Norwegian University of Life Sciences
Faculty of Chemistry, Biotechnology and Food Science

Philosophiae Doctor (PhD)
Thesis 2023:32

Cheese-making efficiency affected by protein source in concentrate feed for dairy cows and their α_{S1}-κ-casein genotypes

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Hvordan ystingseffektivitet blir påvirket av proteinkilde i kraftfôr og genotyper av α_{S1}-κ-casein

Philosophiae Doctor (PhD) Thesis

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Abbreviations and definitions

A30	Firmness after 30 min (mm)
BBAA	α_{S1}-κ-casein BBAA composite genotype (used in Paper 3)
BAR	Barley as protein source in concentrate feed (used in Paper 1 and Paper 2)
BBBB	α_{S1}-κ-casein BBBB composite genotype (used in Paper 3)
BCAA	α_{S1}-κ-casein BCAA composite genotype (used in Paper 3)
β-LGB	β-lactoglobulin
CMP	Caseinomacropeptide
CN	Casein
CCP	Colloidal calcium phosphate
DM	Dry matter
FAA	Free amino acids
FAO	Food and Agriculture Organization
FoN	Foods of Norway
IDF	International Dairy Federation
K20	Time until firmness of 20 mm is achieved (min)
MACY	Moisture-adjusted cheese yield
MCP	Milk coagulation properties
MCPS	Multiple-component pricing system
NMBU	Norwegian University of Life Sciences
NR	Norwegian red
OECD	Organisation for Economic Co-operation and Development
PY	Predicted yield
RCT	Rennet coagulation time (min)
SBM	Soybean meal as protein source in concentrate feed (used in Paper 1 and Paper 2)
SCP	Single cell protein
SDGs	Sustainable Development Goals
The FEED study	Abbreviated title of the study that resulted in Paper 1 and Paper 2 (and Paper 4). An overview over this study is shown in Figure 1

The GPV study	Abbreviated title of the study that resulted in Paper 3. An overview over study is shown in Figure 2
WBCSD	World Business Council for Sustainable Development
Ya	Actual yield
YE	Yield efficiency
YEA	Yeast as protein source in concentrate feed (used in Paper 1 and Paper 2)

Liquid milk	"*Liquid milk is the most consumed, processed and marketed dairy product. Liquid milk includes products such as pasteurized milk, skimmed milk, standardized milk, reconstituted milk, ultra-high-temperature (UHT) milk and fortified milk*". Directly cited from FAO (2023).

List of papers

Paper 1
Olsen, M.A., Ferneborg, S., Vhile, S.G., Kidane, A. & Skeie, S.B. (2023). Different protein sources in concentrate feed for dairy cows affect cheese-making properties and yield. Manuscript accepted by *Journal of Dairy Science,* 24.01.2023.

Paper 2
Olsen, M. A., Vhile, S. G., Porcellato, D., Kidane, A. & Skeie, S. B. (2021). Feeding concentrates with different protein sources to high-yielding, mid-lactation Norwegian Red cows: Effect on cheese ripening. *Journal of Dairy Science*, 104 (4): 4062-4073. doi:10.3168/jds.2020-19226

Paper 3
Olsen, M.A., Ketto, I.A., Øyaas, J., Abdelghani, A., Myhrer, K.S. & Skeie, S.B. (2023). Influence of different genetic polymorphisms of α_{S1}- and κ-casein on Havarti-type cheese: Effects on cheese-making efficiency and cheese quality. Manuscript submitted to *International Dairy Journal*.

Not included in the thesis

Paper 4 (Appendix 1)

Kidane, A., Vhile, S. G., Ferneborg, S., Skeie, S.B., Olsen, M. A., Mydland, L.T., Øverland, M. & Prestløkken, E. (2022). *Cyberlindnera jadinii* yeast as a protein source in early- to mid-lactation dairy cow diets: Effects on feed intake, ruminal fermentation, and milk production. *Journal of Dairy Science*, 105 (3):2343-2353. doi:10.3168/jds.2021-20139

Abstract

Evaluation of cheese-making efficiency is important for the dairy industry for profitability and sustainability reasons. Milk with a beneficial composition that favours good coagulation properties and a higher cheese yield while maintaining cheese quality is therefore of interest.

The dairy industry in Norway aims to increase the proportion of nationally produced feed ingredients for dairy cows. To achieve this, novel protein sources are needed. Forest covers almost 40 % of Norway, and it is possible to produce a protein rich yeast ingredient from spruce wood using technology such as enzymatic hydrolysis and subsequent fermentation of the resulting sugars. However, the impact of yeast microbial protein used in concentrate feed to dairy cows on cheese-making properties, yield and cheese quality is unknown and therefore investigated in a feeding study that resulted in two papers: **Paper 1** (Cheese-making efficiency) and **Paper 2** (Cheese ripening and quality). Norwegian Red dairy cows (n=48) in early/mid lactation were divided in three groups and fed a diet consisting of grass silage and concentrate. The yeast *Cyberlindnera Jadinii* was tested as a protein source in concentrate feed for dairy cows and compared to barley and soybean meal. The concentrates with added soybean meal or yeast contained a higher protein content. In Paper 1, individual milk samples were collected five times during the experiment and a Gouda-type cheese was made from pooled milk from each of the three groups. Milk from cows fed barley-based concentrate contained a lower content of casein and phosphorous, used longer time for renneting and gave a lower cheese yield compared to concentrate with added soybean meal and yeast. Overall, soybean meal and yeast used as a protein source in concentrate feed showed similar cheese-making properties, but for individual milk samples, yeast concentrate showed better coagulation properties. In Paper 2, the cheese ripening and quality of the cheeses were evaluated. Cheeses made from milk from cows fed concentrate with soybean meal had a higher content of DL-pyroglutamic acid and free amino acids than the other cheeses, indicating a faster ripening. There were no differences

in microbiota between the cheeses, and few differences in sensory properties. This feeding experiment showed that it is possible to substitute soybean meal with yeast without compromising cheese-making properties and quality of Norwegian Gouda-type cheese.

Milk protein genetic variants affect the protein/casein composition in milk which again affects cheese-making efficiency. One of the goals for the dairy industry is to optimize the utilization of milk as a raw material for dairy products. One possible strategy is to manipulate the milk composition through breeding, such as obtaining a milk composition tailored to the production of cheese. This can result in a higher cheese yield, reduced manufacturing time and thereby lower energy consumption. Most studies about genetic protein variants have measured coagulation properties at a laboratory scale and have focused on genetic variants of single caseins. However, in **Paper 3**, the effect of composite genotypes of α_{S1}-κ-casein (BBAA, BBBB and BCAA) on the coagulation properties and yield during cheese-making and the quality after ripening of a Havarti-type cheese was investigated. Milk with α_{S1}-κ-casein BCAA obtained a shorter renneting time, while milk with α_{S1}-κ-casein BBAA obtained the highest cheese yield. Different protein profiles in cheese were found between the genotypes. After ripening, cheese with α_{S1}-κ-casein BBBB obtained more free amino acids compared to α_{S1}-κ-casein BCAA. Sensory differences were observed, where cheese with α_{S1}-κ-casein BCAA had a higher intensity of sweetness and lower intensity of hardness compared to cheeses with α_{S1}-κ-casein BBBB and BBAA. Cheeses with α_{S1}-κ-casein BBAA had a higher intensity of sunlight flavour compared to the cheeses with α_{S1}-κ-casein BCAA and it obtained a lower intensity of juiciness compared to α_{S1}-κ-casein BBBB cheeses.

Based on the two experiments presented, both protein source in concentrate feed to dairy cows and the milk protein genetic variants affects cheese-making efficiency and are means that could be used to make the dairy industry more efficient and sustainable.

Norsk sammendrag

Evaluering av ystingseffektiviteten er viktig for meieriindustrien både av økonomiske grunner, men også med tanke på bærekraftig produksjon. Det er av interesse at melka har en gunstig sammensetning som bidrar til å forbedre melkas koaguleringsegenskaper og gir økt utbytte, samtidig som ostekvaliteten opprettholdes.

Meieriindustrien i Norge har som mål å øke andelen norskproduserte fôrråvarer i fôr til melkekyr. For å kunne oppnå dette, så trengs det nye og innovative proteinkilder. Skogen dekker omtrent 40 % av landarealet til Norge, og det er mulig å produsere proteinrik gjær fra grantrær ved bruk av enzymatisk hydrolyse av cellulose fra grantrær etterfulgt av fermentering av sukkere fra denne hydrolysen. Hvilken effekt en slik proteinkilde i kraftfôr til melkekyr har på ysteegenskaper, utbytte og ostekvalitet er ukjent. Derfor ble dette undersøkt i et fôrforsøk som resulterte i to artikler: **Artikkel 1** (Ystingseffektivitet) og **Artikkel 2** (Ostemodning og kvalitet). Melkekyr av rasen Norsk rødt fe (n=48) som var tidlig eller midt i laktasjon ble fordelt i tre grupper og ble gitt en diett bestående av surfôr og kraftfôr. Gjæren *Cyberlindnera Jadinii* ble benyttet som proteinkilde i kraftfôr og sammenliknet med bygg og soyamel benyttet som proteinkilde i kraftfôr. Kraftfôret med gjær og soyamel hadde et høyere innhold av protein. Artikkel 1 omhandler produksjon av Gouda-type ost produsert av melk fra de tre gruppene samt individuelle melkeprøver som ble samlet inn fem ganger i løpet av forsøket. Melk fra kyr som ble fôret med bygg som proteinkilde hadde et lavere innhold av kasein og fosfor, hadde en lengre løpningstid og gav et lavere utbytte sammenliknet med melk fra kyr som ble fôret med kraftfôr med gjær og soyamel. Stort sett gav gjær og soyamel i kraftfôr like ysteegenskaper, men gjær gav bedre koaguleringsegenskaper i de individuelle melkeprøvene. Artikkel 2 omhandler modning og kvalitet av ostene. Ostene produsert av melk fra kyr fôret med soyamel-kraftfôr hadde en høyere konsentrasjon av DL-pyroglutaminsyre og frie aminosyrer sammenliknet med de andre ostene, dette indikerer en raskere modning. Det var ingen forskjell i

mikrobiota mellom ostene og det var små forskjeller i sensoriske egenskaper. Dette fôrforsøket viste at det er mulig å erstatte soyamel med gjær uten at det går ut over ysteegenskaper og kvalitet på norsk Gouda-type ost.

De genetiske proteinvariantene i melk påvirker sammensetningen av protein/kasein i melk, som igjen påvirker ystingseffektivitet. Et av målene til meieriindustrien er å optimalisere bruken av melk som råvare ved produksjon av meieriprodukter. En mulig strategi er å manipulere melkas sammensetning gjennom avl, slik at man får en sammensetning som er bedre tilpasset osteproduksjon. Dette kan resultere i økt utbytte, redusert produksjonstid og dermed et lavere energiforbruk. De fleste studier som omhandler genetiske proteinvarianter har målt koaguleringsegenskaper i labskala og har fokusert på genetiske varianter av enkelt-kaseiner. I **Artikkel 3** ble det undersøkt hvilken effekt genetisk sammensetning av α_{S1}-κ-kasein (BBAA, BBBB and BCAA) har på koaguleringsegenskaper og utbytte, samt kvalitet og modning av en Havarti-type ost. Melk med α_{S1}-κ-kasein BCAA hadde kortest løpningstid, mens melk med α_{S1}-κ-kasein BBAA gav høyest utbytte. Ulik proteinsammensetning i osten ble funnet mellom de ulike genotypene. Etter modning, hadde ost med α_{S1}-κ-kasein BBBB en høyere konsentrasjon av frie aminosyrer sammenliknet med α_{S1}-κ-kasein BCAA. Sensoriske forskjeller ble observert mellom ostene, ost med α_{S1}-κ-kasein BCAA hadde en høyere intensitet av søtsmak og lavere intensitet av hardhet sammenliknet med ost med α_{S1}-κ-kasein BBBB and BBAA. Ost med α_{S1}-κ-kasein BBAA hadde en høyere intensitet av solsmak sammenliknet med ost med α_{S1}-κ-kasein BCAA og hadde en lavere intensitet av saftighet sammenliknet med ost med α_{S1}-κ-kasein BBBB.

Begge forsøkene viser at både proteinkilde i kraftfôr til melkekyr og genetiske proteinvarianter påvirker effektivitet under osteproduksjon og er tiltak som kan benyttes for å gjøre meieriindustrien mer effektiv og bærekraftig.

1 Introduction

On November 15th 2022, the world population reached 8 billion people (United Nations, 2022b). By 2050, it is expected to reach 9.8 billion (United Nations, 2017). It is therefore a challenge for the food industry to feed the increasing world population in a sustainable manner. In order to attain this, drastic and novel changes are needed.

The dairy industry contributes to food security, nutrition, poverty alleviation and economic growth. However, simultaneously, the livestock production system and the dairy industry may have a significant negative effect on the environment (FAO, 2022c). However, Capper et al. (2009) found that modern dairy practice required fewer resources with 21 % of animals, 23 % of feedstuffs, 35 % of water and 10 % of the land needed to produce the same 1 billion kg of milk in 2007 compared to 1944. This shows that the efficiency of dairy production has increased during the last decades. Still, more work is needed. Livestock (including dairy cows) may consume food which is edible for humans and graze on land that could be used for crop production (Mottet et al., 2017). Notwithstanding, 86 % of the diet for livestock today consists of ingredients that are not currently suitable for human consumption (FAO, 2022d). Animals convert these ingredients to high-value food, and animals therefore play a vital role in global food security and nutrition (FAO, 2022d). Work is needed to increase the proportion of non-edible (for humans) ingredients in the diet of livestock animals.

The food system is extremely vulnerable, as the COVID-19 pandemic and the Russian invasion of Ukraine has underlined. The COVID-19 pandemic and the resulting preventive lockdowns led to a sharp reduction in food production, transportation and consumption (Sers & Mughal, 2020). This clearly shows that actions are needed to ensure that the agricultural sector will remain resilient, efficient and sustainable in the future (OECD/FAO, 2021). In addition to possible pandemic outbreaks in the future, political disturbances and wars are also threats to food security. The world is currently witnessing the highest number of violent conflicts since World War II, with one quarter of the global population living in conflict-affected countries. The Russian invasion of Ukraine is an example of how wars can disrupt supply chains. The invasion has caused unprecedented rises in

prices of food, fuel and fertilizer and roiled financial markets. This fuels the threat of a global food crisis and has delayed the urgently needed transition to greener economies (United Nations, 2022c). Russia and Ukraine are some of the largest producers and exporters of wheat, maize, and sunflower seed products, and together export 30 %, 20 % and 80 % of these commodities, respectively. Due to future scenarios of climatic changes, political disturbances and pandemics, every country should maximise their agricultural production with the goal of increasing their degree of self-sufficiency. In order to attain this, every country needs to adjust their agriculture practise to fit the countries topography, seasons, weather etc.

The dairy industry in Norway has a responsibility of adjusting and improving their practice to enable a sustainable development. Therefore, the largest dairy company in Norway, TINE SA, aims to increase the proportion of nationally-produced feed ingredients in dairy cow's feed. Concentrate feed for Norwegian dairy cows currently contain 85 % locally-produced ingredients (TINE SA, 2022b). To increase this proportion, novel protein sources that can be produced in Norway are needed. To enable the projected growth in agricultural production that is needed to feed the increasing population, improvements in productivity and energy efficiency are also required (OECD/FAO, 2021). For the dairy industry, this calls for increased feed efficiency and efficiency in the production of important dairy products such as cheese (e.g. cheese yield, production time), while not compromising product quality.

In addition to reducing the negative environmental effects of production, the dairy industry is continuously interested in making the production as efficient as possible. This is in accordance with the term eco-efficiency, which in short means being an efficient business and at the same time protecting the environment (WBCSD, 2000). Sustainable production can be cost-effective, if the process is made more efficient by reducing manufacturing time, lowering energy consumption, reducing by-products or waste, and if possible, use less raw material.

1.1 Project objectives

The major objectives for this project were to increase knowledge about how the production and quality of cheese is affected by the protein source in concentrate feed to dairy cows and their genetic milk protein variants.

The specific objectives were:

1. To investigate the effect of replacing soybean meal with a novel protein source in concentrate feed for dairy cows in order to attain sustainable dairy production and increase the degree of national self-sufficiency. This may be achieved by using a protein-rich yeast that could grow on Norwegian forestry biomass. This study is hereby abbreviated as "The FEED study". The effect of using a yeast protein source (YEA) in concentrate feed for Norwegian Red (NR) dairy cows, compared to using barley (BAR) or soybean meal (SBM), was assessed by:
    a. Cheese-making efficiency. This resulted in **Paper 1: "Different protein sources in concentrate feed for dairy cows affect cheese-making properties and yield"**.
    b. Cheese ripening and quality. This resulted in **Paper 2: "Feeding concentrates with different protein sources to high-yielding, mid-lactation Norwegian Red cows: Effect on cheese ripening"**.

2. To study the effect of the genetic polymorphism of α_{s1}- and κ-casein on cheese yield and cheese quality. Different milk protein genetic variants are known to affect milk composition and thereby coagulation properties during cheese manufacturing and the resulting cheese yield. These factors are indicators for cheese-making efficiency. The effect of two different variants of α_{S1}-CN (BB and BC) and κ-CN (BB and AA) in milk from NR cows were tested for cheese-making efficiency, protein composition and sensory properties in cheese. This study is hereby abbreviated as "The GPV study". This resulted in **Paper 3: "Influence of different genetic polymorphisms of αs1- and κ-casein on Havarti-type cheese: Effects on cheese-making efficiency and quality of cheese"**.

1.2 The FEED study

The main work in this doctoral thesis is connected to the work in Foods of Norway (FoN), which is a Centre for Research-based Innovation (CRI). The centre develops novel feed ingredients and thereby contributes to growth and increased value creation in Norwegian aquaculture, agriculture and forestry (Foods of Norway, 2022). The goal for FoN is that feed for fish and livestock should be based on renewable resources that do not compete with food for humans. FoN is working with different types of biomass such as trees, seaweed, grass and by-products from animal and fish. A part of the research focuses on how to use natural biomass such as trees and seaweed to produce single cell protein (SCP) as a protein source. Mostly yeast had been evaluated as a protein source in feed to farm animals, specifically the yeast *Cyberlindnera* (*C.*) *Jadinii* (previously known as *Candida utilis*). This yeast ingredient is derived from spruce wood using technology such as enzymatic hydrolysis and subsequent fermentation. More information regarding this technology can be found in the doctoral thesis of David Alpena Gómez, in which one of his research topics was the production of yeast from spruce sugars (Lapeña, 2019).

The yeast has shown promising results when incorporated in feed for piglets and chickens (Cruz et al., 2019; Cruz et al., 2020). They found that growth of the animal was maintained, and the digestive function was either maintained or improved (dependent on the concentration of yeast supplementation) when compared to using a conventional diet with soybean meal. Monogastric animals such as chickens and piglets have a higher proportion of concentrate feed in their diet compared to dairy cows. However, concentrate feed is an important part of the dairy cow's diet to increase feed efficiency and production. The research covered in this part of this doctoral thesis is testing *C. jadinii* in the diet given to dairy cows, and to study how the yeast affects the production and quality of cheese (**Paper 1** and **Paper 2**). In addition to these two papers, another paper concerning cow performance from the FEED study is published (**Paper 4**) (Kidane et al., 2022)(this paper is enclosed in Appendix 1 as it is background material for **Paper 1** and **Paper 2**). No differences in feed uptake, milk yield, body weight or body condition scoring of the cows were found by replacing SBM or BAR with yeast YEA. Figure 1 on the next page shows an overview over the FEED study.

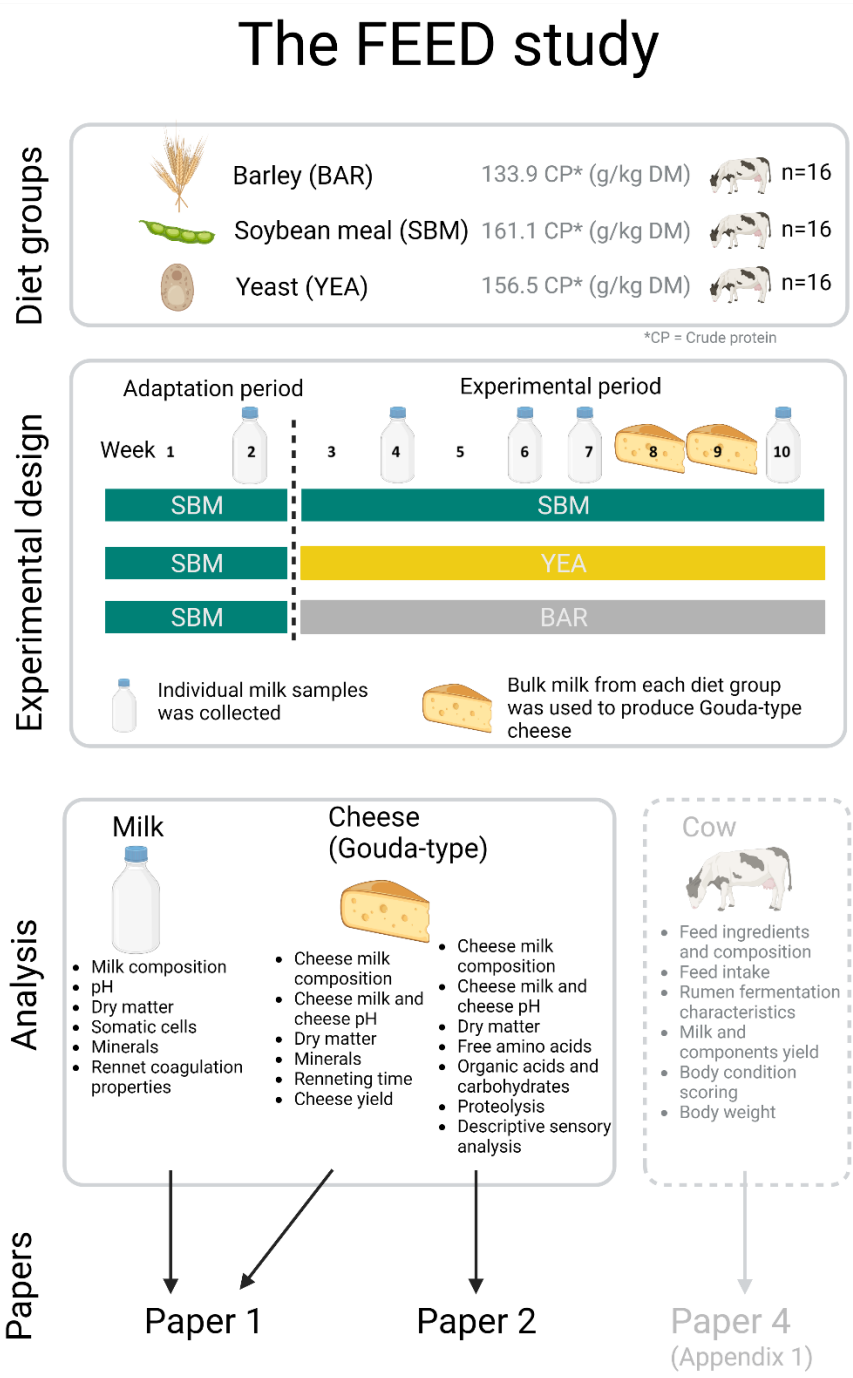

Figure 1 Overview over the FEED study. Diet groups, experimental design, analysis and papers are presented. Figure created with BioRender.com with publishing permission.

1.3 The GPV study

The GPV study is a continuation of the doctoral work by Isaya Ketto (Ketto, 2017). Ketto examined the impact of milk protein genotypes on the acid and rennet coagulation properties of milk. A conclusion from his research was that a focus area on further research should be *"The effects of milk protein genotypes on cheese yield and quality. This will provide evidence for the best alleles in NR cows for efficient cheese processing in Norway"* (Ketto, 2017). **Paper 3** is the first step of focusing on cheese yield and quality regarding milk protein genotypes in NR cows.

Cheese is one of the most important dairy products. Although the consumption of liquid milk is decreasing, cheese consumption is increasing. Casein, the main protein in milk is essential for cheese-making. Thus, the importance of casein content in milk for dairy production is increasing. Genetic protein variants affect protein/casein composition in milk which again affects cheese-making efficiency, such as milk coagulation properties (MCP) and cheese yield. One of the dairy industry goals is to optimize the utilization of milk as a raw material. One possible strategy is to manipulate the milk composition through breeding, such as obtaining a milk composition tailored to the production of cheese. This can result in a higher cheese yield, reduced manufacturing time and thereby lower energy consumption.

The effect of two different genotypes of α_{S1}-CN (BB and BC) and κ-CN (BB and AA) in milk from Norwegian red (NR) cows were investigated. This gave the following α_{s1}-κ-CN composite genotypes: BBAA, BBBB and BCAA.

Figure 2 on the next page shows an overview over the GPV study.

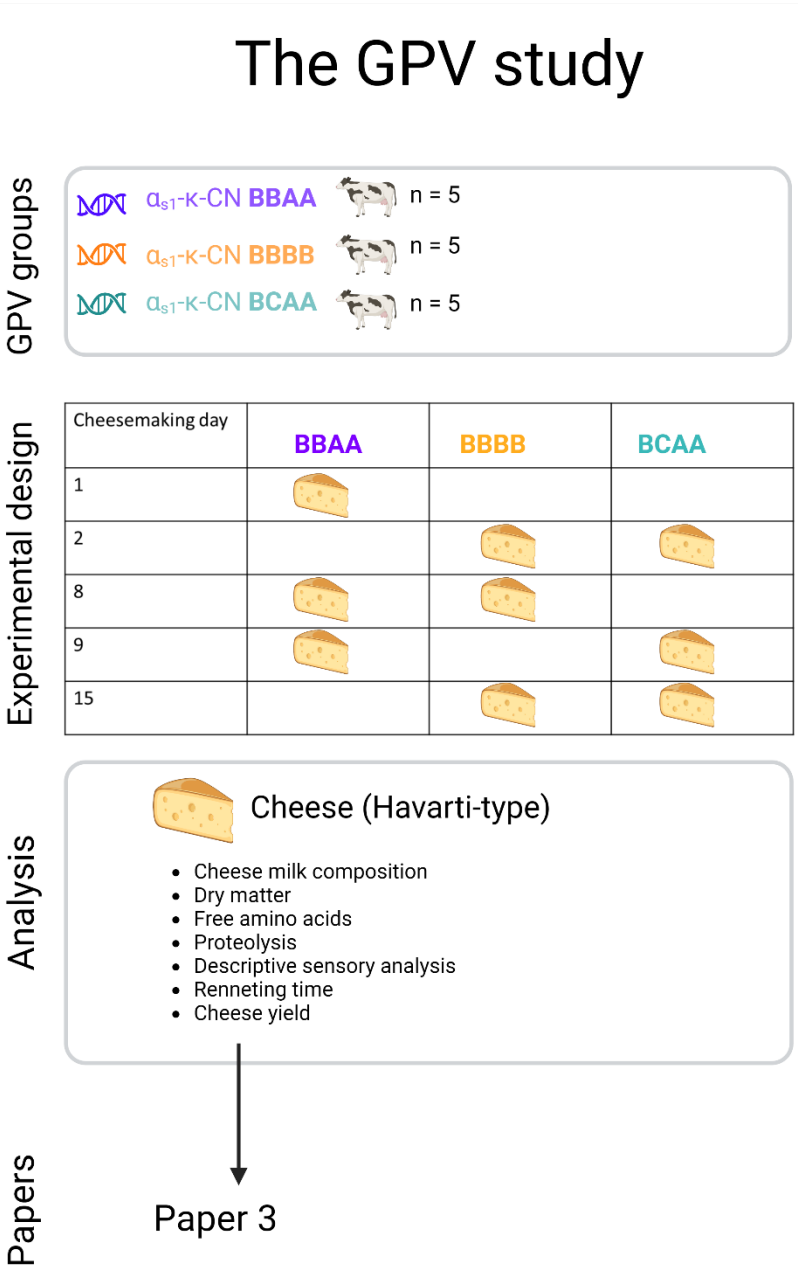

Figure 2 Overview over the GPV study. Diet groups, experimental design, analysis and paper are presented. Figure created with BioRender.com with publishing permission.

2 Literature review

2.1 General introduction

The dairy industry has three main needs that provide the background for this project:

1. The need to feed a growing human population
2. The need to achieve a sustainable dairy production
3. The need for every country to focus on increasing the degree of self-sufficiency

These needs are all important, and all actions or changes in the dairy sector should be evaluated to ensure that changes made are in accordance with these needs, which are based on the Sustainable Development Goals (SDGs).

The United Nations General Assembly (UN-GA) defined 17 interlinked global goals in 2015. These goals are intended to be *"a shared blueprint for peace and prosperity for people and the planet, now and into the future"* (United Nations, 2022a). The intention is to achieve these goals by 2030. One of the SDGs is **to end all forms of hunger and malnutrition by 2030** (goal 2). It is estimated that between 720 and 811 million people in the world suffered from hunger in 2020, which is an increase of 118 million people from 2019 (FAO et al., 2021). Another notable goal, which is relevant to this doctoral work, is goal 12: **Ensure sustainable consumption and production patterns.** Unsustainable consumption and production are the main causes of climate change, biodiversity loss and pollution.

To achieve goal 2 it is necessary to increase the degree of food security. The United Nations define food security: *"Food security exists when all people at all times have physical and economic access to sufficient, safe food for an adequate diet that meets their nutritional needs and preference, and which forms the basis for an active and healthy life"* (World Food Summit, Rome, 1996). The world's population is expected to increase to 9.8 billion people in 2050 (United Nations, 2017) and alongside this increase, food demands will also increase. Baulcombe et al. (2009) and the World Bank (2007) estimated that the global food production has to increase at least by 50-70 % and Tilman et al. (2011) estimated that the global crop production has to

increase by 100-110 % to meet the demands in 2050. Achieving food security in a sustainable way is a huge mission and the global food system needs to make drastic changes to realize this.

Global livestock production has increased over the last 50 years. Milk and meat production has increased by 126 % and 234 %, respectively, from 1970 to 2020 (FAO, 2022b). Livestock production is using 80 % of global agricultural land, if land used for both grazing and crop production is considered (Weindl et al., 2017). As the situation is today, a large fraction of this land is not suitable for anything else than grazing or grass production. The livestock industry is water-demanding and the majority of this water is used for feed production, estimated to 41 % of total agricultural water use. Water used for drinking and servicing (cleaning farm, animals etc.) accounts for only 2 % of the water use in livestock production (Mekonnen & Hoekstra, 2012). Dairy production must without doubt make drastic changes in order to attain sustainable production, especially as the global demand for dairy products continues to grow. The driving factors for the increase in demand for dairy products include an increasing population, improved economic conditions in developing countries and increasing demand for high-quality animal protein (Berry et al., 2020). Dairy protein plays a significant role in the diet globally, representing 10 % of global protein consumption (Boland & Hill, 2020).

This thesis is mainly based on actions that could be taken in Norway in order to attain a more sustainable dairy production. Due to limited cultivated land area, a challenging topography and climate, there is a shortage of nationally-produced feed protein in Norway. This has led to the need to import protein-rich feed ingredients. Soybean meal is one of the most important protein sources in concentrate feeds, and performs well in the dairy cows' diet. Around 75 % of global soy production (measured by weight) is used for livestock feed (FCRN foodsource, 2020). However, due to the global population increase, this is not an optimal use of soy protein since it is also an excellent protein source for human nutrition. Innovative methods are needed to find alternative protein sources for use in feed and thereby increase the national self-sufficiency of feed protein in Norway. It is possible to produce a high protein yeast ingredient by fermentation of sugars derived from lignocellulosic biomass, for example from spruce wood (Lapeña, 2019). This type of protein source is a promising option to soybean meal and other protein sources that cannot be grown in northern countries.

2.2 Feed for dairy cows

Only 3 % of Norway's land area can be used for cultivation, and only a small fraction of this is suitable for production of food grains for human consumption. Therefore, the available land is mainly used for grass and grains intended for feed (Regjeringen.no, 2021).

All of the grass silage and most of the concentrate feed fed to Norwegian animals is normally produced in Norway. For dairy cows, the proportion of feed produced in Norway amounts to 82 %. Both carbohydrate (e.g. wheat, molasses and beet pulp) and protein sources (mainly soybean meal, rapeseed meal and corn gluten meal) are imported to be used in concentrate feed (Landbruksdirektoratet, 2022). In 2021, 42 % of raw materials used in concentrate feed production for farm animals was imported. However, the majority (94 %) of the protein needed for concentrate feed and a smaller fraction (25 %) of the carbohydrate is imported. The need for imported carbohydrates varies, depending on growth and weather conditions in Norway from year to year. The weather greatly affects the quality of the roughage, and in Norway where the temperatures and weather conditions vary widely, the use of concentrate feed can help balance the energy supply of dairy cattle.

The use of concentrate feed to dairy cows has increased in recent decades to ensure effective milk production. Today, most dairy cows in Norway are fed grass silage and concentrates at a ratio around 55:44 (ANIMALIA, 2020). In 1990, Norwegian dairy cows obtained 35.1 % of their energy supply from concentrate feed, and by 2020 this had increased to 44.4 % (H. Volden, Mimiro, Ås, Norway, personal communication). From 1999 to 2021 the number of dairy cows in Norway has decreased by 33 % (TINE SA, 2021). In the same time period, the milk production pr. cow increased by 34 %. This is a result of more efficient milk production, where fewer cows can produce the same volume of milk as a greater number of cows with lower yield.

Feed efficiency, the relative ability to turn feed into food (milk or milk components in the case of dairy cows) is a research area with constant interest because feed costs comprise to up to 60 % of dairy production costs (Connor, 2015). Plant protein is converted quite inefficiently into meat protein since ~ 6 kg of plant protein is needed to produce 1 kg of meat protein (Ritala et al., 2017). Boland and Hill (2020) stated that 1 kg of animal-origin food requires 10 kg of plant-based food. However, the situation is slightly better for dairy production (and eggs) since the animal can

continue to produce these raw materials throughout its life, having a conversion ratio about 4:1 (Boland & Hill, 2020). There is a global increase in consumption of animal products and this has resulted in greater quantities of crops and agricultural products used in feed. This is estimated to increase by an additional 14 % by 2030 (OECD/FAO, 2021), making increased feed efficiency an even more important research area.

The concern for protein deficiency in the future and exploration of novel and unconventional protein sources started in the fifties (Suman et al., 2015), and in 1996 researchers started to grow microorganisms to produce protein biomass, SCP. This term was coined by Carol L. Wilson in 1969. SCP means protein derived from single cells microorganisms such as bacteria, yeast, algae and fungi (Ritala et al., 2017). The production of SCP has many environmental benefits compared to the production of agricultural and animal derived proteins. The production does not require agricultural land areas and is not dependent on seasonal and climatic conditions. In addition, microorganisms grow fast compared to other protein sources (Adedayo et al., 2011). Table 1 presents the efficiency of protein production of beef cattle, soybeans and yeast.

Table 1 Efficiency of protein production of beef cattle, soybeans and yeast in 24 h. Table modified from Israelidis (2003). The numbers allegedly came from agricultural statistics (U.S.D.A) in 1976, but this source was not found.

Organism (1000 kg)	Amount of protein
Beef cattle	1 kg
Soybeans	10 kg
Yeast	100.000 kg

2.2.1 How feed affects milk

Establishing the opportunities and limitations of modifying milk composition through diet manipulation has been an important research area. There are three basic reasons for manipulation milk composition (directly cited from Jenkins and McGuire (2006):

1) Improving the manufacturing and processing of milk and dairy products
2) Altering the nutritional value of milk to conform to dietary guidelines set forth by governmental agencies
3) Using milk as a delivery system for nutraceuticals with known benefits to human health

In the beginning of the early 1980's it was clear that dietary manipulation of milk composition had opportunities but also limitations. Fat content could be increased by 3 % but protein could only be increased by 0.5 % (Jenkins & McGuire, 2006). Due to a targeted focus on health and milk fat, there has been considerably more research on milk fat than milk protein, specifically milk fat content and fatty acid composition.

Research on milk protein content has focused on the effects of forage-to-concentrate ratio and amounts of protein and fat in the cows' diet. By reducing the proportion of forage to 10 % or less of dry matter (DM) in the diet, a 0.4 % increase in milk protein content could be obtained (Jenkins & McGuire, 2006). However, this high proportion of concentrates is not recommended due to risks of digestive and metabolic problems for the cow. Increasing the protein in the cows' diet by 1 %, has been showed to increase milk protein by 0.02 % (Emery, 1978), and the protein transfer efficiency to be 25 to 30 % (Jenkins & McGuire, 2006). This clearly shows that increasing milk protein content can be challenging and costly.

Feed affects milk and cheese in more ways than altering gross milk composition. Low quality feed and/or in insufficient amount leads to milk with inferior technological properties, such as cheese with an undesirable high moisture content due to a lower content of protein, casein or DM (Panthi et al., 2017). Feed composition may also influence the melting point of fat in cheese, cheese produced during the summer and grass-feeding season is usually softer than cheese made from milk produced in the hay feeding season during winter (Fröhlich-Wyder et al., 2017). In addition, both milk and cheese flavour and colour may be affected by feed, since carotenoids and sapid compounds are transferred from feed to milk (Fox et al.,

2017a). As an indirect source of microorganisms, feed may contaminate milk and thereby affect the quality of milk and products made from milk (Montel et al., 2014). Milk buffering capacity (Ong et al., 2017), casein micelle size (Devold et al., 2000) and MCP (Bittante et al., 2012) have also been shown to be affected by feed.

2.3 Milk

Milk is the liquid produced by the mammary glands of all mammals and is produced to meet the nutritional requirements of the neonate. The composition varies widely between different species. The fat content in donkey milk is 1.4 % while being 53.1 % in grey seal, and the protein can vary from 1 % in human milk to more than 11 % in grey seal (Fox et al., 2015b). These variations are linked to the maturity after birth, the growth rate and way of living. In addition to major differences across species, there is also a high variability within each species, both individually and among breeds.

Humans have consumed milk from other species for at least 8000 years (Beja-Pereira et al., 2003) and milk as itself or as dairy products derived from milk provide valuable nutrition. Over 80 % of the world's population consume liquid milk or other dairy products on a regular basis (IDF, 2021). Milk production for commercial use is almost entirely derived from cattle (81 %), buffaloes (15%), goats (2%), sheep (1%) and camels (0.5 %) (FAO, 2022a). Since the research for this thesis is based on milk from cattle, the typical composition of cow's milk is shown in Table 2.

Table 2 Composition (%) of cow's milk. Values for cow's milk are from Fox et al. (2015b). Values for Norwegian cow's milk are from the year 2021 (TINE SA, 2022b).

	Cow's milk	Norwegian cow's milk
Dry matter (%)	12.7	12.6 + ash
Fat (%)	3.7	4.38
Protein (%)	3.4	3.5
Lactose (%)	4.8	4.72
Ash (%)	0.7	-

2.3.1 Milk payment systems

Traditionally, dairy farmers were paid according to volume of delivered milk as fat content. This was because most of the milk was used for butter production and also because it was possible to measure the fat content before analysis of protein content became available. Today, milk payment methods differ across the world and has developed as the range of products and consumers preferences changes (Sneddon et al., 2013). The most common practice today is that farmers are paid according to either protein + fat, milk DM or according to a multiple-component pricing system (MCPS). Quality-based payment systems are a means by which farmers are motivated to deliver milk of high quality. The primary goal of MCPS is that the prices paid reflect the milk value as accurately as possible. Norway is one of the countries that uses MCPS. Farmers are paid a basic price depending on conventional or organic practice and during the year they will get a deduction or addition to the price depending on the month. There are also different price zones depending on the geographical location of the farm, i.e. those located in steep areas by fjords and in mountain areas are paid more than those located in areas for favorable for production. In addition, farmers are paid according to milk quality. If the protein and the fat content exceed or are less than 4 % and 3.2 % respectively, they receive an addition or a reduction for every 0.1 % unit difference. The farmers also receive a payment reduction in the case of high numbers of somatic cells, bacteria, thermostable spore-producing bacteria and free fatty acids, or if remains of antibiotics is found (TINE SA, 2022a).

There is a focus on improving cheese-making efficiency of milk in several areas. In southern Italy, where mozzarella cheese from buffalo milk is produced, the farmers are paid according to milk volume. However, there is work ongoing to switch to a quality-based payment system in order to improve cheese-making efficiency by increasing yield and overall quality of the cheese (Costa et al., 2020). Switching to a quality-based payment system encourages the farmers to improve the hygiene and care of the animals, as well as the feeding practice to improve milk composition for maximum economic return.

In areas where Parmigiano-Reggiano is produced, the farmers are paid by a less conventional method. The coagulation properties of herd milk samples are analysed every 15 days and the resulting values are used to reward or penalize the farmers (Malacarne et al., 2014). There are a number of studies, mentioned by Bittante et al. (2012), both at laboratory and industrial level that confirms that the analysis of MCP

gives information that is relevant for cheese production, such as cheese yield and quality.

As previously stated, protein content is often a part of the payment system today. This has been implemented due to the increased production of dairy products that are reliant on protein content, such as cheese (Fox et al., 2015a). However, in cheese manufacture, the production is not dependent on the total protein content, but on the casein content. Due to the global increase in cheese production and thereby the importance of casein, it is important to evaluate how feeding affects milk casein content. This can lay the foundation for a modified payment system, where the farmers are paid according to the casein content in milk.

2.3.2 Milk processing

Of the milk produced in the European Union (EU), only 11 % is used as liquid milk, and the rest is used for production of other dairy products. Cheese is one of the most important dairy products accounting for 37.7 % of the total use of milk in the EU, while fresh fermented milk products account for 4.3 % (European Union, 2019). The dairy sector in the EU is changing, towards a production that uses a higher fraction of the produced milk for cheese production. The milk production has increased slowly and steadily during the last decade but is now estimated to flatten out towards 2031 (OECD/FAO, 2021). At the same time, the production of cheese has increased more than the milk production. Cheese is estimated to generate the highest value towards 2031, and in general, value-added milk products are set to increase during the next decade.

In Norway, there is also a changed consumption pattern reflected in the processing in milk. The production of liquid milk has decreased by 14 % between 2007 and 2021 (Figure 3). In the same timeframe, the population in Norway has increased by 15 %. Despite this, the production of rennet-coagulated cheeses has increased by almost 13 % (consumption pr. Person). The production of yoghurt (all types) has increased by 19 % (consumption pr. Person). This clearly illustrates a change in the diet of Norwegians whereby they choose other dairy products instead of liquid milk.

