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Feeding behaviour of dairy cows fed a diet with protein derived from yeast (*Candida utilis*) compared with diets containing soybean meal and barley

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Acknowledgements

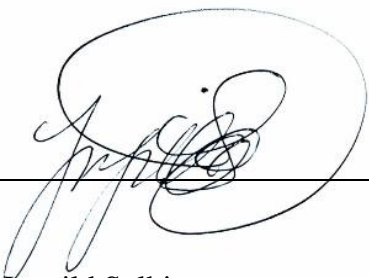
This master thesis concludes five years of study at NMBU. My interest for animals has brought me to unexpected places, and I am happy to say my curiosity has not abated. When choosing a master thesis I looked for something with a forward-thinking, environmental focus, and succeeded. The field of sustainable food production is extremely relevant and hopefully of importance to future generations.

A big thank you to Foods of Norway, Centre for Research-based Innovation at NMBU, for letting me be involved in this project, and to supervisors Egil Prestløykken and Alemayehu Kidane. Another thank you to Bjørg Heringstad at NMBU for very patiently teaching me SAS via Zoom, and to the people who gave me pep-talks along the way.

This thesis was written during a time of home office and isolation, and I would never have seen the end of it without my study friend and 'klemmevenn' Therese Bjørklund Kristiansen. Thank you everyone who cheered me on from the side-lines, with promises of wine at the end (I'll hold you to that).

The end of student life is bittersweet and will be celebrated thoroughly, after which the adventures of the future await!

Ås, 29.10.2020



Ingvild Solhjem

Summary

Norwegian Red dairy cows (NRF) are fed mainly roughage, with supplemented concentrate feed to increase milk production. Concentrate feed contains approximately 50 % nationally sourced ingredients, while the rest are imported, including soy from Brazil. Soy protein holds a high quality while being relatively cheap. Environmental and ethical controversies around the production and import of soy has lead researchers to explore alternative protein sources. Novel protein ingredients should be based on resources that are renewable and can be sustainably sourced, without competing with human food. In Norway, such resources include trees, macroalgae and grass. The yeast strain *Candida utilis* is able to grow on components of cellulose from trees, after which it can be used as a protein source. This trial used *C. utilis*, grown on sugar molasses, as protein source in diets for dairy cows. The purpose of the thesis was to investigate any effects of replacing soy protein with yeast protein from *C. utilis* on the feeding behaviour of dairy cows in a free-stall barn with an AMS (automatic milking system). The trial was performed at NMBU in 2019, where forty-eight NRF dairy cows in 116 ± 33 DIM (days in milk) were divided into three groups of sixteen, blocked by MY (milk yield), parity and DIM. After a two-week adaptation period each group was fed a different concentrate feed over eight weeks: A soy-based feed containing 7 % soybean meal ('Soy'), a yeast-based feed where 7 % of CP from soy was replaced with protein from *C. utilis* ('Yeast'), or a negative control barley-based feed replacing soy protein with barley ('Barley'). All cows were fitted with individual sensor systems from Nedap CowControl to measure their behaviour (eating time, ruminating time, lying time, inactive time). Behaviour data was recorded 24/7 for each cow over ten weeks and collected for analysis. Feed intake and productivity were also measured. Simple data analysis was done in Excel, while statistical analysis was performed in SAS 9.4 using Proc Mixed with a CS covariance structure. Results showed significant correlation ($p \leq 0.05$) between MY and DMI, and behaviour. No significant differences were found between treatments regarding feeding behaviour or productivity. Higher contents of CP (crude protein) and WSC (water-soluble carbohydrates) in Soy may have contributed to its decline in MY being more moderate than in the other groups. *Candida utilis* has shown promise as a protein source in diets for salmon and pigs. Growing *C. utilis* on Norwegian spruce trees for use in animal feed is an unprofitable venture until production costs can be lowered. In conclusion, the results of this study indicate that feeding behaviour in dairy cows is unaffected by replacing soy protein with yeast protein from *C. utilis* in diets, and further research is recommended.

Sammendrag

Norske melkekyr (Norsk Rødt Fe, NRF) spiser hovedsakelig grovfôr, supplert med kraftfôr for å øke melkeproduksjonen. Kraftfôr inneholder ca. 50 % nasjonalt dyrkede ingredienser, mens resten importeres, inkludert soya fra Brasil. Soyaprotein holder høy kvalitet og er relativt billig. Miljømessige og etiske kontroverser rundt produksjon og import av soya har fått forskere til å utforske alternative proteinkilder. Nye proteiningredienser bør være basert på ressurser som er fornybare og kan høstes bærekraftig, uten å konkurrere med mat for mennesker. I Norge omfatter slike ressurser trær, makroalger og gress. Gjærstammen *Candida utilis* kan vokse på bestanddeler av cellulose fra trær, hvoretter den kan brukes som proteinkilde. Denne studien brukte *C. utilis*, dyrket på suktermelasse, som proteinkilde i dietter til melkekyr. Hensikten med oppgaven var å undersøke eventuelle effekter av å erstatte soyaprotein med gjærprotein fra *C. utilis* på spiseatferden til melkekyr i et løsdriftsfjøs med AMS (melkerobot). Studien ble utført ved NMBU i 2019, hvor førtiåtte NRF-melkekyr i 116 ± 33 DIM (dager i melk) ble delt inn i tre grupper på seksten, justert for MY (melkeytelse), laktasjonsnummer og DIM. Etter en to-ukers tilpasningsperiode ble hver gruppe gitt et ulikt kraftfôr over åtte uker: Et soyabasert fôr som inneholdt 7 % soyabønnemel ('Soya'), et gjærbasert fôr hvor 7 % av CP fra soya var erstattet med protein fra *C. utilis* ('Gjær'), eller negativ kontroll, et byggbasert fôr hvor soyaprotein var erstattet med bygg ('Bygg'). Alle kyrne var utstyrt med individuelle sensorsystemer fra Nedap CowControl for å måle deres atferd (spisetid, drøvtyggingstid, liggetid, inaktiv tid). Atferdsdata ble registrert 24/7 for hver ku over ti uker og samlet inn til analyse. Fôrinntak og produktivitet ble også målt. Enkel dataanalyse ble gjort i Excel, mens statistisk analyse ble utført i SAS 9.4 ved hjelp av Proc Mixed med CS-kovariansstruktur. Resultatene viste signifikant korrelasjon ($p \leq 0,05$) mellom MY og DMI (tørrstoffinntak), og atferd. Det ble ikke funnet signifikante forskjeller mellom behandlinger på spiseatferd eller produktivitet. Høyere innhold av CP (råprotein) og WSC (vannløselige karbohydrater) i Soya kan ha bidratt til at nedgangen i MY var mer moderat enn i de andre gruppene. *Candida utilis* har vist seg lovende som proteinkilde i dietter for laks og gris. Dyrking av *C. utilis* på norske grantrær til bruk i dyrefôr er ulønnsomt inntil produksjonskostnadene kan senkes. Som konklusjon indikerer resultatene av denne studien at spiseatferd hos melkekyr ikke påvirkes av å erstatte soyaprotein med gjærprotein fra *C. utilis* i dietten, og videre forskning anbefales.

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List of abbreviations

AMS	Automatic milking system
BW	Body weight
CF	Crude fat
CH ₄	Methane
CHO	Residual carbohydrates
CO ₂	Carbon dioxide
CP	Crude protein
CS	Compound symmetry
DIM	Days in milk
DM	Dry matter
DMI	Dry matter intake
DOT	Day of treatment
FON	Foods of Norway
FPF	Silage fermentation products
GRAS	Generally-regarded-as-safe
ISO	International organisation for standardisation
LAB	Lactic acid bacteria
LS	Least squares
MJ	Megajoule
MP	Metabolizable protein
MY	Milk yield
NDF	Neutral-detergent fibre
NDFOM	Neutral-detergent fibre corrected for organic matter
NEL	Net energy lactation
NMBU	Norwegian University of Life Sciences
NRF	Norwegian Red
PLF	Precision livestock farming
SEM	Standard error means
SHF	Production Animal Experimental Unit
VFA	Volatile fatty acid
WSC	Water-soluble carbohydrates

1.0 Introduction

The Norwegian Red dairy cow (NRF) is a key player in Norwegian agriculture. The breed is used for both milk and meat production, and its genetics are exported to improve health and fertility in other breeds (Vangen, 2019). Like all ruminants it converts grasses into high value protein for humans. As Norway has little arable land suited for growing crops and vegetables, ruminants are used as a tool for utilising feed resources that would otherwise go to waste (Landbruks- og matdepartementet, 2016; MatPrat, 2020). Still, NRF cows do not always graze outdoors, but are indoors large parts of the year due to a cold climate. Indoors the cows are fed harvested grasses like hay and silage, supplemented with concentrate feed. In Norway, the average dairy cow gets 55-60 % roughage in her diet, and 40-45 % concentrates (Felleskjøpet, n.d.). Concentrate feed provides the animal with extra energy and protein, sustaining higher milk yields. On average, one NRF cow has a milk production of over 8000 L per year (Tine Rådgiving, 2019). Based on numbers from 2017, an estimated 55 % of the protein in dairy cow diets comes from roughage during one lactation. When including the dry period, this portion increases to 63 % (Sommersteth, n.d.). According to numbers from 2014, diets for NRF cows contain 80 % nationally sourced ingredients. For concentrates this number is down to 50 % (Nesse, 2019).

