degradability in barley was lower than that of naked oats but depending on the variety of oats, effective CP degradability was either higher or lower in barley than hulled oats.

Studies comparing digestibility of hulled and dehulled oats in ruminants are scarce. Higher in situ effective degradability of DM, CP, and NDF in naked oats than hulled oats was reported by Mustafa et al. (1998). In the study by Petit and Alary (1999), ruminal effective degradability of DM was higher in naked oats than hulled oats but degradability of CP was similar. Finnish national feed tables (LUKE, 2020) report a slightly smaller difference in OM digestibility between hulled and dehulled oats than observed in our study considering that the effect in our study was diluted by inclusion of 60% grass silage. The linear increase in apparent OM digestibility was mostly due to higher NDF digestibility after removal of the hull containing most of the lignin and lignin-associated cellulose and hemicellulose present in whole oat grain (Salo and Kotilainen, 1970). Lignin concentration in hulled grains is negatively related to digestibility (Crosbie et al., 1985; Thompson et al., 2000) and differences in OM digestibility between hulled and dehulled oats will inevitably be dependent on the oat variety as the lignin concentration in oat hulls may vary between 8 and 77 g/kg of DM depending on variety (Crosbie et al., 1985; Thompson et al., 2000).

The slight increase in CH_4E as a ratio of GE intake with increased inclusion of dehulled oats is a direct result of greater GE digestibility. Although feeding the oat diets resulted in greater heat energy losses, the ratio of heat production to ME intake remained unchanged. However, in the case of replacing hulled oats with dehulled oats, lower ratio of heat production to ME intake may be explained by a small part of the ME supply in form of dietary fiber being replaced with more readily degradable starch and fat, as a decrease in dietary fiber content leads to lower heat energy production during digestion in the rumen (Reynolds et al., 1991).

Efficiency of ME use for lactation (average 0.60) was similar to the average value (0.63) in the study by Ramin et al. (2021) when the diets included different proportions of hulled barley and oats. In this study, similar k_l between the barley diet and the oat diets is unexpected based on the observed increase in milk energy on oat diets, although ME intake was similar. Subtraction of the heat energy losses from ME intake gives similar net energy values for the barley diet and for the overall mean of the oat diets. Higher milk energy on the oat diets would thus indicate a shift in energy partitioning on the oat diets toward milk production. When diets were isocaloric on net energy basis, van Knegsel et al. (2007) found that feeding dairy cows with a diet high in lipogenic nutrients caused a shift in energy partitioning toward milk production, increase in milk energy through increased milk fat concentration and yield, and a tendency for higher body fat mobilization compared with a glucogenic diet. Boerman et al. (2015) found similar results when replacing a high-starch diet with a high-fiber and high-fat diet supplying similar NE_L. Nichols et al. (2019) observed that continuous abomasal infusion of glucogenic substrates increased plasma concentrations of glucose and insulin, which favored energy partitioning toward body tissues and decreased milk energy output compared with a control. In addition, compared with the tendency for lower RQ on the oat diets observed in this study, Nichols et al. (2019) reported lower RQ on the lipogenic diet. Based on these studies and the results of this study, it is likely that the lower starch and higher fat concentration of oats compared with barley induces a shift in the energy partitioning of lactating cows to favor milk production, leading to increased milk yield when barley is replaced by oats. In the study by Ramin et al. (2021), milk energy remained constant although ME intake decreased when barley was replaced by hulled oats and no significant effect on k_l was observed. Replacing barley with oats could lead to increased body fat mobilization (van Knegsel et al., 2007) especially if oat diets are supplying less ME than barley diets. Unfortunately, body condition scores were not assessed in this study, but BW of cows fed the oat diets tended to be higher than of cows fed the barley diet. Blood sampling to assess whether glucose and insulin concentrations are affected by replacement of barley with oats could be of interest in future research.

The linear increase in EB together with similar milk energy among the oat diets indicate partitioning of the increased ME supply into body tissues, rather than into milk production, with increasing proportion of dehulled oats in the diet. This would be in line with the discussion related to grain species because increased inclusion of dehulled oats in the diet increased both starch and fat concentration similarly and ratio of fat concentration to starch concentration remained unchanged. The small numerical production response to dehulled oats was mainly related to the fact that cows were in positive EB and therefore not very sensitive to increased ME supply. To draw any further conclusions about the effects of barley and oats on energy utilization and energy partitioning, more investigation into the biological mechanisms is required.

CONCLUSIONS

In this study, cows receiving the oat diets had higher milk and ECM yield compared with cows receiving the barley diet. Replacement of hulled oats with dehulled oats to increase digestibility and ME supply did not significantly increase milk yield or ECM yield. The increased ME supply with increasing inclusion of dehulled oats in the diet seemed to be partitioned toward body energy stores, rather than into milk production when cows were in positive EB. Therefore, based on the results from this study, industrial or on-farm dehulling of oats to improve production performance when fed to dairy cows is an unnecessary process which requires extra labor and increases feed costs. Total daily CH_4 emissions and CH_4 yield were similar between the barley diet and the oat diets but due to the higher ECM yield on the oat diets, CH_4 intensity decreased by 5.7% when the barley diet was replaced by the oat diets. Replacement of hulled oats with dehulled oats increased total daily CH_4 emissions and CH_4 yield, probably because of increased diet digestibility, whereas CH_4 intensity remained unchanged. Therefore, we conclude that replacing hulled oats with dehulled oats in the diet of dairy cows does not mitigate enteric CH_4 emissions.

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