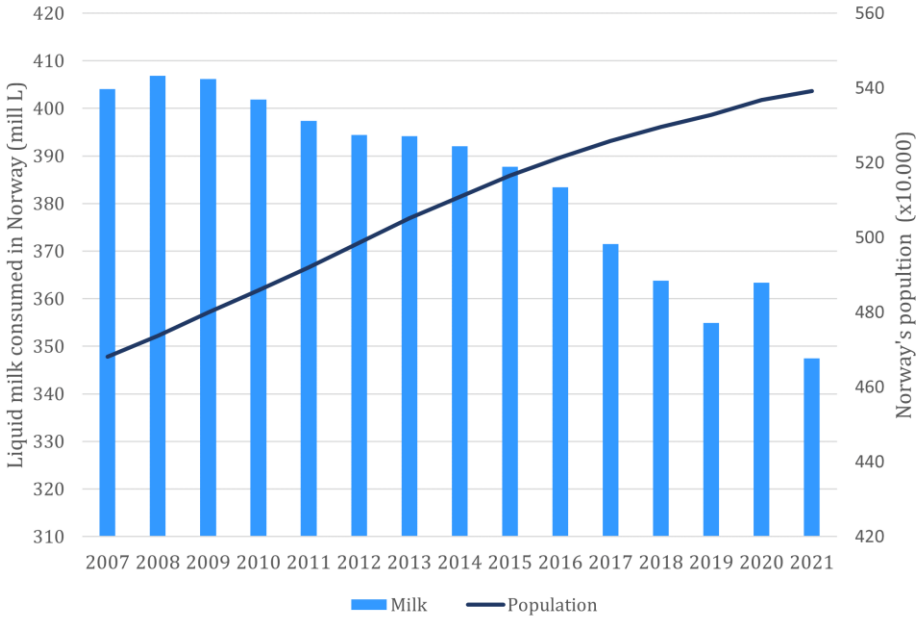

Figure 3 Presentation of the consumed volume of liquid milk (mill L) and the population (in 10.000) in Norway from 2007-2021. Population data from Statistics Norway (2022b) and milk data from Opplysningskontoret for Meieriprodukter (2022).

Compared to liquid milk, in the production of cheese and acidified milk products, the milk components play a technologically vital role. Liquid milk generally undergoes standardization, homogenization and pasteurization; unit processes that do not significantly change the raw material. In contrast, during cheese production, milk is converted to a totally different product where the milk is concentrated and undergoes a coagulation process (Chapter 2.6). Casein (Chapter 2.4) is especially important and affects the coagulation as well as the quality of the finished product (Panthi et al., 2017).

2.4 Casein

Milk protein content comprises about 3.4 % of bovine milk (Table 2). Initially, it was believed that milk contained only one type of protein. However, around the year 1880 the Swedish scientist Olav Hammarsten showed that milk protein could be fractionated into two groups. If the pH of milk is adjusted to 4.6, around 80 % of the total protein is precipitated. This is known as the casein fraction. The proteins that remain soluble at pH 4.6 are referred to as whey proteins (Fox et al., 2015a). The ratio of casein to whey proteins varies considerably between species (Fox et al., 2015a) and also during the lactation period (Fox et al., 2015a). The ratio is also affected by somatic cells and genetic protein variants (Heck et al., 2009).

There are four genes for casein, which code for α_{S1}-CN, β-CN, α_{S2}-CN and κ-CN. These genes are located on the bovine chromosome 6 coded as CSN1S1, CSN2, CSN1S2 and CSN3 respectively (Caroli et al., 2009). They account for approximately 40 %, 35 %, 10 % and 15 % respectively of the casein fraction of bovine milk. Caseins are non-globular phosphoproteins and contain, on average, 0.85 % phosphorus. The phosphate groups are important for many characteristics of casein, both nutritionally but also technologically. In milk, several thousands of casein molecules together with colloidal calcium phosphate (CCP) form aggregates called casein micelles. The structure of the micelle has been extensively investigated over the years and there are still different views on the detailed structure of the micelles. However, there is a common agreement on the general structure and properties of the micelle. The hydrophilic C-terminal of κ-CN protrudes from the micelle surface, provides a steric and electrostatic repulsion which prevents micelles to aggregate. The brush is ~ 7 nm long (Müller-Buschbaum et al., 2007). The casein micelles are polydisperse have an average diameter of ~ 150 nm (Müller-Buschbaum et al., 2007; O'mahony & Fox, 2013) and contain about 2-3.5 g of water per gram of protein (Dalgleish & Corredig, 2012; Goulding et al., 2020), causing its high hydration potential. Even though caseins represent about 2.5 % of the weight of the milk, they occupy around 10 % of the volume due to this high hydration capacity (Dalgleish & Corredig, 2012). Most models that have been proposed over the years have not considered the location of this large amount of water present inside the micelle. The model proposed by Dalgleish and Corredig (2012) has accounted for this large hydration as in shown in Figure 4.

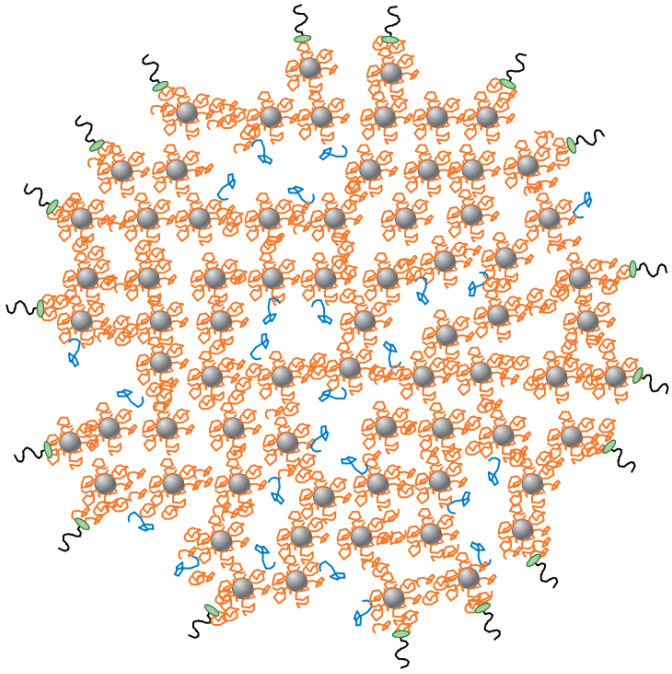

Figure 4 Proposed structure of the casein micelle by Dalgleish and Corredig (2012). The α_s – and β-caseins are represented by orange colour, hydrophobically bound β-caseins is shown by blue colour. Colloidal calcium phosphate (CCP) is represented by grey dots and κ-casein are located at the surface of the micelles with caseinomacropeptide (CMP) as black chains and para-κ-casein as ovoid shaped green dots.

The casein micelle consists of 94 % caseins and 6 % CCP. In addition to CCP, casein micelles also contain phosphate as phosphorylated serine groups in the caseins. All the caseins are phosphorylated, but in different degrees. κ-CN contains the least, with 1-2 phosphate groups while α_{s2}-CN can bind the most from 10-13 phosphate groups. α_{s1}-CN bind 8 or 9 while β-CN binds 4 or 5. CCP is located inside the micelle bound to casein via phosphoserine residues (Horne, 2006), as illustrated in Figure 5. CCP acts like glue to bind the micelle together, and if removed (using i.e. EDTA), the whole micelle collapses (Griffin et al., 1988). Phosphorylated serine groups are classified as organic phosphate, and phosphate in CCP is inorganic phosphate (Fox et al., 2015a).

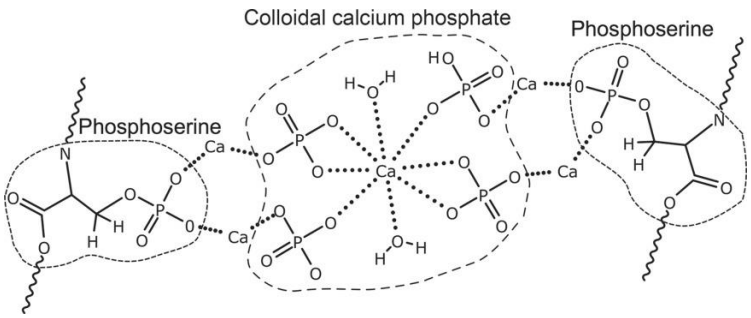

Figure 5 Inter-protein linkages by calcium-bridged phosphoserine to colloidal calcium phosphate (CCP). Figure from Hindmarsh and Watkinson (2017).

Casein micelles have an important role in milk: to deliver important nutrients (calcium, phosphate and protein) to the neonate. In addition, the micelles also have a very important role during the production of many dairy products. They are vital in the coagulation process which occurs when making most cheeses (Chapter 2.6) and fresh fermented milk products such as yogurt and sour cream. The structure and the stability of the micelles is affected by cooling, heat treatment, addition of calcium chelatants, rennet and NaCl, acidification as well as other treatments such as ultrasound and high pressure (Gaucheron, 2005). The casein micelles are quite stable to the regular processing that milk undergoes, including pasteurization and homogenization. However, the controlled destabilization of the casein micelle during production of cheese and acid precipitated dairy products is of primary interest for the dairy industry (Bijjl et al., 2020). Casein is probably the component in milk that contributes most to efficiency and profitability when processing milk.

2.5 Genetic protein variants

Milk proteins are polymorphic, which means that there are variations in the amino acid sequences of the proteins. These types of variations will change the quality, milk composition and physicochemical properties of milk by altering the isoelectric points, electric charge and the hydrophobicity of the proteins. All of the major milk proteins have different numbers of known genetic variants dependent on different breeds (Caroli et al., 2009). The known casein genetic variants in bovine milk are shown in Table 3-6.

Table 3 Changes in bovine α_{S1}-casein variants. DEL: deletion of corresponding sequence. Amino acids in the reference variant is shown by bold text. Table modified from Martin et al. (2013).

	Position							
Variant	14-26	53	51-58	59	64	66	84	192
A	DEL							
B		**Ala**		**Gln**	**SerP**	**SerP**	**Glu**	**Glu**
C								Gly
D		ThrP						
E				Lys				Gly
F					Ser	Leu		
G								
H			DEL					
I							Asp	Gly

Table 4 Changes is bovine β-casein variants. Amino acids in the reference variant is shown by bold text. Table modified from Martin et al. (2013).

	Position												
Variant	18	35	36	37	52	67	72	93	106	122	138	152	?(114-169)
A1						His							
A2	**SerP**	**SerP**	**Glu**	**Glu**	**Phe**	**Pro**	**Gln**	**Met**	**His**	**Ser**	**Pro**	**Pro**	**Gln**
A3									Gln				
B						His				Arg			
C		Ser		Lys		His							
D	Lys												
E			Lys										
F						His						Leu	
G						His					Leu		
H							Glu	Leu					Glu
I								Leu					
J					Ser								

Table 5 Changes in bovine α_{S2}-casein variants. DEL: deletion of corresponding sequence. Amino acids in the reference variant is shown by bold text. Table modified from Martin et al. (2013).

	Position				
Variant	8	33	47	51-59	130
A	**SerP**	**Glu**	**Ala**		**Thr**
B	Phe				
C		Gly	Thr		Ile
D				DEL	

Table 6 Changes in bovine κ-casein variants. INS: insertion of corresponding sequence. Amino acids in the reference variant is shown by bold text. Table modified from Martin et al. (2013).

	Position										
Variant	10	36	97	104	130	135	136	148	148-151	153	155
A	**Arg**	**Pro**	**Arg**	**Ser**	**Pro**	**Thr**	**Thr**	**Asp**		**Ile**	**Ser**
B							Ile	Ala			
B2							Ile	Ala		Thr	
C			His				Ile	Ala			
D			His								
E											Gly
F1								Val			
F2	His										
G1			Cys			Ile					
G2								Ala			
H							Ile				
I				Ala							
J							Ile	Ala			Arg
K		Leu			Arg			Ala			
L								Ala	INS		

One of the most important effects of genetic variants of milk protein is their relation to cheese-making efficiency, which is extremely important for profitability in the dairy industry. The composition of milk proteins varies with lactation, health status of the cow, etc., but is predominantly determined by genetic factors (Heck et al., 2009). Milk protein genetic variants are known to affect milk pH, mineral content, total protein content, casein micelle size and composition of milk protein (Bittante et al., 2012), which are all factors that are known to affect MCP and thereby the efficiency of cheese-making.

The objective of breeding is to identify genetically superior traits in male and female animals and to use this information to select parents for the next generation. Genetic selection has been and is still a successful tool for improvement of dairy cows populations (Brito et al., 2021).There are different genotype frequencies of the different genetic protein variants between herds and breeds. For α_{S1}-CN, the B variant is most common in most European cows, followed by the C variant (Lien et al., 1999). For κ-CN, the A variant is usually more common than the B variant in European cows and the E variant is the least frequent variant (Gai et al., 2021).

I Norway, breeding bulls are genotyped for β- and κ-CN, but this information is not currently used for breeding (B. Heringstad, GENO, Hamar, Norway and Norwegian University of Life Sciences (NMBU), Ås, Norway, personal communication). However, there have been developments in the frequencies of the κ-CN B and E variants during the last 15 years where the B variant has increased significantly while the E variant has decreased (GENO, 2020b). This development is linked to increased milk yield, which has been an important trait to breed for, and κ-CN B is associated with a higher milk yield (Mao et al., 1992). In Italy there has been focus on cattle breeding to get genetic protein variants giving a higher milk casein content since this improves cheese yield (Malacarne et al., 2014).

Ketto et al. (2017b) mapped the genotypes in 118 NR cows at the Animal Production and Experimental Unit at NMBU. This herd is representative for the genetic variation of the NR breed and is the same herd that was used in both the FEED study (spring 2019) and the GPV study (summer 2018). Ketto found that the most frequent variants for the caseins were: α_{S1}-CN B (91.1 %), β-CN A2 (79.7 %) and κ-CN A (48.3 %) closely followed by B (45.7 %).

2.6 Rennet coagulation of milk

One of the first steps during cheese manufacture is the conversion of liquid milk to a milk gel. This is usually achieved by rennet coagulation.

Rennet coagulation of milk can be divided in two overlapping steps

- Primary (enzymatic hydrolysis)
- Secondary (aggregation)

In the **primary** stage, κ-CN is cleaved by rennet at the Phe_{105}-Met_{106} bond. The peptide 1 to 105 is called para-κ-CN (N-terminal) and remains attached to the micelle. The C-terminal is hydrophilic and diffuses from the micelle after hydrolysis. This peptide is called either caseinomacropeptide (CMP) or glycomacropeptide (GMP). During the primary stage, the casein micelle becomes vulnerable to aggregation because when the negatively charged CMP is lost, the surface charge of the micelle is reduced. This reduces the steric repulsion forces between the different micelles permitting a closer approach. The primary stage of rennet coagulation happens independently of the concentration of calcium. However, there is an indirect effect since a higher content of Ca^{2+} in the serum phase of milk gives a lower pH which promotes the rennet activity.

It is during the **secondary** stage of rennet coagulation that aggregation of casein micelles occurs. The aggregation starts before the primary stage is complete, when about 85 % of κ-CN is hydrolyzed. Aggregation is dependent on the milk casein content. If the casein content in increased, the secondary stage starts earlier (Fox et al., 2017c; Horne & Lucey, 2017). Aggregation also starts at a lower degree of κ-CN hydrolysis if the temperature or Ca^{2+} concentration is increased, or if the pH is reduced (Horne & Lucey, 2017). Ca^{2+} is essential for the aggregation of casein micelles.

Both the primary and secondary stages are affected by preheating of milk (pasteurization conditions)(Horne & Lucey, 2017). This effect comes from the denaturation of β-lactoglobulin (β-LGB), that will connect to κ-CN via disulfide bonds. This will inhibit the rennet to come close to the cleaving site, thus prolonging the renneting process.

Figure 6 shows the aggregation of casein micelles following cleavage of κ-CN. The gelation process in the secondary stage starts with single casein micelles colliding

with each other. The casein micelles are gradually connected in a three-dimensional network that encloses milk fat globules and the serum phase (whey). The fat globules are only occluded in the gel and do not contribute to the network that is created. The gel becomes firmer and elastic during the coagulation process.

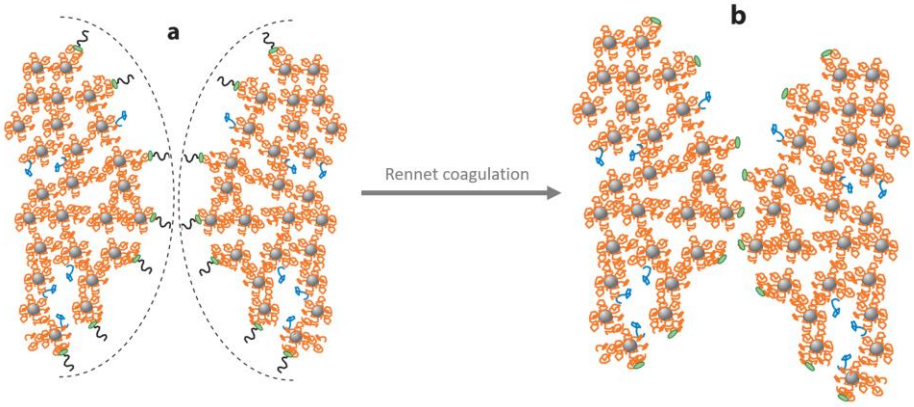

Figure 6 Rennet coagulation. (a) showing that the hydrophilic caseinomacropeptide (CMP) are stabilizing the micelles. (b) When adding rennet and during the coagulation process the structure of the micelles changes. The rennet cuts of the hydrophilic CMP and the micelles lose the stability and bumps together. Modified figure from Dalgleish and Corredig (2012).

Rennet coagulation of milk is of great importance for the dairy industry as it is the foundation for the cheese-making process and affects both the quality of the finished cheese and the cheese yield (Jensen et al., 2012a; Kübarsepp et al., 2005). The first and most important requirement for milk to be used in cheese-making is the ability to coagulate when rennet is added (Malacarne et al., 2014). MCP are affected by milk composition, casein micelle size, milk protein genotypes, milk protein content and composition, ratio of casein and whey proteins, mineral content and composition in addition to the health status of the cow, lactation stage, breed, season and feeding (Gai et al., 2021).

The minerals in milk, especially calcium and phosphate play an important role in the structure of casein micelles and MCP. It is well known that the addition of calcium chloride reduces rennet coagulation time (RCT, min) and increases firmness after 30 min (A30, mm). Milk that contains a higher amount of Ca^{2+} produces firmer gels (Tsioulpas et al., 2007). It has been shown that Ca^{2+} is associated with a shorter time

until firmness of 20 mm is achieved (K20, min) and that Na^+ is associated with a longer RCT and K20 and a lower A30 (Panthi et al., 2017). Phosphorous has been reported to be correlated with good cheese-making traits (Stocco et al., 2021) and in CCP it greatly affects MCP and cheese yield (Lucey & Fox, 1993). However, the direct effect of phosphorous has not been studied to a high degree.

There has been extensive work on the effect of genetic protein variants on the MCP the last decades (Ikonen, 2000; Ketto, 2017; McLean et al., 1984; Ng-Kwai-Hang, 2006; Nilsson, 2020). Most studies has shown that the B variant of κ-CN and β-LGB are associated with the best curd firmness compared to the A variant (Ng-Kwai-Hang, 2006). The BC genotype of α_{S1}-CN has earlier proved to have good MCP (Jensen et al., 2012b; Jõudu et al., 2009; Ketto et al., 2017a; Poulsen et al., 2013).

The latest extensive work collecting data about milk protein variants and coagulation properties in NR cows was done by Ketto (2017) and Swedish Red (SR)(that shares many similarities with NR) by Nilsson (2020). Ketto concluded that κ-CN B, α_{S1}-CN C and β-CN A1 were related to improved rennet coagulation properties in milk from NR. There has been a general lack of focus on the α_{S1}-CN genotypes and their effect on cheese-making properties. According to Ng-Kwai-Hang (2006), this is due to the high frequency of variant B and the lack of variant A and C in most dairy cattle populations. This was the main reason for selecting different genotypes of α_{S1}-CN in the GPV study.

There are few published studies on how feeding affects MCP. However, it is well known that the feeding affects milk quality and composition, which again will influence MCP. Verdier-Metz et al. (1998) compared feeding hay or silage (which was produced from the same forage) to dairy cows in a crossover design. Saint-Nectaire type cheese was produced. Protein content in the resulting milk were similar but casein content was not analysed. Milk from cows fed hay showed a shorter renneting time and this was explained by the lower milk pH. Moreover, a higher cheese yield and DM recovery was attained when producing cheese from milk from cows fed hay. This was explained by the tendency of that milk to have a higher fat content.

2.6.1 Methods of measuring milk coagulation properties

There are various methods that can be used to measure MCP. One common method is called low amplitude oscillation rheometry (LAOR). Using this method is barely non-destructive to the formed gel, but takes only one sample at a time and is therefore quite inconvenient in many research settings where the sample load is large. A method that is more efficient is lacto-dynamographic analysis (Bittante et al., 2012). This method was used in the current work. The Lattodinamografo unit (a digitalized version of the previous Formagraph) is one of the most common dynamic measurement instruments to monitor milk coagulation. It consists of a heated metal block, a sample rack with cavities and a set of pendulums. The movement of the pendulums is measured by a transducer and then electronically captured (Fox et al., 2017c). This analysis measures the viscosity of milk at a fixed temperature following addition of rennet. The primary stage of rennet coagulation is described by the RCT (Kübarsepp et al., 2005). The efficiency of the second stage can be measured by K20 and A30. A typical curve from the Lattodinamografo unit is shown in Figure 7.

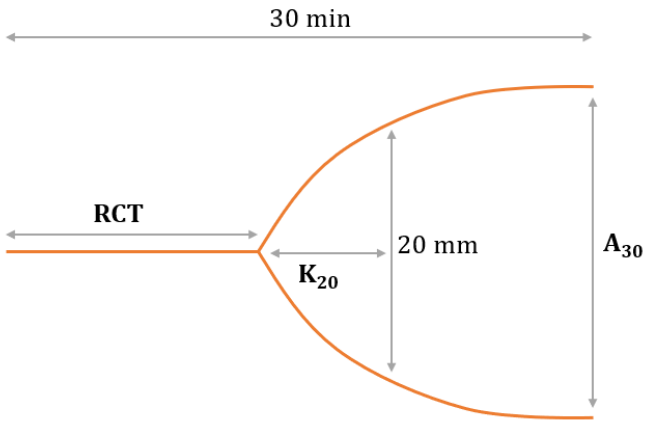

Figure 7 A typical curve from the Lattodinamografo instrument for measuring milk coagulation properties (MCP). RCT = Rennet coagulation time (min), K20 = time until firmness of 20 mm is achieved (min) and A30 = firmness after 30 min (mm). Figure modified from Panthi et al. (2017).

It is considerably easier to measure MCP in laboratory scale when an instrument such as the Lattodinamografo is used where specific values for RCT, K20 and A30 are obtained. There are other instruments that can monitor curd firmness

development in-vat, but are rarely used commercially due to hygienic and practical difficulties (Horne & Lucey, 2017). Instead, the firmness at cutting is usually determined by an experienced cheesemaker. This method does obviously not give specific values for MCP, and it is only the time from rennet addition until cutting that, in most scenarios, can be used as a value for MCP.

2.7 Cheese yield

Cheese yield and the composition of milk are important parameters for the cheese-making industry as they are the basis for milk payment, profitability and the efficiency of the cheese-making (Johnson, 2017). Cheese-making is a dehydration process, where casein and fat are concentrated while water is removed. This means that the fat and casein content in milk will directly affect cheese yield and thus the cheese-making efficiency. Water is also a main component in cheese and greatly affects the actual yield (Ya). Cheeses with a high moisture content will naturally attain a higher cheese yield. It is therefore of economic interest for the dairy industry to increase the water content in cheeses to the designated maximum limit for each cheese variant without it detrimentally affecting cheese quality.

Several factors affect the yield of cheese. The most important of these are the quality and composition of raw milk, milk treatment and storage practices, pre-treatments such as standardization of protein and pasteurization temperature, firmness of the gel at cutting, stirring practice and rate of cooking (Fox et al., 2017b). Casein is the dominant factor affecting milk coagulation, curd firmness, rate of syneresis and moisture retention and thereby affects cheese quality and yield (Jenkins & McGuire, 2006; Jõudu et al., 2008).

Coagulum strength is an important factor for maximum recovery of fat and casein in cheese (Bynum & Olson, 1982; Guinee et al., 1997). Casein micelles that are not a part of the network or fat globules that are not occluded in the network will be lost to the whey after syneresis and whey drainage. This underlines the extent that MCP affects cheese yield.

The casein index is the term given to the proportion of casein in relation to total protein content. Usually this is called the ratio of casein:whey proteins (Visker et al., 2010). This term is useful for comparing different milks with the same total protein content. If the total protein content is high in addition to a high casein index, this will result in a high cheese yield. However, if the casein index is high, but the protein content is low, the term is not useful. Therefore, focus on the casein content should be enough. This is a wider, easier and more adaptable index to use when estimating cheese yield.

Most research about how genetic protein variants affect cheese yield are connected to variants of κ-CN and β-LGB. The BB genotype of κ-CN and β-LGB have been associated with higher cheese yield for a range of cheese varieties when compared to the AA genotype (of both) (Fox et al., 2017b). It has been shown that κ-CN B gives milk with a higher content of total protein and κ-CN and that the micelles are smaller (Bijl et al., 2014; Day et al., 2015; Gai et al., 2021). These are factors that gives good MCP, leading to a strong gel that increases the recovery of casein and fat which results in a higher cheese yield. The BC genotype of α_{S1}-CN has been reported to give a higher yield of Parmesan cheese. However, the BB genotype is associated with a greater milk yield, which results in a higher total cheese yield during the whole lactation compared to milk with α_{S1}-CN BC (Fox et al., 2017b).

A high-quality diet with sufficient DM intake is necessary for attaining high-quality cheese. Previous studies have shown that a low quality diet results in cheese with higher moisture content and a lower cheese yield (Fox et al., 2017b; Kefford et al., 1995). However, there is a limited amount of research on how feeding affects cheese yield, since the main focus during feeding studies is cow performance and the gross milk composition (fat, total protein and lactose) and total milk yield. Soryal et al. (2004) found that a higher concentrate level in feed for goats resulted in a higher cheese yield. This was due to a higher fat, protein and DM content in the milk. Casein was not analysed. Gulati et al. (2018) found that cheese yield increased when cows were grazing on perennial ryegrass pasture or perennial ryegrass and white clover compared to cows that were housed indoors and offered total mixed ration. Milk volume and component masses were not analysed. Zhang et al. (2006) found that ewes fed an supplementation of flaxseeds gave milk with a higher casein yield (due to a higher milk yield) compared to the control diet, but there were no differences in casein content (%). However, both control diet and diet with flaxseed supplementation gave milk with a higher casein content compared to diet with sunflower supplementation.

2.7.1 Cheese yield calculations

The Ya (E1) is simply measured by weighing the milk before cheese-making and then the cheeses after production. This is an easy method for determining cheese yield. However, the Ya only focuses on weight and does not consider possible differences in moisture content in cheese. Moisture-adjusted cheese yield (MACY) (E2) is a formula that eliminates the direct effects of differences in cheese moisture content. This allows the yield of cheeses with different moisture contents to be compared (Fox et al., 2017b). Especially during pilot-scale production where the effect of different treatment factors on cheese yield is evaluated, it might be necessary to use the MACY formula.

In addition to these formulae for calculation how much cheese has been obtained; it is also possible to predict cheese yield before cheese-making. Predicted yield (PY) formulae need to consider many factors: fat and casein content in milk, desired cheese composition and expected loss of fat and casein (Fox et al., 2017b). The recovery of fat and protein is partly determined by the type and condition of the equipment during production. Therefore, every cheese plant should collect data over a period of time and make their own PY formulae for every cheese variety they produce in order to make the formulae as specific as possible. In the pilot plant at NMBU the cheese-making is research-based. Different types of cheese-vats are used depending on the available milk volume. Cheese from both cow's and goat's milk is produced. Often the cheese variety is dependent on the milk volume available, and if new cheeses are to be analysed at each sampling time during ripening, then this may necessitate the production of small cheeses. Due to the high production variability and that the cheese-making is for research purposes, PY formulae for the dairy pilot plant have not been made.

Different PY yield formulae exist, and to be used in commercial cheese-production they should be modified, as previous stated. The first known PY formula only factored in the casein and fat content in the milk. It apparently appeared in a report by Babcock 1895 (source not found)(Emmons & Modler, 2010). The next formula was developed by Van Slyke and Publow (1910) and it included moisture, loss of fat and casein and cheese salt. This formula has been known as the Van Slyke formula and has been widely used. Later, more complicated formulae have been developed that include for example whey solids, and para-casein instead of casein. In the FEED and GPV study, the Van Slyke formula was used for PY (E0) calculation due to practical circumstances.

PY calculations are useful for the industry because they allow for measurement of cheese-making efficiency by comparing Ya and PY (Fox et al., 2017b). These types of calculations are also useful during research, since it is possible to compare the efficiency between different treatments groups. The percentage yield efficiency (% YE) (E3) compares the Ya with the PY (Fox et al., 2017b). The ideal YE is 100 %, however, it is possible to attain a YE both less and higher than 100 %. If the Ya is higher than the PY, this indicates that the cheese has a higher moisture content than predicted and this may suggest that the manufacturing process should be changed to attain a cheese with correct contents of the different components (Emmons et al., 1990). As Emmons and Modler (2010) wrote *"The formula does not identify the problem, but can identify that the problem exists"*. However, usually the opposite happens, that the Ya is lower than the PY. This indicates that the cheese-making needs to be optimized to reduce loss of casein and fat, or that the moisture content has to be increased.

$$\textbf{PY (Van Slyke)} = \frac{(0.93\ F + C - 0.1) \times 1.09}{100 - W} \times 100 \qquad \text{(E0)}$$

Where:
F - Fat in milk (%)
C – Casein in milk (%)
W – Desired water content in cheese
0.1 – Constant for loss of cheese fines in whey
1.09 – Constant representing other solids included in the cheese

$$\textbf{Ya} = \frac{\text{Weight of cheese (kg)}}{\text{Weight of milk (kg)} + \text{weight of starter(kg)}} \times 100 \qquad \text{(E1)}$$

$$\textbf{MACY} = \text{Ya} \times \frac{100 - \text{actual cheese moisture content (\%)}}{100 - \text{reference cheese moisture content (\%)}} \qquad \text{(E2)}$$

$$\textbf{\textit{YE}} = \frac{\text{Ya}}{\text{PY (Van Slyke)}} \times 100 \qquad \text{(E3)}$$

3 Main results and discussion

3.1 Cheese-milk casein content

The casein content in fat-standardized cheese milk, in both the FEED study and the GPV study, was analysed by a MilkoScan FT1 (Foss Electric A/S, Hillerød, Denmark). The results are shown in Figure 8.

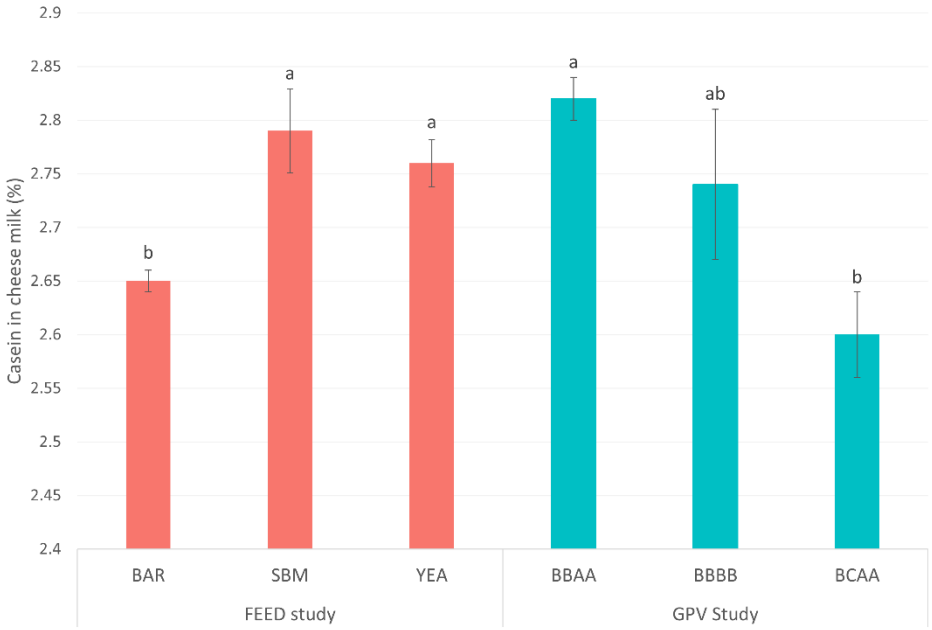

Figure 8 Casein content (%, mean ± SD) in cheese milk in the FEED study (Study explained in Figure 1) (■) and the GPV study (Study explained in Figure 2) (■). Significant differences ($P < 0.05$) within each study is marked with different letters.

Figure 8 clearly shows that both protein source (possible protein content as SBM and YEA concentrates contained a higher protein content than BAR concentrate) in concentrate feed for dairy cows and genetic milk protein variants affect the casein content in milk. One treatment in each experiment shows a particular negative effects: the BAR concentrate in the FEED study and the α_{S1}-κ-casein BCAA genotype

in the GPV study. A casein content below 2.7 % was found in these cheese milks. The genetic protein variants were balanced in the FEED study, and this demonstrates that even if genetic protein variants are one of the main factors affecting milk protein composition, protein content or the protein source in concentrate feed has a significant impact on milk casein content.

It is well known that milk protein genetic variants alter the total protein content, casein content and composition of the different proteins in milk. Therefore, it is not surprising that there are differences in casein content in milk from the GPV study. However, there have been considerably fewer studies relating to how feeding practice affects the content and composition of milk. The reason for this is probably because it was stated early that feeding only had minor effect on protein content (Jenkins & McGuire, 2006). During planning of the FEED study, differences in milk casein content were not an expected outcome of the study. However, some feeding trials in the 1990's analysed the casein:total protein ratio in milk from cows fed different diets. Coulon et al. (1998) found that increasing the energy content in the cow's diet resulted in a higher milk protein content, while the casein:total protein ratio remained unchanged. Malossini et al. (1996) found that the milk protein and casein content increased if the cows were fed with an energy level over the allowances (overfeeding), but the casein:total protein ratio remained unchanged.

The individual milk samples in the FEED study were sent for analysis using the same standard practice by which the commercial dairies operate. This is regular practice in most feeding studies. However, measurement of casein is not a part of the standard analytical procedures and for this reason these results are lacking. The casein results during cheese-making indicate that it is important to focus on casein, and that both feeding studies and commercial dairies should include casein measurement in their standard analyses. Increasing feed efficiency is an important goal, and since casein content plays a significant role in processing of cheese, it is an important component for assessing feed efficiency.

3.2 Milk coagulation properties

3.2.1 During cheese-making

The renneting time during cheese-making in the FEED and the GPV study was measured. The relationship between milk casein content and renneting time is shown in Figure 9. A Havarti-type cheese was made in the GPV study and a Gouda-type cheese in the FEED study. These two cheese varieties have different fat in DM content and the fat content in cheese milk was 4.17 % and 2.72 % for the Havarti- and Gouda-type cheese, respectively. The rennet type (Chy-Max Plus, Chr. Hansen, Copenhagen, Denmark) and rennet addition (mL/L milk) were the same in both studies.

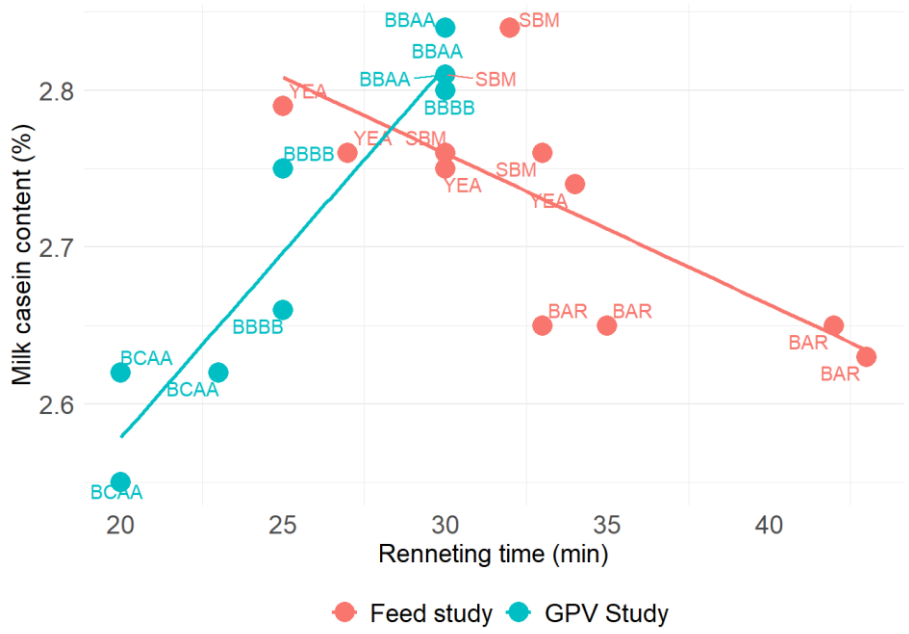

Figure 9 Relationship between milk casein content (%) and renneting time (min) during cheese-making in the FEED study (Study explained in Figure 1) (●) and the GPV study (Study explained in Figure 2) (●). Cheese milk had different fat content (2.71 % in the FEED study and 4.17 % in the GPV study). Figure created using the ggplot2 package (Wickham et al., 2022) in RStudio (R Core Team, 2022).

Figure 9 shows a shorter renneting was seen in the GPV study compared to the FEED study. An increased fat content is known to reduce K20 and increase A30 (Panthi et al., 2017) explaining why the milk in the GPV study seemed to coagulate

faster than the milk in the FEED study. There is a general agreement that MCP significantly improves with an increase in protein or casein content (Jõudu et al., 2008). The results from the FEED study confirms this, with a longer renneting time correlating to lower milk casein content. However, opposite results were seen in the GPV study, as a longer renneting time was experienced when the milk casein content was higher. This is mainly due to the BCAA milk. Milk with α_{S1}-CN BC had a lower casein content but obtained a shorter renneting time compared to the BB genotype with a higher casein content. As stated earlier, the BC genotype is known to have good MCP. Some studies have explained this by a higher milk casein content (Jakob, 1994), but there are conflicting results concerning the effect of α_{S1}-CN BC genotype on casein content (Gai et al., 2021). Both the GPV study and the study by Devold et al. (2000) found a lower casein content in milk from NR cows with α_{S1}-CN BC, this suggests that other factors than casein content have a high impact on renneting behavior of milk, e.g. structure of the caseins.

Casein micelle size was not measured in either the FEED or the GPV study. However, the BC genotype of α_{S1}-CN has earlier been associated with smaller casein micelles (Devold et al., 2000; Ketto et al., 2017b). Ketto found no differences in casein micelle size between k-CN AA and BB. It is generally accepted that smaller casein micelles coagulate faster, and that the resulting gel is firmer compared to milk with larger casein micelles (Glantz et al., 2010; Logan et al., 2015). This is probably due to a larger surface area of the smaller casein micelles and this contributes to a stronger gel-network compared to that formed by larger casein micelles. Ketto et al. (2017b) found that variant C of α_{S1}-CN was associated with a higher concentration of κ-CN, and this probably lead to the formation of smaller casein micelles because it allows for a larger surface area.

The good MCP of the BCAA milk can also be explained by the higher lactose content compared to BBBB and BBAA milk ($P = 0.003$). Higher lactose content has previously been associated with better coagulation properties (Glantz et al., 2010; Ketto et al., 2017b; Malacarne et al., 2014), but the mechanism is not understood. It is possibly due to the contribution of the higher lactose content to the DM content in the milk. DM were not measured in milk from the GPV study, but when adding fat + total protein + lactose (from the MilkoScan FT1 (Foss Electric A/S, Hillerød, Denmark)), BCAA (and BBBB) milk had a lower total content of these components compared to BBAA and this can thereby not explain the good MCP of BCAA milk.

In the FEED study, milk protein genetic variants were balanced between the groups, thus making it possible to reduce the effect that the genetic protein variants have on the cheese-making. The result from this study shows that if the genetic protein variants are balanced between the treatments groups, the milk casein content is a good indicator for renneting time, where a higher milk casein content results in a shorter renneting time. Even though the genetic protein variants were balanced between the groups, 73 % of the cows in the experiment had the AA genotype of κ-CN. This genotype is known to have poor MCP (Gai et al., 2021). Despite this, if the protein content in concentrate feed was high enough (SBM and YEA), the negative effects associated with κ-CN AA were not seen.

A higher content of κ-CN improves MCP (Wedholm et al., 2006). The relative concentrations of the different caseins were analysed in the FEED study but, due to sample size issues, these results were not published. Nevertheless, the differences in the relative concentration of κ-CN (Week 10 – week 2 (adaptation period)) for the diets were: BAR (-0.39), SBM (0.34) and YEA (0.91). This indicates that the relative concentration of κ-CN increased when switching from SBM to YEA, but decreased when switching to BAR. However, as the relative concentration of κ-CN also increased in the SBM group, this can be explained by other factors related to e.g. milk yield and/or lactation stage.

RCT is inversely related to enzyme concentration (Fox et al., 2017c). Since rennet is the active agent leading to coagulation, and because casein is the coagulable material, there should be a balance in the rennet:casein ratio. Rennet addition was not adjusted to the casein content in either the GPV or FEED studies. Industrially, in Norway, rennet is still added according to milk volume (% v/v) and we used this practise during these cheese-makings. In the FEED study, significantly more rennet pr. unit casein was added to the BAR milk compared to the SBM and YEA milk. This has probably given the BAR milk an undeserved short renneting time compared to if the rennet:casein ratio had been standardized between the treatments groups.

Moreover, a possible explanation for the longer renneting time in the BAR milk may also be its higher content of Na and lower content of P (Table 7). As stated in Chapter 2.6, a higher content of Na and a lower content of P have been associated with poorer MCP. The Ca content was similar in milk from all diet groups and can therefore not explain the differences in MCP during cheese-making.

Table 7 Minerals in cheese milk (mean ± SD) obtained from the FEED study (Study explained in Figure 1). Significant differences (P < 0.05) of the means of each concentrate feed are marked with different superscript letters.