Due to roughage being a big part of cattle diets, dairy production is less dependent on concentrate feeds than meat production from pigs and poultry. Nevertheless, concentrate feed is necessary for industrial-scale dairy production, and contains soy protein sourced in Brazil. An increasingly aware consumer mass is protesting the use of imported soy in national animal feed, stating deforestation, loss of biodiversity, increased CO₂ emissions, and soil depletion as consequences of soy production (Randen, 2018; Regnskogfondet, n.d.). To improve the ethical and environmental conditions of food production, researchers are looking for alternatives to soy protein in livestock diets. Foods of Norway (FON) is a project at the Norwegian University of Life Sciences (NMBU) dedicated to the search for novel feed protein ingredients. Their aim is to use national resources to supply protein for livestock (including fish), based on ingredients which can be sustainably sourced without directly competing with human food, such as macroalgae, grass and trees (Øverland, 2015).

This work investigated the effects, if any, on the feeding behaviour of dairy cows when replacing soy protein with yeast protein in concentrate feed. The yeast strain used for protein

production was *Candida utilis*, which can grow on cellulose from trees. Meanwhile, feeding behaviour was recorded by sensor technology from Nedap CowControl. A theoretic chapter introduces feeding behaviour in ruminants, sensor technology, and protein in ruminant diets. The next chapter presents materials & method used in the trial, followed by results and a discussion.

2.0 Theory

2.1 Ruminants

Ruminants, like cattle, sheep and goats, are characterised by their forestomachs, or fermentation chambers, situated between the oesophagus and the stomach. Feed passes from the oesophagus, through the forestomachs (rumen, reticulum and omasum), to the stomach (abomasum) before continuing through the intestines. The forestomachs facilitate anaerobic breakdown, or fermentation, of feed components by microorganisms (microbes). Components in e.g. grass would not be possible to digest without initial microbe fermentation. Microbes are able to break the β -1,4 bonds in cellulose and hemicellulose, something the abomasum enzymes are unable to do. The forestomachs make ruminants capable of utilising grass as their primary energy source, in contrast to monogastric (single-stomached) animals like humans (Sjaastad et al., 2010). After the initial eating, chewing and swallowing, the ruminant regurgitates the food in portions known as boli. Each bolus is then chewed another 40-50 times, breaking the feed into even smaller pieces. The process of regurgitation and re-chewing is called rumination (Nørgaard, 2003). Rumination increases the available surface area for microbes to attack and break down feed in the rumen, making fermentation more effective (McDonald et al., 2011).

2.2 Feeding behaviour

Chewing time is defined as the total time a ruminant spends on eating and ruminating in a day. Lactating cows will typically spend four to seven hours eating and six to ten hours ruminating, making around 28.000-70.000 chewing movements per day. Chewing time is affected by the animal's feed intake, the particle size of the feed, and the level and composition of its fibre fraction, commonly referred to as feed structure. Chewing time generally increases with increased roughage fraction and vice versa; eating time for concentrates is around four minutes per kg, and around thirty minutes per kg for roughage

(Mertens, 1997; Nørgaard, 2003). Chewing time can be used as an indicator of the energy content and nutrient composition of the feed. With *ad libitum* feeding of roughage the cow should eat roughage to satiation, characterised by ten percent leftovers on the feeding tray. At *ad libitum* feeding, a lactating cow with low chewing time (< ten hours per day) indicates a ration that is high in energy. With the combination of high energy and low chewing time, she can develop rumen acidosis (an affliction characterised by low rumen pH) as well as gain unwanted fat deposits. On the other hand, high chewing time (> sixteen hours per day) for a lactating cow indicates a ration that is low in energy, and additional energy sources should be given (Nørgaard, 2003). Total chewing time may vary less than eating and ruminating time separately, because cows can compensate for shorter eating time by ruminating for longer. However, cows will only compensate until reaching their maximum physiological ruminating capacity. Characteristics of the diet which affect ruminating time include NDF intake, particle size, hardness factor (harder feed gives more chewing resistance), and indigestibility of fibre (Beauchemin, 2018).

The Nordic feed evaluation system, NorFor, describes the fibre fraction in feed as neutral-detergent fibre (NDF) (Volden, 2011). Neutral-detergent fibre consists of the cell wall materials cellulose, hemicellulose and lignin. Lignin is a non-degradable, non-carbohydrate polymer which increases with later development of the plant (e.g. late harvested grass). Lignin binds itself to hemicellulose and rearranges cellulose, making the NDF fraction less degradable overall. The size of the lignin fraction therefore affects rumen degradability of NDF (Weisbjerg et al., 2003). All the carbohydrates in feed except for lignin are broken down by rumen microbes (McDonald et al., 2011). Reduced degradability of NDF requires longer ruminating time per kg NDF- and dry matter intake (DMI) for maximal nutrient yield (Nørgaard et al., 2011). Ruminating time, in turn, affects feed intake and rumen environment.

2.2.1 Rumen environment

Microbes including bacteria, fungi and protozoa work in anaerobic conditions to ferment feed components in the rumen. During fermentation, the microbes produce volatile fatty acids (VFAs), microbial protein (microbial body mass), carbon dioxide (CO₂) and methane (CH₄). VFAs are the ruminant's most important energy source and include acetate, butyrate and propionate. When fermentation takes place and VFAs are produced, rumen pH decreases. A stable pH environment is vital for the microbes to function optimally, and should be kept between 5.5 and 6.5. To prevent pH levels from plummeting too low, VFAs are absorbed

through the rumen wall, and buffers are added to the rumen via saliva (McDonald et al., 2011). A lower pH encourages proliferation of lactic acid bacteria (LAB) which produce lactic acid. If pH sinks below 5, LAB will overwhelm the VFA-producing bacteria in the rumen, causing acidosis. This potentially involves damage to the epithelium, and weak or absent rumen contractions: The rumen ceases to function (Sjaastad et al., 2010).

Cattle produce around 150 L of saliva per day. Saliva contains bicarbonate, which has a high pH and acts as a buffer to keep rumen pH stable after feeding. There is a strong link between feed structure, chewing time and saliva secretion. Eating and ruminating stimulate saliva secretion by approximately 150 mL saliva per minute of chewing. Thus, longer chewing time contributes to keeping an appropriate rumen pH level (Nørgaard, 2003), but this effect alone is not enough to prevent rumen acidosis at low pH levels. Additionally, production of saliva per minute of chewing and resting are similar. Chewing stimulates saliva production and flow, but when chewing time increases, resting time decreases, so the total secretion of saliva is not greatly affected by chewing time. Eating rate may be more important for total salivary production than chewing time (Beauchemin, 2018).

The ratio of roughages to concentrate feed in the diet affects rumen environment, and the quantities of VFAs produced. When microbes ferment roughage like grass, hay or silage, acetate is produced in the largest proportion; the more structure, the more acetate. In contrast, fermentation of younger crops or concentrate feed, which are more easily digestible, yields a higher proportion of propionate. With starch-rich diets, breakdown is faster and VFAs are produced relatively quickly, making rumen pH lower than with fibre-rich diets (McDonald et al., 2011).

2.2.2 Feed intake and rate of passage

It is understood that more fibre in the ration will most likely lead to longer chewing time. High inclusions of fibre increase retention time of particles in the rumen, decreasing flow rate through the rumen towards the abomasum and intestines (Weston & Hogan, 1967). Longer retention time allows the microbes more time to break down fibre-rich materials, but also restricts the animal's feed intake due to the physical limits of the rumen. Only when the feed passes on from the rumen will there be space for more feed. Rate of passage through the rumen increases with increased DMI, and is therefore indirectly affected by lactation (increases DMI), and high body condition scores (decreases DMI). Other factors include high

temperatures (reduces DMI) and advancing pregnancy (increases rate of passage by limiting rumen fill capacity) (McDonald et al., 2011). According to Dewhurst et al. (2000), an advantage of increasing rate of passage is microbes spending less time in the rumen, reducing their maintenance costs and in turn improving efficiency of microbial protein synthesis.

With increasing levels of concentrate feed in the ration, cows will eat less roughage *ad libitum* due to the substitution effect. For each extra kg of DMI from concentrate feed, a cow will reduce DMI from roughage by approximately 0.2-1.0 kg, and better quality roughage will cause a greater effect (Randby, 2004). If the ratio of concentrate feed to roughage is high, rumen pH will be lower than ideal, inhibiting NDF fermentation and reducing feed intake (Kristensen & Ingvarsten, 2003).