Minerals	BAR (n=4)	SBM (n=4)	YEA (n=4)	P-value
Na (mg/kg)	338 ± 5.0[a]	325 ± 5.8[b]	335 ± 5.8[ab]	0.041
P (mg/kg)	957 ± 5.0[b]	992 ± 15[a]	985 ± 13.0[a]	0.013
Ca (g/kg)	1.20 ± 0.0	1.20 ± 0.00	1.20 ± 0.00	NS

3.2.2 Individual milk samples (The FEED study)

In the FEED study, individual milk samples were analysed for MCP using a Lattodinamografo. Most of the milk samples demonstrated poor MCP with a long RCT and low A30. Due to a high degree of variation within each of the diet groups, no significant differences in RCT, K20 and A30 were observed. There was a borderline significant difference in RCT ($P = 0.053$) between BAR (19.8) and SBM (17.9) milk. Only 33 % of the total number of samples attained K20 and, due to the low number of samples, this information was instead transformed to a binary dataset: Samples that attained K20 and samples that did not attain K20. This showed that if cows were fed YEA, the milk was more likely to attain K20 compared to BAR and SBM milk.

The inferior MCP observed for individual milk samples in the FEED study has not previously been an issue with milk from the same herd. When grouping the cows in the FEED study, it was decided to use cows with genotypes having the highest frequency at the farm. This herd had a high prevalence of κ-CN AA (73 %) and β-CN A2A2 (63%), both of which are related to inferior MCP (Ketto et al., 2017b). However, during the actual cheese-making, inferior MCP was not observed. The reasons behind the different behaviour of milk during cheese-making and during the Lattodinamografo analysis is not known. Negative effects of individual milk samples are possibly levelled out when using bulk milk during cheese-making. Another possible explanation could be related to the milk volume contribution from each cow during cheese-making. Bulk milk from each group was collected over 2 days, but each cow produces a different volume of milk. It is therefore possible that during cheese-making, the bulk milk had a higher volume of milk with good MCP whereas the Lattodinamografo analysis that uses a fixed volume of milk.

3.3 Cheese yield

When measuring cheese yield it might be more precise to calculate MACY rather than Ya. However, the MACY calculation was not done in the GPV study due to the fact that the weight of the cheese was measured before brining while the cheese DM measured 24 h after start of cheese-making. This would not result in precise numbers when calculating MACY and therefore it was left out in the GPV study. The DM of the cheeses in the GPV study ranged from 41.97 % to 45.19 %, resulting in a high SD (0.98), indicating that cheese yield should have been adjusted for moisture content. However, no differences were found between the treatment groups with regards to cheese DM content within the GPV study (also observed in the FEED study). Due to this, Ya is therefore used and the relationship between Ya and milk casein content in both the FEED study and GPV study is shown in Figure 10.

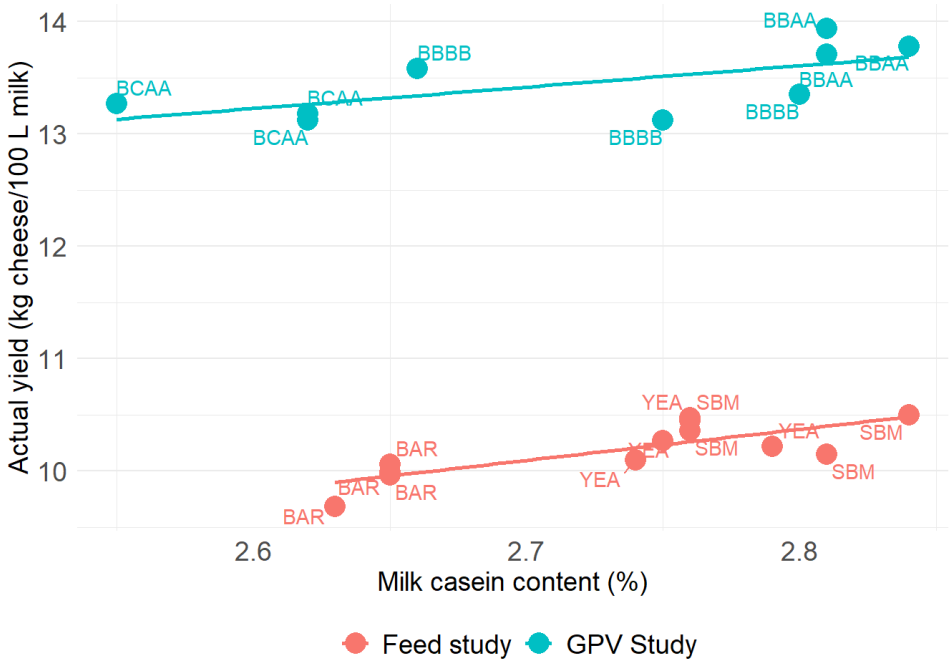

Figure 10 Relationship between milk casein content (%) and Ya (Actual yield) (kg cheese/100 L milk) during cheese-making in the FEED study (Study explained in Figure 1) (●) and the GPV study (Study explained in Figure 2) (●). Cheese milk had different fat content (2.71 % in the FEED study and 4.17 % in the GPV study) and 24 h cheese had different moisture content (48.6 % in the FEED study and 44 % in the GPV study). Figure created using the ggplot2 package (Wickham et al., 2022) in RStudio (R Core Team, 2022).

The higher milk fat content in cheese in the GPV study (Havarti-type cheese) resulted in a higher Ya compared to cheeses in the FEED study (Gouda-type cheese). There was a clear relationship between the content of casein in milk and Ya, as a higher casein content naturally resulted in a higher Ya. In the FEED study, both SBM and YEA cheeses attained a Ya that was significantly higher than BAR cheeses. In the GPV study, BBAA cheeses attained a Ya significantly higher than BCAA cheeses.

YE % was calculated in both studies using Ya and PY. The results from both the FEED study and GPV study are presented in Figure 11. In **Paper 1** (The FEED study), the average moisture content in all cheeses (48.28 %, n=12) was used as the desired moisture content in the PY formula. However, when working on the GPV data for **Paper 3**, it was concluded that a better and more viable approach would be to set a standard moisture content such as the differences between actual and desired moisture content is more visible. Therefore, in Figure 11, moisture content in FEED cheeses is changed from 48.28 % to 49 %.

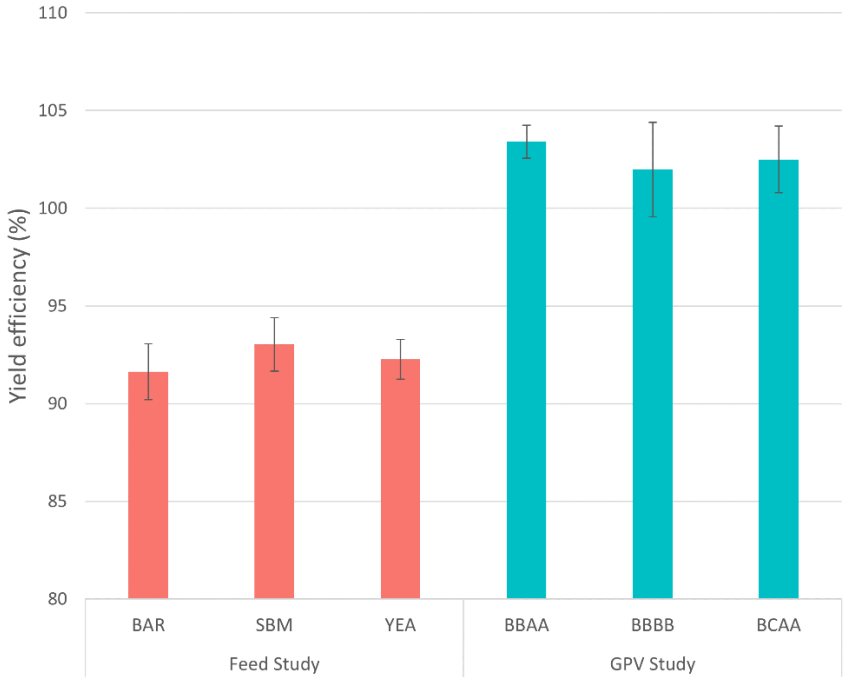

Figure 11 Yield efficiency (YE %) during production of cheeses in the FEED Study (Study explained in Figure 1) (■) and the GPV Study (Study explained in Figure 2) (■). Cheese milk had different fat content (2.71 % in the FEED study and 4.17 % in the GPV study) and 24 h cheese had different moisture content (48.6 % in the FEED study and 44 % in the GPV study).

There were no differences in YE % between the treatment groups within the FEED study or in the GPV study. This shows that the protein source in the FEED study or the α_{S1}-κ-CN variants did not have any effect on the recoveries/losses during cheese production, and that the differences in cheese yield was due to milk composition before the milk was processed (feeding and genetic protein variants factors). However, there was a large difference in YE % between the two studies. Cheese in the GPV study attained YE of > 100 % whereas cheese in the FEED study attained YE < 100 %. This can usually be explained by a too high or too low actual moisture content compared to that predicted. In the FEED study, the cheeses had an average moisture content of 48.3 % while the desired moisture content was 49 %, and the difference is therefore small. In the GPV study, the cheeses had an average moisture content of 44 % while the desired moisture content was 46 %. The attained moisture content was thereby 2 % lower than the predicted, and yet the YE was > 100 %.

The YE < 100 % in the FEED study can partly be explained by a slightly lower moisture content than was predicted. In addition, the curds were not treated gently when transferring them from the cheese vat to the pre-pressing vat, which would probably reduce the recovery of fat and casein and thereby give a lower YE. In the GPV study, the moisture content cannot explain the high YE. The recovery of fat and casein is likely higher in these cheeses compared to cheeses in the FEED study. This can be explained partly by a more gentle transfer of cheese curds because different cheese vats and equipment were used. Also, several processing steps during the production of the Gouda-type cheese were not used during production of the Havarti-type cheese (pre-press, cutting of cheese-mass before molding and pressing). This leading to fewer processing steps in the production of the Havarti-type cheese, where losses of fat and casein can happen. Another possible explanation is that the fat content of the milk was much higher during production of the Havarti-type cheese, which then contributed to a shorter curd firming time and probably stronger cheese curds which would reduce the loss of casein and fat.

The Van Slyke formula was originally designed for Cheddar cheese and can explain the underestimation of PY on the Havarti-type cheese produced in the GPV study, giving a YE > 100 %. The Van Slyke formula might not be the best formula to use while predicting cheese yield for Gouda- and Havarti-type cheeses. However, it was still used because the goal of these studies was not to maximize the efficiency during cheese-making, but to compare how different treatments affected the YE. This was still possible using the Van Slyke formula.

3.4 Cheese quality

Producing cheese of high quality is important for the dairy industry. Cheese quality is determined by many factors, including the milk quality, cheese composition, nutritional value and sensory properties such as texture and flavours (Fox et al., 2017a). The ripening process also determines the quality of cheese and includes activities of many microbial enzymes that change the flavor, morphology and texture of the cheese (Kermasha & Eskin, 2021). When making changes to the cheese-making process, or earlier as in this thesis (genetic protein variants and feed), it is important to evaluate whether these changes affect the ripening process and thereby the quality of the cheese.

3.4.1 Cheese composition

The pH and DM content in cheese 24 h after the start of cheese-making, was measured in cheese in both the FEED study and the GPV study (Table 8). There were no differences between the treatments within each of the studies showing that the cheese-making procedures were standardized.

The protein profile of the 24 h cheese in the GPV study was analysed, and the relative concentrations of the caseins and the large peptides derived from these were calculated. Significant differences in relative concentrations of para-κ-CN, α_{S2}-CN, α_{S1}-CN 8 P, γ1-CN A2, γ3-CN A2 and β-CN A2 were seen between the composite genotypes. The BCAA cheeses had a higher relative concentration of β-CN A2 and a lower relative concentration of the peptide γ1-CN A2 compared to BBBB and BBAA cheeses. This might indicate that the early proteolysis of β-CN happens earlier (or faster) with α_{S1}-CN BB compared to α_{S1}-CN BC. BCAA cheeses also showed a higher content of α_{S1}-CN 8P and a lower content of α_{S2}-CN compared to BBBB and BBAA cheeses. Most of the differences in the protein profile in the 24 h cheese seems therefore to be due to the α_{S1}-CN genotypes (BB vs. BC). The relative concentrations of proteins were not measured in the FEED study, but different protein composition were seen in ripened cheese (**Paper 2**) showing that protein source in concentrate feed affects the protein composition and degradation in cheese.

Table 8 pH and dry matter in 24 h cheese and total content of free amino acids (FAA) and DL-pyroglutamic acid in ripened cheese (mean ± SD) from the FEED study (Study explained in Figure 1) and the GPV study (Study explained in Figure 2). Significant differences (P < 0.05) within each study is marked with different superscript letters.

		24 h cheese		Ripened cheese*	
		pH	Dry matter (%)	Total FAA (µmol/g)	DL-pyroglutamic acid (µmol/g)
FEED Study	BAR (n= 4)	5.34 ± 0.09	51.5 ± 0.26	84.80 ± 4.82	0.96 ± 0.06^{b}
	SBM (n=4)	5.37 ± 0.06	51.6 ± 0.47	92.52 ± 7.16	1.10 ± 0.04^{a}
	YEA (n=4)	5.32 ± 0.04	52.0 ± 0.12	85.03 ± 4.11	0.94 ± 0.06^{b}
GPV Study	BBAA (n=3)	5.10 ± 0.21	56.4 ± 1.63	48.1 ± 8.83^{a}	0.36 ± 0.05^{b}
	BBBB (n=3)	5.03 ± 0.06	55.7 ± 0.56	55.91 ± 8.40^{a}	0.43 ± 0.07^{a}
	BCAA (n=3)	5.06 ± 0.03	55.8 ± 0.70	40.27 ± 5.25^{b}	0.28 ± 0.04^{c}

* The FEED cheese was ripened for 15 weeks and the GPV cheese for 20 weeks

The content of free amino acids (FAA) and organic acids was analysed in ripened cheese in both the FEED and the GPV study. The concentration of FAA in cheese increases during ripening due to proteolysis (Kilcawley, 2017). Proteolysis in cheese is caused by the action of rennet, plasmin and intra- and extracellular proteases and peptidases of lactic acid bacteria (Ardö et al., 2017). In the FEED cheeses, no differences in the concentration of any of the individual FAA or the total FAA content were found. However, the SBM cheeses contained a somewhat higher content of all individual FAA, which naturally resulted in a higher total FAA content. However, due to a high SD, there were no significant differences. In the GPV study, significant differences were seen for almost all the individual FAA (**Paper 3**), and the BCAA cheese contained a significantly lower concentration of total FAA compared to BBAA and BBBB cheese (Table 8), indicating a slower ripening in BCAA cheese.

Of the organic acids analysed, only the concentration of DL-pyroglutamic acid differed between cheeses in the FEED study, the SBM cheese contained a significantly higher concentration than the BAR and YEA cheeses. In the GPV study, BBBB cheese had a significantly higher concentration of DL-pyroglutamic acid than the BBAA and BCAA cheeses (and BBAA was higher than BCAA). Pyroglutamic acid is a derivate from the amino acids glutamine or glutamic acid (Gazme et al., 2019). The transformation can occur from both non-enzymatic (e.g. heat and pressure) and enzymatic processes. The formation of pyroglutamic acid in cheese has been suggested to be due to enzymes released from bacteria during cheese ripening. It has been reported in several studies that the formation is mainly dependent on the

starter culture rather than the raw milk microflora (Gazme et al., 2019). The same cheese culture (CHN-19, Chr. Hansen, Hørsholm, Denmark) was used in all cheese productions in both the FEED and GPV study. In the FEED study, the cheese microbiota was analysed in ripened cheese (**Paper 2**), and the cheeses were not significantly different with respect to protein source in concentrate feed. With this in mind, neither the cheese culture nor the microbiota after ripening can explain the higher concentration of DL-pyroglutamic acid in SBM-cheese. The microbiota was not analysed in cheese from the GPV study. It has been suggested that the concentration of pyroglutamic acid can be used to assess the age of Parmigiano-Reggiano cheese because the concentration increases linearly with the age (Mucchetti et al., 2000). Based on this assumption, and supported by the total FAA contents, this indicates that the ripening is faster in SBM cheese and slower in BCAA cheese.

3.4.2 Sensory properties

Cheese from both the FEED study and the GPV study was analysed using descriptive sensory analysis. The Gouda-type cheese produced in the FEED study was analysed when the cheese was between 11 and 13 weeks (By TINE SA), and the Havarti-type cheese was analysed after 20 weeks of ripening (By the Norwegian Institute of Food, Fisheries, and Aquaculture Research (NOFIMA)). Differences were found in 4 attributes in the GPV study, while only 1 attribute in the FEED study was significantly different (Figure 12).

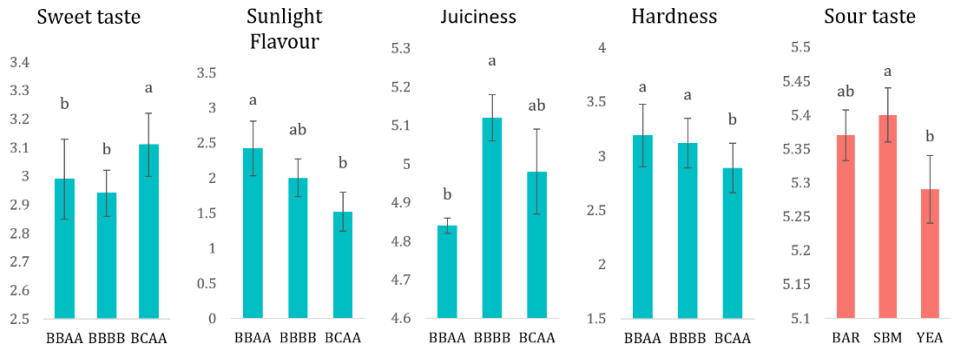

Figure 12 Sensory attributes with significant differences (P < 0.05) in the FEED study (Study explained in Figure 1) (■) and the GPV Study (Study explained in Figure 2)(■). Significant differences (P < 0.05) are marked with different letters. A scale of 1-9 was used (1 = low intensity and 9 = high intensity).

From these results, it appears that the genetic protein variants of α_{S1}-κ-CN affect sensory properties of cheese more than the protein source in the concentrate feed to dairy cows. This is not a surprising result, because different genetic protein variants change the primary structure of the caseins which in turn affect the cheese structure and thereby the sensory properties of cheese. Cheese with α_{S1}-CN BB in the GPV study was experienced as firmer compared to the BC genotype, yet the cheese DM content was not different between cheeses in this study. This has also been previously reported by Nuyts-Petit et al. (1997), as they found that the B variant α_{s1}-CN resulted in Saint-Paulin cheese that was experienced as firmer. Sourness in cheese can be linked directly to organic acids (Kilcawley, 2017), but also some FAA. The higher intensity of sour taste in SBM cheese in the FEED study can be connected to the tendency of higher content of sour-tasting FAA such as histidine, glutamic acid and aspartic acid (Kilcawley, 2017).

The quality of all cheeses in both the FEED and the GPV study was considered as high-quality cheeses despite some differences in the composition and sensory properties.

4 Concluding remarks

For a long time, it has been well known that feeding practices affect milk composition, but feeding with a higher protein content has been shown to be not cost-effective due to the low transfer efficiency. However, casein content is not measured in most feeding studies. Considering the results from the FEED study, protein level or protein source in concentrate feed for dairy cows does not affect the *total protein content*, but it does affect the milk *casein content*. These results show that it is possible that important results have been overseen in previous feeding studies because casein has not been analysed.

Milk casein content is a good indicator for cheese-making efficiency. In a herd with cows that have different genetic protein variants (FEED study), the renneting time was irreversible linear to the milk casein content. A greater milk casein content also resulted in a higher cheese yield. Since casein plays a major role in processing of many important dairy products, casein should be included as a marker for feed efficiency. However, as only a few feeding studies have measured casein, more work on this area must be conducted in order to confirm results from this study. If milk casein content can be increased by dietary manipulation, this would be a step toward a sustainable dairy production, as the production will give more cheese from the same amount of milk. Simultaneously, the energy consumption needed during production would be reduced due to a shorter processing time since a higher content of casein was shown to reduce renneting time. This could be used as an argument for using casein in payment systems to encourage farmers to feed in such a way that a higher milk casein content in achieved.

In the FEED study, cows responded well to YEA as a protein source in concentrate feed and the cheese-making efficiency was improved compared to feeding the BAR diet (which also had a lower protein content in concentrate feed). The YEA protein source is a novel ingredient that has been developed to attain a more ethical and sustainable food production, and to increase the degree of self-sufficiency in countries with a small cultivatable land area. However, if the production of a YEA protein source is to be implemented industrially, there are possible side effects that need to be calculated. Forest covers 37.6 % of the total geographical area in Norway (Statistics Norway, 2022a). The yearly forest growth between 2016 and 2020 was

7.3 million m^3 pr. year and deforestation has decreased yearly from 1950 which has in turn led to a continuously increase of the standing volume (NIBIO, 2021). This represents a great bioresource that can be used for feed protein production. However, biodiversity loss is a concern and work is needed to ensure that the production of YEA protein does not negatively affect the biodiversity to a significant degree.

One of the challenges I met writing this thesis and the papers enclosed is that my knowledge is limited to dairy technology. Several times I wished I had a master's degree in animal nutrition and environmental science in addition to the one I have within food science. The practices in the dairy industry are built on knowledge from both nutrition of dairy cows and dairy technology (among others). Researchers in cow nutrition are interested in analysing milk the same way as the dairies do, because it reflects the payment method which is of interest for the farmers. Dairy researchers are more interested in how the milk behaves during processing after the milk is received. This thesis, however, focuses on the whole value chain, and because of this, important results were found. **Paper 4** (Appendix 1) covered the cow performance part of the trial. Here we found no differences in feed uptake, body condition scoring, body weight or milk yield between BAR, SBM and YEA groups. However, when we analysed milk further and produced cheese (**Paper 1 and Paper 2**), differences were found. This is a clear example that the whole value chain of a product should be evaluated before making large investments or implementations to ensure that it is both economically and sustainably beneficial.

Breeding for specific genetic protein variants to improve cheese-making efficiency has been of interest for many decades. The dairy cattle industry has more than doubled the milk production over the last decades, while the number of cows has been reduced (Emery, 1978; Jenkins & McGuire, 2006). Genetic selection is one of the means used to attain this. However, there are many traits that needs to be considered in breeding. Currently, GENO does not breed directly for genetic protein variants but 17 main characteristics (GENO, 2020a). The most important ones are milk volume, udder health, fertility, meat and health of hoofs. One of the reasons why genetic protein variants are still not used as a breeding characteristic is probably that results from published studies are often contradictory or they have not covered all important aspects.

For example:

- the BC genotype of α_{S1}-CN has superior MCP, but the results of the GPV study and other studies have shown a lower milk casein content when using this genotype. This resulted in a lower cheese yield in the GPV study. MCP and cheese yield is often linked, good MCP results in a higher cheese yield. However, this was not the case in the GPV study. This shows that measuring MCP is not enough when estimating cheese-making efficiency and that cheese yield is just as important as MCP considering indicators for cheese-making efficiency.
- Some variants have been shown to have an increased protein content, but a lower milk yield resulting in a lower protein yield (Gai et al., 2021).
- Ketto (2017) concluded that the genetic protein variants with good rennet coagulation properties had poor acid coagulation properties.

These examples show that selecting the best genotypes is a challenge. However, knowledge of how genetic protein variants affect the processing of milk is valuable when designing experiments with dairy cows. Their genetic protein variants should be analysed to balance the variants between treatment groups.

A change in milk composition and thereby cheese-making efficiency is possible to attain by feed manipulation (FEED study) and by using different genetic protein variants (GPV study). However, feed is a relatively simple means to manipulate milk composition in order to increase the feed efficiency, a change that is also possible to make *much faster* than breeding for specific genetic protein variants. In addition, sensory properties of cheese appeared to be more affected by α_{S1}-κ-CN variants than by the protein source in feed. The differences in sensory properties did not affect the overall quality of cheeses, but it is preferable that the cheese quality remains unchanged or improved when making changes in the process.

The following points summarize the finding in this thesis and recommendations for future studies and actions:

- The whole value chain needs to be considered in future studies involving dairy cows. There might be no differences in cow performance, but both milk and cheese could be affected to a high degree. It would also be both necessary and interesting to focus on other dairy products.

- How feed on a general basis (concentrate level, concentrate protein and level, hay/silage) affects MCP and cheese yield needs to be evaluated. If milk composition can be manipulated by feeding, to a higher degree than assumed, then this method is easier than breeding for specific genetic protein variants.
- Dairies would probably benefit from including casein in the payment system since this type of protein plays a major role for the cheese making efficiency (MCP and cheese yield). The production of cheese is increasing, and thereby emphasis on casein becomes more and more relevant.

5 References

Adedayo, M., Ajiboye, E., Akintunde, J. & Odaibo, A. (2011). Single cell proteins: as nutritional enhancer. *Advances in Applied Science Research*, 2 (5): 396-409.

ANIMALIA. (2020). *Kjøttets tilstand 2020 [The meats condition 2020]*. Status i norsk kjøtt- og eggproduksjon [The status of Norwegian meat- and egg production]. Available at: https://www.animalia.no/no/animalia/aktuelt/kjottets-tilstand-2020/ (accessed: 08.01.2023).

Ardö, Y., McSweeney, P. L., Magboul, A. A., Upadhyay, V. K. & Fox, P. F. (2017). Biochemistry of cheese ripening: Proteolysis. In McSweeney, P. L. H., Fox, P. F., Cotter, P. D. & Everett, D. W. (eds) *Cheese Chemistry, Physics and Microbiology*, pp. 445-482. London: Academic Press.

Baulcombe, D., Crute, I., Davies, B., Dunwell, J., Gale, M., Jones, J., Pretty, J., Sutherland, W. & Toulmin, C. (2009). *Reaping the benefits: science and the sustainable intensification of global agriculture*. Available at: https://royalsociety.org/topics-policy/publications/2009/reaping-benefits/ (accessed: 03.01.2023).

Beja-Pereira, A., Luikart, G., England, P. R., Bradley, D. G., Jann, O. C., Bertorelle, G., Chamberlain, A. T., Nunes, T. P., Metodiev, S. & Ferrand, N. (2003). Gene-culture coevolution between cattle milk protein genes and human lactase genes. *Nature genetics*, 35 (4): 311-313. doi: 10.1038/ng1263.

Berry, S., Sheehy, P., Williamson, P., Sharp, J., Menzies, K., Lefèvre, C., Digby, M., Harland, C., Davis, S. & Snell, R. (2020). Defining the origin and function of bovine milk proteins throgh genomics: The biological implications of manipulation and modification. In Boland, M. & Singh, H. (eds) *Milk Proteins From Expression to Food*, pp. 143-172. London: Academic Press.

Bijjl, E., Holland, J. W. & Boland, M. (2020). Posttranslational modifications of caseins. In Boland, M. & Singh, H. (eds) *Milk Proteins From Expression to Food*, pp. 173-212. London: Academic Press.

Bijl, E., de Vries, R., van Valenberg, H., Huppertz, T. & Van Hooijdonk, T. (2014). Factors influencing casein micelle size in milk of individual cows: Genetic variants and glycosylation of κ-casein. *International Dairy Journal*, 34 (1): 135-141. doi: 10.1016/j.idairyj.2013.08.001.

Bittante, G., Penasa, M. & Cecchinato, A. (2012). Invited review: Genetics and modeling of milk coagulation properties. *Journal of dairy science*, 95 (12): 6843-6870. doi: 10.3168/jds.2012-5507.

Boland, M. & Hill, J. (2020). World supply of food and the role of dairy protein. In Boland, M. & Singh, H. (eds) *Milk Proteins From Expression to Food*. London: Academic Press.

Brito, L., Bédère, N., Douhard, F., Oliveira, H., Arnal, M., Peñagaricano, F., Schinckel, A., Baes, C. F. & Miglior, F. (2021). Genetic selection of high-yielding dairy cattle toward sustainable farming systems in a rapidly changing world. *Animal*, 15: 100292. doi: 10.1016/j.animal.2021.100292.

Bynum, D. & Olson, N. (1982). Influence of curd firmness at cutting on yield and recovery of milk constituents. *Journal of Dairy Science*, 65 (12): 2281-2290. doi: 10.3168/jds.S0022-0302(82)82498-3.

Capper, J. L., Cady, R. A. & Bauman, D. E. (2009). The environmental impact of dairy production: 1944 compared with 2007. *Journal of Animal Science*, 87 (6): 2160-2167. doi: 10.2527/jas.2009-1781.

Caroli, A., Chessa, S. & Erhardt, G. (2009). Invited review: Milk protein polymorphisms in cattle: Effect on animal breeding and human nutrition. *Journal of dairy science*, 92 (11): 5335-5352. doi: 10.3168/jds.2009-2461.

Connor, E. (2015). Invited review: Improving feed efficiency in dairy production: Challenges and possibilities. *Animal*, 9 (3): 395-408. doi: 10.1017/S1751731114002997.

Costa, A., Neglia, G., Campanile, G. & De Marchi, M. (2020). Milk somatic cell count and its relationship with milk yield and quality traits in Italian water buffaloes. *Journal of dairy science*, 103 (6): 5485-5494. doi: 10.3168/jds.2019-18009.

Coulon, J.-B., Hurtaud, C., Rémond, B. & Verite, R. (1998). Factors contributing to variation in the proportion of casein in cows' milk true protein: a review of recent INRA experiments. *Journal of Dairy Research*, 65 (3): 375-387. doi: 10.1017/s0022029998002866.

Cruz, A., Håkenåsen, I. M., Skugor, A., Mydland, L. T., Åkesson, C. P., Hellestveit, S. S., Sørby, R., Press, C. M. & Øverland, M. (2019). Candida utilis yeast as a protein source for weaned piglets: Effects on growth performance and digestive function. *Livestock Science*, 226: 31-39. doi: 10.1016/j.livsci.2019.06.003.

Cruz, A., Sterten, H., Steinhoff, F. S., Mydland, L. T. & Øverland, M. (2020). Cyberlindnera jadinii yeast as a protein source for broiler chickens: effects on growth performance and digestive function from hatching to 30 days of age. *Poultry science*, 99 (6): 3168-3178. doi: 10.1016/j.psj.2020.01.023.

Dalgleish, D. G. & Corredig, M. (2012). The structure of the casein micelle of milk and its changes during processing. *Annual review of food science and technology*, 3: 449-467. doi: 10.1146/annurev-food-022811-101214.

Day, L., Williams, R., Otter, D. & Augustin, M. (2015). Casein polymorphism heterogeneity influences casein micelle size in milk of individual cows. *Journal of Dairy Science*, 98 (6): 3633-3644. doi: 10.3168/jds.2014-9285.

Devold, T. G., Brovold, M. J., Langsrud, T. & Vegarud, G. E. (2000). Size of native and heated casein micelles, content of protein and minerals in milk from Norwegian Red Cattle—effect of milk protein polymorphism and different feeding regimes. *International Dairy Journal*, 10 (5-6): 313-323. doi: 10.1016/S0958-6946(00)00073-X.

Emery, R. (1978). Feeding for increased milk protein. *Journal of Dairy Science*, 61 (6): 825-828. doi: 10.3168/jds.S0022-0302(78)83656-X.

Emmons, D., Ernstrom, C., Lacroix, C. & Verret, P. (1990). Predictive formulas for yield of cheese from composition of milk: a review. *Journal of dairy science*, 73 (6): 1365-1394. doi: 10.3168/jds.S0022-0302(90)78803-0.

Emmons, D. & Modler, H. (2010). Invited review: a commentary on predictive cheese yield formulas. *Journal of Dairy Science*, 93 (12): 5517-5537. doi: 10.3168/jds.2010-3262.

European Union. (2019). *Agriculture, forestry and fishery statistics - 2019 edition*. Luxembourg: Publication Office of the European Union.

FAO, IFAD, UNICEF, WFP & WHO. (2021). *The State of Food Security and Nutrition in the World 2021. Transforming food systems for food security, improved nutrition and affordable healthy diets for all*. Available at: https://www.fao.org/3/cb4474en/online/cb4474en.html (accessed: 11.01.2023).

FAO. (2022a). *Dairy animals*. Available at: https://www.fao.org/dairy-production-products/production/dairy-animals/en/ (accessed: 15.09.2022).

FAO. (2022b). *The FAOSTAT database for Production (Crops and livestock products)*. Available at: https://www.fao.org/faostat/en/#compare (accessed: 30.08.2022).

FAO. (2022c). *Livestock and the environment*. Available at: https://www.fao.org/livestock-environment/en (accessed: 23.09.2022).

FAO. (2022d). *More fuel for the food/feed debate*. Available at: https://www.fao.org/documents/card/en/c/cc3134en/ (accessed: 22.01.2023).

FAO. (2023). *Types and characteristics*. Available at: https://www.fao.org/dairy-production-products/products/types-and-characteristics/en/ (accessed: 11.01.2023).

FCRN foodsource. (2020). *Soy: food, feed and land use change*. Available at: https://www.foodsource.org.uk/building-blocks/soy-food-feed-and-land-use-change.
Foods of Norway. (2022). *About us*. Available at: https://www.foodsofnorway.net/ (accessed: 23.09.2022).
Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H. & O'Mahony, J. A. (2015a). Milk Proteins. In Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H. & O'Mahony, J. A. (eds) *Dairy Chemistry and Biochemistry*, pp. 145-240. London: Springer International Publishing.
Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H. & O'Mahony, J. A. (2015b). Production and Utilization of Milk. In Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H. & O'Mahony, J. A. (eds) *Dairy Chemistry and Biochemistry*, pp. 1-20. London: Springer International Publishing.
Fox, P. F., Cogan, T. M. & Guinee, T. P. (2017a). Factors That Affect the Quality of Cheese. In McSweeney, P. L. H., Fox, P. F., Cotter, P. D. & Everett, D. W. (eds) *Cheese Chemistry, Physics and Microbiology*, pp. 617-642. London: Academic Press.
Fox, P. F., Guinee, T. P., Cogan, T. M. & McSweeney, P. L. H. (2017b). Cheese Yield. In Fox, P. F., Guinee, T. P., Cogan, T. M. & McSweeney, P. L. H. (eds) *Fundamentals of Cheese Science*, pp. 279-331. New York: Springer Science+Business Media LLC.
Fox, P. F., Guinee, T. P., Cogan, T. M. & McSweeney, P. L. H. (2017c). Enzymatic Coagulation of Milk. In Fox, P. F., Guinee, T. P., Cogan, T. M. & McSweeney, P. L. H. (eds) *Fundamentals of Cheese Science*, pp. 185-230. New York: Springer Science+Business Media LLC.
Fröhlich-Wyder, M.-T., Bisig, W., Guggisberg, D., Jakob, E., Turgay, M. & Wechsler, D. (2017). Cheeses with propionic acid fermentation. In McSweeney, P. L. H., Fox, P. F., Cotter, P. D. & Everett, D. W. (eds) *Cheese Chemistry, Physics and Microbiology*, pp. 889-910. London: Academic Press.
Gai, N., Uniacke-Lowe, T., O'Regan, J., Faulkner, H. & Kelly, A. L. (2021). Effect of protein genotypes on physicochemical properties and protein functionality of bovine milk: a review. *Foods*, 10 (10): 2409. doi: 10.3390/foods10102409.
Gaucheron, F. (2005). The minerals of milk. *Reproduction Nutrition Development*, 45 (4): 473-483. doi: 10.1051/rnd:2005030.
Gazme, B., Boachie, R. T., Tsopmo, A. & Udenigwe, C. C. (2019). Occurrence, properties and biological significance of pyroglutamyl peptides derived from different food sources. *Food Science and Human Wellness*, 8 (3): 268-274. doi: 10.1016/j.fshw.2019.05.002.
GENO. (2020a). *Avlsmålet for NRF [The breeding goal for NRF]*. Available at: https://www.geno.no/fagstoff-og-hjelpemidler/avlsprogram-for-norsk-rodt-fe/avlsmalet-for-nrf/ (accessed: 09.02.2023).

GENO. (2020b). *Kaseinvarianter i melk [Genetic variants of casein in milk]*. Available at: https://www.geno.no/produkter-og-tjenester/genotyping-av-nrf-hunndyr/enkeltgener/kaseinvarianter-i-melk/ (accessed: 11.01.2023).

Glantz, M., Devold, T., Vegarud, G., Månsson, H. L., Stålhammar, H. & Paulsson, M. (2010). Importance of casein micelle size and milk composition for milk gelation. *Journal of Dairy Science*, 93 (4): 1444-1451. doi: 10.3168/jds.2009-2856.

Goulding, D. A., Fox, P. F. & O'Mahony, J. A. (2020). Milk proteins: An overview. In Boland, M. & Singh, H. (eds) *Milk Proteins From Expression to Food*, pp. 21-98. London: Academic Press.

Griffin, M. C., Lyster, R. L. & Price, J. C. (1988). The disaggregation of calcium-depleted casein micelles. *European Journal of Biochemistry*, 174 (2): 339-343. doi: 10.1111/j.1432-1033.1988.tb14103.x.

Guinee, T. P., Gorry, C. B., O'Callaghan, D. J., O'Kennedy, B. T., O'Brie, N. & Fenelon, M. A. (1997). The effects of composition and some processing treatments on the rennet coagulation properties of milk. *International Journal of Dairy Technology*, 50 (3): 99-106. doi: 10.1111/j.1471-0307.1997.tb01747.x.

Gulati, A., Galvin, N., Hennessy, D., McAuliffe, S., O'Donovan, M., McManus, J. J., Fenelon, M. A. & Guinee, T. P. (2018). Grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on low-moisture part-skim Mozzarella cheese yield and quality characteristics in mid and late lactation. *Journal of Dairy Science*, 101 (10): 8737-8756. doi: 10.3168/jds.2018-14566.

Heck, J., Schennink, A., Van Valenberg, H., Bovenhuis, H., Visker, M., Van Arendonk, J. & Van Hooijdonk, A. (2009). Effects of milk protein variants on the protein composition of bovine milk. *Journal of Dairy Science*, 92 (3): 1192-1202. doi: 10.3168/jds.2008-1208.

Hindmarsh, J. & Watkinson, P. (2017). Experimental evidence for previously unclassified calcium phosphate structures in the casein micelle. *Journal of Dairy Science*, 100 (9): 6938-6948. doi: 10.3168/jds.2017-12623.

Horne, D. S. (2006). Casein micelle structure: Models and muddles. *Current opinion in colloid & interface science*, 11 (2-3): 148-153. doi: 10.1016/j.cocis.2005.11.004.

Horne, D. S. & Lucey, J. A. (2017). Rennet-Induced Coagulation of Milk In McSweeney, P. L. H., Fox, P. F., Cotter, P. D. & Everett, D. W. (eds) *Cheese Chemistry, Physics and Microbiology*, pp. 115-137. London: Academic Press.

IDF. (2021). *The global impact of dairy*. Available at: https://fil-idf.org/dairys-global-impact/ (accessed: 09.08.2022).

Ikonen, T. (2000). *Possibilities of Genetic Improvement of Milk Coagulation Properties of Dairy Cows*. Doctoral thesis. Helsinki: University of Helsinki. Available at: https://helda.helsinki.fi/handle/10138/20692 (accessed: 12.12.2022).

Israelidis, C. J. (2003). *Nutrition-Single cell protein, twenty years later*. Available at: https://biopolitics.gr/biowp/wp-content/uploads/2013/04/isrealidis.pdf (accessed: 11.01.2023).

Jakob, E. (1994). Genetic Polymorphism of Milk Proteins. *Mljekarstvo*, 44 (3): 197-217.

Jenkins, T. & McGuire, M. (2006). Major advances in nutrition: impact on milk composition. *Journal of Dairy Science*, 89 (4): 1302-1310. doi: 10.3168/jds.s0022-0302(06)72198-1.

Jensen, H., Holland, J., Poulsen, N. & Larsen, L. (2012a). Milk protein genetic variants and isoforms identified in bovine milk representing extremes in coagulation properties. *Journal of Dairy Science*, 95 (6): 2891-2903. doi: 10.3168/jds.2012-5346.

Jensen, H., Poulsen, N., Andersen, K., Hammershøj, M., Poulsen, H. & Larsen, L. (2012b). Distinct composition of bovine milk from Jersey and Holstein-Friesian cows with good, poor, or noncoagulation properties as reflected in protein genetic variants and isoforms. *Journal of Dairy Science*, 95 (12): 6905-6917. doi: 10.3168/jds.2012-5675.

Johnson, M. (2017). A 100-year review: cheese production and quality. *Journal of Dairy Science*, 100 (12): 9952-9965. doi: 10.3168/jds.2017-12979.

Jõudu, I., Henno, M., Kaart, T., Püssa, T. & Kärt, O. (2008). The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. *International Dairy Journal*, 18 (9): 964-967. doi: 10.1016/j.idairyj.2008.02.002.

Jõudu, I., Henno, M., Värv, S., Viinalass, H., Püssa, T., Kaart, T., Arney, D. & Kärt, O. (2009). The effect of milk proteins on milk coagulation properties in Estonian dairy breeds. *Veterinarija Ir Zootechnika*, 68: 14-19.

Kefford, B., Christian, M. P., Sutherland, B. J., Mayes, J. J. & Grainger, C. (1995). Seasonal influences on Cheddar cheese manufacture: influence of diet quality and stage of lactation. *Journal of Dairy Research*, 62 (3): 529-537. doi: 10.1017/s0022029900031228.

Kermasha, S. & Eskin, M. N. A. (2021). Seleced industial enzymes. In Kermasha, S. & Eskin, M. N. A. (eds) *Enzymes - Novel Biotechnological Approaches for the Food Industry*: Academic Press.

Ketto, I. A. (2017). *Impact of milk protein genotypes on milk coagulation properties*. Doctoral thesis. Ås: Norwegian University of Life Sciences. Available at: https://nmbu.brage.unit.no/nmbu-xmlui/handle/11250/2498978 (accessed: 04.11.2022).

Ketto, I. A., Knutsen, T. M., Øyaas, J., Heringstad, B., Ådnøy, T., Devold, T. G. & Skeie, S. (2017a). Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. *Int Dairy J*, 70: 55-64. doi: https://doi.org/10.1016/j.idairyj.2016.10.010.

Ketto, I. A., Knutsen, T. M., Øyaas, J., Heringstad, B., Ådnøy, T., Devold, T. G. & Skeie, S. B. (2017b). Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. *International Dairy Journal*, 70: 55-64. doi: 10.1016/j.idairyj.2016.10.010.

Kidane, A., Vhile, S. G., Ferneborg, S., Skeie, S., Olsen, M. A., Mydland, L. T., Øverland, M. & Prestløkken, E. (2022). *Cyberlindnera jadinii* yeast as a protein source in early-to mid-lactation dairy cow diets: Effects on feed intake, ruminal fermentation, and milk production. *Journal of Dairy Science*, 105 (3): 2343-2353. doi: 10.3168/jds.2021-20139.

Kilcawley, K. N. (2017). Cheese Flavour. In Fox, P. F., Guinee, T. P., Cogan, T. M. & McSweeney, P. L. H. (eds) *Fundamentals of Cheese Science*, pp. 443-474. New York: Springer.

Kübarsepp, I., Henno, M., Kärt, O. & Tupasela, T. (2005). A comparison of the methods for determination of the rennet coagulation properties of milk. *Acta Agriculturae Scandinavica, Section A — Animal Science*, 55 (4): 145-148. doi: 10.1080/09064700500453377.

Landbruksdirektoratet. (2022). *Kraftfôrstatistikk [Statistics concentrate feed]*. Available at: https://www.landbruksdirektoratet.no/nb/statistikk-og-utviklingstrekk/utvikling-i-jordbruket/kraftforstatistikk?resultId=1.0&searchQuery=kraftf%C3%B4r (accessed: 23.09.2022).

Lapeña, D. G. (2019). *Production of yeast from spruce sugars and hydrolysates of protein-rich by-products as feed ingredient*. Doctoral thesis. Ås: Norwegian University of Life Scienses. Available at: https://nmbu.brage.unit.no/nmbu-xmlui/handle/11250/2824135 (accessed: 12.01.2023).

Lien, S., Kantanen, J., Olsaker, I., Holm, L. E., Eythorsdottir, E., Sandberg, K., Dalsgard, B. & Adalsteinsson, S. (1999). Comparison of milk protein allele frequencies in Nordic cattle breeds. *Animal Genetics*, 30 (2): 85-91. doi: 10.1046/j.1365-2052.1999.00434.x.

Logan, A., Leis, A., Day, L., Øiseth, S. K., Puvanenthiran, A. & Augustin, M. A. (2015). Rennet gelation properties of milk: Influence of natural variation in milk fat globule size and casein micelle size. *International Dairy Journal*, 46: 71-77. doi: 10.1016/j.idairyj.2014.08.005.

Lucey, J. & Fox, P. (1993). Importance of calcium and phosphate in cheese manufacture: A review. *Journal of Dairy Science*, 76 (6): 1714-1724. doi: 10.3168/jds.S0022-0302(93)77504-9.

Malacarne, M., Franceschi, P., Formaggioni, P., Sandri, S., Mariani, P. & Summer, A. (2014). Influence of micellar calcium and phosphorus on rennet coagulation properties of cows milk. *Journal of Dairy Research*, 81 (2): 129-136. doi: 10.1017/s0022029913000630.

Malossini, F., Bovolenta, S., Piras, C., Dalla Rosa, M. & Ventura, W. (1996). Effect of diet and breed on milk composition and rennet coagulation properties. *Annales de zootechnie*, 45 (1): 29-40. doi: 10.1051/animres:19960103.

Mao, I., Buttazzoni, L. & Aleandri, R. (1992). Effects of polymorphic milk protein genes on milk yield and composition traits in Holstein cattle. *Acta Agriculturae Scandinavica A-Animal Sciences*, 42 (1): 1-7. doi: 10.1080/09064709209410101.

Martin, P., Bianchu, L., Cebo, C. & Miranda, G. (2013). Genetic Polymorphism of Milk Proteins. In McSweeney, P. L. H. & Fox, P. F. (eds) vol. 1A *Advanced Dairy Chemistry*, pp. 463-514. New York: Springer Science+Business Media.

McLean, D. M., Graham, E. B., Ponzoni, R. W. & McKenzie, H. A. (1984). Effects of milk protein genetic variants on milk yield and composition. *Journal of Dairy Research*, 51 (4): 531-546. doi: 10.1017/S0022029900032854.

Mekonnen, M. M. & Hoekstra, A. Y. (2012). A global assessment of the water footprint of farm animal products. *Ecosystems*, 15 (3): 401-415. doi: 10.1007/s10021-011-9517-8.

Montel, M.-C., Buchin, S., Mallet, A., Delbes-Paus, C., Vuitton, D. A., Desmasures, N. & Berthier, F. (2014). Traditional cheeses: rich and diverse microbiota with associated benefits. *International journal of food microbiology*, 177: 136-154. doi: 10.1016/j.ijfoodmicro.2014.02.019.

Mottet, A., de Haan, C., Falcucci, A., Tempio, G., Opio, C. & Gerber, P. (2017). Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. *Global Food Security*, 14: 1-8. doi: 10.1016/j.gfs.2017.01.001.

Mucchetti, G., Locci, F., Gatti, M., Neviani, E., Addeo, F., Dossena, A. & Marchelli, R. (2000). Pyroglutamic Acid in Cheese: Presense, Origin, and Correlation with Ripening Time of Grana Padano Cheese. *Journal of Dairy Science*, 83: 659-665. doi: 10.3168/jds.S0022-0302(00)74926-5.

Müller-Buschbaum, P., Gebhardt, R., Roth, S., Metwalli, E. & Doster, W. (2007). Effect of calcium concentration on the structure of casein micelles in thin films. *Biophysical Journal*, 93 (3): 960-968. doi: 10.1529%2Fbiophysj.107.106385.

Ng-Kwai-Hang, K. (2006). Genetic variants of milk proteins and their effects on the yield and quality of cheese. *CABI reviews*. doi: 10.1079/PAVSNNR20061056.

NIBIO. (2021). *Tilvekst og skogavvikrkning [growth and deforestation]*. Available at: https://www.skogbruk.nibio.no/tilvekst-og-skogavvirkning (accessed: 28.01.2023).

Nilsson, K. (2020). *Non-coagulation milk from Swedish Red Dairy cattle*. Doctoral Thesis. Lund: Lund University. Available at: https://www.dissertations.se/dissertation/a694f0ba69/ (accessed: 27.01.2023).

Nuyts-Petit, V., Delacriox-Buchet, A. & Vassal, L. (1997). Influence de trois haplotypes des caséines asl' pet K fréquents en race bovine Normande sur la composition du lait et l'aptitude à la fabrication fromagère. *Lait*, 77: 625-39. doi: 10.1051/LAIT%3A1997545.

O'mahony, J. A. & Fox, P. E. (2013). Milk Proteins: Introduction and Historical Aspects. In McSweeney, P. L. H. & Fox, P. F. (eds) vol. 1A *Advanced Dairy Chemistry* pp. 43-85. New York: Springer Science+Business.

OECD/FAO. (2021). *OECD-FAO Agricultural Outlook 2021-2030*. Available at: https://www.oecd-ilibrary.org/agriculture-and-food/oecd-fao-agricultural-outlook-2021-2030_19428846-en (accessed: 04.11.2022).

Ong, L., Lawrence, R. C., Gilles, J., Creamer, L. K., Crow, V. L., Heap, H. A., Honoré, C. G., Johnston, K. A., Samal, P. K. & Powell, I. B. (2017). Cheddar cheese and related dry-salted cheese varieties. In McSweeney, P. L. H., Fox, P. F., Cotter, P. D. & Everett, D. W. (eds) *Cheese Chemistry, Physics and Microbiology*, pp. 829-863. London: Academic Press.

Opplysningskontoret for Meieriprodukter. (2022). *Statistikk - tall og fakta [Statistics - numbers and facts]*. Available at: https://www.melk.no/Statistikk (accessed: 24.09.2022).

Panthi, R. R., Jordan, K. N., Kelly, A. L. & Sheehan, J. J. (2017). Selection and Treatment of Milk for Cheesemaking. In McSweeney, P. L. H., Fox, P. F., Cotter, P. D. & Everett, D. W. (eds) *Cheese Chemistry, Physics & Microbiology*, pp. 23-52. London: Academic Press.

Poulsen, N., Bertelsen, H., Jensen, H., Gustavsson, F., Glantz, M., Månsson, H. L., Andrén, A., Paulsson, M., Bendixen, C. & Buitenhuis, A. (2013). The occurrence of noncoagulating milk and the association of bovine milk coagulation properties with genetic variants of the caseins in 3 Scandinavian dairy breeds. *Journal of Dairy Science*, 96 (8): 4830-4842. doi: 10.3168/jds.2012-6422.

R Core Team. (2022). *R: A language and environment for statistical computing.* R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Regjeringen.no. (2021). *Jordvern [Soil conservation]*. Available at: https://www.regjeringen.no/no/tema/mat-fiske-og-landbruk/landbrukseiendommer/innsikt/jordvern/jordvern/id2009556/ (accessed: 30.12.2022).

Ritala, A., Häkkinen, S. T., Toivari, M. & Wiebe, M. G. (2017). Single cell protein—state-of-the-art, industrial landscape and patents 2001–2016. *Frontiers in microbiology*, 8: 2009. doi: 10.3389/fmicb.2017.02009.

Sers, C. F. & Mughal, M. (2020). Covid-19 outbreak and the need for rice self-sufficiency in West Africa. *World Development*, 135: 105071. doi: 10.1016/j.worlddev.2020.105071.

Sneddon, N., Lopez-Villalobos, N., Hickson, R. & Shalloo, L. (2013). *Review of milk payment systems to identify the component value of lactose. Proceedings of the New Zealand Society of Animal Production*: New Zealand Society of Animal Production. Available at: https://www.nzsap.org/proceedings/2013/review-milk-payment-systems-identify-component-value-lactose (accessed: 04.01.2023).

Soryal, K., Zeng, S., Min, B., Hart, S. & Beyene, F. (2004). Effect of feeding systems on composition of goat milk and yield of Domiati cheese. *Small Ruminant Research*, 54: 121-129. doi: 10.1016/j.smallrumres.2003.10.010.

Statistics Norway. (2022a). *Land use and land cover*. Available at: https://www.ssb.no/en/natur-og-miljo/areal/statistikk/arealbruk-og-arealressurser (accessed: 28.01.2023).

Statistics Norway. (2022b). *Population*. Available at: https://www.ssb.no/en/statbank/table/05803/ (accessed: 23.09.2022).

Stocco, G., Summer, A., Cipolat-Gotet, C., Malacarne, M., Cecchinato, A., Amalfitano, N. & Bittante, G. (2021). The mineral profile affects the coagulation pattern and cheese-making efficiency of bovine milk. *Journal of Dairy Science*, 104 (8): 8439-8453. doi: 10.3168/jds.2021-20233.

Suman, G., Nupur, M., Anuradha, S. & Pradeep, B. (2015). Single cell protein production: a review. *International Journal of Current Microbiology and Applied Sciences*, 4 (9): 251-262.
Tilman, D., Balzer, C., Hill, J. & Befort, B. L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the national academy of sciences*, 108 (50): 20260-20264. doi: 10.1073/pnas.1116437108.
TINE SA. (2021). *Statistikksamling for ku- og geitekontrollen 2021 [Statistics from the control of milk from cows and goats 2021]*. Available at: https://medlem.tine.no/fag-og-forskning/statistikksamling-for-ku-og-geitekontrollen-2021 (accessed: 11.02.2023).
TINE SA. (2022a). *Melkepris for ku [Price of cow's milk]*. Available at: https://medlem.tine.no/melk/regelverk-og-melkepris/melkepris-og-satser/melkepris-for-ku (accessed: 04.10.2020).
TINE SA. (2022b). *Årsrapport 2021 [Yearly report 2021]*. Available at: https://www.tine.no/om-tine/TINE-aarsrapport-2021-digital.pdf (accessed: 23.01.2023).
Tsioulpas, A., Lewis, M. J. & Grandison, A. S. (2007). Effect of minerals on casein micelle stability of cows' milk. *Journal of Dairy Research*, 74 (2): 167-173. doi: 10.1017/s0022029906002330.
United Nations. (2017). *World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100*. Available at: https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html (accessed: 10.01.2023).
United Nations. (2022a). *The 17 Goals*. Available at: https://sdgs.un.org/goals (accessed: 29.08.2022).
United Nations. (2022b). *Day of Eight Billion*. Available at: https://www.un.org/en/dayof8billion (accessed: 22.01.2023).
United Nations. (2022c). *The Sustainable Development Goals Report 2022*. Available at: https://unstats.un.org/sdgs/report/2022/ (accessed: 09.01.2023).
Van Slyke, L. & Publow, C. (1910). *The Science and Practice of Cheese-Making*. New York: Orange Judd Company.
Verdier-Metz, I., Coulon, J.-B., Pradel, P., Viallon, C. & Berdaguè, J.-L. (1998). Effect of forage conservation (hay or silage) and cow breed on the coagulation properties of milks and on the characteristics of ripened cheeses. *Journal of Dairy Research*, 65 (1): 9-21. doi: 10.1017/S0022029997002616.
Visker, M. H. P. W., Schopen, G. C. B., van Arendonk, J. & Bovenhuis, H. (2010). Genome Wide Association For Casein Index In Milk Of Dairy Cattle. *Wageningen University & Research Publications*. doi: core.ac.uk/reader/29239319.

WBCSD. (2000). *Eco-efficiency - creating more value with less impact*. Available at: http://www.ceads.org.ar/downloads/Ecoeficiencia.%20Creating%20more%20value%20with%20less%20impact.pdf (accessed: 10.02.2023).

Wedholm, A., Larsen, L., Lindmark-Månsson, H., Karlsson, A. & Andrén, A. (2006). Effect of protein composition on the cheese-making properties of milk from individual dairy cows. *Journal of Dairy Science*, 89 (9): 3296-3305. doi: 10.3168/jds.S0022-0302(06)72366-9.

Weindl, I., Popp, A., Bodirsky, B. L., Rolinski, S., Lotze-Campen, H., Biewald, A., Humpenöder, F., Dietrich, J. P. & Stevanović, M. (2017). Livestock and human use of land: Productivity trends and dietary choices as drivers of future land and carbon dynamics. *Global and Planetary Change*, 159: 1-10. doi: 10.1016/j.gloplacha.2017.10.002.

Wickham, H., Chang, W., Henry, L., Pedersen, T., Takahashi, K., Wilke, C., Woo, K., Yutani, H. & Dunnington, D. (2022). *ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics*. URL https://cran.r-project.org/web/packages/ggplot2/index.html

World Bank. (2007). *World development report 2008: Agriculture for development*. Available at: https://openknowledge.worldbank.org/handle/10986/5990 (accessed: 11.11.2022).

Zhang, R., Mustafa, A. & Zhao, X. (2006). Effects of feeding oilseeds rich in linoleic and linolenic fatty acids to lactating ewes on cheese yield and on fatty acid composition of milk and cheese. *Animal Feed Science and Technology*, 127 (3-4): 220-233. doi: 10.1016/j.anifeedsci.2005.09.001.

Paper 1

DIFFERENT PROTEIN SOURCES IN CONCENTRATE FEED FOR DAIRY COWS AFFECT CHEESE-MAKING PROPERTIES AND YIELD

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ABSTRACT

Soybean meal (SBM) is a commonly used protein source in feed. Yeast microbial protein could be used as a substitute for SBM, but its impact on cheese-making properties and yield is not known. Norwegian Red dairy cows (n = 48) in early/mid lactation were divided in three groups and fed a ration consisting of grass silage and concentrate, where the concentrates were barley based but with different additional protein sources. These were: completely barley-based with no additional protein source (BAR), additional protein from soybean meal (SMB) or additional protein from yeast (*Cyberlindnera jadinii*) (YEA). The SBM and YEA concentrates had a higher protein content than the barley concentrate. Four batches of cheese were made from pooled milk from each of the three groups of dairy cows. Milk samples were collected five times during the experiment.

Milk from cows fed BAR concentrate showed inferior cheese-making properties (lower casein content, longer renneting time, lower content of phosphorus and lower cheese yield) compared to SBM and YEA concentrates. Overall, SBM or YEA bulk milk had similar cheese-making properties, but when investigating individual milk samples, YEA milk showed better coagulation properties.

Key words

Cheese-making efficiency, effect of feed on cheese, cheese yield, feed concentrate

1. INTRODUCTION

Cheese-making efficiency is highly influenced by milk composition, which again is affected by the source and type of feed. The main indicators for cheese-making efficiency are renneting time, cheese yield and loss of fat and protein in the whey. Curd structure, curd firmness, cheese yield and renneting time are all directly related to the casein content of milk (Jenkins and McGuire, 2006, Jõudu et al., 2008).

Manipulating milk composition by adjusting the dairy cow diet has been of interest for many years, and already in the early 1980s it was clear that dietary control of milk composition had opportunities, but also restrictions (Jenkins and McGuire, 2006). Protein content is more responsive to diet than lactose, but less responsive than fat. A review by Jenkins and McGuire (2006) stated that the transfer efficiency of dietary protein to milk protein is only 25-30 %, which explains the inability of the diet to markedly increase milk protein content. Since fat is the easiest milk component to manipulate, and considering human health issues connected to saturated fat, most research on the dairy cow diet has been concerned with fat content and fatty acid composition.

Due to sustainability issues and also to increase food security, the feed industry needs to develop novel non-food protein sources. Countries located above ~55° north have limited areas of cultivated land and a challenging climate, and this has led to the need to import protein-rich feed ingredients. However, our research has shown that it is possible to use new bio-refining technology to make protein-rich yeast biomass from cellulose (Lapeña et al., 2020). If this technology can be upscaled to substitute or partially substitute, for example, soy in feed (Kidane

et al., 2022) whilst maintaining the quality of cheese and other dairy products (Olsen et al., 2021) , this can both reduce the climate footprint of animal feeds and increase food security.

Soryal et al. (2004) tested the effect of pasture feeding in combination with different levels of concentrate feeds (0.66, 0.33 or 0 kg/day pr. 1.5 kg milk) on Domiati cheese from goat milk. They found that milk from goats fed a high concentrate level (0.66 kg/day) during pasture feeding gave a higher yield of Domiati cheese. They attributed this to a higher fat, protein and total solids content in milk from goats given concentrate feed compared to milk from goats kept on pasture without concentrate feed or under a confined feeding system with hay and concentrate.

Testroet et al. (2018) compared two diets given to mid-lactation Holstein cows. Diet 1 contained 13.5% of DM from soybean meal and diet 2 contained 19.5% of DM from reduced-fat dried distillers' grain. No differences were found between the diets regarding the suitability of milk for cheese- making (Baby Swiss cheese). Ferreira et al. (2017) studied the effect of replacing soybean meal and ground corn with licuri cake (a biodiesel by-product) at different concentrations (0, 200, 400 and 600 g/kg DM). They found a linear increase in milk fat content when ground corn and soybean meal were replaced with licuri cake, which led to a higher fat content in Minas frescal cheese. No differences were found between the feeds regarding cheese yield, protein, lactose, total solids and solids non-fat contents of either milk or cheese.

A lower milk casein content, but better coagulation properties were observed following pasture feeding during spring and summer in Poland compared to feeding silage or hay during autumn

and winter (Teter et al., 2020). Kälber et al. (2013) found that feeding buckwheat silage to dairy cows resulted in shorter coagulation time and increased curd firmness compared to feeding chicory or ryegrass. However, the milk protein content did not differ between the treatments and casein was not analyzed.

The use of yeast as a protein source in rations to dairy cows and the subsequent effect on feed efficiency, milk yield and metabolic status of the cow has been studied by several authors (Sabbia et al., 2012, Neal et al., 2014, Manthey et al., 2016, Kidane et al., 2022). Their results showed no clear differences in milk composition due to the different feed treatments. However, these studies have only to a minor extent focused on how the yeast influences milk quality more extensively than by only measuring the crude milk composition. None of these studies analyzed milk casein content, which is an important parameter for the dairy industry with regard to cheese-making.

The present work is based on results from the same feeding experiment as described by Kidane et al. (2022) and Olsen et al. (2021). These papers examined the effects of feeding different protein sources (barley, soybean meal and yeast) in concentrate feed for dairy cows, hypothesizing that *Cyberlindnera jadinii* yeast protein can replace soybean meal or barley in early- to mid-lactation Norwegian Red (NR) dairy cow diets without adverse effects on milk yield, milk composition and cheese quality. The results of Kidane et al. (2022) indicated that yeast could be used as a protein source for NR dairy cows without a negative effect on milk yield, and Olsen et al. (2021) found that all three protein sources resulted in cheeses of good quality. In addition to the quality of cheese, the cheese-making efficiency is highly important for

the cheese maker. Therefore in the present study, the performance of the milk during the cheese-making process was studied.

The main objective of this study was, therefore, to evaluate the effect of total substitution of soybean meal in concentrate feeds by *C. jadinii* yeast protein in grass silage-based rations of early- to mid-lactation NR cows on milk coagulation properties, cheese-making and cheese yield. Furthermore, as barley can be produced in Norway and is the most used concentrate feed ingredient, a diet with barley replacing both yeast protein and soybean meal in the concentrate feed was compared to those two other protein sources.

2. MATERIALS AND METHODS

2.1 Experimental setup, animal and feeding

The feeding experiment was performed at the Animal Production and Experimental Unit (SHF) at the Norwegian University of Life Sciences (NMBU, Ås, Norway) with all animal procedures approved by the national animal research authority of the Norwegian Food Safety Authority (FOTS ID 18038).

The feeding experiment is described in detail by Kidane et al. (2022) and lasted for 10 weeks comprising two weeks of adaptation and eight weeks of experimental diet. In short, forty-eight early- to mid-lactation Norwegian Red dairy cows were allocated into three treatment groups with 16 replicates per treatment based on parity, milk yield at start of the experiment (measured in the milking robot), days in milk (DIM) and milk protein genetic variants. An overview over the milk protein genetic variants is given in Olsen et al. (2021). The cows were fed a ration consisting of grass silage and concentrate. The concentrates were barley-based, but with different additional protein sources. These were: no additional protein source and completely barley-based (BAR), additional protein from soybean meal (SMB) or additional protein from yeast (*Cyberlindnera jadinii*) (YEA). The composition of concentrate feed and grass silage is shown in Table 1.

Table 1 Composition of concentrate feeds (Barley, BAR; soybean meal, SBM; and yeast, YEA) and grass silage. List of ingredients in shown in Kidane et al. (2022)

Chemical composition †	Concentrate feed			Grass silage
	BAR	SBM	YEA	
Dry matter, g/kg	875	875	881	300
Ash, g/kg DM	69.6	65.9	67.5	75.8
Crude protein (CP), g/kg DM ‡	134	161	157	181
Neutral detergent fiber (NDF), g/kg DM	187	186	169	533
Starch, g/kg DM	406	385	365	-
Fat, g/kg DM	38.0	38.3	36.9	46.3
Water soluble carbohydrate, %	5.68	6.15	5.85	1.67

† The reported chemical composition is based on a minimum of 3 analysis on composite samples
‡ CP was calculated as: N*6.25

During the adaptation period (2 weeks), cows in all three treatment groups were fed the concentrate feed with SBM. During the experimental period (the following 8 weeks) the cows in each treatment group were given either the same SBM concentrate feed as in the adaptation period, or BAR or YEA concentrate feed.

The chemical composition of the basal diet (grass silage) and concentrate feed together with basic cow information is provided by Olsen et al. (2021). The experimental concentrate feeds were prepared in such a way that the SBM and YEA were iso-nitrogenous with a somewhat higher protein content compared to the BAR concentrate (161, 157 and 134 g protein/kg of DM respectively) and all three feeds were approximately iso-energetic.

2.2 Milk sampling

Individual milk samples were collected in weeks 2, 4, 6, 7 and 10. The samples (50 mL) were collected automatically at each milking in a Delaval Classic milking robot system (DeLaval International AB, Tumba, Sweden). The cows had access to the milking robot every 6th hour and on average, 5 samples were obtained from each cow during a 48 h period and kept cold until

164 further preparation. On arrival at the analytic laboratory, all samples from the same cow were
165 mixed and these pooled samples were used for further analysis.
166
167 ***2.3 Cheese-making***
168 Gouda-type cheeses were made during weeks 8 and 9 of the feeding experiment, in the
169 University dairy pilot plant. Milk from the specific cows of each group (BAR, SBM and YEA)
170 was collected in a separate milk tank over 2 days.
171
172 It was only possible to sample milk separately from one experimental group at a time; therefore,
173 cheese was produced over six production days, two days for each type of milk. At each
174 production day, two vats of cheese were made, and these were considered as replicates. This
175 resulted in four cheese vats produced from the same type of milk (BAR, SBM or YEA) and in
176 total 12 vats of cheese were made. Cheeses were made as described by Olsen et.al (2020).
177 ***2.4 Analysis of individual milk samples and cheese milk***
178 Both the individual milk samples and the fat-standardized cheese milk prior to cheese-making
179 were analyzed for gross composition. Samples for analysis of gross composition were preserved
180 with bronopol (2-bromo-2-nitropane-1,3 diol, Broad-Spectrum Microtabs II, Advanced
181 Instruments, Norwood, MA, USA) and were analyzed by TINE S/A (Heimdal, Norway) for fat,
182 protein, lactose and somatic cell count using a DairySpec Combi (Bentley Instruments Inc.,
183 Chaska, MN, USA). The cheese milk was analyzed for fat, protein, casein and lactose using
184 MilkoScan FT1 (Foss Electric A/S, Hillerød, Denmark) in the University dairy pilot plant. The
185 pH was measured using a PHM 92 Lab pH meter (Radiometer, Copenhagen, Denmark).
186

187 The mineral content of individual milk samples and cheese milk was analyzed according to the
188 method described by Jørgensen et al. (2015) using SRM 1549A (National Institute of Standards
189 & Technology, Gaithersburg, MD, USA) as reference material.
190
191 Rennet coagulation properties (i.e., RCT, rennet clotting time; K20, time until 20 mm width
192 between the pendulums is achieved in the Lattodinamografo; and A30, firmness after 30 min) of
193 the individual milk samples were analyzed using Lattodinamografo (LAT; Foss-Italia SpA,
194 Padova, Italy) according to the method described by Inglingstad et al. (2014). This analysis was
195 made on the same day as the samples arrived at the laboratory. The K20 results were transformed
196 to binary data (0 = samples that did not attain firmness of 20 mm and 1 = samples that did attain
197 firmness of 20 mm).

198 ***2.5 Cheese Analysis and calculations***

199 Renneting time during production of cheese was defined as the time from adding the rennet until
200 cutting the coagulum. Curd firmness and time to cut was evaluated by an experienced
201 cheesemaker. The amount of cheese milk (L) and weight of cheese (kg) after brining was
202 measured.
203
204 Twenty-four hours after the start of cheese-making, cheese was analyzed for dry matter (IDF
205 standard 50C (IDF, 1995)) and pH (using a PHM 92 Lab pH meter (Radiometer, Copenhagen,
206 Denmark)).
207
208 Predicted cheese yield (PY) was calculated by using the Van Slyke formula (Fox et al., 2017a)
209 using the fat and casein content and a constant for loss of fines and other solids included in the

cheese (E0). Actual Yield (Ya) (E1) and moisture adjusted cheese yield (MACY) (E2) were calculated according to Banks (2007). Yield efficiency (YE) using Ya and PY was calculated (E3) according to Fox et al. (2017a).

$$\textbf{PY (Van Slyke)} = \frac{(0.93\ \text{F}+\text{C}-0.1)\ \text{x}\ 1.09}{100-\text{W}}\ \text{x}\ 100 \qquad \text{(E0)}$$

Where:

F - Fat in milk (%)

C – Casein in milk (%)

W – Desired water content in cheese*

0.1 – Constant for loss of cheese fines in whey

1.09 – Constant representing other solids included in the cheese

* The mean moisture content of all 24 hour cheeses (n=12)

$$\textbf{Ya} = \frac{\text{Weight of cheese (kg)}*}{\text{Weight of milk (kg)}+\text{weight of starter(kg)}} \times 100 \qquad \text{(E1)}$$

*weight of cheese after brining

$$\textbf{MACY} = \text{Ya} \times \frac{100-\text{actual cheese moisture content (\%)}}{100-\text{reference cheese moisture content (\%)}*} \qquad \text{(E2)}$$

* The mean moisture content of all 24 hour cheeses (n=12)

$$\textbf{\textit{YE}} = \frac{\text{Ya}}{\text{PY (Van Slyke)}}\ \text{x}\ 100 \qquad \text{(E3)}$$

2.6 Statistical analysis

Data for milk composition and coagulation properties (RCT and A30) were analyzed using the mixed procedure of SAS (SAS Enterprise Guide 7.1, SAS, Cary, NC, USA). Somatic cell counts were log10 transformed prior to analysis because of non-normal distribution. The model included the fixed effects of concentrate feed (BAR, SBM or YEA), weeks (4, 6, 7, 10), parity (primiparous or multiparous), a covariate value (the respective variables from each cow from the

end of the adaption period (first two weeks)), and the interaction between concentrate feed and week, as well as the repeated effect of week and random effect of cow nested within concentrate feed and parity. Tukey-Kramer was used to test for pairwise differences between least square means. Data are presented as least square means, with statistical significance declared at $P < 0.05$.

The effect of concentrate feed on K20 was tested using the logistic procedure in SAS Enterprise Guide 7.1. As many of the samples did not attain K20, data were converted to a binary format (samples that did attain K20 and samples that did not attain K20), as previously described. The model used included the fixed effects of concentrate feed (BAR, SBM or YEA), week (4, 6, 7, 10), parity (primiparous or multiparous), covariate (week 2), and the interaction between concentrate feed and week.

Significant effects ($P \leq 0.05$) of experimental factors on the cheese milk, cheeses and production parameters were found using the mixed procedure of SAS Enterprise Guide 7.1. The experimental factors used were concentrate feed as the main factor ($n = 3$) and cheese-making day ($n = 6$) as a random factor. Least Square Post Hoc (Tukey) was used to test differences between means (all pairwise differences).

3. RESULTS

All data used in this paper can be found under NMBU Open research Data (Olsen, 2022).

3.1 Individual milk samples

Gross composition and coagulation properties (RCT and A30) of individual milk samples are shown in Table 2. The concentrate feed did not affect the gross composition of milk or its content of somatic cells, but milk protein content increased towards the end of the experiment. YEA milk had a significantly higher content of phosphorus than to the BAR milk, and BAR milk contained a significantly more selenium and iodine compared to YEA and SBM milk.

Most of the milk samples demonstrated poor coagulation properties, showing a long RCT and low A30. The RCT of the milk was not influenced by the types of feed, although SBM milk had a borderline significantly shorter RCT compared to BAR milk ($P = 0.051$) (17.9 vs. 19.8 min respectively). The A30 was considered weak as most of the milk gels had a firmness well below 20 mm. BAR milk obtained the least firm gel with a mean A30 of 12.33±1.04 mm, while YEA milk obtained the highest A30 with a mean of 15.29±1.04 mm. Out of a total of 236 analyzed samples, only 77 samples (33%) attained K20.

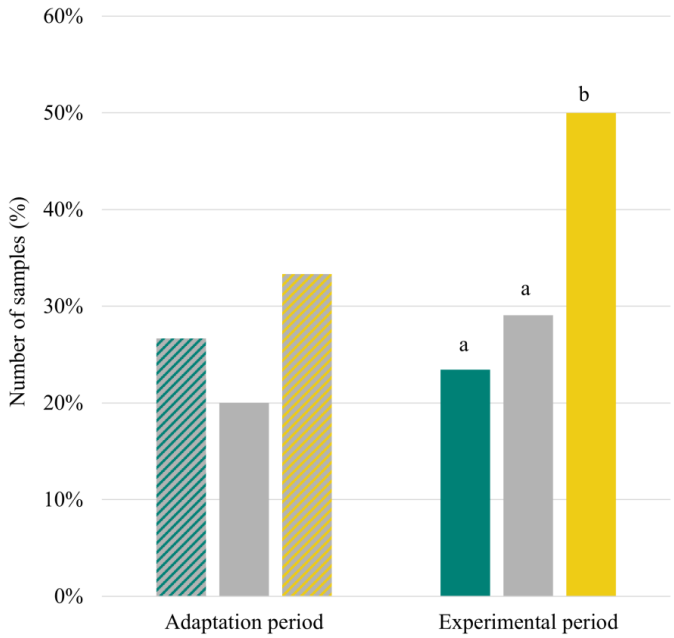
There was a greater probability that the milk gel would attain a firmness of at least 20 mm if the cows were fed YEA concentrate feed compared to both SBM and BAR milk (Figure 1). If they were fed SBM concentrate feed, it was more likely that the milk gel would attain K20 compared to feeding BAR concentrate feed. Although no treatment*week interaction was found, it appears as the proportion of samples attaining K20 increased gradually (except for week 10) for the YEA treatment (44 %, 50 %, 63 % and 44 % in week 4, 6, 7 and 10 respectively). Milk from

277 primiparous cows was less likely to attain K20 than milk from multiparous cows (results not
278 shown).
279
280 In total, 13 milk samples from 9 cows (distribution: BAR = 2, SBM = 5 and YEA = 2) were non-
281 coagulating, i.e., they did not form a curd within 30 minutes. The SBM group had a higher
282 proportion of non-coagulating samples, but this group included two cows that gave milk that did
283 not coagulate at 3 out of the 5 samplings. This indicates more of an individual cow problem
284 rather than a feed problem.
285

Table 2 Milk composition and coagulation properties of individual milk samples from dairy cows fed concentrate feed based on 3 different protein sources (Barley, BAR; soybean meal, SBM; and yeast, YEA). Values are presented as LS means (n= 240; on some occasions the amount of milk was not sufficient for all analysis). Significant differences (P<0.05) of the LS means of each concentrate feed are marked with different letters.

	Concentrate feed				Statistics (P-value)		
	BAR	SBM	YEA	SE	Concentrate feed	Week	Concentrate feed*Week
Milk composition							
Fat, %	4.45	4.40	4.42	0.074	NS	0.012	<0.001
Protein, %	3.50	3.61	3.61	0.054	NS	NS	NS
Lactose, %	4.79	4.79	4.79	0.019	NS	0.007	NS
pH	6.78	6.78	6.78	0.008	NS	<0.001	NS
Dry matter, %	13.3	13.3	13.3	0.108	NS	0.001	<0.001
SCC[1], log cells/mL	4.79	4.79	4.79	0.019	NS	0.007	NS
Minerals							
Ca, g/kg	1.18	1.20	1.22	0.013	NS	NS	NS
K, g/kg	1.73	1.76	1.76	0.012	NS	0.009	NS
Mg, g/kg	0.12	0.12	0.12	0.001	NS	0.053	NS
Na, mg/kg	341	320	324	8.247	NS	NS	NS
P, mg/kg	974[b]	995[ab]	1005[a]	8.981	0.037	NS	NS
Zn, mg/kg	3.52	3.66	3.56	0.072	NS	0.001	NS
Se, µg/kg	11.1[a]	10.4[b]	10.2[b]	0.176	0.003	0.024	NS
I, mg/kg	0.33[a]	0.28[b]	0.27[b]	0.010	<0.001	<0.001	<0.001
Coagulation properties							
RCT[2], min	19.8	17.9	18.5	0.558	0.053	0.038	NS
A30[3], mm	12.3	14.6	15.3	1.043	NS	<0.001	NS
K20*[4], min	9.59	9.40	8.30	0.712	NS	NS	NS

a-b Different superscript letter represents significant differences between the different concentrate feeds at P ≤ 0.05 for the diet variable
* 33 % of the analyzed samples obtained K20
[1] Somatic cell count
[2] Rennet clotting time
[3] Time before firmness 20 mm is achieved
[4] Firmness after 30 min



300

301 Figure 1 Proportion of milk samples, in %, that attained a firmness of at least 20 mm (K20: time
302 in min taken for the width of the curves to increase to 20 mm) during the 30 minutes run using a
303 Lattodinamografo for each diet group (■ Barley, BAR; ■ soybean meal, SBM; and ■ yeast,
304 YEA) in the adaption and experimental periods (Results in the experimental period is the
305 calculated mean of week 4, 6, 7 and 10). Different letters indicate significant differences
306 ($p<0.05$) between the concentrate feeds in the experimental period. In the adaptation period all
307 groups were fed soybean meal, the color mixed with grey shows which feeding each group were
308 further allocated to in the experimental period.
309

310

311 ***3.2 Cheese milk and cheese-making***

312 The composition of fat-standardized cheese milk and of cheese the day after production is shown
313 in Table 3, while different yield parameters are shown in Table 4. Gross composition and pH of
314 individual milk samples were not influenced by the different concentrate feeds used. However,
315 YEA and SBM milk had a significantly (P= 0.0005) higher content of casein compared to BAR
316 milk, and this resulted in >0.44 kg more casein in the SMB and YEA cheese vats compared to
317 the BAR cheese vats.
318
319 BAR cheese milk differed from SMB and YEA milk with regard to the content of several
320 minerals (Table 3). The BAR cheese milk had a significantly lower concentration of
321 phosphorous than YEA and SBM milk. In addition, the BAR cheese milk had a higher
322 concentration of sodium compared to SBM cheese milk and a higher concentration of iodine
323 compared to YEA and SBM cheese milk.
324
325 Due to differences in the casein content, the rennet to casein ratio differed between the
326 experimental groups, as the rennet was added according to volume of milk and not according to
327 kg of casein. Significantly more rennet in relation to casein (ml/kg casein) was added to the BAR
328 milk vats compared to YEA and SBM milk vats. Despite this, the BAR milk had a significantly
329 longer renneting time compared to the YEA milk. Due to the higher content of casein in YEA
330 and SBM milk, the predicted cheese yield (PY) from cheese vats from these groups was
331 significantly higher than cheese made from BAR milk. Both the Ya and MACY confirmed the
332 results calculated for the PY. There were no significant differences in YE due to high standard

333 deviations, but a tendency indicated that it could be more efficient to make cheeses from SBM
334 cheese milk compared to BAR cheese milk.

335 Table 3 Gross composition of cheese milk and renneting properties of the produced cheeses
336 within each diet group (Barley, BAR; soybean meal, SBM; and yeast, YEA). Values are
337 presented as mean of four replicates ± standard deviation. Significant differences (P<0.05) of the
338 means of each concentrate feed are marked with different letters

	Concentrate feed			
	BAR (n=4)	SBM (n=4)	YEA (n=4)	P-value
Cheese milk				
Gross composition				
Fat, %	2.71 ± 0.026	2.71 ± 0.032	2.74 ± 0.040	NS
Protein, %	3.66 ± 0.142	3.74 ± 0.060	3.69 ± 0.040	NS
Lactose, %	4.68 ± 0.043	4.66 ± 0.066	4.68 ± 0.022	NS
Casein				
Casein, %	2.65 ± 0.010^{b}	2.79 ± 0.039^{a}	2.76 ± 0.022^{a}	<0.001
Casein/Protein, %	72.4 ± 2.646	74.8 ± 0.303	74.9 ± 0.249	NS
Casein cheese milk, kg/vat	8.56 ± 0.099^{b}	9.01 ± 0.135^{a}	9.00 ± 0.071^{a}	0.002
Minerals				
Ca, g/kg	1.20 ± 0.000	1.20 ± 0.000	1.20 ± 0.000	NS
K, g/kg	1.80 ± 0.000	1.73 ± 0.050	1.73 ± 0.058	NS
Mg, g/kg	0.12 ± 0.000	0.13 ± 0.005	0.12 ± 0.005	NS
Na, mg/kg	338 ± 5.0^{a}	325 ± 5.8^{b}	335 ± 5.8^{ab}	0.041
P, mg/kg	957 ± 5.0^{b}	992 ± 15^{a}	985 ± 13.0^{a}	0.013
Zn, mg/kg	3.40 ± 0.000^{b}	3.63 ± 0.096^{a}	3.53 ± 0.050^{a}	0.007
Se, µg/kg	10.5 ± 0.577	10.0 ± 0.000	9.83 ± 0.096	NS
I, mg/kg	0.31 ± 0.032^{a}	0.22 ± 0.006^{b}	0.22 ± 0.015^{b}	0.008
pH	6.83 ± 0.119	6.84 ± 0.077	6.80 ± 0.085	NS
Coagulation				
Rennet added, ml/kg casein	9.45 ± 0.036^{a}	8.96 ± 0.126^{b}	9.06 ± 0.071^{b}	<0.001
Renneting time, min	38.3 ± 4.992^{a}	31.3 ± 1.500^{ab}	29.0 ± 3.916^{b}	0.031
Cheese 24-hours after starter addition				
pH	5.34 ± 0.085	5.37 ± 0.056	5.32 ± 0.035	NS
Dry matter, %	51.5 ± 0.258	51.6 ± 0.466	52.0 ± 0.120	0,107

339

340 Table 4 Different cheese yield parameters from the production of cheeses within each diet group
341 (Barley, BAR; soybean meal, SBM; and yeast, YEA). Values are presented as mean of four
342 replicates ± standard deviation. Significant differences ($P<0.05$) of the means of each concentrate
343 feed are marked with different letters

	Concentrate feed			
	BAR (n=4)	SBM (n=4)	YEA (n=4)	P-value
Predicted yield (PY) (kg cheese/100 L milk) [1]	10.8 ± 0.056^{b}	11.1 ± 0.037^{a}	11.1 ± 0.065^{a}	0.0003
Actual yield (Ya) (kg cheese/100 L milk)[2]	9.92 ± 0.168^{b}	10.4 ± 0.152^{a}	10.3 ± 0.153^{a}	0.0284
MACY (kg cheese/100 L milk)[3]	9.87 ± 0.172^{b}	10.33 ± 0.241^{a}	10.35 ± 0.151^{a}	0.03797
YE (%)[4]	91.7 ± 1.444	93.1 ± 1.378	92.3 ± 1.026	NS

344 [1] PY (E0)
345 [2] Ya (E1)
346 [3] MACY (E2)
347 [4] YE (E3)

348

4. DISCUSSION

This study showed that feeding YEA concentrate feed gave a higher probability that the individual milk samples attained good coagulation properties, and in the cheese vat, the YEA cheese milk was superior to the BAR cheese milk. This can probably be attributed to the higher casein content in the YEA milk compared to the BAR cheese milk. Higher casein content is correlated with better coagulation properties and has also been shown to be more important than total protein content (Auldist et al., 2002, Jõudu et al., 2008).