According to Mertens (1997) the intake of NDF increases in proportion with the animal's live weight, with *ad libitum* feeding. However, this is not taking into consideration the body condition score of the animal. When body condition score increases from 3 to 4, live weight increases by 12-16 %, without making the animal's forestomachs better equipped to receive larger amounts of NDF (Nørgaard, 2003).

2.3 Precision Livestock Farming

Precision Livestock Farming (PLF) is technology used for the continuous (24/7) monitoring of animal health, welfare, production, reproduction, and environmental impact. Technology includes cameras, microphones, and sensors on or around the animal. Collected data is analysed to produce valuable feedback, and give warning if something is out of the ordinary. Benefits of PLF include better control of each individual animal in large herds, catching diseases early before symptoms are visible, and generally aiding the farmer in monitoring the animals (Berckmans, 2017). Objective detection of changes in behaviour, before more obvious symptoms of e.g. illness present themselves, is useful for herd management purposes (Weary et al., 2009). For instance, cows experiencing rumen acidosis might decrease ruminating time per kg DMI (DeVries et al., 2009), and cows experiencing ketosis or lameness might decrease their daily eating time (González et al., 2008). Continuous recording of feeding behaviour may detect irregularities and indicate rumen health of the herd.

Some methods that can be used for recording eating time include connecting a weighing scale to the feeding trough for tie-stall cows, or by utilising computer-controlled feeders for loose-housed cows. Meanwhile, ruminating time can be measured through registration of

forestomach motility (Dado & Allen, 1993). Alternatively, they can be measured by individual sensor technology. This involves attaching sensors directly to each animal, around the jaw or nose, where they register and characterise jaw movements. Such systems have been used and developed for decades, like the transducer converting jaw movements into electrical signals by Beauchemin et al. (1989). Individual sensor-based systems are often lower in cost than stationary systems, and allow for recording chewing behaviour on pasture as well as in tie-stall or free-stall conditions (Ruuska et al., 2016). Different sensors for measuring chewing activity have varying accuracy and precision. Their accuracy, however, is less important than the relative changes they record in animal behaviour. As long as the sensor detects significant changes in e.g. ruminating time, this information can be used to detect illness, oestrus, and parturition (Beauchemin, 2018).

There is a plethora of commercially available technologies for PLF in dairy production (Borchers et al., 2016). One of these is the individual sensor-based system RumiWatch (Itin + Hoch GmbH, Liestal, Switzerland). It measures rumination, feed intake, water intake and locomotion through a noseband sensor. The noseband is filled with liquid and has a built-in pressure sensor, a data logger and a battery. The movements are registered through the pressure sensor, while data is logged and evaluated by a software (Zehner et al., 2012; Zehner et al., 2017). However, drinking time has proved difficult to measure due to its low time budget of just a few minutes per day. There have been found systematic overestimates in measures of eating time by RumiWatch, in free-stall barns in particular. This may be due to more mouth-related behaviour in free-stall conditions, which the sensor wrongly construes as eating behaviour (Ruuska et al., 2016).

The sensor system used in this trial was Nedap CowControl (Nedap Livestock Management, Groenlo, the Netherlands). Nedap sensors include a neck collar (“SmartTag Neck”) and a leg band (“SmartTag Leg”) intended to be worn by each cow. Nedap SmartTags have built-in accelerometers, which register changes in direction and speed in real-time, 24/7 (Andresen, 2018; Nedap Livestock Management, 2018). The tags register movements up- and downwards, for- and backwards, and left and right (Van Erp-Van der Kooij et al., 2016). As observed by Benaissa et al. (2019), collar-mounted accelerometers performed on a level with RumiWatch noseband sensors regarding classification of behaviours like eating and ruminating. Health monitoring functions of Nedap SmartTags include detection of eating, ruminating, and inactive (neither eating nor ruminating) behaviour by the neck tag, while the

leg tag detects standing, lying and walking behaviour. Data from the SmartTags are processed and uploaded to a cloud system, from which they can be viewed and downloaded as illustrated in figure 1 (Nedap Livestock Management, 2018; Nedap Livestock Management, 2020).



Figure 1. Illustration of Nedap, where the cow on the left is wearing a Smarttag Leg and the cow on the right is wearing a Smarttag Neck. Registration at cow level is uploaded to an internet cloud, from which the data can be viewed and downloaded. (Nedap Livestock Management, n.d.)

2.4 Protein in ruminant diets

Ruminants get the most concentrate feed in Norway based on sales numbers from 2019: 1.060.040 tons for ruminants out of 2.018.086 tons in total for ruminants, swine and poultry (Landbruksdirektoratet, 2020b). Norway annually imports approximately 200.000 tons soy and soybean meal for use in concentrate feed for land livestock. When including farmed fish in this number, it rises to approximately 850.000 tons (MatPrat, 2019). Concentrate feed for

Norwegian livestock (excluding fish) contained 8.4 % soy protein in 2019, which constitutes 44 % of the total protein fraction (Landbruksdirektoratet, 2020a).

2.4.1 Soy protein

Soy is a plant in the pea flower family with seed-bearing pods, which are called soybeans. They are grown in tropical and sub-tropical environments, where the U.S. is the largest producer followed by Brazil and Argentina. Soybean meal is an attractive feed ingredient due to its high contents of fat (18 %), vitamins A and B, and true protein (38 %) (Holtet, 2020). Meanwhile, the crude protein fraction makes up approximately 50-55 % of dry matter content, which in turn constitutes about 88 % of the total (Heuzé et al., 2020).

Soy is a controversial feed ingredient, as its production contributes to deforestation of forest areas in the Amazon, Cerrado, Atlantic Forest, and Pampa biomes, which are areas important for biodiversity in South America. Conservation policies are in place to limit the impact of soy production, although the effectiveness of such policies has been questioned (Lima et al., 2019). Soy can be fit for direct human consumption as a protein source, heightening its controversy as a feed ingredient for livestock (Vennet et al., 2016). Soy production may also contribute to socioeconomic issues like land distribution inequality and income inequality, and the debate around soy production is more complex than what is often presented in the media (Martinelli et al., 2017).

Only 2 % of the world's soy produce is certified sustainable, of which Norway imports approximately $\frac{1}{6}$. Switzerland and Norway are currently the only countries in the world which import soy that can be traced back to each individual farm (MatPrat, 2019). Even with these considerations, pressure from consumers to eliminate the use of soy in Norwegian livestock diets remains high. Soy production in Europe is not financially feasible when competing with imported soy, and alternative protein sources are being researched, including microalgae, macroalgae, duckweed, yeast, bacteria, alfalfa leaves, and insects. These novel protein sources are more sustainable than soy production in terms of reduced agricultural land use and greenhouse gas emissions. A higher level of self-sufficiency of feed protein in Europe will increase food security, which is vulnerable to changes in global trade and market prices (Tallentire et al., 2018).

Production of monogastric animals is more dependent on soy protein than ruminant production systems (de Visser et al., 2014), and research with yeast protein for pigs has

shown promise (Cruz et al., 2019). Novel protein ingredients are also of interest in aquaculture, as soybean meal causes enteritis in Atlantic salmon (*Salmo salar*) (Van den Ingh et al., 1991), and because the traditionally used fishmeal is an expensive ingredient in limited supply (Øverland & Skrede, 2017).

2.4.2 Yeast protein

Yeasts are single-cell eukaryotic microorganisms, classified as fungi (Montes de Oca et al., 2016). Yeast cell composition depends on strain, growth medium, growing conditions, and downstream processing after fermentation. Yeasts have been used in livestock diets for over a hundred years, and have been commercially available as livestock supplements for over 70 years (Bush, 1960; Stone, 2006; Øverland & Skrede, 2016). Yeast production has become cheaper and more efficient with modern technology (Øverland et al., 2013), and three different strains of yeast that are commonly grown on industrial by-products include *Saccharomyces cerevisiae* (aka Brewer's or Baker's yeast), *Kluyveromyces marxianus* (aka Whey yeast) and *Candida utilis* (aka Torula yeast) (Habeeb, 2017).

Of the global market for animal feed protein, yeast protein constitutes approximately 4.3 %. Further, 88.0 % of this was, per 2017, derived from *Saccharomyces cerevisiae*. Meanwhile, *Kluyveromyces marxianus* made up 10 %, and the remaining 2 % was accounted for by other yeast strains like *Candida utilis* (Skogli et al., 2019). Yeasts can utilise waste products from various industries and turn them into a valuable resource. *Saccharomyces cerevisiae* is typically a by-product of the beer-brewing industry, and is efficient in fermenting hexose sugars like glucose. *Candida utilis*, formerly *Torulopsis utilis*, can utilise waste from the paper industry for growth, while *K. marxianus* can grow on lactose waste from whey production (Stone, 2006). Both *C. utilis* and *K. marxianus* are able to co-ferment hexoses (glucose, mannose) and pentoses (xylanose, arabinose), which are components of lignocellulosic biomass. *Kluyveromyces marxianus* can ferment substrates in an anaerobic state, producing both ethanol and yeast biomass, whereas *C. utilis* will ferment under aerobic conditions, producing only yeast biomass (Øverland & Skrede, 2016).