The coagulation properties of the individual milk samples were in general poor, and may be due to factors such as late lactation, high somatic cell count, casein content and polymorphism of the milk proteins, among others (Fox et al., 2017b, a). Those factors of relevance for this experiment are discussed further. None of the cows were in late lactation during this experiment and the somatic cell counts were low, therefore the whey protein:casein ratio in the individual milk samples was most probably fairly constant (not analyzed). The non-coagulating samples came from all the diet-groups, suggesting it is unlikely that the feed type caused the difference. When dealing with coagulation experiments the genetic variants of the milk proteins for the cows used in the experiment should be balanced, since these genetic variants affect cheese-making properties such as coagulation properties and cheese yield (Ng-Kwai-Hang, 2006, Gustavsson et al., 2014, Ketto et al., 2017). When grouping the cows, it was decided to use those cows with genotypes having the highest frequency at SHF (Olsen et al., 2021), and these cows unfortunately had a high prevalence of genetic protein variants related to inferior milk coagulation properties, like κ-CN AA and β-CN A2A2 (Ketto et al., 2017). The occurrence of these variants was high within the experimental herd, in total 35 out of 48 cows (73 %) had the

372 AA-variant of κ-CN in this experiment, which in NR is associated with poorer coagulation
373 properties than the BB variant of κ-CN (Ketto et al., 2017). All of the non-coagulating milk
374 samples had the AA-variant of κ-CN. This may explain the poor coagulation properties of the
375 individual milk samples in this experiment.
376
377 During cheese-making, rennet was added at a concentration of 25 mL/100 L milk without any
378 adjustment for casein concentration, as is normal practice in Norway. However, since the milk in
379 this experiment had greater differences in casein concentration than the normal variation, an
380 adjustment of the rennet addition should preferably have been done. Previously, the average
381 casein content in milk from cows at SHF has been 2.65 % and this has been used to standardize
382 the rennet:casein ratio. This gives 9.38 mL of rennet used (Chy-Max Plus, Chr. Hansen,
383 Hørsholm, Denmark) pr. kg casein which corresponds to using 25 mL of rennet used pr. 100 L of
384 milk. When comparing the actual amount of rennet added in this experiment with the calculated
385 amount of rennet needed if 9.38 mL rennet/kg casein should be used, the correct amount of
386 rennet was added to BAR cheese milk, but less rennet than optimal was added to YEA and SBM
387 cheese milk. Probably, if the amount of rennet had been adjusted to the casein content, an even
388 greater difference in renneting time would have been found between BAR milk and the two
389 others (SBM and YEA). Moreover, as the casein content was not analyzed in the individual milk
390 samples, and there were probably differences in casein concentration in those samples as well,
391 then the same rennet:casein situation would also apply for the individual samples.
392
393

It is well known that casein content and composition of bovine milk influences the cheese-making efficiency and is therefore of great importance for profitability. An increase of the casein content in milk does not only normally result in better coagulation properties, but also in a higher cheese yield (Bobe et al., 1999, Banks, 2007, Fox et al., 2017a). This was also observed in this trial and may be attributed to the casein content and also mineral content as discussed further. The milk salts, especially calcium and phosphate, play a vital role in the structure of casein micelles and affect not only milk coagulation but also other aspects of cheese-making such as buffer capacity and cheese texture (Lucey and Fox, 1993). Stocco et al. (2021) studied the effect of minerals on milk coagulation properties and yield of model cheeses. They found that phosphorus was associated with good cheese-making traits and an increased cheese yield (curd solids), and that a higher concentration of sodium in milk was associated with lower protein recovery in model cheese. Therefore, the higher content of phosphorus and casein in SBM and YEA cheese milks compared to BAR milk may have affected the coagulation and cheese-making properties and contributed to the higher cheese yield. In addition, the higher sodium content of BAR cheese milk compared to SBM cheese milk might have contributed to an undesirable longer renneting time and a lower protein recovery, thereby resulting in a lower Ya and MACY compared to the other groups. Although no differences in YE were found between the groups due to the high standard deviation, BAR milk showed a tendency to be less efficient for cheese-making. This may be due to the higher sodium content. Several other authors have also found a link between rennet coagulation properties and the mineral content of milk, both Malacarne et al. (2014) and Jensen et al. (2012) showed that milk with good coagulation properties had a higher content of calcium, phosphorous and magnesium, compared to poorly coagulating and non-coagulating milk. The mineral concentration in milk is affected by the mineral composition in

417 the feed and by soil conditions where the feed is grown (Alothman et al., 2019). This study
418 shows that changing the protein content or protein source in feed to dairy cows can have several
419 side-effects additional to changing the gross composition of milk, and that these changes can
420 influence milk properties during the processing of different dairy products.
421
422 During feeding trials with dairy cows, milk protein, fat, lactose and milk yield are usually
423 measured, but casein is not usually analyzed. Normally, feeding trials do not include a cheese-
424 making experiment, and we have managed to identify only a few feeding studies where cheese
425 has also been made.
426
427 Our results indicates that if casein content was measured in the studies of for example Sabbia et
428 al. (2012), Manthey et al. (2016) and Neal et al. (2014), where alternative protein sources for
429 dairy cows were investigated, differences could actually have been obtained.
430
431 In this study we used grass silage which is the commonly used silage type in northern countries.
432 Further work is needed to see if similar results would be obtained by using other types of silage,
433 such as in example maize silage, used in regions suitable for such crops. In addition, an
434 interesting approach would be further testing of different protein sources and protein levels in
435 concentrate feeds also in relation to the cheese-making efficiency and cheese quality.
436
437

438 ## 5. CONCLUSIONS

439 With increasing global population and climate change, it is necessary to find alternative non-food
440 protein sources for farm animal feed and to allocate food-grade protein to human consumption.

Yeast production, using cellulose as raw material, is a possible alternative in countries with limited cultivated land. By using such resources, more countries could have a self-sufficient supply of feed ingredients and therefore limit long distance transportation due to export/import. This experiment shows that it is possible to substitute or partly substitute soybean meal with yeast as a protein source in concentrate feed to dairy cows, without negative effects on cheese-making properties. However, when comparing yeast or soy with barley, the cheese-making properties of the milk were clearly different, Therefore, the protein source and protein content of the feed are of importance when addressing cheese-making properties and cheese yield. This should be considered when planning dairy cow rations, as it influences the economy of the entire dairy chain.

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6. REFERENCES

Alothman, M., S. A. Hogan, D. Hennessy, P. Dillon, K. N. Kilcawley, M. O'Donovan, J. Tobin, M. A. Fenelon, and T. F. O'Callaghan. 2019. The "grass-fed" milk story: understanding the impact of pasture feeding on the composition and quality of bovine milk. Foods. 8:350. https://doi.org/10.3390/foods8080350

Auldist, M., C. Mullins, B. O'Brian, B. O'Kennedy, and T. Guinee. 2002. Effect of cow breed on milk coagulation properties. Milchwissenschaft. 57:140-143.

Banks, J. M. 2007. Cheese yield. Pages 100-114 in Cheese Problems Solved. P. L. H. McSweeney, ed. Woodhead Publishing Limited, Cambridge, England.

Bobe, G., D. Beitz, A. Freeman, and G. Lindberg. 1999. Effect of milk protein genotypes on milk protein composition and its genetic parameter estimates. J Dairy Sci. 82:2797-2804. https://doi.org/10.3168/jds.s0022-0302(99)75537-2

Ferreira, A. C., J. F. Vieira, A. M. Barbosa, T. M. Silva, L. R. Bezerra, N. G. Nascimento, J. E. de Freitas, S. M. P. L. Jaeger, P. d. A. Oliveira, and R. L. Oliveira. 2017. Effect of replacing ground corn and soybean meal with licuri cake on the performance, digestibility, nitrogen metabolism and ingestive behavior in lactating dairy cows. CABI Agric Biosci. 11:1957-1965. https://doi.org/10.1017/S175173111700074X

Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney. 2017a. Cheese Yield. Pages 279-331 in Fundamentals of Cheese Science. P. F. Fox, T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney, ed. Springer Science+Business Media LLC, New York.

Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney. 2017b. Enzymatic Coagulation of Milk. Pages 185-230 in Fundamentals of Cheese Science. P. F. Fox, T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney, ed. Springer Science+Business Media LLC, New York.

Gustavsson, F., A. Buitenhuis, M. Glantz, H. Stålhammar, H. Lindmark-Månsson, N. Poulsen, L. Larsen, A. Andrén, and M. Paulsson. 2014. Impact of genetic variants of milk proteins on chymosin-induced gelation properties of milk from individual cows of Swedish Red dairy cattle. Int Dairy J. 39:102-107. https://doi.org/10.1016/j.idairyj.2014.05.007

IDF. 1995. Milk and Milk Products: Guidance on Sampling. in IDF Standard 50C. International Dairy Federation, Brussels, Belgium.

Inglingstad, R., H. Steinshamn, B. Dagnachew, B. Valenti, A. Criscione, E. Rukke, T. Devold, S. Skeie, and G. Vegarud. 2014. Grazing season and forage type influence goat milk composition and rennet coagulation properties. J Dairy Sci. 97:3800-3814. https://doi.org/10.3168/jds.2013-7542

Jenkins, T. and M. McGuire. 2006. Major advances in nutrition: impact on milk composition. Journal of Dairy Science. 89:1302-1310. 10.3168/jds.s0022-0302(06)72198-1

Jensen, H., N. Poulsen, K. Andersen, M. Hammershøj, H. Poulsen, and L. Larsen. 2012. Distinct composition of bovine milk from Jersey and Holstein-Friesian cows with good, poor, or noncoagulation properties as reflected in protein genetic variants and isoforms. J Dairy Sci. 95:6905-6917. https://doi.org/10.3168/jds.2012-5675

Jõudu, I., M. Henno, T. Kaart, T. Püssa, and O. Kärt. 2008. The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. Int Dairy J. 18:964-967. 10.1016/j.idairyj.2008.02.002

Jørgensen, C. E., R. K. Abrahamsen, E.-O. Rukke, A.-G. Johansen, R. B. Schüller, and S. B. Skeie. 2015. Improving the structure and rheology of high protein, low fat yoghurt with undenatured whey proteins. Int Dairy J. 47:6-18. https://doi.org/10.1016/j.idairyj.2015.02.002

Ketto, I. A., T. M. Knutsen, J. Øyaas, B. Heringstad, T. Ådnøy, T. G. Devold, and S. B. Skeie. 2017. Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. Int Dairy J. 70:55-64. 10.1016/j.idairyj.2016.10.010

Kidane, A., S. G. Vhile, S. Ferneborg, S. Skeie, M. A. Olsen, L. T. Mydland, M. Øverland, and E. Prestløkken. 2022. *Cyberlindnera jadinii* yeast as a protein source in early-to mid-lactation dairy cow diets: Effects on feed intake, ruminal fermentation, and milk production. Journal of Dairy Science. 10.3168/jds.2021-20139

Kälber, T., M. Kreuzer, and F. Leiber. 2013. Effect of feeding buckwheat and chicory silages on fatty acid profile and cheese-making properties of milk from dairy cows. J Dairy Res. 80:81-88. https://doi.org/10.1017/S0022029912000647

Lapeña, D., P. M. Olsen, M. Ø. Arntzen, G. Kosa, V. Passoth, V. G. Eijsink, and S. J. Horn. 2020. Spruce sugars and poultry hydrolysate as growth medium in repeated fed-batch fermentation processes for production of yeast biomass. Bioprocess Biosyst Eng. 43:723-736. https://doi.org/10.1007/s00449-019-02271-x

Lucey, J. and P. Fox. 1993. Importance of calcium and phosphate in cheese manufacture: A review. Journal of Dairy Science. 76:1714-1724. 10.3168/jds.S0022-0302(93)77504-9

Malacarne, M., P. Franceschi, P. Formaggioni, S. Sandri, P. Mariani, and A. Summer. 2014. Influence of micellar calcium and phosphorus on rennet coagulation properties of cows milk. Journal of Dairy Research. 81:129-136. 10.1017/s0022029913000630

528 Manthey, A., K. Kalscheur, A. Garcia, and K. Mjoun. 2016. Lactation performance of dairy
529 cows fed yeast-derived microbial protein in low-and high-forage diets. J Dairy Sci. 99:2775-
530 2787. https://doi.org/10.3168/jds.2015-10014

531 Neal, K., J. Eun, A. Young, K. Mjoun, and J. Hall. 2014. Feeding protein supplements in alfalfa
532 hay-based lactation diets improves nutrient utilization, lactational performance, and feed
533 efficiency of dairy cows. J Dairy Sci. 97:7716-7728. https://doi.org/10.3168/jds.2014-8033

534 Ng-Kwai-Hang, K. 2006. Genetic variants of milk proteins and their effects on the yield and
535 quality of cheese. CABI reviews. 10.1079/PAVSNNR20061056

536 Olsen, M. A. 2022. Replication Data for "Different protein sources in concentrate feed to dairy
537 cows affects cheese making properties and yield". DataverseNO.
538 https://doi.org/10.18710/2LXWNW

539 Olsen, M. A., S. G. Vhile, D. Porcellato, A. Kidane, and S. B. Skeie. 2021. Feeding concentrates
540 with different protein sources to high-yielding, mid-lactation Norwegian Red cows: Effect on
541 cheese ripening. J Dairy Sci. 104:4062-4073. https://doi.org/10.3168/jds.2020-19226

542 Sabbia, J., K. Kalscheur, A. Garcia, A. Gehman, and J. Tricarico. 2012. Soybean meal
543 substitution with a yeast-derived microbial protein source in dairy cow diets. J Dairy Sci.
544 95:5888-5900. https://doi.org/10.3168/jds.2011-5237

545 Soryal, K., S. Zeng, B. Min, S. Hart, and F. Beyene. 2004. Effect of feeding systems on
546 composition of goat milk and yield of Domiati cheese. Small Ruminant Research. 54:121-129.
547 10.1016/j.smallrumres.2003.10.010

548 Stocco, G., A. Summer, C. Cipolat-Gotet, M. Malacarne, A. Cecchinato, N. Amalfitano, and G.
549 Bittante. 2021. The mineral profile affects the coagulation pattern and cheese-making efficiency
550 of bovine milk. Journal of Dairy Science. 104:8439-8453. 10.3168/jds.2021-20233

551 Testroet, E., D. Beitz, M. O'Neil, A. Mueller, H. Ramirez-Ramirez, and S. Clark. 2018. Feeding
552 reduced-fat dried distillers grains with solubles to lactating Holstein dairy cows does not alter
553 milk composition or cause late blowing in cheese. J Dairy Sci. 101:5838-5850.
554 https://doi.org/10.3168/jds.2017-13699

555 Teter, A., M. Kędzierska-Matysek, J. Barłowska, J. Król, and A. Brodziak. 2020. Nutritional
556 value and coagulation properties of milk from local cow breeds, including the selected macro-
557 and micronutrients and trace elements. Mljekarstvo: časopis za unaprjeđenje proizvodnje i
558 prerade mlijeka. 70:210-220. https://doi.org/10.15567/mljekarstvo.2020.0307
559

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Feeding concentrates with different protein sources to high-yielding, mid-lactation Norwegian Red cows: Effect on cheese ripening

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ABSTRACT

Soybean meal is one of the most important protein sources in concentrate feeds for dairy cows. The objective of the present study was to provide knowledge on the effects of using a novel yeast microbial protein source (*Candida utilis*) in concentrate feed for dairy cows on the production and quality of a Gouda-type cheese. Forty-eight Norwegian Red dairy cows in early to mid lactation were fed a basal diet of grass silage, which was supplemented with 3 different concentrate feeds. The protein source of the concentrates was based on conventional soybean meal (SBM), novel yeast (*C. utilis*; YEA), or barley (BAR; used as negative control because barley has a lower protein content). The experiment was carried out for a period of 10 wk, with the first 2 wk as an adaptation period where all dairy cows were fed grass silage and the SBM concentrate. The cows were then randomly allocated to 1 of the 3 different compound feeds: SBM, yeast, or barley. Cheeses were made during wk 8 and 9 of the experiment, with 4 batches of cheese made from milk from each of the 3 groups. The cheeses made from milk from cows fed SBM concentrate (SBM cheese) had a higher content of DL-pyroglutamic acid and free amino acids than the other cheeses, indicating a faster ripening in the SBM cheeses. Despite these differences, the sensory properties, the microbiota, and the *Lactococcus* population at 15 wk of ripening were not significantly different between the cheeses. This experiment showed that although the raw materials used in the concentrate feed clearly influenced the ripening of the cheeses, this did not affect cheese quality. Yeast (*C. utilis*) as a protein source in concentrate feed for dairy cows can be used as a replacement for soybean meal without compromising the quality of Norwegian Gouda-type cheeses.

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Key words: novel concentrate feed, cheese ripening, cheese quality

INTRODUCTION

The feed industry needs to develop novel, sustainable, nonfood protein sources to increase food security and to have more choices in an unpredictable future. Due to limited cultivatable land and a challenging climate, there is a shortage of nationally produced protein feed sources in countries above approximately 55° N, which necessitates the import of protein-rich feed ingredients (de Visser et al., 2014; Øverland and Skrede, 2017). Diets for high-yielding dairy cows in Norway commonly consist of grass silage and concentrates at a ratio of 60/40 (Animalia, 2019). Today, it is difficult to locally produce sufficient amounts of protein from grass silage and cereals such as barley to cover the nutritional needs of high-yielding dairy cows. The Norwegian dairy industry therefore needs to find novel and alternative protein sources that can be added as a supplement to barley in feed concentrates.

Around 75% of the soy produced worldwide (measured by weight) is used as feed for livestock (FCRN Foodsource, 2020). Because the world's population is expected to increase to 9.8 billion people by 2050 (United Nations, 2017), this is not an optimal use of soy protein. Soybeans are rich in protein, making them an excellent protein source for human nutrition. However, the feed influences the milk composition (Sutton, 1989) and, therefore, most likely the cheese quality. Several studies have evaluated the effect of different protein sources used in feed for dairy cows on milk and cheese quality; however, none of these studies used yeast as a protein source in the feed. Sankarlal et al. (2015) fed dried distillers grains at 0, 10, and 20% of a TMR diet to mid-lactation Holstein cows and found an increase in long-chain unsaturated fatty acids and a decrease in most medium-chain and all short-chain fatty acids in Baby Swiss cheeses. Testroet et al. (2018) compared 2 different isonitrogenous and isoenergetic diets given to

mid-lactation Holstein cows: a diet containing 13.5% of DM from soybean meal versus 19.5% of DM from reduced-fat dried distillers grains. They found no differences in the suitability of milk for cheese making (Baby Swiss cheese), as the quality of the produced cheeses was similar. Ferreira et al. (2017) studied the effect of partially replacing ground corn and soybean meal with licuri cake (a biodiesel by-product) at different concentrations (0, 200, 400, and 600 g/kg in DM).They reported a linear increase in milk fat concentration, which resulted in a higher fat content in the Minas Frescal cheese. They observed no differences between the feeds in relation to yield, protein, lactose, total solids, and solids nonfat in either the milk or the cheese.

Innovative methods are needed to increase national self-sufficiency of livestock feed and to reallocate nutritious soy protein from feed to food protein. Recent developments in biorefining technologies have made it possible to produce yeast biomass by fermentation of sugars derived from lignocellulosic biomass, such as spruce wood (Øverland and Skrede, 2017; Lapeña et al., 2020), where enzyme technology has been used to convert the cellulose and hemicellulose into sugars. Today, single-cell protein from yeast, bacteria, and algae are obtained by commercially growing them on molasses from different sources (e.g., sugar cane, sugar beets, corn). It is, however, possible to use by-products from agriculture and raw materials from forestry. Many countries have substantial areas of forest that traditionally have been of low value as feed for livestock. Forested areas in Norway account for 37.4% of the mainland (Statistisk Sentralbyrå, 2019) and therefore represent a large bioresource. Production of biomass from wood may make it possible in the future for the livestock industry in areas with low self-sufficiency of protein-rich feed to use locally produced nonfood protein sources such as yeast in animal feed.

Several studies investigated the effect of yeast as a protein source in feed for dairy cows on feed efficiency, milk yield, and the metabolic status of the cow (Sabbia et al., 2012; Neal et al., 2014; Manthey et al., 2016). No clear differences in milk composition could be attributed to the different feed treatments. However, these studies have only to a minor extent focused on whether use of yeast influences milk quality more extensively than the crude milk composition. To our knowledge, products made from such milk (i.e., cheese) have not been studied. About 37.7% of milk is processed into cheese in the European Union (Eurostat, 2019). Therefore, there is a need to ensure that the use of novel yeast microbial protein sources for concentrate feed for dairy cows does not compromise the production and quality of cheese. The objective of this study was to compare the effect of a novel concentrate feed for dairy cows based on yeast (*Candida utilis*) with a conventional concentrate feed based on soybean meal or barley on the quality of a Gouda-type cheese.

MATERIALS AND METHODS

Animals, Feed Composition, and Feeding Regimens

The feeding experiment was performed at the Animal Production and Experimental Unit at the Norwegian University of Life Sciences (Ås, Norway). All animal procedures were approved by the national animal research authority of the Norwegian Food Safety Authority (FOTS ID 18038).

Forty-eight Norwegian Red dairy cows in early to mid lactation were allocated into 3 treatment groups with 16 replicates per treatment based on parity, milk yield at start of the experiment, DIM, and milk protein genetic variants. An overview of the milk protein genetic variants is shown in Table 1. Milk samples from each individual cow were collected in wk 7 and analyzed for SCC by flow cytometry (Bentley Instruments, Chaska, MN). The feeding experiment lasted for 10 wk whereby the first 2 wk were an adaptation period and during the remaining 8 wk the cows were given the experimental diets (cheesemaking was done in wk 6 and 7 of the experimental period).

Table 1. Milk protein genetic variants (no.)[1]

Protein and genotype	BAR	SBM	YEA
α_{S1}-CN			
BB	10	14	11
BC	5	2	5
CC	1	0	0
β-CN			
A1A1	0	1	1
A2A2	12	8	10
A2B	0	0	1
A1A2	4	7	4
κ-CN			
BB	0	1	1
HH	0	1	1
AA	14	12	9
AE	1	1	3
AB	1	1	2
β-LG			
BB	6	9	6
AB	8	4	8
AA	1	1	1
Unknown	1	2	1

[1]The experiment was carried out for a period of 10 wk, with the first 2 wk as an adaptation period where all dairy cows were fed grass silage and soybean meal (SBM) concentrate. The cows were then randomly allocated to 1 of the 3 different compound feeds: SBM (n = 16), novel yeast (*Candida utilis*; YEA; n = 16), or barley (BAR; n = 16).

During the entire experiment, the cows had free access to good-quality grass silage from individual automatic feeders. Mean daily silage DMI was 14.5 kg/cow during the experimental period. During the 2-wk adaption period, cows in all 3 treatment groups were fed the soybean meal (**SBM**) concentrate feed. During the experimental period (8 wk), the cows in each treatment group received concentrate feed prepared with SBM (as in the adaption period), *C. utilis* (**YEA**), or barley (**BAR**; negative control diet where soybean meal or yeast were replaced by barley, which has a lower protein content).

The amount of concentrate feed for each individual cow was calculated using the Nordic feeding standard (Volden, 2011). This was on average 7.7 kg of DM/cow per day during the experimental period. The daily portions of the concentrate feed were fed from automatic feeders on split portions with a maximum of 4 kg/cow per visit.

Characterization of the Experimental Feeds

The chemical composition of the basal diet (grass silage) and concentrate feed is provided in Table 2. The experimental concentrate feeds were prepared in such a way that the SBM and YEA were iso-proteinaceous and all 3 feeds were roughly iso-energetic. This was achieved by substituting yeast and barley for the soybean meal in the respective diets. The *C. utilis* used in this experiment was produced by Danstar Ferment (Fredericia, Denmark) with sugar cane molasses as the growth medium.

Collection of Milk and Cheese Making

Cheeses were made during wk 8 and 9 of the feeding experiment. The cows were milked by a milking robot system (De Laval, Lund, Sweden), and the milk from the specific cows of each group (SBM, BAR, and YEA) was collected in a separate milk tank over 2 d.

It was only possible to sample milk from one experimental group at a time. Therefore, cheese was produced over 6 production days, 2 randomly selected days for each type of milk. At each production day, 2 vats of cheese were made and these were considered to be replicates. This resulted in 4 cheese vats produced from the same type of milk (SBM, BAR, or YEA); in total, 12 vats of cheese were made.

Full-fat Gouda-type cheese was produced using the method described by Porcellato and Skeie (2016) with minor adjustments. In short, cheeses were made from 300 L of pasteurized (72°C for 15 s) milk (standardized to 2.7% fat). The milk was analyzed for fat, protein, casein, and lactose using a MilkoScan FT1 (Foss Elec-

Table 2. Information (DIM at start, milk yield at start, and parity) on the dairy cows distributed in the 3 diet groups and the composition of the 3 concentrate feeds

Item	Concentrate feed[1]			Grass silage
	BAR	SBM	YEA	
Cows (mean ± SD)				
DIM at start (wk 0)	99.7 ± 35.43	101.2 ± 31.48	100.6 ± 37.04	
Milk yield at start (L/d)	31.7 ± 7.97	30.2 ± 7.61[2]	31.8 ± 11.64[2]	
Parity	1.7 ± 0.95	2.0 ± 1.63	1.7 ± 0.98	
SCC (log cells/mL)	4.44 ± 0.40[4]	4.37 ± 0.34[5]	4.64 ± 0.68	
Chemical composition[6]				
DM (g/kg)	875.3	875.2	881.3	300
Ash (g/kg of DM)	69.6	65.9	67.5	75.8
CP[7] (g/kg of DM)	133.9	161.1	156.5	181.4
NDF (g/kg of DM)	187.0	186.4	169.3	532.5
Starch (g/kg of DM)	406.1	385.0	364.9	—
Fat (g/kg of DM)	38.0	38.3	36.9	46.3
Water-soluble carbohydrate (%)	5.7	6.2	5.9	1.7

[1]The experiment was carried out for a period of 10 wk, with the first 2 wk as an adaptation period where all dairy cows were fed grass silage and soybean meal (SBM) concentrate. The cows were then randomly allocated to 1 of the 3 different compound feeds: SBM (n = 16), novel yeast (*Candida utilis*; YEA; n = 16), or barley (BAR; n = 16).

[2]n = 15 (milk yield not measured for 1 cow).

[3]The reported SCC is based on individual milk samples from wk 7 of the experiment.

[4]n = 14 (2 cows were taken out of the experiment in wk 7–10).

[5]n = 15 (1 cow was taken out of the experiment in wk 7–10).

[6]The reported chemical composition is based on a minimum of 3 analyses on composite samples.

[7]Calculated as N × 6.25.

tric A/S, Hillerød, Denmark). The bulk starter was prepared by inoculating a freeze-dried CHN-19 starter (Chr. Hansen, Hørsholm, Denmark) in UHT milk and incubated at 20°C for 20 h. The cheese milk was inoculated with 1% (vol/vol) of the prepared starter. After 30 min of preripening at 32°C, rennet (Chy-Max Plus, Chr. Hansen) was added at 25 mL/100 L of milk. After cutting, the curd was stirred for 18 min before whey drainage and water addition, the temperature was increased for 10 min to a scalding temperature of 38°C, and further scalding and stirring proceeded for 35 min. Whey drainage was 40% (vol/vol) and water addition was 40% (vol/vol). After whey drainage and pressing, the cheeses were salted in saturated brine (26%, wt/wt) for 10 h. The cheeses were ripened for 10 d at 11°C at 60 to 65% RH and then for 14 d at 19°C at 65 to 70% RH before further ripening at 4°C until 15 wk. During the first 10 d of ripening, the cheeses were coated with 2 layers of Ceska-WL plastic emulsion containing 0.025% natamycin (CSK Food Enrichment, Leeuwarden, the Netherlands). After 24 d, when moved to the 4°C room, the cheeses were vacuum-packed in Cryovac cheese vacuum bags (Cryovac, Elmwood Park, NJ).

Cheese Analysis

Cheese was sampled according to IDF standard 50C (International Dairy Federation, 1995).

Chemical Analysis of Cheese. Cheese pH was measured using a PHM 92 Lab pH meter (Radiometer, Copenhagen, Denmark). Dry matter content was determined according to IDF standard 50C (International Dairy Federation, 1995). Grated cheese samples for analysis of free AA (**FAA**) were stored at −20°C until analysis by HPLC as described by Martinovic et al. (2013). Quantification of organic acids and carbohydrates was done by HPLC, according to a method described by Skeie et al. (2008). Total protein was determined according to IDF standard 20B (International Dairy Federation, 1993). The citrate water slurry used for determination of total protein was analyzed by capillary electrophoresis as described previously (Jørgensen et al., 2016; Ketto et al., 2017) using an Agilent G1600AX equipped with Agilent ChemStation software (Agilent Technologies, Waldbronn, Germany).

Sensory Analysis. Descriptive analysis of the cheeses was performed when the cheeses were between 11 and 13 wk by a panel of 6 trained assessors, according to the method described by Kraggerud et al. (2008). The analysis followed ISO standards 8589, 5492, and 4121 (ISO, 1988, 1992, 2003, respectively) for the design of the test room, vocabulary, and response scale (1–9), respectively. The means between the panelists for each attribute were used in further calculations.

Statistical Analysis

Significant effects ($P < 0.05$) of the experimental factors for all responses (outside of the microbiota data) were found using the SAS Mixed models procedure (SAS Institute Inc., Cary, NC). Feed was used as the main factor (n = 3) and cheesemaking day (n = 6) as a random factor. A least squares post hoc test (Tukey) was used to test differences between means (all pairwise differences).

Principal component analysis (**PCA**) of the organic acids, FAA, and sensory profiling data was performed using The Unscrambler X version 10.4 (https://www.camo.com/unscramblersuite/). The data (except for the sensory data) were weighted by dividing each response variable by the standard deviation of the variable.

Statistical analysis of the microbiota data was done similarly to that described by Skeie et al. (2019). Briefly, the sequence variants tables were normalized using the cumulative-sum scaling method using the R package "metagenomeSeq" (Paulson et al., 2013). Permutational multivariate ANOVA between the cheeses in the different diet groups was performed using the Bray-Curtis dissimilarity matrixes (no. of permutations = 999). The nonmetric multidimensional scaling was chosen as ordination method using the Bray-Curtis distance matrix.

RESULTS

Gross Composition of Cheese Milk and Cheese

Gross composition of fat-standardized cheese milk and cheese is shown in Table 3. The casein content of the cheese milk was significantly ($P = 0.0005$) influenced by the concentrate feed, whereby the BAR milk showed a lower casein content than the YEA and SBM milks. The difference in casein content led to a significantly higher rennet-to-casein ratio in the BAR cheese milk compared with the YEA and SBM cheese milks.

After 15 wk of ripening, only minor compositional differences were found between the cheeses. Although not significant, the SBM cheese had a higher average number of viable presumptive lactococci counts (log) on M17 agar compared with the BAR and YEA cheeses.

Organic Acids

Principal components analysis of the organic acids present in the cheeses after 15 wk of ripening (Figure 1) showed that the SBM and YEA cheeses were located opposite each other along principal component 1 with a higher content of orotic acid and DL-pyroglutamic

Table 3. Gross composition of cheese milk, 24-h cheese, and cheese ripened for 15 wk within each diet group[1]

Item	Concentrate feed[2]		
	BAR	SBM	YEA
Cheese milk			
Fat (%)	2.71 ± 0.03	2.71 ± 0.03	2.74 ± 0.04
Protein (%)	3.66 ± 0.14	3.74 ± 0.06	3.69 ± 0.04
Casein (%)	2.65 ± 0.01[b]	2.79 ± 0.04[a]	2.76 ± 0.02[a]
Lactose (%)	4.68 ± 0.04	4.66 ± 0.07	4.68 ± 0.02
Casein/protein (%)	72.39 ± 2.65	74.77 ± 0.30	74.90 ± 0.25
pH	6.83 ± 0.12	6.84 ± 0.08	6.80 ± 0.08
Rennet-to-casein ratio (mL/kg)	9.45 ± 0.04[a]	8.96 ± 0.13[b]	9.06 ± 0.07[b]
Cheese 24 h after starter addition			
pH	5.34 ± 0.09	5.37 ± 0.06	5.32 ± 0.04
DM (%)	51.45 ± 0.26	51.56 ± 0.47	51.99 ± 0.12
Counts on M17[3] (log cfu/mL)	7.51 ± 0.49	7.68 ± 0.28	7.45 ± 0.18
Cheese ripened for 15 wk			
pH	5.53 ± 0.01	5.56 ± 0.05	5.50 ± 0.02
DM (%)	58.15 ± 0.25	57.89 ± 0.34	57.48 ± 0.95
Counts on M17[3] (log cfu/mL)	5.80 ± 0.12	6.10 ± 0.26	5.72 ± 0.13
Protein in DM (%)	53.11 ± 0.37	52.71 ± 0.94	52.92 ± 1.22
DL-Pyroglutamic acid (mmol/kg)	0.96 ± 0.06[b]	1.1 ± 0.04[a]	0.94 ± 0.06[b]
Total free AA (μmol/g)	84.80 ± 4.82	92.52 ± 7.16	85.03 ± 4.11

[a,b]Means within a row with different superscripts differ significantly ($P < 0.05$).

[1]Values are presented as mean of 4 replicates ± SD.

[2]The experiment was carried out for a period of 10 wk, with the first 2 wk as an adaptation period where all dairy cows were fed grass silage and soybean meal (SBM) concentrate. The cows were then randomly allocated to 1 of the 3 different compound feeds: SBM (n = 4), novel yeast (*Candida utilis*; YEA; n = 4), or barley (BAR; n = 4).

[3]Presumptive lactococci.

acid associated with the SBM cheeses, whereas the YEA cheeses in addition to BAR cheeses 3 and 4 were associated with a higher content of lactic acid; YEA cheese 4 was an outlier due to a higher concentration of lactic acid compared with the other cheeses. The SBM cheeses showed a clear clustering, whereas the YEA and BAR cheeses showed a greater variation and a less clear clustering. The SBM cheeses contained a significantly higher concentration of DL-pyroglutamic acid (1.1 ± 0.04 mmol/kg) than the BAR (0.96 ± 0.06 mmol/kg) and YEA (0.94 ± 0.06 mmol/kg) cheeses, which is in accordance with their positioning in the PCA (Figure 1).

Protein, Proteolysis, and FAA

The electropherograms showed that the composition of the cheeses differed for some proteins and large peptides (Figure 2). The BAR cheeses had a higher peak of intact β-CN A1 and γ_1-CN A2 compared with the SBM and YEA cheeses. The SBM cheeses had a higher peak of intact α_{S1}-CN 8P and 9P compared with the BAR and YEA cheeses. The YEA cheeses had a protein or peptide (denoted as "x" in the figure) that was not apparent in the BAR and SBM cheeses.

The SBM cheeses contained a slightly higher (but not significant) content of total FAA (92.52 ± 7.16 μmol/g) compared with the BAR (84.80 ± 4.82 μmol/g) and YEA (85.03 ± 4.11 μmol/g) cheeses after 15 wk of ripening. This derives from a slightly higher content of almost every individual AA (Figure 3), but no significant differences were found for any of the individual FAA between the 3 experimental ripened cheeses. However, for arginine, the SBM cheeses contained a concentration (0.41 ± 0.03 μmol/g) similar to that of the BAR (0.38 ± 0.03) and YEA (0.42 ± 0.05) cheeses.

The PCA of FAA (Figure 3) showed no clear grouping, but the cheeses produced from the second day of SBM milk were characterized by a higher content of most of the FAA, whereas arginine was more associated with all cheeses.

Sensory Analysis

Sensory analysis was carried out by an industrial trained panel at the ripening stage at which the industry normally evaluates Norwegian Gouda-type cheese. The sensory analysis showed that the SBM cheeses had a significantly ($P = 0.028$) higher intensity of sour taste (5.4 ± 0.04) than the YEA cheeses (5.29 ± 0.05). The PCA analysis (Figure 4) did not show any clear grouping of the cheeses, but the texture attributes "grainy," "pasty," "elasticity," "cohesiveness," and "shear firmness" explained most of the variation between the

cheeses, and most of the taste and smell attributes were clustered in the middle near origo and thereby did not explain any of the variation between the cheeses. Most of the YEA cheeses were associated with a pasty texture, whereas the SBM and BAR cheeses were more associated with a grainy texture.

Microbiota After 15 wk of Ripening

Analysis of the cheese microbiota after 15 wk of ripening showed that the cheeses were not significantly different with respect to the source of feed (Adonis P-value = 0.483). The main genus identified was *Lactococcus* (98.3% of all the reads) followed by *Lactobacillus* (1%) and *Leuconostoc* (0.15%). The *Lactococcus* population (as sequenced by the *epsD* gene) identified 22 sequence variants with abundance greater than 0.3%, and no significant influence of the feed was found on the composition of the *Lactococcus* population after 15 wk of ripening (Adonis P-value = 0.424). Nonmetric multidimensional scaling did not show any clear clustering of the samples with regards to the microbiota, and although small differences in *Lactococcus* population composition were detected between the BAR and SBM feeds, no significant differences between the groups was detected (Figure 5).

DISCUSSION

Concentrate feeds based on 3 different protein sources (BAR, SBM, and YEA) were fed to 48 mid-lactation Norwegian Red cows during a period of 10 wk. Gouda-type cheeses were made during wk 8 and 9 of the feeding experiment.

The capillary electrophoresis uncovered differences in protein composition and degradation during ripening between the cheeses. However, although the cows were grouped to balance the genetic variants, we did not succeed completely and the grouping became somewhat unbalanced with some differences between the experimental groups. The SBM group contained more A1 cows than the BAR and YEA groups. These differences might explain some of the differences between the cheeses as observed by capillary electrophoresis. Although the BAR cows showed the lowest prevalence of the β-CN A1 allele, the BAR cheeses had the highest peak of intact β-CN A1 after 15 wk of ripening, showing a much lower degradation of β-CN A1. The BAR cows showed the highest prevalence of the β-CN A2 allele, but the peak height of intact β-CN A2 did not differ between the cheeses of the different experimental groups. However, BAR cheeses had a higher peak of γ_1-CN A2, and one possible explanation could be that the degradation of β-CN A2 was faster in the BAR cheeses. The SBM cheeses showed higher peaks of intact α_{S1}-CN 8P and 9P, and because the rennet-to-casein ratio was lowest in this cheese (although not significantly different from the YEA cheese), a lower retention of rennet and hence a lower rennet activity could be expected in the SBM cheeses (Fox et al., 2017). This could further lead to a lower degradation of α_{S1}-CN in the SBM cheeses as observed in this experiment. Therefore, if the rennet-

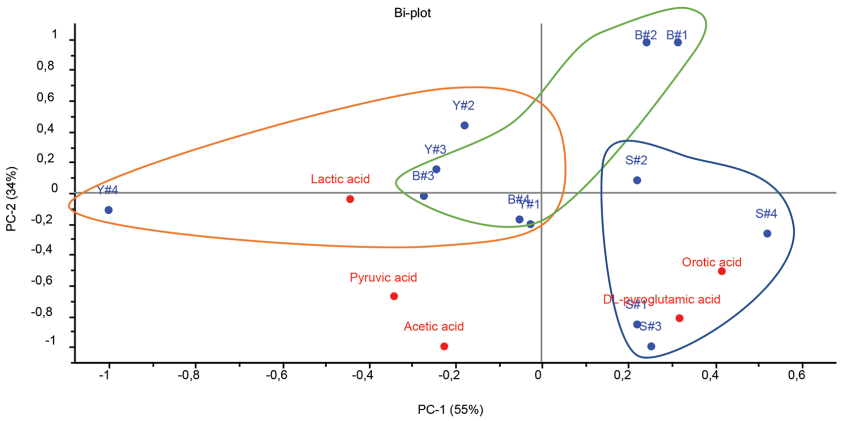

Figure 1. Principal component analysis of organic acids in cheeses ripened for 15 wk. The letter represents the concentrate feed treatment (B = barley; Y = yeast; S = soybean meal), and the number after each letter indicates which replicate (cheeses 1 and 2 within the same diet group were made on cheesemaking d 1, and cheeses 3 and 4 were made on cheesemaking d 2). Principal components (PC) 1 and 2 explain 55 and 34% of the variation, respectively. The experimental groups are outlined.

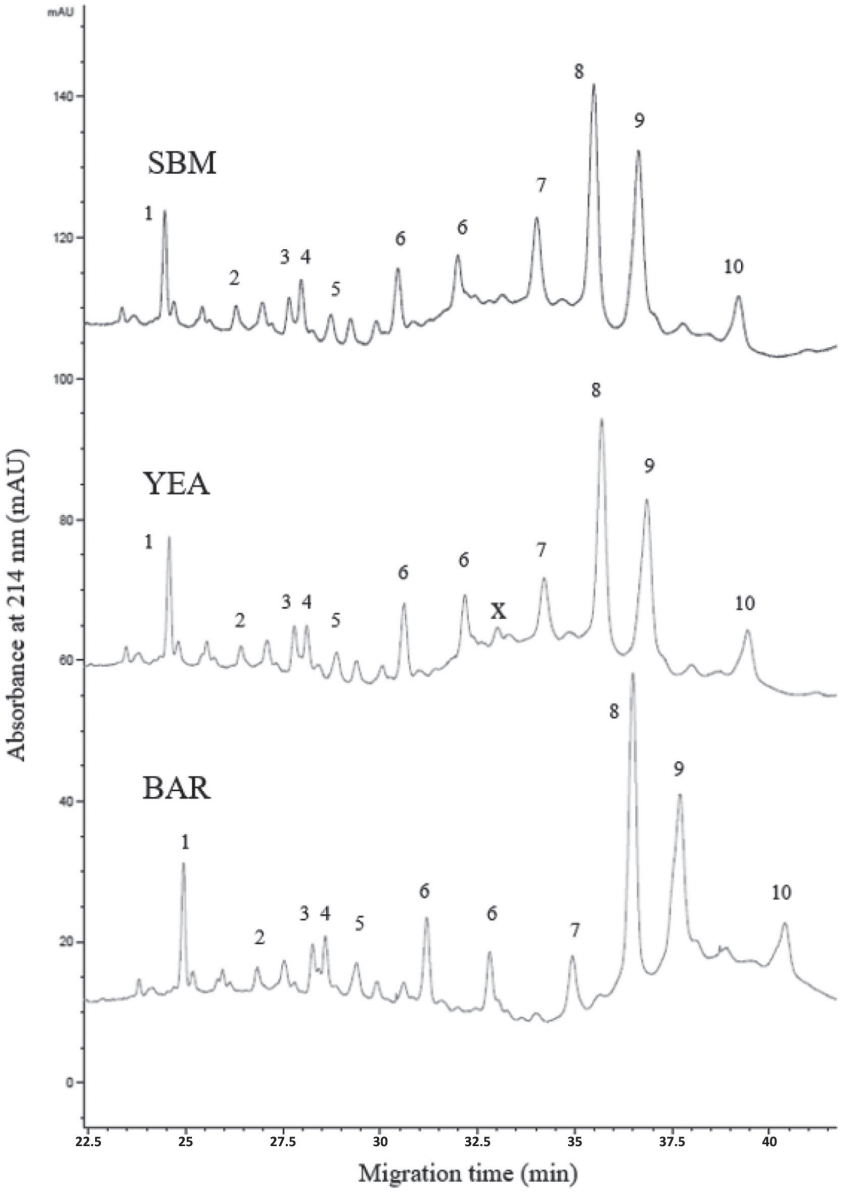

Figure 2. Protein profiles of cheeses after 15 wk of ripening representing the 3 diet groups (BAR = barley; YEA = yeast; SBM = soybean meal) analyzed by capillary electrophoresis. All 4 replicates from each group were identical, and 1 cheese from each group was chosen to represent the whole group. Identification of the peaks was based on previous findings (Andersen, 2009; Ardö et al., 2017): 1 = para-κ-CN, 2 = γ_2-CN, 3 = γ_1-CN A1, 4 = α_{S1}-CN 8P, 5 = γ_1-CN A2, 6 = γ_3-CN, 7 = α_{S1}-CN 9P, 8 = β-CN A1, 9 = β-CN A2, and 10 = α_{S1}-I CN 8P. X = unknown peak visible only in YEA.

to-casein ratio had been adjusted, these differences in α_{S1}-CN degradation might have been less. In the present experiment, rennet was added per volume of milk as that is the normal practice in the Norwegian cheese industry (there are normally very small variations in protein and casein content of milk). However, when using new feed sources that might alter the casein content of the milk, it may be important for the cheese industry to adjust the amount of rennet according to the content of casein in milk to make sure that the rennet-to-casein ratio is constant and thereby standardize the initial proteolysis of α_{S1}-CN in the produced cheese.