Candida utilis has a generally-regarded-as-safe (GRAS) status, and can utilise sugars from lignocellulosic biomass with added growth media like nitrogen, inorganic phosphate and sulphate (Cruz et al., 2019; Sharma et al., 2018). Lignocellulosic by-products from forestry is a renewable and cheap resource otherwise non-edible for livestock and humans, found in

abundance in Norway (Øverland & Skrede, 2016). A national production of feed protein can increase food security and improve the sustainability of national food production, as well as address environmental and ethical concerns around feed crops that compete with human food (Sharma et al., 2018).

Trimblings of spruce trees can be pre-treated by delignification (separation of the lignin fraction), before exposing the remaining fraction, cellulose and hemicellulose, to enzymatic hydrolysis. This yields sugar monomers to be fermented by yeast, which is finally processed to give a protein-rich ingredient. Downstream processing involves washing, cell disruption to increase nutrient availability, and drying. The pre-treatment and enzymatic hydrolysis make the biomass ready for fermentation, and will vary with type of biomass. Wood biomass like spruce trimblings differs from non-wood biomass (e.g. agricultural crop residues) in requiring more effort to free the monomers, as does hard wood vs. soft wood (Sharma et al., 2018; Øverland & Skrede, 2016). The process of converting lignocellulosic biomass into protein-rich yeast biomass is presented in the flow chart below (figure 2).

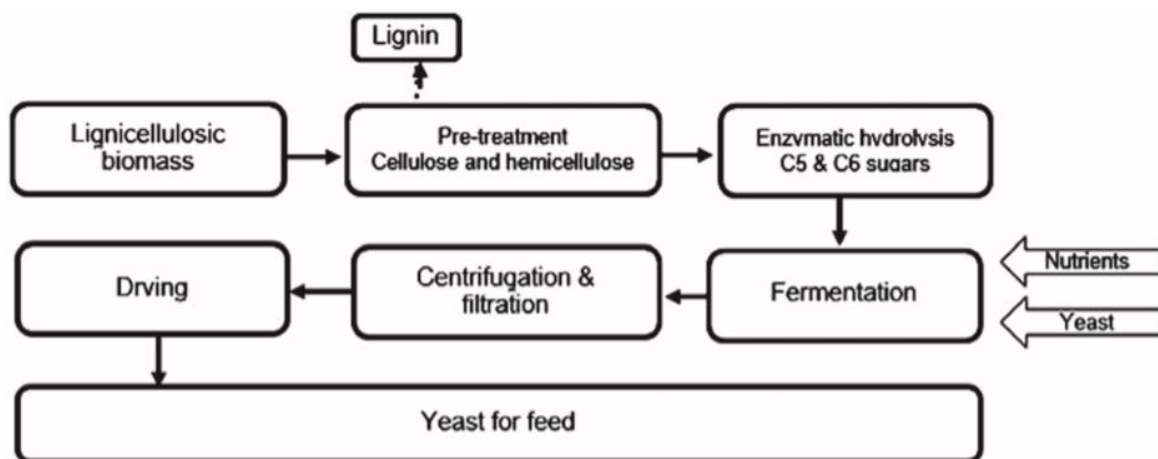


Figure 2. The processing of lignocellulosic biomass into yeast protein for livestock (Øverland & Skrede, 2016).

Vohra et al. (2016) summarised research of yeast in ruminant diets and suggested the following modes of action for yeast: Rumen pH regulation; oxygen scavenging by live yeast, benefiting the anaerobic process of rumen microbes; production of growth factors such as B-vitamins, peptides and amino acids; promoter of protozoa growth in the rumen; improved NDF digestion; and methane reduction. The function and benefits of yeast in livestock diets are influenced by yeast form (dry, live, etc.) and processing. Rumsey et al. (1991) found that

a disruption of the yeast cell wall significantly improved nutritional value of the yeast (*S. cerevisiae*) in aquaculture diets. Several trials have shown higher digestibility of yeast protein when the yeast cell wall material has been removed. Still, the cell wall contents include mannan-oligosaccharides and beta-glucans. Considering the positive health effects seen by including these components in livestock diets, the cell wall material is still a valuable ingredient (Øverland & Skrede, 2016).

2.4.3 Protein requirements

Protein requirements for ruminants are expressed as the need for metabolizable protein (MP). This is protein which is broken down and absorbed in the intestines, without first being used by microbes in the rumen. Protein requirements will vary with milk yield (MY), feed intake and forage quality. Good quality forage might cover most of the ruminant's MP requirements (Steinshamn et al., 2019).

During carbohydrate breakdown in the rumen, simple sugars are taken up by microbes and metabolised intracellularly. Production of microbial protein, which can be digested and absorbed by the animal post-rationally, is affected by the energy content of the diet including digestible organic matter and rumen-degradable carbohydrates. Forages rich in water-soluble carbohydrates (WSC) will yield more microbial protein than forages that are more fibrous (approximately 260 and 200 g microbial protein/kg organic matter, respectively) (McDonald et al., 2011). Consequently, levels of fermentable carbohydrates in the diet affect synthesis of microbial protein, which covers 60-80 % of the ruminant's MP requirements (Strøm, 2011). Nonetheless, microbial protein yield is not simply a matter of available energy for microbes, and there are high standard errors for predicting microbial protein yield this way. Factors including microbe maintenance requirements and rumen pH should be taken into account. Microbial protein yield is determined by both the available energy and protein for microbes, either of which can be in limited supply (McDonald et al., 2011). Utilisation of protein from forage can be maximised by reducing its crude protein content, producing silage with high fermentation quality, optimal harvest time of grasses (not too early), and avoiding high levels of nitrogen fertilisation (Huhtanen & Broderick, 2016).

Amino acids

The content of essential amino acids in grains is unbalanced, i.e. one or more amino acids are limiting for use in livestock feed (Åssveen, 2009). Securing the protein needs of dairy cows

therefore requires thought and planning. It is possible to mix grains whose amino acid levels complement each other, and for high-producing animals it can be necessary with additional supplements of the first limiting amino acids.

Limiting amino acids for lactating dairy cows include methionine, lysine and histidine.

Which amino acid is the first limiting depends on the feed protein source. When the main source of rumen-undegradable protein is soybean meal, methionine is the first limiting amino acid; when maize is the main protein source, lysine is the first limiting amino acid; when grass silage constitutes the main part of the diet, with barley and oats as energy supplements, histidine is the first limiting amino acid (Schwab et al., 2005). In Scandinavia, cattle are fed mostly grass and grain-based (barley, oats, wheat) concentrate feeds. Vanhatalo et al. (1999) investigated the effects of abomasal infusions of histidine, lysine and methionine to dairy cows on a grass silage-based diet. Milk and milk protein yields were increased as a response to the infusion of histidine, but additional lysine or methionine had no such effect.

Table 1 shows the content of limiting amino acids in common feed ingredients. Values show that torula yeast has a lysine content to rival soybean meal, but contains less histidine and methionine. Meanwhile, oats have the highest level of methionine, and more histidine than torula yeast. Barley has neither the highest nor lowest levels of any of the amino acids presented in table 1, but from Wollenweber (2002) we know that the amino acid composition of barley varies with available nitrogen levels in different growth stages.

Table 1. Content of amino acids in some commonly used feed ingredients (Schwab et al., 2005) and in torula yeast (Lagos & Stein, 2020). Values given in % of crude protein (CP).

	Amino acids (% of CP)		
	Histidine	Lysine	Methionine
Grass silage	1.7	3.3	1.2
Barley	2.3	3.6	1.7
Maize	3.1	2.8	2.1
Oats	2.4	4.2	2.9
Soybean meal	2.8	6.3	1.4
Torula yeast	1.8	6.7	1.1

Torula yeast barely contains more histidine than grass silage, thus may be insufficient as the only protein supplement to grass silage-based diets. It is also important to note that microbial

protein from the rumen is lower in histidine than milk protein and most feed ingredients, highlighting the importance of histidine supplementation for Scandinavian dairy cows (Giallongo et al., 2016). From table 1 we might surmise that adding soybean meal or torula yeast to a maize-based diet could balance lysine, and supplementing maize and soybean meal to a grass silage diet could balance histidine. Meanwhile, oats are the richest source of methionine and could supplement diets of e.g. soybean meal, torula yeast and grass silage.

Cruz et al. (2019) found that methionine content in experimental diets to weaned piglets increased with increasing inclusion of *Candida utilis* protein. Even so, Habeeb (2017) recommends a methionine supplement in addition to the torula yeast, due to its generally low levels of sulphur-containing amino acids, i.e. methionine, cysteine, homocysteine and taurine (Brosnan & Brosnan, 2006).

Supplemented amino acids are best utilised when broken down in the small intestine and absorbed through the intestinal wall directly to the animal itself. As such, amino acids require protection from microbial breakdown in the rumen. Rumen-protected supplements are able to bypass the rumen and travel unharmed to the intestines, but can be challenging to produce (Schwab & Broderick, 2017). The most widely used rumen-protected amino acids on a commercial scale are methionine and lysine (Wu & Papas, 1997).

3.0 Materials & method

The current study used yeast protein from *Candida utilis*, grown on sugar molasses, in concentrate feed for dairy cows as replacement for soy protein. The objective was to evaluate any effects of replacing soy protein with yeast protein on feeding behaviour. Measured parameters included feed intake, eating time, ruminating time, lying time, and inactive time. Additionally, body weight (BW) and milk yield (MY) were recorded.

3.1 Experimental design

The trial was performed by Foods of Norway (FON) at the Production Animal Experimental Unit (Senter for Husdyrforsk, SHF) at the Norwegian University of Life Sciences (NMBU) in Ås, Norway from January 31st to April 11th 2019.

3.2 Experimental animals

Forty-eight lactating NRF dairy cows, in 116 ± 33 (mean \pm standard deviation) days in milk (DIM) at start, were used in the experiment. The experiment was conducted over ten weeks: The first two weeks were used for adaptation ('covariate period') during which all cows were fed similar diets; the following eight weeks ('trial period'), cows were given different diets. Cows were divided into three groups of sixteen after being balanced for initial milk yield (MY), parity and DIM. Each group was then randomly allocated to one of three different concentrate feeds along with *ad libitum* fed grass silage. They were housed in a free-stall barn using an automatic milking system (AMS) from De Laval, which they had access to every 6th hour. The animals were milked a maximum of four times per day.

3.3 Experimental feed

Roughage feed: Throughout the experiment, all cows were given the same grass silage, fed *ad libitum* from automatic feeders (BioControl AS, Rakkestad, Norway) which identified the individual cows during feeding. The grass silage was provided by SHF, cut from one area in one harvest, and ensiled in the same horizontal silo. The silage had an energy content of 6.45 MJ NEL20/kg DM (dry matter) and crude protein content of 17.3 % CP/kg DM.

Concentrate feeds: During the first two weeks, the covariate period, all animals were given the same concentrate feed with soy protein for the purpose of establishing a baseline. For the next eight weeks, the three groups were each given a different concentrate feed (treatment):

Treatment 1: 'Soy' – 7 % inclusion of soybean meal per kg concentrate.

Treatment 2: 'Yeast' – 7 % inclusion of *Candida utilis* yeast, replacing 7 % of the CP from soybean meal.

Treatment 3: 'Barley' – negative control where soybean meal was replaced by barley (lower in CP).

To ensure sufficient protein supply, all forty-eight cows were offered 1 kg of soy-based concentrate feed per day in the milking robots throughout the trial period, regardless of which treatment they received.

The concentrate feeds were produced by Felleskjøpet Agri (FKA, Vestnes, Norway) through an expander process. All three concentrates were iso-energetic (same level of energy), while the soy and yeast concentrates were iso-nitrogenous (same level of CP). The rest of the

ingredients were kept as constant as possible. A composition of ingredients is shown below in table 2. The amounts of concentrate feed needed for each animal was calculated using the online tool TINE Optifôr, with a ratio of silage to concentrates 60:40 on a DM basis. Concentrate feed was reduced for all animals over time, with the expectation of a decline in MY and thus energy demand with advancing lactation stage. Feed was given in several portions per day from an automatic feeding system.

Table 2. Ingredient composition in % of each concentrate feed.

	Soy (treatment 1)	Yeast (treatment 2)	Barley (treatment 3)
Barley	49.5	49.9	55.8
Corn gluten meal	2.0	2.0	2.0
Oats	5.0	5.0	5.0
Wheat	10.0	10.0	10.0
Molasses	5.0	5.0	5.0
Beet pulp	15.0	15.0	15.0
Soybean meal	7.0	0.0	0.0
Yeast	0.0	7.0	0.0
Vegetable oil	3.1	2.8	3.0
Others	3.4	3.3	4.2

3.4 Data registration and sampling

Feed intake: Daily feed intakes for individual cows were recorded in the automatic feeding system, and retrieved each morning. Mean daily feed intake was registered as total DMI (the sum of DMI, dry matter intake, from concentrate feed and silages), and an assumption was made of zero concentrate feed refuse.

Silage feed: Samples were analysed once a week for DM contents by drying in an oven at 60 °C for forty-eight hours, or at 103 °C overnight. Additional pooled silage samples (from weeks 1-2, weeks 3-6, and weeks 7-10) were taken and kept at -20 °C until chemical analysis at the end of the experiment, at which point they were freeze-dried and milled through a 1.0 mm sieve in a cutting mill (Retsch SM 200, Retsch GmbH, Germany).

Concentrate feeds: 300-400 grams of all three concentrate feeds were sampled once a week. The samples were stored at -20 °C before being analysed.

Chemical analyses of feed: For determination of DM content, samples were dried at 103 °C overnight. Ash content was determined through the ISO 5984 method, at 550 °C for four hours minimum. Kjeldahl-N crude protein content ($CP = N \times 6.25$) was determined with Kjeltex 2400/2460 Auto Sampler System (Foss Analytical, Hilleroed, Denmark), using Method 2001.11 (Thiex et al., 2002). Starch content was determined using the AACCI Method 76-13.01 (Megazyme amyloglucosidase/ α -amylase). NDF content was determined with ANKOM220 fiber analyser (ANKOM Technology, Fairport, NY), using sodium sulphite and α -amylase (Mertens, 2002).

Automatic milking system (AMS): Body weight (BW) was recorded for each animal every time they went into the AMS. The AMS also measured somatic cell count in the milk, as well as conductivity and MY.

Milk: Separate samples were taken in trial weeks 2, 4, 6, 7, and 10, at each milking from Sunday evening to Wednesday morning. All samples were mixed together at individual cow level. Milk samples were preserved with bronopol tablets (2-bromo-2-nitropane-1,3-diol, Broad Spectrum Microtabs® II) and stored at 4 °C until analysis at TINE (TINE Meieriet, Brumunddal, Norway), where chemical, sensory and composition analyses were performed.

Sick animals: In the case of illness, the animal was separated from the others. Sick animals were milked manually twice per day and given their allotted concentrate three times per day. Data from sick animals were recorded manually on a form.

Behaviour: Before the trial, all cows were fitted with Nedap sensors SmartTag Neck and SmartTag Leg. The neck sensor recorded movements through accelerometer technology, measuring movement in three-dimensional space, on an x-y-z-axis. The angle to the floor determined positions and movements of the cow's head, which were matched to eating, ruminating, lying and inactive behaviours. The leg sensor detected "lying down" and "standing up" movements; the time between lying down and standing up was categorised as lying time (Theije, 2017). The sensors had a time registration overlap caused by "lying time" coinciding with other behaviours. Both inactive and ruminating behaviours may occur while the cow is lying down, and the sensors measured each behaviour individually also when they occurred simultaneously.

Behaviour data were recorded by the sensors for each cow 24/7 throughout the trial. Data from the past twenty-four hours were summarised for each behaviour and for each cow, then downloaded and saved for later processing and analysis.

3.5 Data analysis

Behaviour data recorded by the Nedap sensors were downloaded to either a computer or a mobile phone each day of the trial. Formatting of the downloaded data differed based on which unit they were downloaded to, and was therefore changed to one format using Microsoft Excel. Behavioural data was then imported from Excel into the software SAS 9.4 (SAS, 2012) for statistical analysis.

Behavioural data collected over the experimental days were analysed as repeated measurements using SAS Proc Mixed with a compound symmetry (CS) covariance structure as described below:

$$Y_{ijk} = \mu + T_i + C_j + Day_k + Cov_j + b_1 * MY + b_2 * DMI + \varepsilon_{ijkl}$$

where Y_{ijk} = response variable (behaviour), μ = overall mean, T_i = effect of treatment ($i = 1, 2, 3$), C_j = the random effect of cow in a treatment, Day_k = the fixed effect of day of measurement, Cov_j = the fixed effect pre-experimental recorded behaviour for a cow, $b_1 * MY$ = effect of level of milk yield, $b_2 * DMI$ = effect of total DMI, and ε_{ijkl} = residual error term.

Qualitative variables included in the model were cow ID, day of treatment (DOT), DIM, and treatment. Fixed effects included in the model were treatment, DOT, total DMI, and MY. Data were grouped by treatment, and it was assumed that total DMI and MY had a correlation with behaviour response variables. Dependent variables included in the model were the behaviour parameters: Chewing time (calculated from eating time + ruminating time), eating time, ruminating time, lying time, inactive time, as well as these mentioned variables calculated in min/kg total DMI. Cow ID was defined as both a random effect and a repeated measure, and values from the two-week adaptation period were included as a covariate in the mixed model.