It is well known that proteolysis in cheese is important and decisive for texture development in cheese, and the differences we observed in the proteolysis pattern can explain why the sensory attributes related to texture explained most of the variation between the cheeses. Further work is needed to gain more knowledge about how degradation of specific proteins and peptides influences the development of texture during cheese ripening. The SBM cheeses had a significantly higher intensity of sour taste compared with the YEA cheeses, which may be explained by the higher concentrations of the FAA histidine, glutamic acid, and aspartic acid, which can contribute to a sour taste (Kilcawley, 2017).

Most LAB are auxotrophic for several AA (Christiansen et al., 2008), and to grow and fulfil their nutritional requirement they degrade casein to small peptides and AA. The SBM cheeses had a higher total concentration of FAA, and, together with a significantly higher

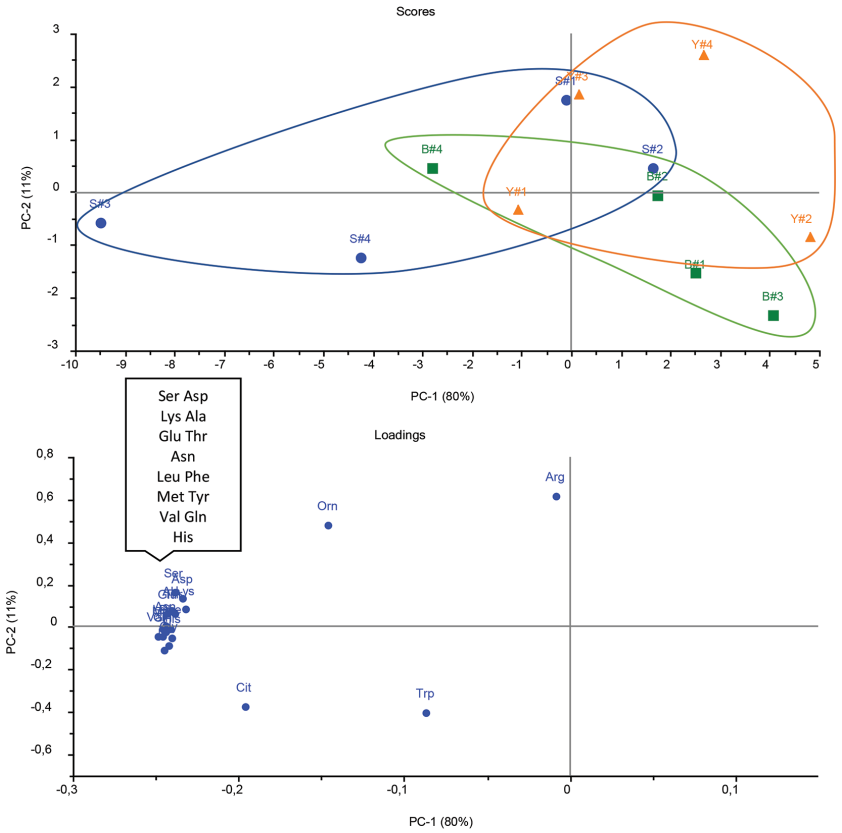

Figure 3. (A) Scores and (B) loadings of the principal component analysis of free AA in cheeses after 15 wk of ripening. The letter represents the concentrate feed treatment (B = barley; Y = yeast; S = soybean meal), and the number after each letter indicates which replicate (cheeses 1 and 2 within the same diet group were made on cheesemaking d 1, and cheeses 3 and 4 were made on cheesemaking d 2). Principal components (PC) 1 and 2 explain 80 and 11% of the variation, respectively.

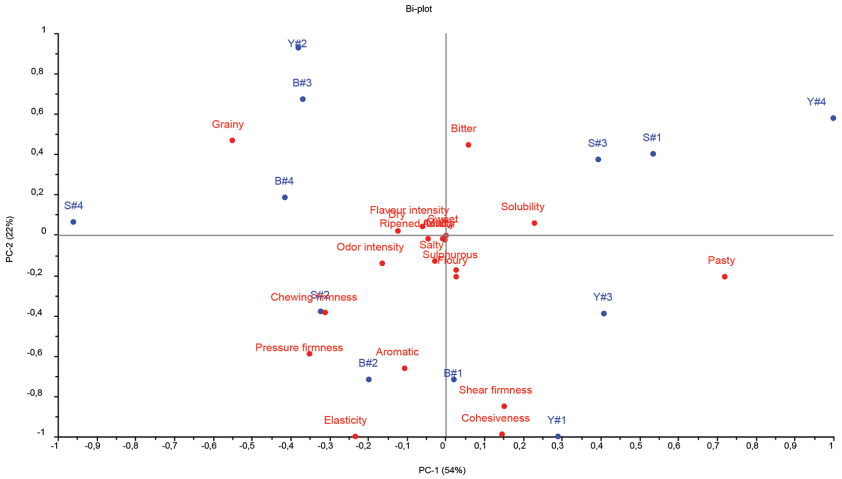

Figure 4. Principal component analysis of sensory analysis in cheese after 11 to 13 wk of ripening. The letter represents the concentrate feed treatment (B = barley; Y = yeast; S = soybean meal), and the number after each letter indicates which replicate (cheeses 1 and 2 within the same diet group were made on cheesemaking d 1, and cheeses 3 and 4 were made on cheesemaking d 2). Principal components (PC) 1 and 2 explain 54 and 22% of the variation, respectively. The experimental groups are shown in different gray colors.

content of DL-pyroglutamic acid, this indicates that the overall ripening occurred somewhat faster in these cheeses. Moreover, the somewhat higher content of *Lactococcus* spp. observed in the SBM cheeses both 24 h after starter addition and after 15 wk of ripening may have contributed to increased enzymatic activity and may thereby explain the faster ripening in these cheeses. After 15 wk of ripening, DL-pyroglutamic acid was the only organic acid that was significantly different between the cheeses. Pyroglutamic acid can be formed from either glutamic acid or glutamine, where the FAA cyclizes from a lactam (Tschager and Jager, 1988; RCSB PDB, 2020). The SBM cheeses obtained a higher content of both glutamic acid and glutamine while also having a significantly higher concentration of DL-pyroglutamic acid. This supports the suggestion that ripening proceeded faster in the SBM cheeses. Pyroglutamic acid is a common compound in many cheese varieties, but it is particularly present in long-ripened cheeses such as Grana Padano and Parmigiano Reggiano. Mucchetti et al. (2000) found that the concentration of pyroglutamic acid was positively correlated with the ripening time of Grana Padano cheeses. Most likely, variations in the microbiota of the cheese influence the content of DL-pyroglutamic acid (Mucchetti et al., 2002). Nevertheless, in this experiment all other factors except the protein source in the concentrate feed were held constant during cheese production. A mesophilic starter culture was used, and the cheese milk was pasteurized at 72°C for 15 s. Moreover, as shown by the 16S rDNA sequencing made after 15 wk of ripening to map the microbiota, no differences were observed between the cheeses, and it is therefore not likely that differences in the microbiota could explain the differences in ripening between the SBM, YEA, and BAR cheeses. Based on these observations, it seems that the development of pyroglutamic acid during cheese ripening is dependent not only on the starter culture or the raw milk microflora but also on factors influenced by the milk and the concentrate feed that the dairy cow has been fed.

Further work is required to reveal the influence of feed on cheese ripening. A study by Inglingstad et al. (2016) tested the effect of adding saturated and unsaturated lipids in concentrate feed to Norwegian goats on milk composition, coagulation properties, and cheese quality. The cheese made with milk from goats fed concentrate with saturated fat had a higher total solids content compared with cheese made with milk from goats fed concentrate with unsaturated fat. They also uncovered a faster ripening and a better texture in cheese made with milk from goats that received concentrate supplemented with saturated lipids. These results show that the feed may influence the ripening of the cheese.

Even if the different concentrate feeds seemed to affect the ripening of cheese in this study, the effect on individual sensory attributes was minor. Nevertheless,

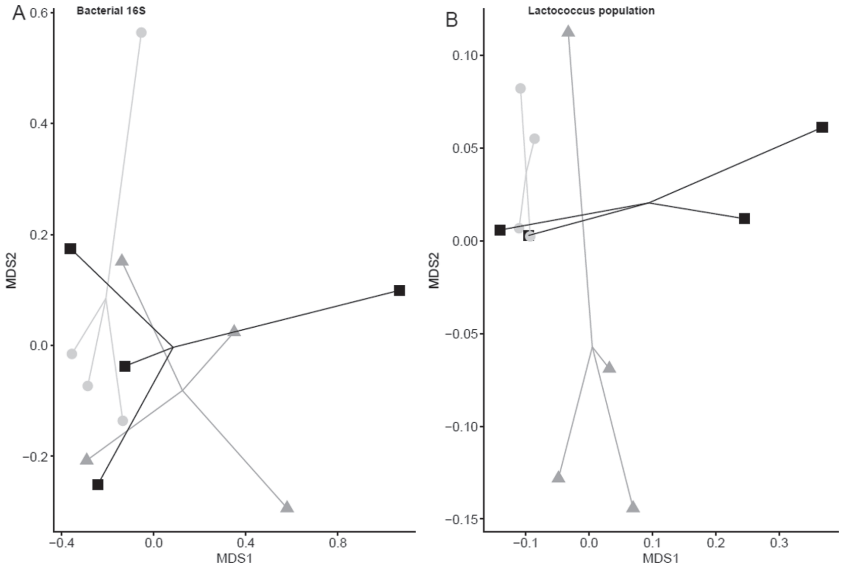

Figure 5. Microbiota of cheeses after 15 wk of ripening using nonmetric multidimensional scaling (MDS) of (A) microbial composition (bacteria 16S, stress value = 0.02) and (B) the *Lactococcus* population (obtained from amplicon sequencing of the lactococcal *epsD* gene, stress value = 0.01). ● = barley; ▲ = YEA; ■ = SBM.

these results show that a switch is possible from a barley- or soy-based concentrate (mostly used in Norway today) to a more novel and sustainable yeast-based concentrate while maintaining the overall quality of the Gouda-type cheese produced.

The robustness of the experiment was tested by making cheese with milk from the same group of cows over 2 cheese-making days, thereby using different milk, which is more applicable to industrial production. However, this led to quite high standard deviations for some of the milk and cheese variables; this could be one reason why we often see a trend in the results but few significant differences. The cheeses were ripened for only 15 wk when analyzed, and we could possibly expect a higher deviation between the cheeses if they were ripened for a longer time. However, the bulk amount of cheese produced in Norway is sold after 3 mo of ripening (J. Øyaas, TINE SA, Trondheim, Norway, personal communication); therefore, the results obtained in this experiment are very relevant for the industry.

CONCLUSIONS

The main finding in this experiment was that the SBM cheeses seemed to have a somewhat faster ripening process than the YEA and BAR cheeses as indicated by the significantly higher concentration of DL-pyroglutamic acid and a higher content of FAA after 15 wk of ripening. However, beyond these results, there are few clear differences between the cheeses, and the sensory analysis did not show any clear indications that support our interpretation of faster ripening in the SBM cheeses. All cheeses were judged to be of good quality, and these results thus suggest that the yeast *C. utilis* can replace soy or be added as a supplement to barley in concentrate feed for dairy cows without compromising the quality of cheese.

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REFERENCES

Andersen, L. T. 2009. A chemical and sensory study of water-soluble compounds in mature Cheddar cheese with savory flavour. PhD Thesis. Department of Food Science, Faculty of Life Sciences, University of Copenhagen, Copenhagen, Denmark.

Animalia. 2019. Hva spiser husdyra? Accessed Apr. 23, 2020. https://www.animalia.no/no/samfunn/kjottproduksjon/hva-spiser-husdyra/.

Ardö, Y., P. L. McSweeney, A. A. Magboul, V. K. Upadhyay, and P. F. Fox. 2017. Biochemistry of cheese ripening: Proteolysis. Pages 445–482 in Cheese Chemistry, Physics and Microbiology. P. L. H. McSweeney, P. F. Fox, P. D. Cotter, and D. W. Everett, ed. Academic Press, San Diego, CA.

Christiansen, J. K., J. E. Hughes, D. L. Welker, B. T. Rodríguez, J. L. Steele, and J. R. Broadbent. 2008. Phenotypic and genotypic analysis of amino acid auxotrophy in *Lactobacillus helveticus* CNRZ 32. Appl. Environ. Microbiol. 74:416–423. https://doi.org/10.1128/AEM.01174-07.

de Visser, C. L. M., R. Schreuder, and F. Stoddard. 2014. The EU's dependency on soya bean import for the animal feed industry and potential for EU produced alternatives. Oilseeds and fats. Crops Lipids 21:D407. https://doi.org/10.1051/ocl/2014021.

Eurostat. 2019. Agriculture, forestry and fishery statistics. Accessed Jun. 25, 2020. https://ec.europa.eu/eurostat/documents/3217494/10317767/KS-FK-19-001-EN-N.pdf/742d3fd2-961e-68c1-47d0-11cf30b11489.

FCRN Foodsource. 2020. Soy: Food, feed and land use change. Accessed Apr. 23, 2020. https://www.foodsource.org.uk/building-blocks/soy-food-feed-and-land-use-change.

Ferreira, A. C., J. F. Vieira, A. M. Barbosa, T. M. Silva, L. R. Bezerra, N. G. Nascimento Jr., J. E. de Freitas Jr., S. M. P. L. Jaeger, P. A. Oliveira, and R. L. Oliveira. 2017. Effect of replacing ground corn and soybean meal with licuri cake on the performance, digestibility, nitrogen metabolism and ingestive behavior in lactating dairy cows. Animal 11:1957–1965. https://doi.org/10.1017/S175173111700074X.

Fox, P. F., T. M. Cogan, and T. P. Guinee. 2017. Factors that affect the quality of cheese. Pages 617–640 in Cheese Chemistry, Physics and Microbiology. P. L. H. McSweeney, P. F. Fox, P. D. Cotter, and D. W. Everett, ed. Academic Press, San Diego, CA.

Inglingstad, R., M. Eknæs, L. Brunborg, T. Mestawet, T. Devold, G. Vegarud, and S. Skeie. 2016. Norwegian goat milk composition and cheese quality: The influence of lipid supplemented concentrate and lactation stage. Int. Dairy J. 56:13–21. https://doi.org/10.1016/j.idairyj.2015.12.010.

International Dairy Federation. 1993. Determination of the nitrogen (Kjeldahl method) and calculation of the crude protein content. IDF Standard 20B. International Dairy Federation, Brussels, Belgium.

International Dairy Federation. 1995. Milk and milk products: Guidance on sampling. Standard 50C. International Dairy Federation, Brussels, Belgium.

ISO. 1988. Sensory analysis—General guidance for the design of test rooms. ISO Standard 8589. International Organization for Standardization, Geneva, Switzerland.

ISO. 1992. Sensory analysis—Vocabulary. ISO Standard 5492. International Organization for Standardization, Geneva, Switzerland.

ISO. 2003. Sensory analysis—Guidelines for the use of quantitative response scales. ISO Standard 4121. International Organization for Standardization, Geneva, Switzerland.

Jørgensen, C. E., R. K. Abrahamsen, E.-O. Rukke, A.-G. Johansen, R. B. Schüller, and S. B. Skeie. 2016. Optimization of protein fractionation by skim milk microfiltration: Choice of ceramic membrane pore size and filtration temperature. J. Dairy Sci. 99:6164–6179. https://doi.org/10.3168/jds.2016-11090.

Ketto, I. A., T. M. Knutsen, J. Øyaas, B. Heringstad, T. Ådnøy, T. G. Devold, and S. Skeie. 2017. Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. Int. Dairy J. 70:55–64. https://doi.org/10.1016/j.idairyj.2016.10.010.

Kilcawley, K. N. 2017. Cheese flavour. Pages 443–474 in Fundamentals of Cheese Science. 2nd ed. P. F. Fox, T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney, ed. Springer, New York, NY.

Kraggerud, H., S. Skeie, M. Høy, L. Røkke, and R. Abrahamsen. 2008. Season and ripening temperature influence fatty acid composition and sensory properties of semi-hard cheese during maturation. Int. Dairy J. 18:801–810. https://doi.org/10.1016/j.idairyj.2007.11.003.

Lapeña, D., G. Kosa, L. D. Hansen, L. T. Mydland, V. Passoth, S. J. Horn, and V. G. Eijsink. 2020. Production and characterization of yeasts grown on media composed of spruce-derived sugars and protein hydrolysates from chicken by-products. Microb. Cell Fact. 19:19. https://doi.org/10.1186/s12934-020-1287-6.

Manthey, A. K., K. Kalscheur, A. Garcia, and K. Mjoun. 2016. Lactation performance of dairy cows fed yeast-derived microbial protein in low-and high-forage diets. J. Dairy Sci. 99:2775–2787. https://doi.org/10.3168/jds.2015-10014.

Martinovic, A., K. M. Moe, E. Romeih, B. Aideh, F. K. Vogensen, H. Østlie, and S. Skeie. 2013. Growth of adjunct *Lactobacillus casei* in Cheddar cheese differing in milk fat globule membrane components. Int. Dairy J. 31:70–82. https://doi.org/10.1016/j.idairyj.2013.02.009.

Mucchetti, G., F. Locci, M. Gatti, E. Neviani, F. Addeo, A. Dossena, and R. Marchelli. 2000. Pyroglutamic acid in cheese: Presense, origin, and correlation with ripening time of Grana Padano cheese. J. Dairy Sci. 83:659–665. https://doi.org/10.3168/jds.S0022-0302(00)74926-5.

Mucchetti, G., F. Locci, P. Massara, R. Vitale, and E. Neviani. 2002. Production of pyroglutamic acid by thermophilic lactic acid bacteria in hard-cooked mini-cheeses. J. Dairy Sci. 85:2489–2496. https://doi.org/10.3168/jds.S0022-0302(02)74331-2.

Neal, K., J. Eun, A. Young, K. Mjoun, and J. Hall. 2014. Feeding protein supplements in alfalfa hay-based lactation diets improves nutrient utilization, lactational performance, and feed efficiency of dairy cows. J. Dairy Sci. 97:7716–7728. https://doi.org/10.3168/jds.2014-8033.

Øverland, M., and A. Skrede. 2017. Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture. J. Sci. Food Agric. 97:733–742. https://doi.org/10.1002/jsfa.8007.

Paulson, J. N., O. C. Stine, H. C. Bravo, and M. Pop. 2013. Differential abundance analysis for microbial marker-gene surveys. Nat. Methods 10:1200–1202. https://doi.org/10.1038/nmeth.2658.

Porcellato, D., and S. B. Skeie. 2016. Bacterial dynamics and functional analysis of microbial metagenomes during ripening of Dutch-type cheese. Int. Dairy J. 61:182–188. https://doi.org/10.1016/j.idairyj.2016.05.005.

RCSB PDB (Research Collaboratory for Structural Bioinformatics Protein Data Bank). 2020. PDB ID DB11245. Accessed Feb. 19, 2020. http://www.rcsb.org/ligand/PCA.

Sabbia, J. A., K. Kalscheur, A. Garcia, A. Gehman, and J. Tricarico. 2012. Soybean meal substitution with a yeast-derived microbial protein source in dairy cow diets. J. Dairy Sci. 95:5888–5900. https://doi.org/10.3168/jds.2011-5237.

Sankarlal, V. M., E. Testroet, D. Beitz, and S. Clark. 2015. Dried distillers grains with solubles do not always cause late blowing in baby Swiss cheese. J. Dairy Sci. 98:8545–8553. https://doi.org/10.3168/jds.2015-9983.

Skeie, S. B., M. Håland, I. Thorsen, J. Narvhus, and D. Porcellato. 2019. Bulk tank raw milk microbiota differs within and between farms: A moving goalpost challenging quality control. J. Dairy Sci. 102:1959–1971. https://doi.org/10.3168/jds.2017-14083.

Skeie, S., A. Kieronczyk, S. Eidet, M. Reitan, K. Olsen, and H. Østlie. 2008. Interaction between starter bacteria and adjunct *Lactobacillus plantarum* INF15D on the degradation of citrate, asparagine and aspartate in a washed-curd cheese. Int. Dairy J. 18:169–177. https://doi.org/10.1016/j.idairyj.2007.09.002.

Statistisk Sentralbyrå. 2019. Land use and land cover. Accessed Jun. 10, 2020. https://www.ssb.no/en/natur-og-miljo/statistikker/arealstat.

Sutton, J. 1989. Altering milk composition by feeding. J. Dairy Sci. 72:2801–2814. https://doi.org/10.3168/jds.S0022-0302(89)79426-1.

Testroet, E. D., D. Beitz, M. O'Neil, A. Mueller, H. Ramirez-Ramirez, and S. Clark. 2018. Feeding reduced-fat dried distillers grains with solubles to lactating Holstein dairy cows does not alter milk composition or cause late blowing in cheese. J. Dairy Sci. 101:5838–5850. https://doi.org/10.3168/jds.2017-13699.

Tschager, E., and H. Jager. 1988. Pyroglutamic acid in milk and milk products with specific consideration of cheese. Milchwirtsch Berichte 95:79–83.

United Nations. 2017. World population projected to reach 9.8 billion in 2050, and 11.2 billion in 2100. Accessed May 20, 2020. https://www.un.org/development/desa/en/news/population/world-population-prospects-2017.html.

Volden, H. 2011. NorFor: The Nordic feed evaluation system. Vol. 30. Wageningen Academic Publishers, Wageningen, the Netherlands.

Paper 3

Influence of different genetic polymorphisms of α_{s1}- and κ-casein on Havarti-type cheese: Effects on cheese-making efficiency and cheese quality.

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Abstract

The effect of the composite genotypes of α_{S1}-κ-casein (BBAA, BBBB and BCAA) on the coagulation properties and yield during cheese-making of a Havarti-type cheese was investigated. Milk with α_{S1}-κ-CN BCAA showed a shorter renneting time, while milk with α_{S1}-κ-CN BBAA obtained the highest cheese yield. 24 h after starter addition, different protein profiles were found between the cheeses with different α_{S1}-κ-CN genotypes. After 5 months of ripening, cheese with α_{S1}-κ-CN BBBB contained more free amino acids compared to α_{S1}-κ-CN BCAA. Furthermore, cheese with α_{S1}-κ-CN BCAA expressed a higher intensity of sweetness and lower intensity of hardness compared to the α_{S1}-κ-CN BBBB and BBAA cheeses. Cheeses with α_{S1}-κ-CN BBAA had a higher intensity of sunlight flavour compared to the cheeses with the two other α_{S1}-κ-CN genotypes and was assessed to be less juicy than α_{S1}-κ-CN BBBB cheeses.

Keywords

Genetic protein variants, cheese-making efficiency, cheese yield, sensory properties

1 Introduction

Numerous investigations have been made on the effects of milk protein genetic polymorphism on the rennet coagulation properties of individual milk samples at laboratory scale (Gustavsson et al., 2014; Hallén, Allmere, Näslund, Andrén, & Lundén, 2007; Jensen et al., 2012; Jõudu, Henno, & Värv, 2007; Jõudu et al., 2009a; Ketto et al., 2017; Poulsen et al., 2013). From these studies, there is a general agreement that the B-variant of κ-casein (κ-CN) and β-lactoglobulin (β-LGB) and the BC genotype of α_{S1}-casein (α_{S1}-CN) are associated with better coagulation properties. The B-variant of β-casein (β-CN) is usually represented at a low frequency in the cow population, but in the studies mentioned above, several report that milk with this variant show good coagulation properties. The BB genotype of κ-CN and β-LGB are associated with higher cheese yield compared to the AA genotype (Fox, Guinee, Cogan, & McSweeney, 2017a).

Renneting time and cheese yield are important indicators of cheese-making efficiency. Cheese yield calculations are, on principle, based on how much cheese is obtained from a specific amount of milk, and expressed as the amount of cheese (kg) produced from 100 kg of milk. It is also possible to predict cheese yield based on milk composition and the desired composition of cheese. These kind of calculation are useful both for the industry to keep track of the efficiency and profitability of their production, and also for researchers to study the efficiency when making changes in the raw material or in the cheese-making process (Lucey & Kelly, 1994).

A review by Skeie (2007) stated that identification of the casein genotypes which are correlated with both improved cheese-making properties, cheese yield and cheese quality would provide possibilities for selective breeding of cows producing milk for cheese production. Some studies on the effect of single-protein loci on the cheese-making properties of various cheeses have been established i.e., Cheddar, Mozzarella, Parmigiano-Reggiano, Sevicia, Asiago, Caciotta and

Montansio cheeses (Bonfatti et al., 2011; Walsh et al., 1998a; Zambrano, Eraso, Solarte, & Rosero, 2010). However, there is limited published information on the effects of casein composite genotypes (α_{s1}-κ-CN) on the cheese-making properties, cheese yield, and especially cheese quality. Mayer, Ortner, Tschager, and Ginzinger (1997) made Edam type cheese from pooled milk of Brown cattle with genotype combinations of β-CN, κ-CN and β-LGB. Dependent on the composite genetic variants, they observed large differences in the fat content of whey, curd fines and cheese yield. Milk with the composite genotype (β-κ-CN, β-LGB) A^2B-AA-AA showed the highest cheese yield, whereas milk with the composite genotype (β-κ-CN, β-LGB) A^2A^2-AA-AA gave the lowest cheese yield. In milk from Norwegian Red (NR) the A^2-variant is dominating with a frequency of 79.7 %, while the B-variant has a frequency of 1.2 % (Ketto et al., 2017). Moreover, the B-variant is most frequent for α_{S1}-CN while the C-variant represent 8.9 % of the frequency in the NR population. For κ-CN, the A and B variants are equally distributed. In the above-mentioned studies, focus has been on β-CN, κ-CN and β-LGB, and very few studies have focused on the influence of α_{S1}-CN on cheese yield and cheese quality.

The objective of the current study was therefore to make a Havarti-type cheese from pooled milk with the A^2A^2 genotype of β-CN in combination with the two most frequent genotypes of α_{S1}-CN (BB and BC) and κ-CN (AA and BB) in milk from NR. This gave three different α_{s1}-κ-CN composite genotypes (BBAA, BCAA and BBBB) and the effects on cheese-making properties, yield, and sensory quality were investigated.

2 Materials and methods

The cows were genotyped according to the procedure described by Ketto et al. (2017).

2.1 Experimental design

Evening and morning milk from fifteen individual NR cows were collected from the Animal Production and Experimental Unit (SHF) at the Norwegian University of Life Sciences (NMBU, Ås, Norway).

The cows had the same genetic polymorphism for β-CN (A^2A^2) but differed in α_{s1}-CN (BB and BC) and κ-CN (AA and BB), resulting in three groups of α_{s1}-κ-CN composite genotypes (BBAA, BBBB, and BCAA). A similar genetic polymorphism for β-LGB within the three groups was aimed for. However, this was not possible within the university herd and therefore the number of cows within the three α_{s1}-κ-CN composite genotype groups had the following β-LGB genotype: Group BBAA (1 AA, 2 AB, 2 BB), group BBBB (0 AA, 4 AB, 1 BB) and group BCAA (1 AA, 1 AB, 3 BB).

The milk from the individual cows was transported from SHF to the dairy pilot plant at NMBU and pooled according to their α_{S1}- and κ-CN composite genotypes (BBAA, BBBB, and BCAA) before treatment and cheese-making. Milk and cheese with the different α_{S1}- and κ-CN composite genotypes are further denoted as BBAA, BBBB, and BCAA milk or cheese.

2.2 Cheese production

A Havarti-type cheese was manufactured over a period of 15 days, with in total 5 production days with one or two vats produced on each production day, in total 9 vats of cheese. This resulted in 3 cheese vats (defined as replicate blocks 1-3) produced from each of three types of milk (BBAA,

BBBB and BCAA). For each replicate block, the milk with the specified composite genotypes was randomized between the two 200 L cheese vats used. In each vat, 100 L of cheese-milk was standardized to 4.18 ± 0.04 % fat and pasteurized at 62 °C for 30 min. The cheese-milk was cooled to 32 °C, before addition of starter culture (CHN-19, Chr. Hansen, Copenhagen, Denmark) at 2 % (v/v) concentration. Rennet (Chy-Max Plus, Chr. Hansen, Copenhagen, Denmark) was added 30 minutes after starter addition, at 25 mL 100 L^{-1}. The coagulum was cut when optimum firmness was obtained, this was determined by an experienced cheesemaker, and the time from rennet addition to the time when the coagulum was cut was recorded as renneting time (min). After cutting, the grains were stirred for 50 min at 32 °C, then 45 % whey was drained off and replaced with 35 % water. The temperature of the curd was increased to 38 °C and the curd was scalded for 20 min, with a double stirring intensity. After scalding, the cheese curd was drained and transferred to the moulds (1 L) and turned every 20 minutes, in total 4 times. The moulds were removed, and the cheeses were salted in brine (25 °Be) for 1 hr. The cheese was stored overnight at room temperature and then vacuum sealed in plastic bags. The cheese was ripened at 16 °C for 4 weeks and then at 5 °C for 4 months. The pH profile during cheese-making was monitored using a pH meter (PHM61; Radiometer, Copenhagen, Denmark).

2.3 Milk and cheese analyses

The chemical composition (fat, lactose, total protein and casein) of the cheese-milk was analysed by using a MilkoScan FT1 (Foss Electric A/S, Hillerød, Denmark). Cheese dry matter (DM) was measured 24 h after start of cheese-making according to IDF standard 4A (IDF, 1982) with a slight modification as the samples were pre-dried at room temperature (not using sand) for 20 h prior to drying in the oven.

Predicted cheese yield (PY) was calculated using the Van Slyke formula (E0) (Fox, Guinee, Cogan, & McSweeney, 2017a). Actual cheese yield (Ya) was calculated according to Banks (2007) where Ya was determined as the ratio of the cheese weight (in kg) before brining to the sum of the weight of cheese milk and starter culture (E1). Yield efficiency (YE) (E2) using Ya and PY (E2) was calculated according to Fox, Guinee, Cogan, and McSweeney (2017a).

$$\mathbf{PY\ (Van\ Slyke)} = \frac{(0.93\ F + C - 0.1)\ x\ 1.09}{100 - W}\ x\ 100 \qquad \text{(E0)}$$

Where:

F - Fat in milk (%)

C – Casein in milk (%)

W – Desired water content in cheese (46 %)

0.1 – Constant for loss of cheese fines in whey

1.09 – Constant representing other solids included in the cheese

$$\mathbf{Ya} = \frac{\text{Weight of cheese}}{(\text{Weight of milk} + \text{weight of starter})} \times 100 \qquad \text{(E1)}$$

$$\mathbf{YE} = \frac{\text{Ya}}{\text{PY (Van Slyke)}}\ x\ 100 \qquad \text{(E2)}$$

Samples for analysis of free amino acids (FAA) were frozen and stored at -20 °C until analysis by HPLC as described by Martinovic et al. (2013). Analysis of organic acids was done according to the method described by Skeie et al. (2008). The protein fractions and their relative concentrations in 24 h cheese and ripened cheese were determined by Capillary Electrophoresis (CE) (Ketto et al., 2017), where peak identification was achieved by comparing with previously

146 published electropherograms (Ardö, McSweeney, Magboul, Upadhyay, & Fox, 2017; Otte,
147 Zakora, Kristiansen, & Qvist, 1997; Otte, Ardö, Weimer, & Sørensen, 1999).

148 **2.4 Descriptive sensory analysis**

149 Cheeses were evaluated by a trained sensory panel of 10 trained assessors at the Norwegian
150 Institute of Food, Fisheries, and Aquaculture Research (NOFIMA, Aas, Norway) after 5 months
151 of ripening. Descriptive sensory profiling was made according to the Generic Descriptive Analysis
152 as described by Lawless and Heymann (2010). The analysis was done according to ISO standards
153 8589 and 8586 (ISO, 2007, 2012) for the design of the test room and for the selection and training
154 of assessors, respectively. The sensory panel is trained, controlled, and tested prior to every project.
155 During the term generation phase, assessors developed a vocabulary describing the samples, and
156 they agreed upon a list of 8 aromas, 11 taste/flavour attributes and 7 texture attributes as described
157 in supplementary Table S1. In a pre-test session, as described in Lawless and Heymann (2010),
158 the judges were trained in the definition of the attributes by testing samples that were considered
159 extreme with respect to selected attributes typical for the cheese.

160 The samples were presented in cubes 2 x 2 x 1 cm, and each assessor was served two cheese cubes
161 holding the temperature of 18 °C ± 1 °C. Each assessor made a monadic evaluation of the samples
162 at individual speed. Sensory attributes were evaluated using a 15 cm non-structured continuous
163 line scale with the left side of the scale corresponding to the lowest intensity and the right side
164 corresponding to the highest intensity. During analysis, 9 samples were evaluated in two replicates,
165 in 5 serving sessions. All samples and replicates were served in a randomized order. EyeQuestion
166 (Logic8, Elst, The Netherlands) was used for direct recording of data and the software transformed

the responses into numbers between 1 = low intensity and 9 = high intensity. The panel was asked to rinse their palates between the samples using hot and cold water in addition to unsalted crackers.

2.5 Statistical analysis

Significant effects ($P < 0.05$) on the milk composition, renneting time, cheese yield, cheese composition, proteolytic (caseins and amino acids) and sensory data due to the α_{S1}-κ-CN composite genotype (BBAA, BCAA, and BBBB) were found using the mixed procedure of SAS (SAS Enterprise Guide 8.3, SAS, Cary, USA). The composite genotype (n=3) was used as fixed factor, while replicate block (n=3) and vat number (n=2) were used as random factors. The covariance structure used was variance components. Least Square Post Hoc (Tukey) was used to test differences between means (all pairwise differences). For the sensory data the mean of 10 assessors were used in the statistical calculations.

Principal Component Analysis (PCA) was used to analyse the relationship between the α_{s1}-κ-CN composite genotypes and the protein profile in 24 h cheese and FAA composition in ripened cheese using the factoextra package (Kassambara & Mundt, 2020) in RStudio (R Core Team, 2022)(RStudio 2022.02.2, Boston, USA).

3 Results

The α_{s1}-κ-CN composite genotype significantly affected the composition of cheese milk (Table 1). BCAA milk had a higher content of lactose and lower content of protein compared to both BBAA and BBBB milk. Moreover, BCAA milk had a lower content of casein compared to BBAA milk. BBAA milk had a higher DM content (sum of fat, lactose and protein) than BBBB and BCAA milk.

Table 1. Effects of the α_{s1}-κ-CN composite genotypes (BBAA, BBBB and BCAA) on milk composition (mean and SD). Significant differences between means are shown by different superscript letters ($P < 0.05$).

α_{s1}-κ-CN genotypes	BBAA		BBBB		BCAA		
	mean	SD	mean	SD	mean	SD	P-value
Fat (%)	4.19	0.01	4.14	0.04	4.17	0.06	NS
Lactose (%)	4.68^{b}	0.02	4.59^{b}	0.07	4.85^{a}	0.04	0.003
Protein (%)	3.78^{a}	0.03	3.66^{a}	0.06	3.43^{b}	0.05	0.006
Casein (%)	2.82^{a}	0.02	2.74^{ab}	0.07	2.60^{b}	0.04	0.02
DM* (%)	12.65^{a}	0.05	12.40^{b}	0.10	12.45^{b}	0.05	0.0105

* Dry matter (DM) as fat + lactose + protein from the MilkoScan FT1 (Foss Electric A/S, Hillerød, Denmark)

The pH development during cheese-making was similar in all vats, average pH in milk at start of cheese-making was 6.59 (± 0.02) and pH in cheese 24 h after starter addition was 5.06 (± 0.11). The mean DM of the cheeses 24 h after starter addition was 55.96 (± 0.98). No significant difference was found in pH and DM content, which indicates that the cheese-making procedure was standardized between the treatments and replicates.

Table 2. Effects of the α_{s1}-κ-CN composite genotypes (BBAA, BBBB and BCAA) on experienced renneting time and different yield calculations (mean and SD). Significant differences between means are shown by different superscript letters ($P < 0.05$).

α_{s1}-κ-CN genotypes	BBAA		BBBB		BCAA		
	mean	SD	mean	SD	mean	SD	P-value
Renneting time (min)[1]	30.00^{a}	0.00	26.67^{ab}	2.88	21.00^{b}	1.73	0.02
PY (kg cheese 100 L^{-1} milk)[2]	12.88^{a}	0.02	12.63^{b}	0.08	12.41^{b}	0.14	0.007
Ya (kg cheese 100 L^{-1} milk)[3]	13.81^{a}	0.11	13.35^{ab}	0.23	13.18^{b}	0.07	0.016
YE (%)[4]	103.41	0.85	101.97	2.41	102.50	1.70	NS

[1] Time from rennet addition to cutting of the gel as observed by an experienced cheese-maker.
[2] Equation E0
[3] Equation E1
[4] Equation E2

The renneting time was significantly ($P < 0.02$) longer (~ 9 min) for BBAA milk compared to BCAA milk (Table 2). The calculated PY gave an estimate that milk with BBAA would attain a higher cheese yield compared to milk with BCAA and BBBB and this was confirmed by Ya. BBAA milk gave 0.6 kg more cheese per 100 L milk than BCAA milk ($P < 0.02$) and 0.5 kg more cheese per 100 L milk than BBBB milk ($P = 0.05$). Differences were found in the protein profile of the 24 h cheeses, and the PCA plot (Figure 1) shows a clear grouping between the cheeses. BCAA cheeses are located on the left side and were associated with higher levels of α_{S1}-CN 9P- and 8P ($P < 0.05$) and β-CN A2 ($P < 0.01$) while BBBB and BBAA cheeses are located on the right side and were associated with higher levels of α_{S2}-CN ($P < 0.01$), para-κ-CN (BBAA > BBBB/BCAA) and γ-caseins.

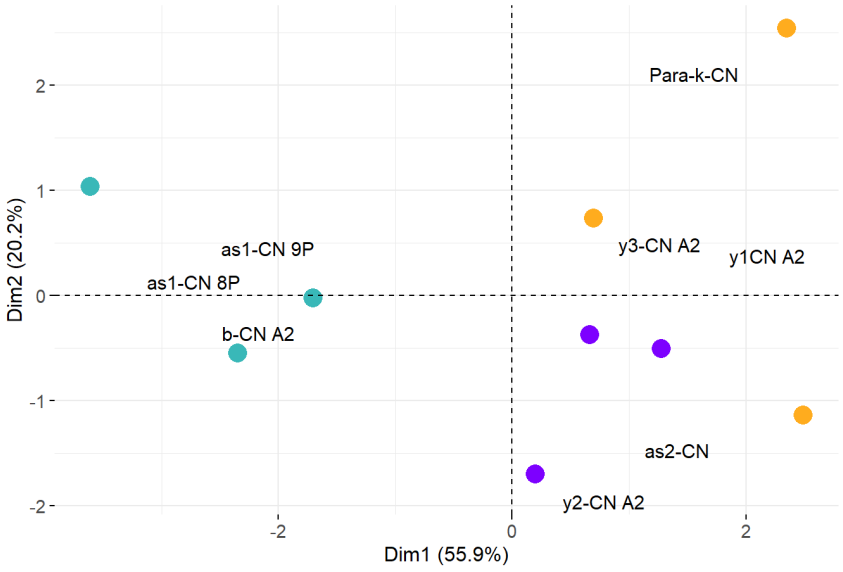


Figure 1. Principal Component analysis of caseins and their peptides in 24 h cheeses with different composite genotypes of α_{s1}-κ-CN (BBAA●, BBBB● and BCAA●). Principal components 1 and 2 explain 55.9 and 20.2 % of the variation, respectively. (Dim = Dimension of the PCA).

Further, in ripened cheese, cheeses of the same composite genotype groups still had a remarkably similar protein profile (Figure 2), while there were clear differences between cheeses with different composite genotypes.