The Proc Mixed analysis assumes the data are normally distributed, and the means linear. It also assumes that variances and covariances exhibit a certain pattern, described by an error

correlation structure. Compound symmetry (CS) was used to explain correlations between residual errors of repeated measures, and correlated errors between timepoints within each cow were presumed to be the same for each set of timepoints. Since measures were repeatedly taken on each cow, at different points in time, observations were not assumed to be independent (Penn State University, n.d.).

Least Squares Means (LS-means), predicted population margins, were estimated for each treatment and variable. Standard Error Means (SEM) were estimated for each variable and given as the arithmetic average of treatments, along with p-values for treatment effects on each response variable. Computed differences of LS-means were used to assess whether or not treatments differed significantly from each other: $Pr > |t|$ for $H_0: LS\text{Mean}(i) = LS\text{Mean}(j)$, where i and j are treatments (SAS Institute Inc, 2019). P-values of ≤ 0.05 were considered significant, and $\geq 0.05 \leq 0.10$ regarded as tendencies.

4.0 Results

The following chapter presents results from measurements and sampling, starting with analyses of the feed. Feed intake, milk yield (MY) and body weight (BW) are covered, before moving on to the data recorded by Nedap sensors on the animals. Finally, statistical analyses will determine correlations and significance of results.

4.1 Feed analyses

Analyses of the feed used in the covariate period and in the trial period are given in tables 3-4 below. From table 3 we see that of the concentrates given, Soy had the highest content of CP and WSC, and the least DM and ash; Yeast had the highest content of fibre (NDF and NDF corrected for organic matter: NDFOM), and the least fat; Barley contained the least CP, NDF and WSC.

Table 3. Analysis of concentrate feeds given in the covariate period and for each treatment in the trial period.

	g/kg	g/kg DM					
	DM	Ash	CP	NDF	NDFOM	Fat	WSC
Soy*	920.2	65.6	164.43	180.4	173.7	41.7	60.0
Soy	923.2	63.9	160.12	187.7	180.7	41.6	63.1
Yeast	936.2	67.4	154.29	193.2	185.1	40.7	58.5
Barley	936.4	68.2	134.03	181.2	174.6	41.9	56.8

* Soy concentrate used in the covariate period (weeks 1-2).

DM = dry matter; CP = crude protein; NDF = neutral-detergent fibre; NDFOM = neutral-detergent fibre corrected for organic matter; WSC = water-soluble carbohydrates.

Table 4 shows the analysis of silage given. Analyses are split into the covariate period (weeks 1-2) and the trial period (weeks 3-10). In the covariate period, silage had less CP, NDF and NDFOM, than the following weeks, while CHO and DM contents were higher.

Table 4. Analysis of silages given in weeks 1-2, 3-6, and 7-10. The mean values are arithmetic for all weeks in total.

	g/kg	g/kg DM							
	DM	Ash	CP	NDF	NDFOM	CF	WSC	FPF	CHO
W 1-2	303	79.68	179.89	524.55	513.67	45.73	17.94	98.90	64.19
W 3-6	295	67.89	184.76	539.57	528.93	48.52	18.98	96.40	54.52
W 7-10	303	73.99	182.48	533.27	522.42	44.75	13.19	104.30	58.86
Mean	300	73.85	182.38	532.46	521.67	46.33	16.70	99.87	59.19

DM = dry matter; CP = crude protein; NDF = neutral-detergent fibre; NDFOM = neutral-detergent fibre corrected for organic matter; CF = crude fat; WSC = water-soluble carbohydrates; FPF = silage fermentation products; CHO = residual carbohydrates.

4.2 Feed intake, milk yield, body weight

From the covariate period to the trial period, average daily DMI (dry matter intake) decreased for all groups, shown in table 5. The difference was bigger for concentrates DMI than silage DMI, which was by design (ref. chapter 3.3).

Intake of NDF decreased for all groups between the two periods. The reduction was larger for the barley group than for the soy and yeast groups, shown in table 5.

Table 5. Average values of feed intake, as arithmetic means. Values for DMI are given in kg DM per day, while NDF intake is given as g/kg DM. Numbers are divided into covariate period ('cov') and trial period ('trial'), for each treatment.

	Concentrate DMI		Silage DMI		Total DMI		NDF intake	
	Cov	Trial	Cov	Trial	Cov	Trial	Cov	Trial
Soy	8.79	7.46	14.80	14.61	23.59	22.08	9466.5	9179.7
Yeast	9.03	7.59	15.17	14.94	24.21	22.57	9706.9	9421.3
Barley	9.29	7.94	13.70	13.51	23.00	21.46	8971.1	8632.1

Milk yield was relatively stable between the treatment groups, at approximately 30 kg/day on average as shown in table 6. All treatment groups reduced their MY during the trial period, as expected with progressing lactation stage. Body weight increased for the soy and yeast groups by 13.79 and 15.17 kg, respectively, while decreasing for the barley group by -5.87 kg.

Table 6. Average MY per day and body weight BW given as arithmetic means, divided into covariate and trial periods for each treatment.

	MY (L)		BW (kg)	
	Cov	Trial	Cov	Trial
Soy	31.23	29.56	632.43	646.22
Yeast	32.79	30.15	636.04	651.21
Barley	33.93	30.84	623.20	617.33

4.3 Behaviour (Nedap data)

Figure 3 shows the group averages in behaviours as recorded by Nedap sensors on the animals, for the covariate and trial periods. Eating time decreased slightly for all groups, but remained relatively stable, where the soy group had the highest eating time and yeast the lowest. Ruminating time saw a minor increase for all groups. The soy and yeast groups were almost equal in ruminating time, during both periods. Chewing time represents the sum of

eating + ruminating time, and we see that total chewing time decreased slightly for the yeast and barley groups (by five and seven minutes, respectively), while the soy group was stable.

All groups had a minor increase in lying time. The highest lying time was seen in the yeast group, in both periods; the lowest was seen in the soy group. Inactive time was stable for the yeast and barley groups, with a decrease for the soy group by nine minutes.

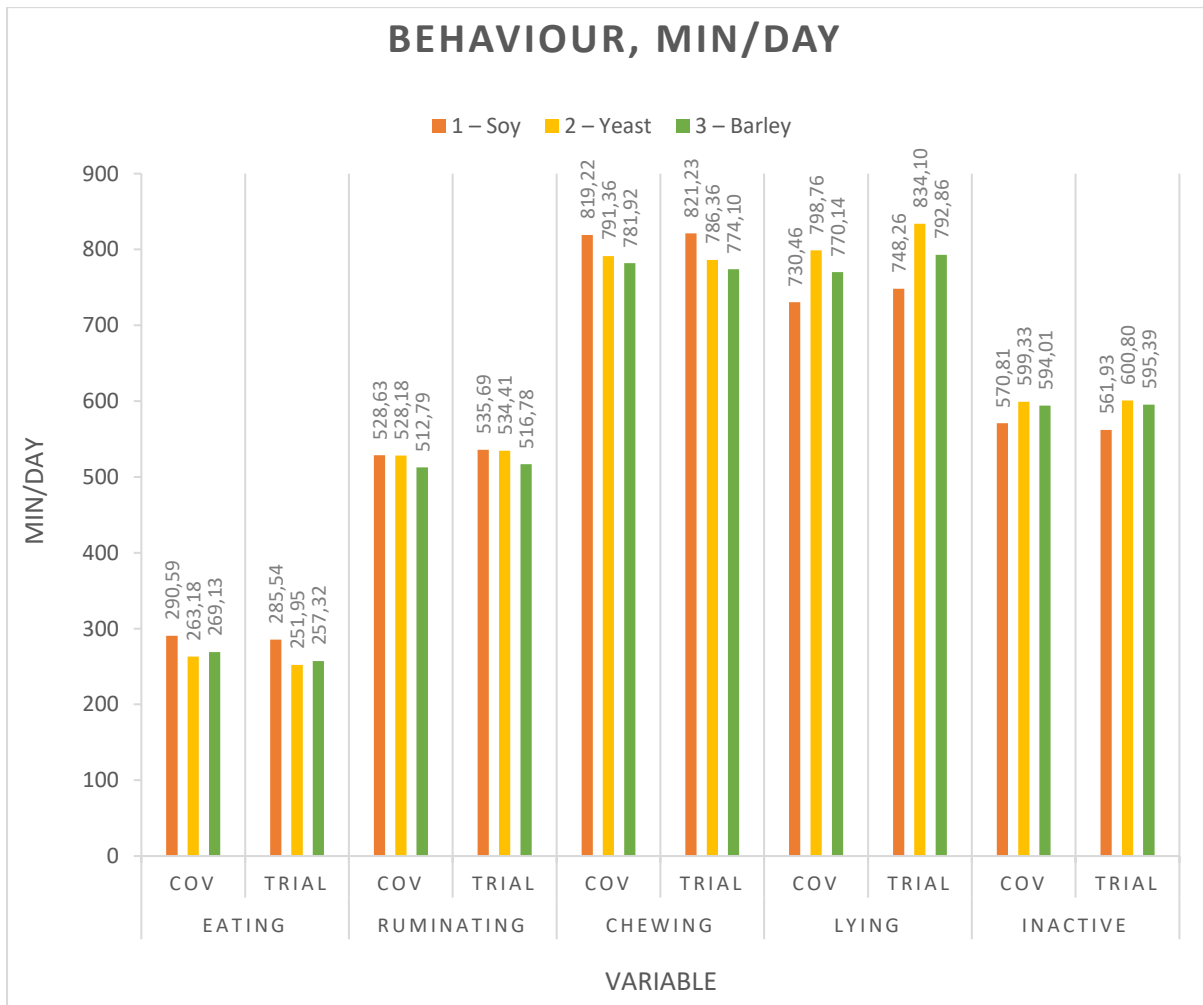


Figure 3. Behaviour variables in minutes per day, for the covariate and trial periods. Values are given as arithmetic means for each treatment.

Figure 4 shows that per kg DMI, ruminating and chewing times were lowest for the yeast group. The barley group showed a slight increase in eating, lying and inactive times relative to the other groups. Overall, changes from the covariate to the trial period were minimal.

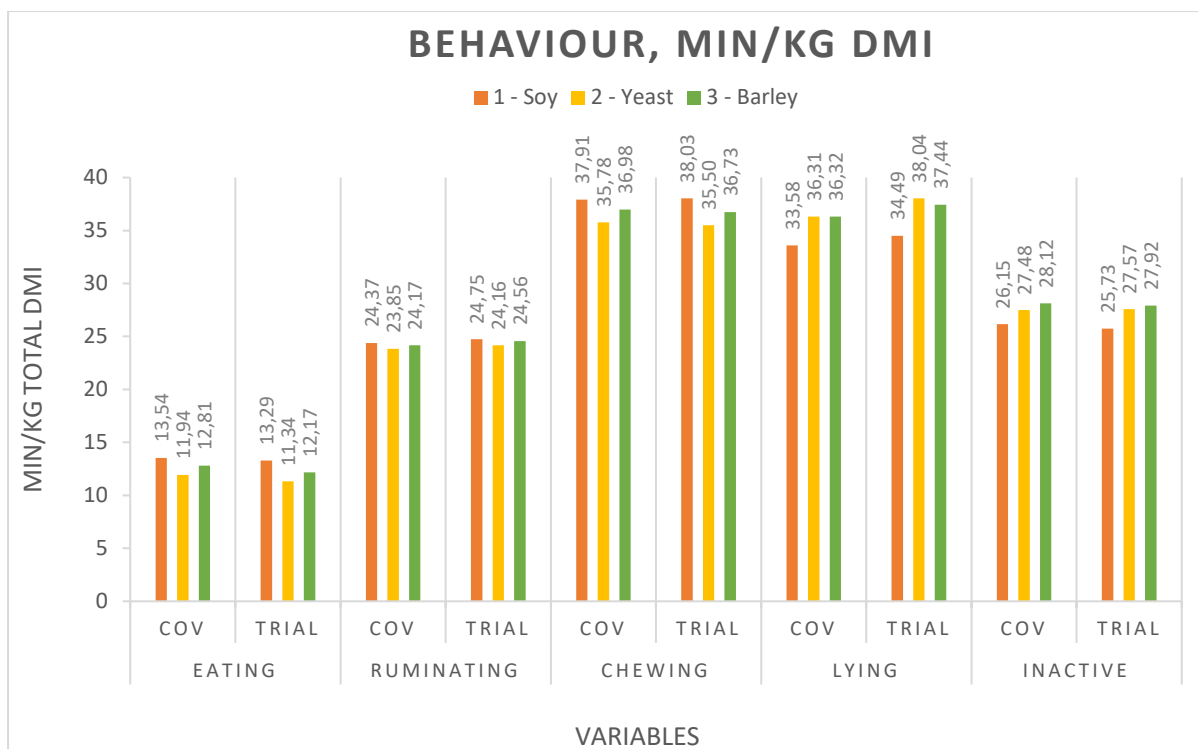


Figure 4. Behaviour variables calculated as minutes per kg total DMI, for the covariate and trial periods. Values are given as arithmetic means for each treatment.

Table 7 shows Nedap behaviour data, with values converted to hours. All treatment averages were within the recommended eating and ruminating times (ref. chapter 2.2). Eating times were on the low end at under five hours, while ruminating times were on the high end at over eight hours. Total chewing time for all groups was between twelve and fourteen hours. The lowest chewing time was seen in the barley group; the highest chewing time was seen in the soy group.

Table 7. Average number of hours spent on each behaviour, as arithmetic means per day of the covariate and trial periods.

	Eating		Ruminating		Chewing		Lying		Inactive	
	Cov	Trial	Cov	Trial	Cov	Trial	Cov	Trial	Cov	Trial
Soy	4.84	4.76	8.81	8.93	13.65	13.69	12.17	12.47	9.51	9.37
Yeast	4.39	4.20	8.80	8.91	13.19	13.11	13.31	13.90	9.99	10.01
Barley	4.49	4.29	8.55	8.61	13.03	12.90	12.84	13.21	9.90	9.92

The sum of “chewing + inactive” time should explain all feeding behaviours or absence of such behaviours taking place within a day. Chewing time involves “eating or ruminating” while inactive time involves “not eating or ruminating”. As figure 5 shows, the sum of chewing + inactive time was around twenty-three hours for all treatments, leaving approximately one hour uncategorised by Nedap, per twenty-four hours.

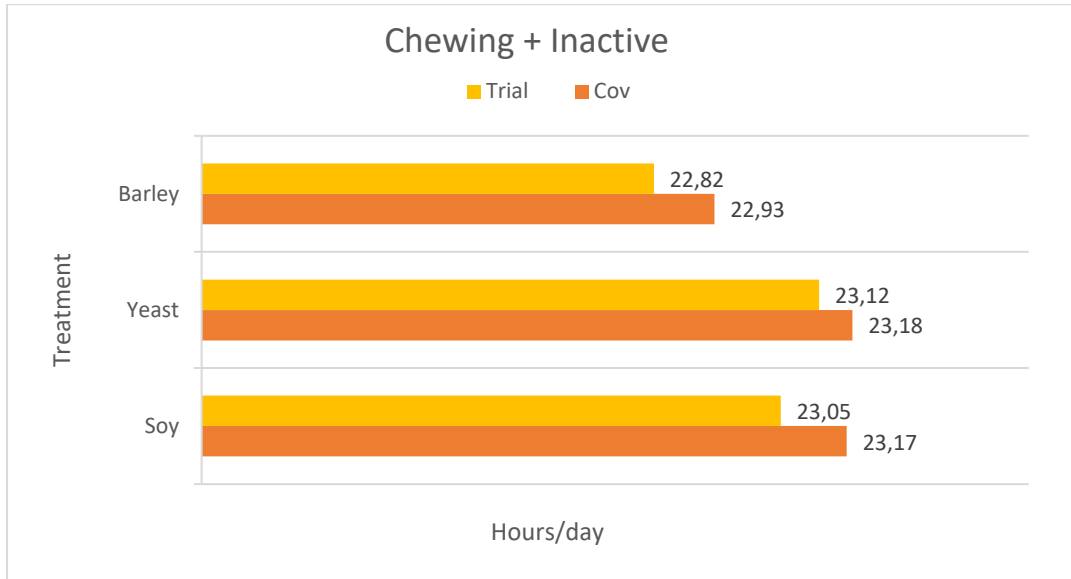


Figure 5. The total average number of hours spent on chewing and inactive behaviours, as arithmetic means per day of the covariate and trial periods.

As shown in figure 6 below, the barley group was the only one where BW decreased, and MY decreased by 9 %, more than for the other two groups. The animals in the barley group had a lower BW than the other two groups from the start, as well as higher MY. The yeast group had the highest BW throughout the trial, and gained the most weight. Their MY was slightly higher than for the soy group, and decreased by 8 %. The soy group had the lowest MY through the trial. However, MY decreased by 5 %, less than for the other two groups. The soy group also gained weight during the trial, nearly as much as the yeast group.