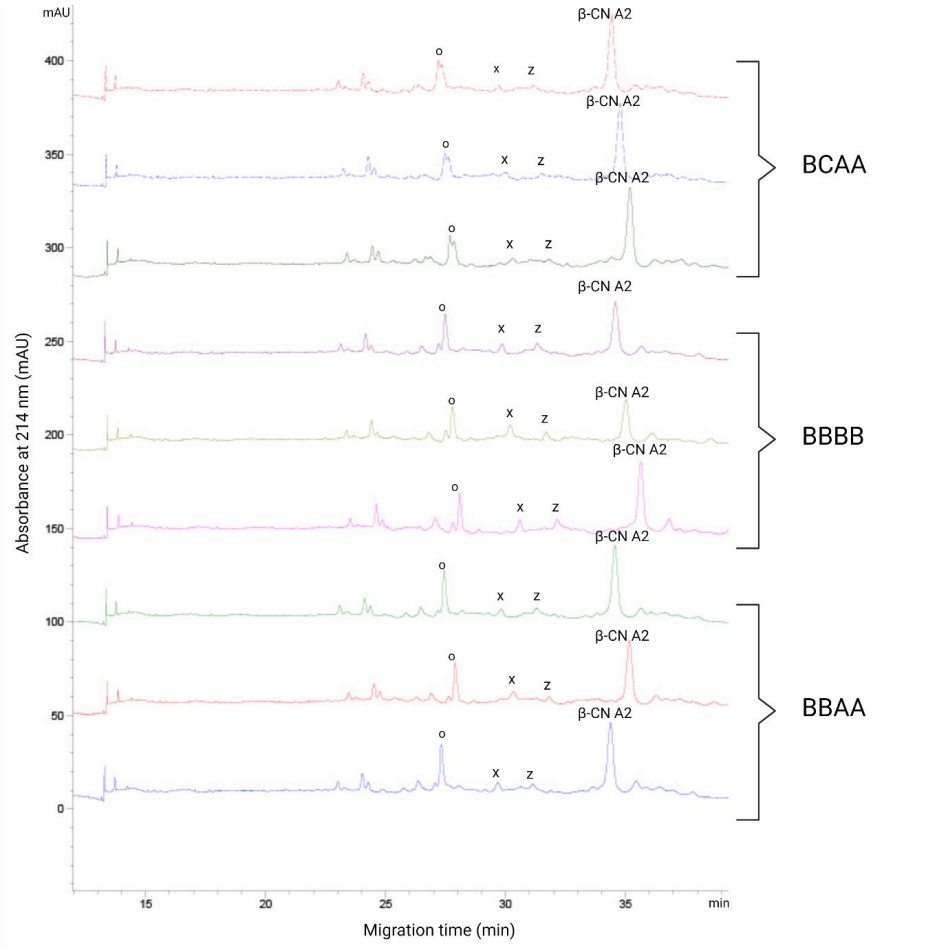
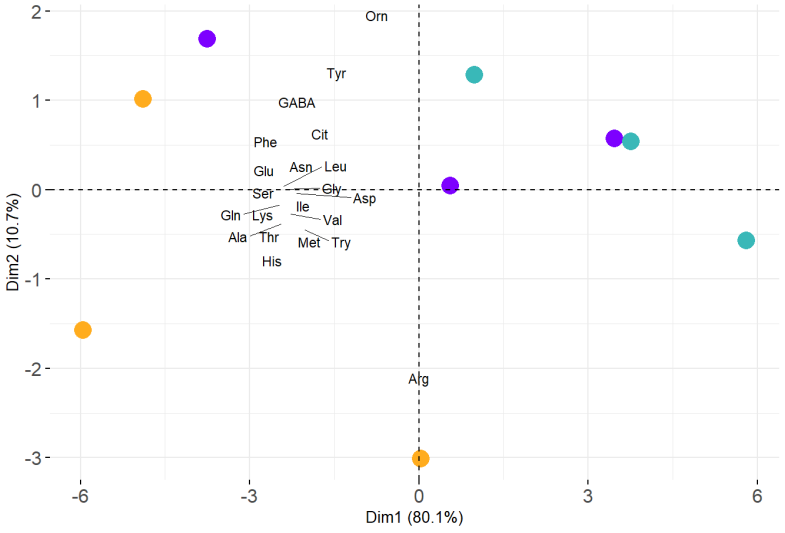


Figure 2. Protein profiles of cheeses after 5 months of ripening representing the 3 composite genotypes (BBAA, BBBB and BCAA) analysed by capillary electrophoresis.

230 BCAA cheeses had higher area of intact β-CN A2, lower peak area for two proteins/peptides
231 which were most probably α_{S1}-CN 9P and γ-CN (marked with "x" and "z" respectively).
232 Moreover, BCAA cheese had a different profile of the protein/peptides marked with "o", this is
233 possibly γ-CN and α_{S1}-CN 8P. Differences in the content of FAAs in ripened cheese were found
234 between the cheeses as illustrated in the PCA shown in Figure 3.



235

236 Figure 3. Principal Component analysis of free amino acids in cheese with different composite
237 genotypes of α_{s1}-κ-CN (BBAA●, BBBB● and BCAA●) ripened for 5 months. Principal
238 components 1 and 2 explain 80.1 and 10.7 % of the variation, respectively. (Dim = Dimension of
239 the PCA).

240

241 BCAA cheese had a significantly lower content of total FAA (40.27 µmol g^{-1}) compared to
242 BBAA cheese (48.06 µmol g^{-1}) and BBBB cheese (55.91 µmol g^{-1}) (P = 0.03). BCAA is located
243 on the right side and BBBB cheese is located on the left side in. All differences in the content of
244 FAA between the cheeses are shown in supplementary Table S2, but briefly, cheese with κ-CN

BB (BBBB cheese) differed from cheese with κ-CN AA (BBAA and BCAA) due to significantly higher concentrations of five FAAs (Ser, Gln, Thr, Ala and Met). Cheese with α_{S1}-CN BC (BCAA) differed from cheese with α_{S1}-CN BB (BBBB and BBAA) by having a significantly lower concentrations of six FAAs (Ser His, Gly, Ile, Leu and Lys). In accordance with the FAA results, the content of DL-pyroglutamic acid also differed significantly between the cheeses: with BCAA<BBAA<BBBB (0.28, 0.36 and 0.43 µmol g^{-1} respectively).

The BCAA cheese were perceived by the sensory panel to have a higher intensity of sweet taste than BBAA and BBBB cheeses ($P < 0.01$) but had a lower intensity of hardness ($P < 0.05$) (Figure 4). There was more sunlight flavour in BBAA cheese compared to BCAA cheese ($P < 0.05$). Moreover, the BBBB cheese did not seem to differ from the two other cheeses (BBAA and BCAA) in most attributes except that it had a higher degree of juiciness ($P < 0.05$) compared to the BBAA cheese.

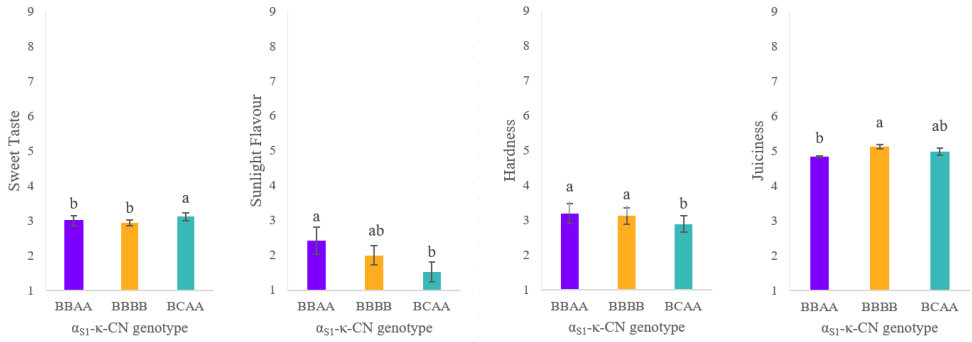


Figure 4. Effects of the α_{s1}-κ-CN composite genotypes (BBAA ■, BBBB ■ and BCAA ■) on the sensorial attributes sweet taste, sunlight flavour, hardness and juiciness. Different letters (Tukey groupings) between the α_{s1}-κ-CN composite genotypes indicate significant difference at $P < 0.05$.

4 Discussion

In the present experiment, a Havarti-type cheese was made from milk with different composite genotypes of α_{S1}-κ-CN (BBAA, BBBB and BCAA). Milk composition, cheese-making properties, yield, ripening and sensory properties of the cheeses were evaluated.

Even though the BCAA milk had a lower casein content than BBAA milk, the BCAA milk coagulated significantly faster. Usually, a higher milk protein/casein content leads to better coagulation properties (Fox, Guinee, Cogan, & McSweeney, 2017b; Horne & Lucey, 2017; Jõudu, Henno, Kaart, Püssa, & Kärt, 2008). The BC genotype of α_{S1}-CN has earlier been reported to have good renneting properties (Jensen et al., 2012; Jõudu et al., 2009b; Ketto et al., 2017; Poulsen et al., 2013). However, conflicting results of casein content in milk with α_{S1}-CN BC are reported. Devold, Brovold, Langsrud, and Vegarud (2000) found a lower casein content while Jakob (1994) reported a higher content. The result from the current study suggests that other important factors than casein content affect the coagulation properties of milk. Factors such as protein composition, casein micelle size and the higher lactose content may have an influence, as explained further. Ketto et al. (2017) found that milk with α_{S1}-CN BC contained smaller micelles compared to α_{S1}-CN BB. Smaller casein micelles are known to have a positive effect on coagulation properties (Glantz et al., 2010; Logan et al., 2014; Walsh et al., 1998b), probably due to a greater total surface area of the micelles, which then leads to the formation of a stronger network during coagulation. Ketto et al. (2017) also found that milk with α_{S1}-CN BC contained a higher relative concentration of κ-CN, which forms the stabilizing layer of the casein micelles, and this might explain why the micelles were smaller in milk with α_{S1}-CN BC.

The lactose content has also been shown to affect the rennet coagulation of milk, where a higher lactose content in milk was associated with a shorter renneting time and a firmer coagulum

(Glantz et al., 2010; Ketto et al., 2017; Malacarne et al., 2014). However, the mechanism of this is not understood. Malacarne et al. (2014) suggested that it was the contribution of lactose to a higher DM content in milk that improved the MCP. In the present experiment, the sum of fat, lactose and protein from the MilkoScan FT1 was used as an indication of the DM content in cheese milk. BBAA milk had the highest DM content, but BCAA milk had the highest lactose content, which indicates that it is not necessarily the higher DM content that contributes to better coagulation properties.

In addition to coagulation properties, cheese yield is another important parameter for cheese-making efficiency. Good coagulation properties are usually connected to a higher cheese yield (Fox, Guinee, Cogan, & McSweeney, 2017a) since the coagulum gets stronger and limits the loss of casein and fat. However, milk with good coagulation properties cannot alone estimate a high cheese yield. This current study confirms the good coagulation properties of α_{S1}-CN BC, however, BCAA milk resulted in a significantly lower cheese yield compared to BBAA. These results underline the importance of analysing more broadly and not only focus on coagulation properties when selecting genotypes of casein when breeding for an increased efficiency of the cheese-making process. The most important factor affecting cheese yield is the milk composition, more specifically fat and casein (Fox, Guinee, Cogan, & McSweeney, 2017a), therefore the milk casein content directly influences cheese yield. It was predicted by PY that BBAA milk, which had the highest casein content, would give a significantly higher yield compared to the two other genotypes, and BBAA did actually show a higher Ya than BCAA.

The differences found in PY and Ya in this study seems to be due to the α_{S1}-CN genotype, since the BBAA and BBBB cheeses obtained the highest yield. Aleandri, Buttazzoni, Schneider, Caroli, and Davoli (1990) found that the BB genotype of α_{S1}-CN resulted in higher cheese yield

309 compared to the BC genotype, which confirms the results of the present study, while others have
310 found no differences in cheese yield when comparing the B and C variant (Ng-Kwai-Hang,
311 2006). This current study did not find any evidence that the κ-CN genotype affected Ya, but
312 other studies (Walsh, Guinee, Harrington, Murphy, & FitzGerald, 1995; Walsh et al., 1998a)
313 have reported a higher cheese yield with the κ-CN BB genotype compared to the AA genotype
314 on pilot-scale Cheddar cheese production. In this study, the κ-CN genotype AA in combination
315 with α_{S1}-CN BC resulted in a lower cheese yield, while in combination with α_{S1}-CN BB the yield
316 gets higher. This underlines the necessity to evaluate the composite genotypes rather than the
317 individual ones.

318 The protein composition in 24 h cheese is usually reflected by the protein profile of the milk
319 since the degradation of proteins is limited this early in the ripening process. However, there are
320 already a difference in degradation of β-CN, since cheese with α_{S1}-CN BB are associated with a
321 higher relative concentration of γ-CN. This indicates that the early proteolysis of β-CN might be
322 affected by differences in α_{S1}-CN genotypes (BC vs. BB).

323 In ripened cheese, differences were observed in protein degradation, especially between cheeses
324 with different genotypes of α_{S1}-CN. However, the electropherogram of ripened cheese is difficult
325 to compare with that of the 24 h cheese as the retention times is different, therefore the peaks are
326 difficult to identify. If the anticipations made on the identification of the peaks are correct,
327 cheese with α_{S1}-CN BB (BBAA and BBBB) have a higher degradation of β-CN, while cheese
328 with α_{S1}-CN BC (BCAA) have a higher degradation of α_{S1}-CN. The left part of the peak marked
329 with "o" in the electropherogram of the BCAA cheese is probably γ-CN that have not further
330 been degraded as seen in BBAA and BBBB cheese while the right part of the peak most
331 probably is α_{S1}-CN 8P which is more degraded than that of the BBAA and BBBB cheese.

DL-pyroglutamic acid is a derivate from glutamine or glutamic acid (Gazme, Boachie, Tsopmo, & Udenigwe, 2019). It is present in many cheese varieties, but especially in long-ripened Italian cheeses such as Grana Padano and Parmigiano Reggiano (Mucchetti et al., 2000). Pyroglutamic acid can be used as an indicator of ripening (Mucchetti et al., 2000), as can the total content of FAA. Since BCAA cheese had a lower concentration of both total FAA and DL-pyroglutamic acid and considering the differences in protein profiles of the ripened cheeses, it is reasonable to assume that the degradation of β-CN is most important for the ripening process of this cheese and that the ripening proceeded slower in the BCAA cheeses.

The formation of pyroglutamic acid is believed to be more dependent on the starter culture than on the raw milk microflora (Gazme, Boachie, Tsopmo, & Udenigwe, 2019; Mucchetti et al., 2000). However, the same starter culture was used for all cheeses in this current study. There are some indications that there are other factors in addition to the microflora that can affect the formation of pyroglutamic acid. Olsen, Ferneborg, Vhile, Kidane, and Skeie (2023) found that the protein source in concentrate feed affected the content of pyroglutamic acid in a Gouda-type cheese. Also, in this experiment the same starter culture was used for the cheese-makings and there were no differences in cheese microbiota. This indicates that both milk protein genetic variants and the feed for dairy cows can affect the formation of pyroglutamic acid and thereby the cheese ripening.

BCAA cheese was experienced by the sensory assessors to be less firm compared to BBAA and BBBB cheeses, even though they did not differ in DM content. This has also previously been reported by Nuyts-Petit, Delacriox-Buchet, and Vassal (1997) who found that Saint-Paulin

355 cheese made with the B variant of α_{s1}-CN was associated with firmer cheese after 45 days
356 ripening. Moreover, the differences in firmness and juiciness could most probably be related to
357 differences in proteolysis between the cheeses. Although significant differences in some sensory
358 attributes were observed by a trained professional sensory panel, the intensities of these were
359 low. Therefore, consumers can probably not differentiate between the cheeses.

360

361

362 **5 Conclusions**

363 Despite that BCAA milk had a lower casein content, making cheeses with this genotype showed
364 a shorter renneting time, but a lower cheese yield compared to BBAA milk. BBAA milk had the
365 highest casein content giving the highest cheese yield. This shows that the superior renneting
366 properties of α_{s1}-CN BC, which has also been reported in previous studies, might not contribute
367 to a higher cheese-making efficiency in total. The results of this experiment add to previous
368 research showing that the genetic polymorphism of casein needs to be taken into consideration
369 with regards to cheese-making properties, cheese yield and most probably also cheese quality.
370 Which variant to choose would depend on what properties to emphasise. Using the results
371 obtained in this present experiment to calculate the influence of the genetic variants on the
372 revenue of a cheese plant producing 30 vats (processing 20000 kg milk pr vat) pr day, 126 kg
373 more cheese could be obtained pr day using BBAA milk compared to BCAA milk. However,
374 BCAA milk used 9 minutes less to coagulate to sufficient firmness than BBAA milk and would
375 result in 4.5 h daily reduced processing time. The cheesemaker needs therefore to decide what to
376 emphasize.

377 The BCAA cheeses had a lower content of FAA and were less firm than BBAA and BBBB
378 cheeses. Cheese with α_{s1}-CN BB genotype contained the highest concentration of FAA and a
379 higher content of peptides from proteolysis, indicating a faster ripening. However, it is important
380 to note that the current research was carried out at pilot scale, using only 100 L of milk, and
381 these results therefore need to be confirmed at larger scale.

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391

References

Aleandri, R., Buttazzoni, L., Schneider, J., Caroli, A., & Davoli, R. (1990). The effects of milk protein polymorphisms on milk components and cheese-producing ability. *Journal of Dairy Science, 73*, 241-255.

Ardö, Y., McSweeney, P. L., Magboul, A. A., Upadhyay, V. K., & Fox, P. F. (2017). Biochemistry of cheese ripening: Proteolysis. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter & D. W. Everett (Eds.), *Cheese Chemistry, Physics and Microbiology* (pp. 445-482). London: Academic Press.

Banks, J. M. (2007). Cheese yield. In P. L. H. McSweeney (Ed.), *Cheese Problems Solved* (pp. 100-114). Cambridge, England: Woodhead Publishing Limited.

Bonfatti, V., Cecchinato, A., Di Martino, G., De Marchi, M., Gallo, L., & Carnier, P. (2011). Effect of κ-casein B relative content in bulk milk κ-casein on Montasio, Asiago, and Caciotta cheese yield using milk of similar protein composition. *Journal of Dairy Science, 94*, 602-613.

Devold, T. G., Brovold, M. J., Langsrud, T., & Vegarud, G. E. (2000). Size of native and heated casein micelles, content of protein and minerals in milk from Norwegian Red Cattle—effect of milk protein polymorphism and different feeding regimes. *International Dairy Journal, 10*, 313-323.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2017a). Cheese Yield. In P. F. Fox, T. P. Guinee, T. M. Cogan & P. L. H. McSweeney (Eds.), *Fundamentals of Cheese Science* (pp. 279-331). New York: Springer Science+Business Media LLC.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2017b). Enzymatic Coagulation of Milk. In P. F. Fox, T. P. Guinee, T. M. Cogan & P. L. H. McSweeney (Eds.), *Fundamentals of Cheese Science* (pp. 185-230). New York: Springer Science+Business Media LLC.

Gazme, B., Boachie, R. T., Tsopmo, A., & Udenigwe, C. C. (2019). Occurrence, properties and biological significance of pyroglutamyl peptides derived from different food sources. *Food Science and Human Wellness, 8*, 268-274.

Glantz, M., Devold, T., Vegarud, G., Månsson, H. L., Stålhammar, H., & Paulsson, M. (2010). Importance of casein micelle size and milk composition for milk gelation. *Journal of Dairy Science, 93*, 1444-1451.

Gustavsson, F., Buitenhuis, A., Glantz, M., Stålhammar, H., Lindmark-Månsson, H., Poulsen, N., Larsen, L., Andrén, A., & Paulsson, M. (2014). Impact of genetic variants of milk proteins on chymosin-induced gelation properties of milk from individual cows of Swedish Red dairy cattle. *International Dairy Journal, 39*, 102-107.

Hallén, E., Allmere, T., Näslund, J., Andrén, A., & Lundén, A. (2007). Effect of genetic polymorphism of milk proteins on rheology of chymosin-induced milk gels. *International Dairy Journal, 17*, 791-799.

Horne, D. S., & Lucey, J. A. (2017). Rennet-Induced Coagulation of Milk In P. L. H. McSweeney, P. F. Fox, P. D. Cotter & D. W. Everett (Eds.), *Cheese Chemistry, Physics and Microbiology* (pp. 115-137). London: Academic Press.

IDF. (1982) *Cheese and Processed Cheese : Determination of the Total Solids Content. IDF standard 4A*. Brussels, Belgium: International Dairy Federation.

ISO. (2007) *Sensory analysis - general guidance for the design of test rooms. ISO Standard 8589*. Genève, Switzerland: International Organization for Standardization.

ISO. (2012) *Sensory Analysis – General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors. ISO Standard 8586*. Genève, Switzerland: International Organisation for Standardisation.

Jakob, E. (1994). Genetic Polymorphism of Milk Proteins. *Mljekarstvo, 44*, 197-217.

Jensen, H., Poulsen, N., Andersen, K., Hammershøj, M., Poulsen, H., & Larsen, L. (2012). Distinct composition of bovine milk from Jersey and Holstein-Friesian cows with good, poor, or noncoagulation properties as reflected in protein genetic variants and isoforms. *Journal of Dairy Science, 95*, 6905-6917.

Jõudu, I., Henno, M., & Värv, S. (2007). Milk protein genotypes and milk coagulation properties of Estonian Native cattle. *Agricultural and Food Science, 16*, 222-231.

Jõudu, I., Henno, M., Kaart, T., Püssa, T., & Kärt, O. (2008). The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. *International Dairy Journal, 18*, 964-967.

Jõudu, I., Henno, M., Värv, S., Viinalass, H., Püssa, T., Kaart, T., Arney, D., & Kärt, O. (2009a). The effect of milk proteins in milk coagulation properties in Estonian dairy breeds. *Veterinarija ir Zootechnika, 46*.

Jõudu, I., Henno, M., Värv, S., Viinalass, H., Püssa, T., Kaart, T., Arney, D., & Kärt, O. (2009b). The effect of milk proteins on milk coagulation properties in Estonian dairy breeds. *Veterinarija ir Zootechnika, 68*, 14-19.

Kassambara, A., & Mundt, F. (2020) *factoextra: Extract and Visualize the Results of Multivariate Data Analyses*. https://cran.r-project.org/web/packages/factoextra/index.html

Ketto, I. A., Knutsen, T. M., Øyaas, J., Heringstad, B., Ådnøy, T., Devold, T. G., & Skeie, S. B. (2017). Effects of milk protein polymorphism and composition, casein micelle size and salt distribution on the milk coagulation properties in Norwegian Red cattle. *International Dairy Journal, 70*, 55-64.

Lawless, H. T., & Heymann, H. (2010). *Sensory evaluation of food: Principles and practices* (2nd ed.). New York: Springer.

Logan, A., Day, L., Pin, A., Auldist, M., Leis, A., Puvanenthiran, A., & Augustin, M. A. (2014). Interactive effects of milk fat globule and casein micelle size on the renneting properties of milk. *Food and Bioprocess Technology, 7*, 3175-3185.

Lucey, J., & Kelly, J. (1994). Cheese yield. *International Journal of Dairy Technology, 47*, 1-14.

Malacarne, M., Franceschi, P., Formaggioni, P., Sandri, S., Mariani, P., & Summer, A. (2014). Influence of micellar calcium and phosphorus on rennet coagulation properties of cows milk. *Journal of Dairy Research, 81*, 129-136.

Martinovic, A., Moe, K. M., Romeih, E., Aideh, B., Vogensen, F. K., Østlie, H., & Skeie, S. (2013). Growth of adjunct Lactobacillus casei in Cheddar cheese differing in milk fat globule membrane components. *International Dairy Journal, 31*, 70-82.

Mayer, H., Ortner, M., Tschager, E., & Ginzinger, W. (1997). Composite milk protein phenotypes in relation to composition and cheesemaking properties of milk. *International Dairy Journal, 7*, 305-310.

Mucchetti, G., Locci, F., Gatti, M., Neviani, E., Addeo, F., Dossena, A., & Marchelli, R. (2000). Pyroglutamic Acid in Cheese: Presense, Origin, and Correlation with Ripening Time of Grana Padano Cheese. *Journal of Dairy Science, 83*, 659-665.

Ng-Kwai-Hang, K. (2006). Genetic variants of milk proteins and their effects on the yield and quality of cheese. *CABI Reviews*.

Nuyts-Petit, V., Delacriox-Buchet, A., & Vassal, L. (1997). Influence de trois haplotypes des caséines asl' pet K fréquents en race bovine Normande sur la composition du lait et l'aptitude à la fabrication fromagère. *Lait, 77*, 625-639.

Olsen, M. A., Ferneborg, S., Vhile, S. G., Kidane, A., & Skeie, S. (2023). Different protein sources in concentrate feed for dairy cows affect cheese-making properties and yield. *Accepted manuscript, Journal of Dairy Science, 24.01.2023*.

Otte, J., Zakora, M., Kristiansen, K., & Qvist, K. (1997). Analysis of bovine caseins and primary hydrolysis products in cheese by capillary zone electrophoresis. *Le Lait, 77*, 241-257.

Otte, J., Ardö, Y., Weimer, B., & Sørensen, J. (1999). Capillary electrophoresis used to measure proteolysis in cheese. *International Dairy Federation*.

Poulsen, N., Bertelsen, H., Jensen, H., Gustavsson, F., Glantz, M., Månsson, H. L., Andrén, A., Paulsson, M., Bendixen, C., & Buitenhuis, A. (2013). The occurrence of noncoagulating milk and the association of bovine milk coagulation properties with genetic variants of the caseins in 3 Scandinavian dairy breeds. *Journal of Dairy Science, 96*, 4830-4842.

R Core Team. (2022) *R: A language and environment for statistical computing.* Vienna, Austria: R Foundation for Statistical Computing. https://www.R-project.org/

Skeie, S., Kieronczyk, A., Eidet, S., Reitan, M., Olsen, K., & Østlie, H. (2008). Interaction between starter bacteria and adjunct Lactobacillus plantarum INF15D on the degradation of citrate, asparagine and aspartate in a washed-curd cheese. *International Dairy Journal, 18*, 169-177.

Skeie, S. B. (2007). Characteristics in milk influencing the cheese yield and cheese quality. *Journal of Animal and Feed Sciences, 16*, 130-142.

Walsh, C. D., Guinee, T. P., Harrington, D., Murphy, J. J., & FitzGerald, R. J. (1995). Cheddar cheese-making and rennet coagulation of bovine milks containing K-CN AA or BB genetic variants. *Milchwissenschaft, 50*, 492-496.

Walsh, C. D., Guinee, T. P., Harrington, D., Mehra, R., Murphy, J., & Fitzgerald, R. J. (1998a). Cheesemaking, compositional and functional characteristics of low-moisture part-skim Mozzarella cheese from bovine milks containing κ-casein AA, AB or BB genetic variants. *Journal of Dairy Research, 65*, 307-315.

Walsh, C. D., Guinee, T. P., Reville, W. D., Harrington, D., Murphy, J. J., O'Kennedy, B. T., & FitzGerald, R. J. (1998b). Influence of κ-casein genetic variant on rennet gel microstructure, cheddar cheesemaking properties and casein micelle size. *International Dairy Journal, 8*, 707-714.

Zambrano, B. G. L., Eraso, C. Y. M., Solarte, P. C. E., & Rosero, G. C. Y. (2010). Kappa casein genotypes and curd yield in Holstein cows. *Revista Colombiana de Ciencias Pecuarias, 23*, 422-428.

515 Supplementary Table S1. Definition of the sensory attributes used by the Norwegian Institute of
516 Food and Aquaculture Research (NOFIMA).

Attribute	Description
Odour(O)	
Sour-O	Related to a fresh, balanced odour due to the presence of organic acids
Butter-O	Related to the odour of butter
Milk-O	Related to the odour of milk
Mature-O	Related to the odour of a matured, well-developed cheese
Sour milk-O	Related to the odour from dairy products that are acidified with bacterial culture
Metallic-O	Related to a metallic odour
Sunlight-O	Related to photo oxidation, as when milk has been exposed to light
Cloying-O	Unfresh and/or sickeningly sweet odour
Taste (T) and flavour (F)	
Sour-F	Related to a fresh, balanced flavour due to the presence of organic acids
Sweet-T	Related to the basic taste sweet (Sucrose)
Acidic-T	Related to the basic taste acidic (Citric acid)
Salty-T	Related to the basic taste salty (Sodium chloride)
Bitter-T	Related to the basic taste bitter (Caffeine)
Umami-T	Related to the basic taste umami (Monosodium glutamate)
Butter-F	Related to the flavour of butter
Milk-F	Related to the flavour of milk
Mature-F	Related to the flavour of a matured, well-developed cheese
Metallic-F	Related to a metallic flavour
Sunlight-F	Related to photo oxidation, as when milk has been exposed to light
Tart-F	Related to a tart flavour
Cowshed-F	Related to the flavour of a cowshed
Texture	
Hardness	Mechanical textural attribute relating to the force required to achieve a given deformation or penetration of a product
Juiciness	Surface textural attribute which describes the perception of water absorbed by released from the product
Fattiness	Surface textural attribute relating to perception of the quantity of fat in the product
Stickiness	Mechanical textural attribute relating to the force required to remove material that adheres to the mouth
Granularity	Geometrical textural attribute relating to the perception of the size and shape of particles in a product.
Rubbery	Mechanical textural attribute related to the cohesiveness of a tender product. In the mouth, it is related to the effort required to disintegrate the product to the state ready for swallowing.
Astringency	Organoleptic attribute of pure substances or mixtures which produces the astringent sensation

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Supplementary Table S2. Effects of the α_{s1}-κ-casein composite genotypes (BBAA, BBBB and BCAA) on free amino acids (FAA, μmol g^{-1}) (mean and SD). Significant differences between means are shown by different superscript letters ($P < 0.05$).

α_{s1}-κ-CN genotypes	BBAA		BBBB		BCAA		
	mean	SD	mean	SD	mean	SD	P value
Glu	7.27^{ab}	1.15	8.40^{a}	0.89	6.34^{b}	0.84	0.04
Asn	3.79^{ab}	0.75	4.44^{a}	0.62	3.17^{b}	0.42	0.04
Ser	1.50^{b}	0.32	1.91^{a}	0.39	1.12^{c}	0.23	0.01
Gln	2.02^{b}	0.47	2.66^{a}	0.43	1.61^{b}	0.32	0.01
His	0.32^{a}	0.05	0.38^{a}	0.06	0.27^{b}	0.02	0.03
Gly	1.06^{a}	0.17	1.26^{a}	0.17	0.90^{b}	0.12	0.03
Thr	0.71^{b}	0.18	1.02^{a}	0.14	0.50^{b}	0.08	0.01
Arg*	0.97	0.09	1.13	0.16	0.99	0.10	0.05
Ala	1.91^{b}	0.32	2.32^{a}	0.25	1.57^{b}	0.23	0.01
Val*	2.96	0.71	3.67	0.19	2.52	0.25	0.05
Met	0.87^{b}	0.16	1.24^{a}	0.22	0.65^{b}	0.09	0.006
Ile	0.47^{a}	0.16	0.66^{a}	0.14	0.32^{b}	0.04	0.02
Leu	9.51^{a}	1.79	11.04^{a}	0.99	8.14^{b}	0.97	0.04
Lys	3.22^{a}	0.59	3.98^{a}	0.66	2.34^{b}	0.31	0.01
Total FAA	48.06^{a}	8.83	55.91^{a}	8.40	40.27^{b}	5.25	0.03

* Tukey's test did not find differences

Appendix 1

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Cyberlindnera jadinii yeast as a protein source in early- to mid-lactation dairy cow diets: Effects on feed intake, ruminal fermentation, and milk production

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ABSTRACT

We examined the effects of substituting soybean meal with either yeast protein from *Cyberlindnera jadinii* or barley in concentrate feeds on feed intake, ruminal fermentation products, milk production, and milk composition in Norwegian Red (NRF) dairy cows. The concentrate feeds were prepared in pellet form as soy-based (SBM; where soybean meal is included as a protein ingredient), yeast-based (YEA; soybean meal replaced with yeast protein), or barley-based (BAR; soybean meal replaced with barley). The SBM contained 7.0% soybean meal on a dry matter (DM) basis. This was replaced with yeast protein and barley in the YEA and BAR concentrate feeds, respectively. A total of 48 early- to mid-lactation [days in milk ± standard deviation (SD): 103 ± 33.5 d] NRF cows in their first to fourth parity and with initial milk yield of 32.6 kg (SD = 7.7) were allocated into 3 groups, using a randomized block design, after feeding a common diet [SBM and good-quality grass silage: crude protein (CP) and neutral detergent fiber (NDF) content of 181 and 532 g/kg of DM, respectively] for 14 d (i.e., covariate period). The groups (n = 16) were then fed one of the dietary treatments (SBM, YEA, or BAR) for a period of 56 d (i.e., experimental period). The concentrate feeds were offered in split portions from 3 automatic feeders using electronic identification, with ad libitum access to the same grass silage. Dietary treatments had no effect on daily silage intake, total DM intake, or total NDF intake. Dietary CP intake was lower and starch intake was higher in the BAR group compared with the other groups. Ruminal fluid pH, short-chain volatile fatty acid (VFA) concentrations, acetate-to-propionate ratio, and non-glucogenic to glucogenic VFA ratio were not affected by dietary treatments. No effects of the dietary treatments were observed on body weight change, body condition score change, milk yield, energy-corrected milk yield, milk lactose and fat percentages, or their yields. In conclusion, yeast protein can substitute conventional soybean meal in dairy cow diets without adverse effect on milk production and milk composition, given free access to good-quality grass silage.

Key words: amino acid, dietary nitrogen, milk composition, soybean, barley

INTRODUCTION

Sustainable meat and milk production is essential for future agricultural production. Growing environmental concerns surrounding food and feed production, and sustainability issues due to increasing population and demand for food (Foley et al., 2011; Notarnicola et al., 2017), necessitate the search for local feed resources (Åby et al., 2014). Diets for high-yielding dairy cows in the Nordic countries commonly consist of grass silage (Huhtanen et al., 2013) augmented with concentrate feeds based on barley and a relatively high proportion of imported protein feed ingredients such as soybean meal, corn gluten meal, and rapeseed meal (Åby et al., 2014).

Norway has a challenging climate for agriculture, with a typical grassland of only about 3% cultivated land and limited potential to grow food crops. Therefore, a growing need exists to develop novel, sustainable, nonfood protein sources that can be used in animal diets to allocate food protein to the increasing human population. Recent efforts, in Norway and elsewhere, have focused on the effects of partial or complete substitution of imported protein ingredients with alternative protein sources in animal feeds (Neal et al., 2014; Dalle Zotte et al., 2019; Cruz et al., 2020a,b). Yeast-derived microbial protein is one such emerging protein ingredient, with favorable AA composition in animal feeds (Øverland and Skrede, 2017). With a forest cover of about 38% of the total land area (Government of

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Norway, 2014) and the country experiencing a large accumulation of forest biomass with steady increase in net growth over the recent years (Solberg et al., 2021), yeast produced using wood biomass can provide high-quality protein. For instance, a typical *Candida utilis* grown on biomass hydrolysate with ammonium sulfate as a nitrogen source (Sharma et al., 2018) had an AA profile comparable to that of soybean meal (Cavins et al., 1972). Sabbia et al. (2012) reported that DEMP—a yeast-derived microbial protein based on *Saccharomyces cerevisiae* (Alltech Inc.)—had an AA profile similar to that of ruminal microbial protein. As a result, Sabbia et al. (2012) reported that DEMP could replace plant protein in dairy cow diets without negative effects on milk production when used from 1.14 to 3.41% of the diet DM. Neal et al. (2014) reported a tendency for increased milk production when adding 1.15% of the diet DM as yeast microbial protein. In addition, Higginson et al. (2017) reported improved metabolic status (e.g., reduced metabolic stress and adipose tissue mobilization) in transition cows during the postpartum period when fed yeast-derived microbial protein. However, Manthey et al. (2016) reported reduced feed efficiency (energy-corrected milk per kilogram of DMI) and milk fat yield for cows fed 2.25% of diet DM as yeast-derived microbial protein. These studies were based on corn silage and alfalfa forages, in contrast with the Scandinavian grass-based silage, with expected differences in nutrient composition and density. Such differences, especially in the carbohydrate fraction of the diet, would be expected to influence the utilization efficiency of the dietary protein in dairy cow diets (Hristov et al., 2005). Furthermore, the microbial proteins used in these studies were largely based on *Saccharomyces cerevisiae*, which might differ from other yeasts in the level of CP, AA profile, and other nutrients (Øverland and Skrede, 2017).

We hypothesize that *Cyberlindnera jadinii* yeast protein can replace soybean meal or barley in early- to mid-lactation Norwegian Red (**NRF**) dairy cow diets without adverse effects on milk yield and milk composition. The main objective of this study was, therefore, to evaluate the effects of total substitution of soybean meal in concentrate feeds by *C. jadinii* yeast protein in grass silage-based rations of early- to mid-lactation NRF cows on feed intake, ruminal fermentation parameters, milk yield, and milk composition. Furthermore, as barley can be produced in Norway and is the most used concentrate feed ingredient, a diet with barley replacing both yeast protein and soybean meal in the concentrate feed was compared against those 2 protein sources.

MATERIALS AND METHODS

Experimental Animals, Diets, and Design

This experiment was performed at the Livestock Production Research Center of the Norwegian University of Life Sciences (Ås, Norway), with all animal procedures approved by the national animal research authority of the Norwegian Food Safety Authority (FOTS ID no. 18038).

A total of 48 NRF dairy cows of mixed parity (primiparous = 27, second lactation = 10, and third lactation and above = 11) in their early- to mid-lactation period, averaging (mean ± SD) 103 ± 33.5 DIM, 623 ± 72.7 kg of BW, and 32.6 ± 7.7 kg milk yield at the beginning of the experiment were used in a completely randomized block design (Figure 1). All animals had free access to the same grass silage, prepared from primary growth using a bunker silo, for a period of 70 d. Chemical composition of the grass silage is presented in Table 1. The silage was distributed through 40 automatic feeders (BioControl AS) equipped with vertically moving gates with electronic cow identification and feed intake registration for each individual cow. All cows had free access to the 40 automatic feeders. The feed troughs were filled twice every day (between 0800 and 1000 h, and between 1500 and 1600 h) with fresh grass silage. The silage was chopped using a Siloking chopping and mixing machine (DUO1814, Siloking Kverneland, Kverneland Group Ireland Ltd.) until uniform mixture and particle length was achieved, to restrict feed selection by cows.

The first 14 d were considered a covariate period, during which all 48 cows were fed a soybean-based concentrate feed (**SBM**) in addition to grass silage (Figure 1). The ratio between grass silage and concentrate was 39:61 (CP and NDF shown in Table 1). The amount of concentrate feed for each animal was calculated to meet requirements for maintenance and production at the start of the experiment using the NorFor feeding system (TINE OptiFôr; NorFor, 2011).

At the end of the covariate period, the 48 cows were randomly assigned to 1 of 3 treatment groups blocked for parity (i.e., first and second or greater lactation) and balanced for DIM and milk yield, giving 16 cows in each treatment group. The groups were then randomly allocated to 1 of the 3 different concentrate feeds: SBM (continuation of covariate period feeding), yeast (**YEA**), or barley (**BAR**; see Figure 1 for experimental layout). The SBM concentrate feed contained 7.0% (on DM basis) soybean meal. This was quantitatively replaced by yeast and barley in the YEA and BAR

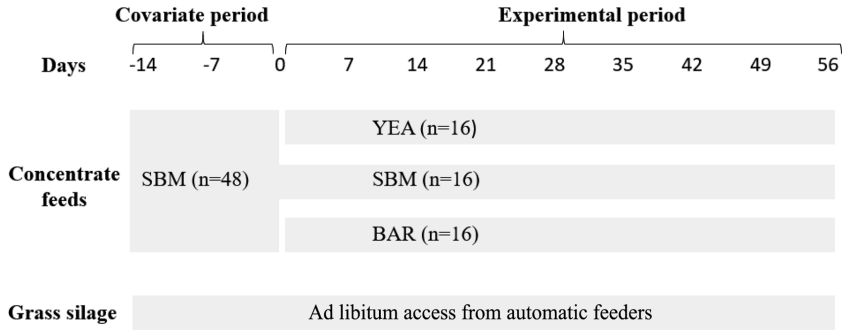

Figure 1. Schematic of the experiment, with dairy cows fed grass silage augmented with 3 different concentrate feeds, where soybean meal (SBM) was substituted with either yeast protein from *Cyberlindnera jadinii* (YEA) or barley (BAR) over an experimental period of 56 d.

concentrate feeds, respectively. Ingredient and chemical compositions of the feeds used are provided in Table 1, whereas data on AA composition of the feeds are provided in Table 2. The yeast protein was supplied by Lallemand (produced by Danstar Ferment A.G.), and all concentrate feeds were prepared at Felleskjøpet Agri (FKA, Vestnes, Norway). A brief summary of the chemical composition of *C. jadinii* is provided in the footnotes of Table 1. All 3 concentrate feeds were formulated to be isoenergetic (Table 1). The SBM and YEA concentrate feeds were formulated to be isonitrogenous, whereas the BAR concentrate feed was formulated to ensure the dietary protein supply needed for the good-quality grass silage. The concentrate feeds were offered in split portions daily, maximum of 4 kg per cow per visit, from 3 FSC40 DeLaval feeding stations, with additional small portions (~1.0 kg of SBM split over 3 visits) fed in a milking robot. All cows had free access to their feeding stations. The level of concentrate feeds offered was adjusted twice over the experimental period (reduced 15% on d 28, and an additional 10% on d 50, relative to covariate-period feeding) for all groups, to account for the increasing stage of lactation and declining yield.

Individual feed intake of grass silage and concentrate feeds as well as milk yield were measured daily for 70 d. The cows were housed in a freestall with concrete slatted floors and lying cubicles with rubber mats and sawdust bedding. Cow BW and BCS (on a scale from 1.0 = emaciated to 5.0 = obese) were recorded multiple times (mean = 2.5; SD = 0.85) per cow per day when cows visited the milking robot. The BCS was recorded by a DeLaval BCS camera mounted on a DeLaval sort gate (DeLaval VMS Classic). The camera took a 3-dimensional image of the lower back of cows, which was then analyzed with DeLaval BCS software, determining the amount of fat covering the loin, rump tailhead, hooks, pins, and short ribs to calculate the automated BCS, as recently described by Mullins et al. (2019). The BW of cows was recorded just after milking with a BioControl weighing scale (BioControl AS). Changes in BW and BCS over the experimental period, calculated as the difference between mean BW and BCS in the last week of the experiment (i.e., d 50–56) relative to the covariate period BW and BCS, in respective order, were later used in the statistical analysis.

Feed Sampling and Analyses

About 400 g of each of the 3 concentrate feeds and 500 g of grass silage samples were taken once every week and stored at −20°C pending further processing. At completion of the experiment, the grass silage samples were pooled at 3 time points (i.e., covariate period, first 28 d, and last 28 d of the experimental period), whereas the concentrate feeds were pooled at the latter 2 time points. The samples were then dried in duplicates at 45°C for 48 h in preparation for milling. The duplicates were mixed and milled using a cutting mill (SM 200, Retsch GmbH) at different sieve sizes for the planned analyses as subsequently described.

Concentrate feed samples for starch analysis were milled through a 0.5-mm sieve, whereas both concentrate feed and silage samples for other analysis were milled through a 1.0-mm sieve. The DM content of the samples was determined by drying at 103°C overnight (ISO, 1999), whereas the ash content was determined by incinerating the samples at 550°C (ISO, 2002). The nitrogen content of the feeds was analyzed using AOAC method 2001.11 (Thiex et al., 2002), with a Kjeltec 2400/2460 Auto Sampler System (Foss Analytical). Total starch content of the concentrate feed samples was analyzed using AACC method 76-13.01 (Megazyme amyloglucosidase/α-amylase method; AACC, 2000)

Table 1. Ingredients (%, DM basis) and chemical composition of grass silage and 3 concentrate feeds (SBM = soybean meal-based; YEA = yeast-based; BAR = barley-based, with barley replacing both yeast and soybean meal)

Item	Grass silage	SBM	YEA	BAR
Ingredient composition				
Barley		48.9	49.2	55.4
Corn gluten meal		2.14	2.13	2.15
Oat		4.94	4.93	4.97
Wheat		9.89	9.85	9.94
Molasses		4.20	4.19	4.23
Beet pulp		15.3	15.3	15.4
Soybean meal		7.00	—	—
Yeast[1]		—	7.29	—
Calcium soap of fatty acids[2]		3.38	3.04	3.29
Limestone		0.30	0.53	0.31
Monocalcium phosphate		0.66	0.37	0.77
Sodium bicarbonate		1.16	1.27	1.35
Magnesium oxide		0.51	0.51	0.51
Sodium sulfate		0.12	0.03	0.20
Salt (NaCl), feed-grade		1.00	1.00	1.01
Micromineral premix[3]		0.11	0.11	0.11
Selenpremiks[4]		0.14	0.14	0.15
Vitamin premix[5]		0.09	0.09	0.09
Agolin Ruminant[6]		0.07	0.07	0.07
Chemical composition[7] and energy value				
DM content (g/kg)	300	875	881	875
CP	181	161	157	134
NDF[8]	522	175	169	174
Fat	46.3	38.3	36.9	38.0
Starch	—	385	365	406
Ash	75.8	65.9	67.5	69.2
FPF[9]	99.9	—	—	—
WSC[10]	16.7	61.5	58.5	56.8
Residual CHO[11]	58.4	113	146	122
NE_L (MJ/kg of DM)[12]	6.6	7.1	7.1	7.0

[1]*Cyberlindnera jadinii* yeast (composition on DM basis): CP (N × 6.25) 479 g/kg; ash 69 g/kg; crude fat 54 g/kg; total carbohydrates 397 g/kg; and macrominerals P 14.4 g/kg, Na 0.86 g/kg, Mg 1.34 g/kg, and Ca 1.03 g/kg.

[2]Calcium soap of palm fatty acids provided as Akofeed Kalkfett (AAK).

[3]Micromineral premix (mg/kg of feed): 19.3 Cu; 0.25 Co; 5.3 I; 86 Zn; 40 Mn; and 100 Fe.

[4]Selenium premix (Vilomix) providing 0.4 mg Se per kg of feed.

[5]Vitamin premix providing 5,004 IU of vitamin A, 2,010 IU of vitamin D, and 80 mg of vitamin E per kilogram of feed.

[6]Agolin Ruminant is a feed additive produced by Agolin SA; https://agolin.ch/.

[7]Mean values for chemical composition are based on a minimum of triplicate analysis.

[8]NDF in feeds corrected for ash.

[9]Sum of fermentation products (silage fermentation acids and alcohols).

[10]Water-soluble carbohydrates in feeds.

[11]Calculated residual carbohydrates (difference between DM content and sum of all analytical values) according to NorFor (2011).

[12]Estimated NE_L at 20 kg of DMI (NorFor, 2011).

with starch hydrolyzed to glucose and determining the concentration of glucose colorimetrically using an RX DaytoNa$^+$ spectrophotometer (Randox Laboratories Ltd.). The content of NDF was determined with an Ankom 220 fiber analyzer (Ankom Technology) according to Mertens (2002), using sodium sulfite and α-amylase, and further corrected for residual ash. Water-soluble carbohydrate content was determined as described in Randby et al. (2010), whereas residual carbohydrate content was determined as the difference between DM and analytical components (sum of starch, CP, NDF, crude fat, and ash for concentrate feeds, with additional adjustment for silage fermentation products for the grass silage) according to the Nordic feed evaluation system (NorFor, 2011). The AA contents (except for tryptophan; not analyzed) of the silage and concentrate feeds were determined by ion-exchange chromatography according to commission regulation no. 152/2009

of the European Communities (EC, 2009; Table 2). Silage fermentation products and ammonia nitrogen were analyzed on fresh silage samples at Eurofins (Eurofins Agro Testing Norway AS, Moss, Norway) as recently described by Randby et al. (2020).

Ruminal Fluid Samples

Ruminal fluid samples were taken from all animals at 3 time points: at the end of the covariate period (d −2 to 0), at the middle of the experimental period (d 26–28), and at the end of the experimental period (d 54–56; see Figure 1 for explanation of days). Each sampling point constituted 3 consecutive days (covering all 48 cows) with roughly one-third of the cows in each group included per sampling day. The cows within each feeding group were randomly assigned to 1 of 3 sampling days for the first sampling, and the same groupings were used accordingly for the later samplings. On the sampling days, the cows were moved to the holding area before the morning feed distribution (between 0800 and 0830 h). The samples were taken between 0900 and 1030 h by aspiration using manually operated esophageal tubing (Akselsens Agenturer A/S) fitted with a perforated steel endpoint to restrict suction of large particles. The first portion of the ruminal fluid (approximately 200–300 mL) was discarded to avoid saliva contamination, and an equivalent volume was withdrawn for analysis. This was strained through 4 layers of cheesecloth, and 9.5 mL was preserved with 0.5 mL of concentrated formic acid (98%; vol/vol) and stored in a cold room (4°C) until completion of the experiment. The pH of the remaining ruminal fluid was measured using a digital pH 3310 meter (Xylem Analytics Germany GmbH). The stored samples were later analyzed for ruminal fluid VFA by GC (TRACE 1300 Gas Chromatograph equipped with a Stabilwax-DA column, 3 m, 0.53-mm internal diameter, 0.25 μm; Thermo Scientific) and for ruminal fluid ammonia-N using AOAC method 2001.11 (Thiex et al., 2002) with a modification that block digestion was not carried out.

Table 2. Amino acid composition (% of total AA) and total AA content of grass silage and the 3 concentrate feeds (SBM = soybean meal-based; YEA = yeast-based; BAR = barley-based)

Amino acid	Grass silage	SBM	YEA	BAR
Ala	7.97	4.33	4.87	4.48
Arg	4.26	5.59	5.03	5.01
Asp	12.3	8.11	7.58	6.95
Cys	0.77	1.72	1.61	1.84
Glu	13.4	26.7	26.9	28.6
Gly	5.31	3.72	3.78	3.71
His	2.40	2.70	2.60	2.64
Ile	5.09	3.98	4.07	3.81
Leu	9.04	8.19	8.26	8.31
Lys	6.19	4.22	4.34	3.67
Met	1.62	1.31	1.33	1.39
Phe	5.75	5.41	5.20	5.03
Pro	7.18	9.24	8.90	10.6
Ser	4.63	4.69	4.75	4.60
Thr	5.30	3.77	4.12	3.69
Tyr	3.03	2.10	2.21	1.24
Val	5.73	4.25	4.49	4.37
Total AA[1]	115.1	124.5	114.6	100.2

[1]Total amino acid content of the feeds (g/kg of DM) excluding Trp, which was not analyzed.

Milk Yield and Milk Sample Registration

The cows were milked using a robotic milking system (DeLaval VMS Classic) with the minimal milking interval set to 5.5 h. Daily milk yield was summed from multiple milkings (mean ± SD: SBM = 2.86 ± 0.66, YEA = 2.64 ± 0.70, and BAR = 2.80 ± 0.75) per cow per day. Milk samples were taken at the end of the covariate period (i.e., d 0), and on d 14, 28, 35, and 56 of the experimental period. On the milk sampling days, 1 composite milk sample per cow was taken from 1900 h to 0700 h the next morning. The samples were preserved with a bronopol tablet (2-bromo-2-nitropane-1,3 diol, Broad-Spectrum Microtabs II, Advanced Industries Inc.) and stored in a cold room (4°C) until analysis for milk protein, fat, lactose, and urea using a Bentley FTS/FCM instrument (Bentley Instruments Inc.). The ECM yield over the experimental period was calculated for each individual cow based on mean milk chemical composition and milk yield according to Sjaunja et al. (1991).

Statistical Analysis

One cow from the BAR group had mastitis during the experimental period and was separated from the group until she completed medication (i.e., 14 d). No data collected on this cow for this period were included in the statistical analysis. The data were analyzed using the PROC MIXED procedure of SAS (SAS for Windows 9.4; SAS Institute Inc.). The respective variables from the covariate period (d −14 to 0) were used as covariates. The covariate structure that minimized Akaike's information criterion was used, primarily Toeplitz, compound symmetry, or autoregressive. Cow (diet × parity) was considered a repeated subject in all models. The full model for the effect of different concentrate feeds on feed intake variables, milk yield, milk component yields, and milk N efficiency (**NUE**, expressed as N secreted in milk as a percentage of N intake) was as follows:

$$Y_{ijkl} = \mu + Diet_i + Day_j + Parity_k + (Diet \times Day)_{ij} + DIM + covX_{ikl} + Cow_{ikl} + e_{ijkl},$$

where Y_{ijkl} = response variable (e.g., milk yield); μ = overall mean; $Diet_i$ = fixed effect of concentrate feed type (i.e., BAR, SBM, YEA); Day_j = fixed effect of day of measurement (j = 1–56); $Parity_k$ = fixed effect of parity (k = primiparous or multiparous); DIM = effect of days in lactation of each individual cow at the start of the covariate period; $covX_{ikl}$ = effect of covariate period data for each cow within diet and parity (e.g., covariate period milk yield for milk yield data); $(Diet \times Day)_{ij}$ = interaction effect of concentrate feed type and day of measurement; Cow_{ikl} = random effect of cow nested within diet and parity (l = 1–16); and e_{ijkl} = residual error. Models for VFA and ruminal fluid pH also included the number of minutes since the last feeding occasion. The effects of dietary treatments on BW and BCS changes were tested using the general linear model in SAS (PROC GLM) with diet, parity, DIM, and covariate period BW and BCS values included in their respective model. Tukey-Kramer was used to test for differences between means. Data are presented as least squares means, with statistical significance declared at $P \leq 0.05$ and tendencies discussed at $0.05 < P < 0.1$.

RESULTS

Intake, BCS, and BW

Data on daily DM and nutrient intake are presented in Table 3. Daily total DMI, silage DMI, concentrate feed DMI, and NDF intake were not affected by the dietary treatment (i.e., concentrate feed type). Cows in all 3 dietary treatments achieved a similar level of NDF intake per unit BW (13.8 ± 0.29 g/kg of BW). However, total starch intake was higher and total dietary CP intake was lower in the BAR group. The proportions of silage and concentrate feed in the DMI of all groups were calculated to be roughly 65% and 35%, respectively.

The effect of dietary treatments on BW change was not significant, although BW increased over the experimental period (22.3, 29.8, and 25.4 kg in SBM, YEA, and BAR, respectively) over the experimental period. Similarly, BCS change was not affected by the dietary treatments over the experimental period.

Rumen Fermentation Products

Data on rumen fermentation characteristics are presented in Table 4. Dietary treatment did not influence ruminal ammonia nitrogen, total ruminal fluid VFA, or molar proportions of individual VFA. All ruminal fermentation parameters were affected ($P < 0.04$) by sampling day, except the molar proportion of butyrate. As a result, total ruminal fluid VFA and molar proportions of acetate were higher in the middle of the experimental period than at the end. Conversely, ruminal fluid ammonia nitrogen concentration, molar proportions of propionate, valerate, isobutyrate, and isovalerate were lower in the middle than at the end of the experimental period. The effects of dietary treatments on ruminal fluid pH, acetate-to-propionate ratio, and non-glucogenic to glucogenic VFA ratio were not significant. These variables were significantly higher on samples taken in the middle than at the end of the experimental period.

Milk Yield, Milk Composition, and Component Yields

Data on milk yield, milk composition, and component yields are presented in Table 5. Both daily milk yield and ECM yield were not affected by the dietary treatments. Similarly, milk fat and lactose contents did not differ among the dietary treatments, but a significant interaction effect of sampling day by treatment was found for milk fat content ($P < 0.01$). This was observed with YEA showing the highest fat content (mean ± SEM, g/kg of milk: YEA = 45.1 ± 0.79; SBM = 43.7 ± 0.82; BAR = 44.2 ± 0.80) with samples taken on d 35. Furthermore, milk protein content ($P = 0.10$) and MUN ($P = 0.06$) were marginally lower in the BAR group than in the other 2 dietary groups. Milk component yields and dietary NUE were not affected by the dietary treatments.

DISCUSSION

For most parameters, a significant effect of day was observed. This effect is most likely due to the change in amount of concentrate at d 28 and 50. Except for fat concentration in milk, no diet day interaction was observed, and the day effect is not further discussed.

Feed Intake

Feed intake was not affected by substituting yeast or barley for soybean meal in dairy cow diets. The concentrate feeds were offered in restriction based on individual cow requirements as calculated by NorFor, the Nordic feed evaluation system (TINE OptiFôr; NorFor, 2011), and hence were expected to remain similar between the groups. However, grass silage was offered ad libitum, allowing variations in DMI between cows based on individual cow intake capacity. Despite this, the ratio of silage to total DMI remained similar between the groups. Previous studies with yeast-based

Table 3. Feed and nutrient intake with dairy cows fed grass silage augmented with 3 different concentrate feeds containing soybean meal (SBM), yeast (YEA), and barley (BAR)

	Treatment				Statistics (*P*-value)		
Item	SBM	YEA	BAR	SE	Diet	Day	Diet × Day
Feed intake (kg/d)							
Total DMI	22.1	22.0	21.9	0.17	0.80	<0.01	0.32
Silage DMI	14.5	14.5	14.1	0.18	0.18	<0.01	0.12
Concentrate feed DMI[1]	7.74	7.60	7.68	0.05	0.20	<0.01	0.41
Other parameters							
CP intake (kg/d)	3.84[a]	3.81[a]	3.60[b]	0.03	<0.01	<0.01	0.24
Starch intake (kg/d)	2.98[b]	2.77[c]	3.13[a]	0.02	<0.01	<0.01	0.21
NDF intake[2] (kg/d)	8.92	8.85	8.70	0.10	0.27	<0.01	0.20
DMI/kg of BW (g/kg)	35.2	35.4	34.2	0.65	0.41	<0.01	0.22
DMI/kg of $BW^{0.75}$ (g/kg)	176.5	177.3	171.7	3.23	0.43	<0.01	0.24
Mean achieved AAT intake and others[3]							
Total AAT (g/d)	2,233	2,216	2,159				
Met/AAT intake (%)	2.12	2.13	2.16				
His/AAT intake (%)	2.49	2.47	2.47				
Lys/AAT intake (%)	6.63	6.68	6.63				

[a–c]Different superscript letters within a row indicate significant differences between treatments at $P \leq 0.05$.

[1]The concentrate feeds were offered in split portions, maximum of 4 kg per cow per visit, each day from 3 DeLaval FSC40 feeding stations, with additional small portions (~1.0 kg of SBM split over 3 visits) fed in a milking robot.

[2]NDF in the feed corrected for ash.

[3]Estimated achieved total amino acids absorbed in the intestine (AAT) and intakes of Met, Lys, and His as percentage of the total AAT calculated using NorFor feeding standards (TINE OptiFôr; NorFor, 2011) at a diet level.

protein on dairy cow diets produced mixed results. Neal et al. (2014) reported decreased intake of DM and nutrients with dairy cows fed total mixed ration supplemented with yeast-based microbial protein (**YMP**). They stated that the observed effect was unexpected and was difficult to explain. With dairy cows fed high-forage diets containing increasing levels of YMP (i.e., 0, 1.14, 2.28, and 3.41% DM of YMP replacing soybean meal), Sabbia et al. (2012) reported a cubic response on DMI over the YMP inclusion range, with the 2.28% YMP inclusion level producing DMI similar to the control diet. This is comparable to our YEA diet (about 7.0% yeast in the concentrate feed, which constituted 35% of the achieved DMI, producing approximately 2.45% inclusion of yeast in the total diet). In our study, because all the concentrate feeds were roughly isoenergetic, and cows were fed one quality silage over the experimental period, energy intake would not have differed between the groups. Furthermore, the early-cut grass silage used here was above average quality based on the chemical composition (e.g., high in CP and intermediate in NDF), and hence intake limitation due to rumen fill would have been minimal. Indeed, dietary NDF is heterogeneous in nature, and

Table 4. Ruminal fermentation parameters from dairy cows fed grass silage augmented with 3 different concentrate feeds from soybean meal (SBM), yeast (YEA), and barley (BAR)

	Treatment				Statistics (*P*-value)		
Item	SBM	YEA	BAR	SE	Diet	Day	Diet × Day
NH_3-N (mg/L)	79.5	74.8	91.0	15.3	0.66	<0.01	0.23
Total VFA (m*M*)	70.2	67.4	77.9	4.7	0.35	0.04	0.42
Individual VFA (molar % of total VFA)							
Acetate	66.9	65.2	64.6	1.0	0.44	<0.01	0.54
Propionate	16.8	17.9	17.9	0.89	0.74	0.01	0.66
Butyrate	13.3	13.6	14.4	0.31	0.13	0.50	0.83
Valerate	1.10	1.20	1.29	0.091	0.47	<0.01	0.78
Isobutyrate	0.85	0.83	0.87	0.032	0.68	<0.01	0.40
Isovalerate	1.03	1.02	1.09	0.054	0.47	<0.01	0.52
Ruminal fluid pH	7.21	6.80	6.87	0.094	0.11	<0.01	0.67
Acetate:propionate	3.99	3.67	3.64	0.027	0.73	<0.01	0.48
NGR[1]	5.15	4.85	4.85	0.032	0.82	<0.01	0.54

[1]Non-glucogenic to glucogenic VFA ratio, calculated according to Morvay et al. (2011) as [acetate + (2 × butyrate) + (2 × branched-chain VFA)]/[propionate + branched-chain VFA].

Table 5. Milk yield, milk composition, milk component yields and dietary milk nitrogen efficiency from dairy cows fed grass silage augmented with 3 different concentrate feeds containing protein from soybean meal (SBM), yeast (YEA), and barley (BAR)

	Treatment				Statistics (*P*-value)		
Item	SBM	YEA	BAR	SE	Diet	Day	Diet × Day
Milk yield							
Milk yield (kg/d)	30.8	30.0	29.7	0.45	0.20	<0.01	0.62
ECM[1] (kg/d)	32.6	32.8	31.6	0.58	0.32	<0.01	0.66
Milk composition							
Fat (g/kg)	43.7	45.1	44.2	0.81	0.45	<0.01	<0.01
Protein[2] (g/kg)	36.0	36.2	34.9	0.47	0.10	0.01	0.37
Lactose (g/kg)	47.9	48.0	47.8	0.19	0.75	<0.01	0.66
MUN (mg/dL)	14.7	14.8	14.2	0.19	0.06	<0.01	0.13
Milk component yields							
Fat (kg/d)	1.32	1.36	1.31	0.031	0.56	<0.01	0.32
Protein (kg/d)	1.09	1.09	1.04	0.024	0.23	<0.01	0.24
Lactose (kg/d)	1.48	1.44	1.42	0.022	0.26	<0.01	0.19
NUE[3]	28.4	28.5	29.5	0.49	0.24	<0.01	0.16

[1]ECM = milk yield (kg) × [(38.3 × fat (g/kg) + 24.2 × protein (g/kg) +16.54 × lactose (g/kg) +20.7)/3,140], according to Sjaunja et al. (1991).
[2]Milk true protein.
[3]NUE = gross dietary milk nitrogen efficiency (nitrogen secreted in milk as a percentage of nitrogen intake).

equating rumen NDF pool based on NDF intake has limitations (Huhtanen et al., 2016). Here, over 97.5% of the NDF intake originated from a common NDF pool (65% from the common grass silage, and 32.5% from the concentrate feed component, as barley, yeast, and soybean meal substitutions accounted for about 7.0% of the concentrate feed ingredients). Therefore, NDF intake expressed per kilogram of BW could be used as an indicator of rumen fill (NorFor, 2011; Huhtanen et al., 2016). To this end, calculated NDF intake per kilogram of BW was similar between the treatments.

Ruminal Fluid VFA and pH

Marked changes in the molar proportions of the concentrations of VFA in the ruminal fluid can be observed in response to dietary manipulations (Chalupa, 1977; Sutton et al., 2003). Here, we did not observe any difference between the 3 dietary treatments on ruminal fluid VFA. The observed VFA levels were lower than those reported for dairy cows fed nonrestrictive diets (Sabbia et al., 2012; Neal et al., 2014; Kidane et al., 2018). It has been reported that method of sampling (i.e., via rumen canula vs. esophageal tubing) could affect the total VFA content, with esophageal tubing underestimating the VFA content (Raun and Burroughs, 1962; Geishauser and Gitzel, 1996; Shen et al., 2012; van Gastelen et al., 2019). However, the molar percentages of specific VFA have been reported to be unbiased by the method of sampling (Raun and Burroughs, 1962; van Gastelen et al., 2019) and also were not influenced by the dietary treatments in our experiment. Furthermore, ruminal fluid acetate-to-propionate ratio and non-glucogenic to glucogenic VFA ratio were not altered by the dietary treatments. For both ratios, the values are higher than those observed in cows fed TMR (Kidane et al., 2018) using samples taken at multiple time points over a 24-h cycle.

Ruminal fluid pH was not affected by the dietary treatments, despite our expectation that the BAR diet would decrease rumen pH compared with the other treatments because of increased and rapid starch degradation (Nikkhah, 2012). Ruminal fluid pH usually oscillates depending upon, among other factors, meals and feeding times (Palmonari et al., 2010; Kidane et al., 2018). Our samples were collected before morning feeding, and the observed elevated ruminal fluid pH would suggest low VFA concentration, due to active uptake and reduced fermentable OM in the rumen. High ruminal fluid pH could also be partly due to saliva contamination (Grünberg and Constable, 2009), despite our attempts to avoid this.

Milk Yield, Milk Composition, and Milk Nitrogen Efficiency

Milk yield and milk composition were not affected by the dietary treatments. Achieved dietary CP levels were not restrictive, with the lowest for the BAR group being 164 g/kg of DM. With early- to mid-lactation Holstein dairy cows, Law et al. (2009) demonstrated a tendency toward a greater milk yield response when increasing dietary CP from 114 to 144 g/kg of DM than from 144 to 173 g/kg of DM. Others (Cunningham et al.,1996; Leonardi et al., 2003) observed no improvement in milk yield when dietary CP increased over a range (e.g., 161–189 g/kg of DM) that contained what was achieved in our experiment. The supply of amino

acids absorbed in the intestine (**AAT**) for milk synthesis is mainly contributed by rumen microbial protein and rumen undegraded dietary protein absorbed in the small intestine. These are, in turn, influenced by both rate of protein degradation in the rumen and rate of passage. As a result, differences in the rate of degradation of different types of protein and rate of passage from the rumen make it difficult to compare the bypass protein level of different feeds given comparable dietary CP (Owens and Bergen, 1983). To our knowledge, values of ruminal degradation and passage rate of the yeast protein used here are unknown. Our effort to compare ruminal degradation rates of the yeast protein and soybean meal ingredients using an in sacco technique (38-μm pore size; NorFor, 2011) was not successful because of substantial particle loss (over 80% on DM basis) upon washing with the yeast protein. Sabbia et al. (2012) speculated that yeast-derived microbial protein would flow with the liquid phase out of the rumen, rendering it some degree of protection due to a high rumen escape rate. This was observed with a linear decrease in ruminal ammonia concentrations with increasing yeast-derived microbial protein in the diets. Our YEA concentrate feed created only numerical difference compared with SBM on ruminal fluid ammonia concentration, failing to support the above hypothesis. However, Owens and Bergen (1983) argued that plant proteins, including soybean, have a higher degree of protein degradation in the rumen compared with other protein sources with a high bypass fraction (e.g., distillers products).

Lysine, methionine, and histidine have been identified most often as the limiting AA for milk production (Schwab and Broderick, 2017). Which AA is the first limiting depends on the feed protein source. Here, calculated dietary intakes of total AAT and these 3 AA fell within a narrow range for all groups, with Lys and Met intake (percentage of AAT) close to milk yields allowable by the achieved AAT intake (NRC, 2001). Thus, the observed milk yield, milk protein content, and protein yield from the SBM and YEA diets suggested that the diets supplied comparable levels of AA absorbed in the small intestine.

It has been reported that His could be the first limiting AA for milk production when grass silage constituted the main part of the diet with barley- and oat-based concentrate feeds (Kim et al., 1999; Schwab et al., 2005). This was more pronounced when rumen microbial protein provided most of the MP supply to the small intestine (Lee et al., 2012). However, the observed numeric differences in milk and milk protein yields between the dietary groups here were not as large as expected. It has been reported that endogenous reserves (e.g., carnosine, anserine, and hemoglobin) can release His to sustain metabolic needs during periods of deficiency (Clemens et al., 1984; Lapierre et al., 2008), indicating some degree of phenotypic plasticity in His-deficient diets. Therefore, it can be argued that, only with an extended period of feeding, shortage of dietary His in the BAR diet would have penalized milk protein synthesis.

Furthermore, microbial protein supplies a large portion of the AAT (Storm and Ørskov, 1983; Clark et al., 1992), with an AA profile comparable to that of milk. Thus, increasing the concentration of rumen-fermentable carbohydrates would be expected to influence microbial protein synthesis in the rumen (Meyer et al., 1967) and improve milk production at a given dietary CP intake (Broderick, 2003). Our barley-based diet had higher starch but lower dietary CP content relative to the SBM and YEA diets. Given the proportion of grass silage in the total DMI and its high CP content (with 550 g of soluble CP per kg of CP), and the high starch intake from barley in the concentrate feed, microbial CP synthesis would be expected to be higher (Keady et al., 1998; Cone and Becker, 2012) in the BAR group. Therefore, even with the observed lower CP intake in the group relative to the other 2 diets, it might be that an increased microbial CP synthesis in the BAR could have compensated for this. This could explain the observed similar milk yield across treatments, in contrast to our hypothesis.

In dairy cow feeding, dietary nitrogen intake, nitrogen secretion in milk, and excretion in manure regulate environmental impacts. As a result, efforts are being made to improve NUE and reduce nitrogen loss. About 25 to 35% of dietary nitrogen is captured and secreted in milk (Broderick, 2003; Kidane et al., 2018). A large part of the remaining nitrogen is lost in manure, which is undesirable both in terms of cost and from the environment perspective. In our experiment, NUE was numerically higher in the BAR group compared with the others, but the absence of contrasting difference among the dietary treatments could be explained by the narrow range of dietary CP in the DMI.

CONCLUSIONS

Our results indicate that yeast can be used as a protein source in diets for early- to mid-lactation NRF dairy cows, without negative effects on milk yield and milk composition. Replacement of soybean meal and yeast with barley, in combination with a grass silage of good quality, showed a tendency for decreased milk protein content. Further research on the long-term effects of these diets, in combination with varying silage qualities, may be required to adequately describe effects on milk production and milk composition, without

interfering effects of metabolic plasticity in response to changes in nutrient supply.

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REFERENCES

Åby, B. A., J. Kantanen, L. Aass, and T. Meuwissen. 2014. Current status of livestock production in the Nordic countries and future challenges with a changing climate and human population growth. Acta Agric. Scand. A Anim. Sci. 64:73–97. https://doi.org/10.1080/09064702.2014.950321.

AACC. 2000. Approved Methods of the AACC. 10th ed. Am. Assoc. Cereal Chem.

Broderick, G. A. 2003. Effects of varying dietary protein and energy levels on the production of lactating dairy cows. J. Dairy Sci. 86:1370–1381. https://doi.org/10.3168/jds.S0022-0302(03)73721-7.

Cavins, J. F., W. F. Kwolek, G. E. Inglett, and J. C. Kowan. 1972. Amino acid analysis of soybean meal: Interlaboratory study. J. AOAC 55:686–691. https://doi.org/10.1093/jaoac/55.4.686.

Chalupa, W. 1977. Manipulating rumen fermentation. J. Anim. Sci. 45:585–599. https://doi.org/10.2527/jas1977.453585x.

Clark, J. H., T. H. Klusmeyer, and M. R. Cameron. 1992. Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. J. Dairy Sci. 75:2304–2323. https://doi.org/10.3168/jds.S0022-0302(92)77992-2.

Clemens, R. A., J. D. Kopple, and M. E. Swendseid. 1984. Metabolic effects of histidine-deficient diets fed to growing rats by gastric tube. J. Nutr. 114:2138–2146. https://doi.org/10.1093/jn/114.11.2138.

Cone, J. W., and P. M. Becker. 2012. Fermentation kinetics and production of volatile fatty acids and microbial protein by starchy feedstuffs. Anim. Feed Sci. Technol. 172:34–41. https://doi.org/10.1016/j.anifeedsci.2011.12.006.

Cruz, A., H. Sterten, F. S. Steinhoff, L. T. Mydland, and M. Øverland. 2020a. *Cyberlindnera jadinii* yeast as a protein source for broiler chickens: Effects on growth performance and digestive function from hatching to 30 days of age. Poult. Sci. 99:3168–3178. https://doi.org/10.1016/j.psj.2020.01.023.

Cruz, A., A.-H. Tauson, C. F. Matthiesen, L. T. Mydland, and M. Øverland. 2020b. *Cyberlindnera jadinii* yeast as a protein source for growing pigs: Effects on protein and energy metabolism. Livest. Sci. 231:103855. https://doi.org/10.1016/j.livsci.2019.103855.

Cunningham, K. D., M. J. Cecava, T. R. Johnson, and P. A. Ludden. 1996. Influence of source and amount of dietary protein on milk yield by cows in early lactation. J. Dairy Sci. 79:620–630. https://doi.org/10.3168/jds.S0022-0302(96)76407-X.

Dalle Zotte, A., Y. Singh, J. Michiels, and M. Cullere. 2019. Black soldier fly (*Hermetia illucens*) as dietary source for laying quails: Live performance, and egg physico-chemical quality, sensory profile and storage stability. Animals (Basel) 9:115. https://doi.org/10.3390/ani9030115.

EC (European Communities). 2009. Commission regulation no. 152/2009 of 27 January 2009, laying down the methods of sampling and analysis for the official control of feed. Off. J. Eur. Union L 54:24–27.

Foley, J. A., N. Ramankutty, K. A. Brauman, E. S. Cassidy, J. S. Gerber, M. Johnston, N. D. Mueller, C. O'Connell, D. K. Ray, P. C. West, C. Balzer, E. M. Bennett, S. R. Carpenter, J. Hill, C. Monfreda, S. Polasky, J. Rockström, J. Sheehan, S. Siebert, D. Tilman, and D. P. M. Zaks. 2011. Solutions for a cultivated planet. Nature 478:337–342. https://doi.org/10.1038/nature10452.

Geishauser, T., and A. Gitzel. 1996. A comparison of rumen fluid sampled by oro-ruminal probe versus ruminal fistula. Small Rumin. Res. 21:63–69. https://doi.org/10.1016/0921-4488(95)00810-1.

Government of Norway. 2014. Forestry. Accessed Aug. 2, 2021. https://www.regjeringen.no/en/topics/food-fisheries-and-agriculture/skogbruk/innsikt/skogbruk/id2009516/.

Grünberg, W., and P. D. Constable. 2009. Chapter 6: Function and dysfunction of the ruminant forestomach. Pages 12–19 in Food Animal Practice, 5th ed. D. E. Anderson and D. M. Rings, ed. W. B. Saunders.

Higginson, V., B. Baurhoo, Y. Schuermann, M. Taibi, A. St-Yves, R. Duggavathi, and A. Mustafa. 2017. Effects of yeast-derived rumen escape microbial protein on lactational performance and metabolic status of transition dairy cows. J. Anim. Sci. 95(Suppl. 2):39. https://doi.org/10.2527/asasmw.2017.083.

Hristov, A. N., J. K. Ropp, K. L. Grandeen, S. Abedi, R. P. Etter, A. Melgar, and A. E. Foley. 2005. Effect of carbohydrate source on ammonia utilization in lactating dairy cows. J. Anim. Sci. 83:408–421. https://doi.org/10.2527/2005.832408x.

Huhtanen, P., E. Detmann, and S. J. Krizsan. 2016. Prediction of rumen fiber pool in cattle from dietary, fecal, and animal variables. J. Dairy Sci. 99:5345–5357. https://doi.org/10.3168/jds.2015-10842.

Huhtanen, P., S. Jaakkola, and J. Nousiainen. 2013. An overview of silage research in Finland: From ensiling innovation to advances in dairy cow feeding. Agric. Food Sci. 22:35–56. https://doi.org/10.23986/afsci.6632.

ISO. 2002. 5984: Animal feeding stuffs—Determination of crude ash. International Organization for Standardization.

ISO. 1999. 6496: Animal feeding stuffs—Determination of moisture and other volatile matter content. International Organization for Standardization.

Keady, T. W. J., C. S. Mayne, and M. Marsden. 1998. The effects of concentrate energy source on silage intake and animal performance with lactating dairy cows offered a range of grass silages. Anim. Sci. 66:21–33. https://doi.org/10.1017/S1357729800008808.

Kidane, A., M. Øverland, L. T. Mydland, and E. Prestløkken. 2018. Interaction between feed use efficiency and level of dietary crude protein on enteric methane emission and apparent nitrogen use efficiency with Norwegian Red dairy cows. J. Anim. Sci. 96:3967–3982. https://doi.org/10.1093/jas/sky256.

Kim, C.-H., J.-J. Choung, and D. G. Chamberlain. 1999. Determination of the first-limiting amino acid for milk production in dairy cows consuming a diet of grass silage and a cereal-based supplement containing feather meal. J. Sci. Food Agric. 79:1703–1708. https://doi.org/10.1002/(SICI)1097-0010(199909)79:12<1703::AID-JSFA424>3.0.CO;2-5.

Lapierre, H., D. Ouellet, L. Doepel, G. Holtrop, and G. Lobley. 2008. Histidine, lysine and methionine: From metabolism to balanced dairy rations. Pages 14–31 in Proc. Eastern Nutrition Conference of Animal Nutrition Association of Canada. University of Guelph.

Law, R. A., F. J. Young, D. C. Patterson, D. J. Kilpatrick, A. R. Wylie, and C. S. Mayne. 2009. Effect of dietary protein content on animal production and blood metabolites of dairy cows during lactation. J. Dairy Sci. 92:1001–1012. https://doi.org/10.3168/jds.2008-1155.

Lee, C., A. N. Hristov, K. S. Heyler, T. W. Cassidy, H. Lapierre, G. A. Varga, and C. Parys. 2012. Effects of metabolizable protein supply and amino acid supplementation on nitrogen utilization, milk

production, and ammonia emissions from manure in dairy cows. J. Dairy Sci. 95:5253–5268. https://doi.org/10.3168/jds.2012-5366.
Leonardi, C., M. Stevenson, and L. E. Armentano. 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. J. Dairy Sci. 86:4033–4042. https://doi.org/10.3168/jds.S0022-0302(03)74014-4.
Manthey, A. K., K. F. Kalscheur, A. D. Garcia, and K. Mjoun. 2016. Lactation performance of dairy cows fed yeast-derived microbial protein in low- and high-forage diets. J. Dairy Sci. 99:2775–2787. https://doi.org/10.3168/jds.2015-10014.
Mertens, D. R. 2002. Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: Collaborative study. J. AOAC Int. 85:1217–1240.
Meyer, R. M., E. E. Bartley, C. W. Deyoe, and V. F. Colenbrander. 1967. Feed processing. I. Ration effects on rumen microbial protein synthesis and amino acid composition. J. Dairy Sci. 50:1327–1332. https://doi.org/10.3168/jds.S0022-0302(67)87624-0.
Morvay, Y., A. Bannink, J. France, E. Kebreab, and J. Dijkstra. 2011. Evaluation of models to predict the stoichiometry of volatile fatty acid profiles in rumen fluid of lactating Holstein cows. J. Dairy Sci. 94:3063–3080. https://doi.org/10.3168/jds.2010-3995.
Mullins, I. L., C. M. Truman, M. R. Campler, J. M. Bewley, and J. H. C. Costa. 2019. Validation of a commercial automated body condition scoring system on a commercial dairy farm. Animals (Basel) 9:287. https://doi.org/10.3390/ani9060287.
Neal, K., J. S. Eun, A. J. Young, K. Mjoun, and J. O. Hall. 2014. Feeding protein supplements in alfalfa hay-based lactation diets improves nutrient utilization, lactational performance, and feed efficiency of dairy cows. J. Dairy Sci. 97:7716–7728. https://doi.org/10.3168/jds.2014-8033.
Nikkhah, A. 2012. Barley grain for ruminants: A global treasure or tragedy. J. Anim. Sci. Biotechnol. 3:22. https://doi.org/10.1186/2049-1891-3-22.
NorFor. 2011. NorFor: The Nordic Feed Evaluation System. H. Volden, ed. EAAP Scientific Series, No. 30. Wageningen Academic Publishers.
Notarnicola, B., G. Tassielli, P. A. Renzulli, V. Castellani, and S. Sala. 2017. Environmental impacts of food consumption in Europe. J. Clean. Prod. 140:753–765. https://doi.org/10.1016/j.jclepro.2016.06.080.
NRC. 2001. Nutrient Requirements of Dairy Cattle. 7th ed. National Academy Press.
Øverland, M., and A. Skrede. 2017. Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture. J. Sci. Food Agric. 97:733–742. https://doi.org/10.1002/jsfa.8007.
Owens, F. N., and W. G. Bergen. 1983. Nitrogen metabolism of ruminant animals: historical perspective, current understanding and future implications. J. Anim. Sci. 57(Suppl. 2):498–518. https://doi.org/10.2527/animalsci1983.57Supplement_2498x.
Palmonari, A., D. M. Stevenson, D. R. Mertens, C. W. Cruywagen, and P. J. Weimer. 2010. pH dynamics and bacterial community composition in the rumen of lactating dairy cows. J. Dairy Sci. 93:279–287. https://doi.org/10.3168/jds.2009-2207.
Randby, Å. T., H. N. Halvorsen, and A. K. Bakken. 2020. Losses and grass silage quality in bunker silos compacted by tractor versus wheel loader. Anim. Feed Sci. Technol. 266:114523. https://doi.org/10.1016/j.anifeedsci.2020.114523.
Randby, Å. T., P. Nørgaard, and M. R. Weisbjerg. 2010. Effect of increasing plant maturity in timothy-dominated grass silage on the performance of growing/finishing Norwegian Red bulls. Grass Forage Sci. 65:273–286. https://doi.org/10.1111/j.1365-2494.2010.00745.x.
Raun, N. S., and W. Burroughs. 1962. Suction strainer technique in obtaining rumen fluid samples from intact lambs. J. Anim. Sci. 21:454–457. https://doi.org/10.2527/jas1962.213454x.
Sabbia, J. A., K. F. Kalscheur, A. D. Garcia, A. M. Gehman, and J. M. Tricarico. 2012. Soybean meal substitution with a yeast-derived microbial protein source in dairy cow diets. J. Dairy Sci. 95:5888–5900. https://doi.org/10.3168/jds.2011-5237.
Schwab, C. G., and G. A. Broderick. 2017. A 100-Year Review: Protein and amino acid nutrition in dairy cows. J. Dairy Sci. 100:10094–10112. https://doi.org/10.3168/jds.2017-13320.
Schwab, C. G., P. Huhtanen, C. Hunt, and T. Hvelplund. 2005. Nitrogen requirements of cattle. In Nitrogen and Phosphorus Nutrition of Cattle: Reducing the Environmental Impact of Cattle Operations. E. Pfeffer and A. N. Hristov, ed. CABI Publishing.
Sharma, S., L. Hansen, J. Ø. Hansen, L. Mydland, S. Horn, M. Øverland, V. Eijsink, and K. Vuoristo. 2018. Microbial protein produced from brown seaweed and spruce wood as a feed ingredient. J. Agric. Food Chem. 66:8328–8335. https://doi.org/10.1021/acs.jafc.8b01835.
Shen, J. S., Z. Chai, L. J. Song, J. X. Liu, and Y. M. Wu. 2012. Insertion depth of oral stomach tubes may affect the fermentation parameters of ruminal fluid collected in dairy cows. J. Dairy Sci. 95:5978–5984. https://doi.org/10.3168/jds.2012-5499.
Sjaunja, L. O., L. Baevre, L. Junkarinen, J. Pedersen, and J. Setälä. 1991. A Nordic proposal for an energy corrected milk (ECM) formula. Pages 156–157 in Performance Recording of Animals: State of the Art, 1990: Proceedings of the 27th Biennial Session of the International Committee for Animal Recording (ICAR), Paris, France. Wageningen Academic Publishers.
Solberg, B., A. Moiseyev, J. Ø. Hansen, S. J. Horn, and M. Øverland. 2021. Wood for food: Economic impacts of sustainable use of forest biomass for salmon feed production in Norway. For. Policy Econ. 122:102337. https://doi.org/10.1016/j.forpol.2020.102337.
Storm, E., and E. Ørskov. 1983. The nutritive value of rumen micro-organisms in ruminants: 1. Large-scale isolation and chemical composition of rumen micro-organisms. Br. J. Nutr. 50:463–470. https://doi.org/10.1079/BJN19830114.
Sutton, J. D., M. S. Dhanoa, S. V. Morant, J. France, D. J. Napper, and E. Schuller. 2003. Rates of production of acetate, propionate, and butyrate in the rumen of lactating dairy cows given normal and low-roughage diets. J. Dairy Sci. 86:3620–3633. https://doi.org/10.3168/jds.S0022-0302(03)73968-X.
Thiex, N. J., H. Manson, S. Anderson, J.-Å. Persson, S. Anderson, E. Bogren, G. Bolek, D. Budde, C. Ellis, S. Eriksson, G. Field, E. Frankenius, C. Henderson, C. Henry, M. Kapphahn, L. Lundberg, H. Manson, J. Moller, M. Russell, J. Sefert-Schwind, and M. Spann. 2002. Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: Collaborative study. J. AOAC Int. 85:309–317. https://doi.org/10.1093/jaoac/85.2.309.
van Gastelen, S., F. Schumacher, J. W. Cone, J. Dijkstra, and W. F. Pellikaan. 2019. In dairy cattle, the stomach tube method is not a feasible alternative to the rumen cannulation method to examine in vitro gas and methane production. Anim. Feed Sci. Technol. 256:114259. https://doi.org/10.1016/j.anifeedsci.2019.114259.

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