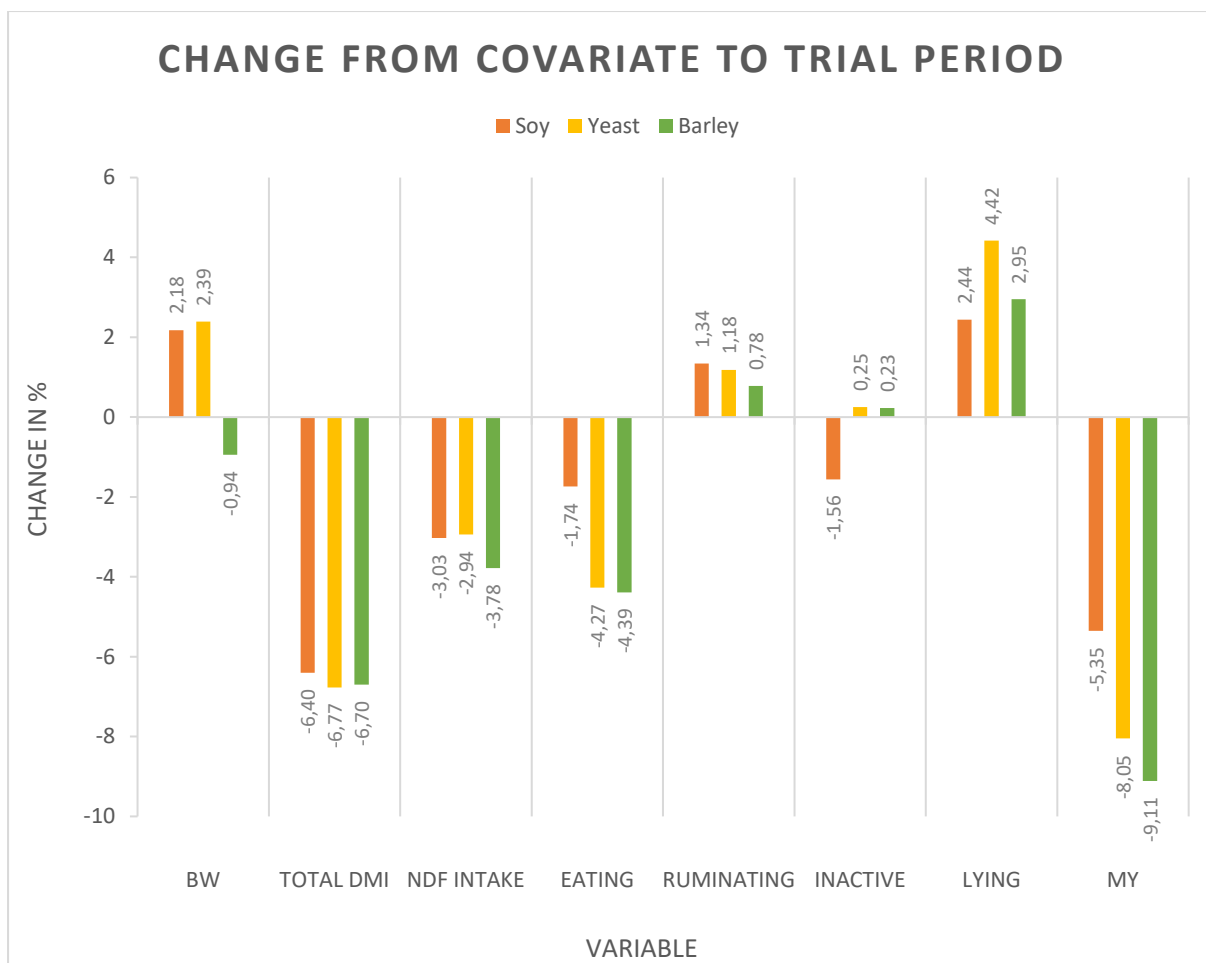


Figure 6. Values for behaviour, feed intake and productivity given as % change from the covariate to the trial period. Percentages are based on arithmetic means.

BW = body weight; total DMI = total dry matter intake; MY = milk yield.

4.4 Statistical analysis

4.4.1 Least squares means and fixed effects

Results from statistical analysis in SAS are presented below, with significant p-values ≤ 0.05 shown in *italics*.

Table 8 shows the following results for all variables and treatments: LS-means estimates, SEM given as the arithmetic average of treatments, and p-values ($\text{Pr} > |t|$) for correlations between treatments and each response variable. P-values were found using type 3 tests of fixed effects. The five bottom response variables represent behaviour in minutes per kg of total DMI. According to the results in table 8, the method of LS-means showed no significant

treatment correlations with any variables. The highest p-value was seen for Ruminating, and the lowest for Lying.

Table 8. Least squares means (LS-means), SEM and p-values for treatments and behaviour response variables.

LS-MEANS	Soy	Yeast	Barley	SEM	Pr > t
Eating	270.5	258.1	266.7	7.77	0.520
Ruminating	531.6	528.8	530.1	4.98	0.925
Chewing	804.0	785.2	796.5	10.91	0.469
Lying	774.1	811.2	786.0	15.77	0.260
Inactive	576.0	597.6	578.9	11.28	0.342
Eating min/kg DM	12.7	11.8	12.3	0.39	0.269
Ruminating min/kg DM	24.6	24.5	24.4	0.25	0.896
Chewing min/kg DM	37.4	36.2	36.6	0.54	0.322
Lying min/kg DM	36.0	37.5	36.4	0.79	0.402
Inactive min/kg DM	27.2	27.3	26.7	0.70	0.805

Table 9 below shows p-values for the fixed effects in the model, using type 3 tests of fixed effects. P-values are shown for all fixed effects on all variables, including behaviour in minutes per kg of total DMI. The fixed effects include the covariate adaptation period ('COV'), DOT, total DMI, and MY.

There are significant p-values seen for COV and DOT on all variables. Total DMI shows significant correlation with all variables except for Chewing min/kg DM and Inactive min/kg DM. However, significant effects are seen on both Ruminating min/kg DM and Eating min/kg DM. Milk yield (MY) shows significant correlation with Eating, Lying, Eating min/kg DM and Lying min/kg DM.

Table 9. Type 3 tests of fixed effects on all behaviour response variables.

FIXED EFFECTS	COV	DOT	Total DMI	MY
Eating	< 0.0001	< 0.0001	< 0.0001	0.015
Ruminating	< 0.0001	< 0.0001	< 0.0001	0.351
Chewing	< 0.0001	< 0.0001	< 0.0001	0.309
Lying	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Inactive	< 0.0001	< 0.0001	< 0.0001	0.607
Eating min/kg DM	< 0.0001	< 0.0001	< 0.0001	0.014
Ruminating min/kg DM	< 0.0001	< 0.0001	< 0.0001	0.539
Chewing min/kg DM	< 0.0001	< 0.0001	0.144	0.205
Lying min/kg DM	< 0.0001	< 0.0001	< 0.0001	0.0001
Inactive min/kg DM	< 0.0001	< 0.0001	0.237	0.984

4.4.2 Differences of least squares means

Tables 10-14 show Differences of LS-means for each behaviour response variable. This method compares two and two treatments through estimates, SEM and p-values.

Table 10 shows Differences of LS-means for the variable Chewing (eating time + ruminating time). P-values show no significant differences between treatments on Chewing, and SEM are similar.

Table 10. Differences of LS-means for response variable Chewing.

		Chewing		
Treatments		Estimate	SEM	Pr > t
Barley	Soy	-7.54	15.64	0.63
Barley	Yeast	11.34	15.35	0.46
Soy	Yeast	18.88	15.49	0.22

Table 11 shows Differences of LS-means for the variable Eating. P-values show no significant differences between treatments, and SEM are similar.

Table 11. Differences of LS-means for response variable Eating.

		Eating		
Treatments		Estimate	SEM	Pr > t
Barley	Soy	-3.75	11.05	0.73
Barley	Yeast	8.62	10.93	0.43
Soy	Yeast	12.37	11.13	0.27

Table 12 shows Differences of LS-means for the variable Ruminating. P-values show no significant differences between treatments, and SEM are similar.

Table 12. Differences of LS-means for response variable Ruminating.

		Ruminating		
Treatments		Estimate	SEM	Pr > t
Barley	Soy	-1.48	7.08	0.83
Barley	Yeast	1.28	7.09	0.86
Soy	Yeast	2.76	7.01	0.69

Table 13 shows Differences of LS-means for the variable Lying. P-values show no significant differences between treatments. Soy vs. Yeast had a slightly higher SEM than the other comparisons.

Table 13. Differences of LS-means for response variable Lying.

		Lying		
Treatments		Estimate	SEM	Pr > t
Barley	Soy	11.95	22.31	0.59
Barley	Yeast	-25.12	22.10	0.26
Soy	Yeast	-37.07	23.15	0.11

Table 14 below shows Differences of LS-means for the variable Inactive. P-values show no significant differences between treatments, and SEM are similar.

Table 14. Differences of LS-means for response variable Inactive.

Treatments		Inactive		
		Estimate	SEM	Pr > t
Barley	Soy	2.88	16.01	0.86
Barley	Yeast	-18.70	15.91	0.24
Soy	Yeast	-21.58	16.06	0.18

5.0 Discussion

The following chapter discusses results with relevant literature. Possible sources of measurement errors are illuminated, followed by an evaluation of the statistical method used. Assessments of feed compositions precede a discussion of the main treatment effects.

5.1 Sources of error

5.1.1 Animals

During the trial, one of the cows suffered an injury and was euthanised. Replacement cows had been prepared, receiving the same treatment as the experimental cows. Upon losing one cow, a replacement cow was rotated into the trial in its place. As such, the results should not be affected when looking at treatment effects.

5.1.2 Feeding

The automatic feeding systems did not weigh leftovers of concentrate feed, hence the assumption is made that cows ate all the proffered concentrates every day of the trial. Moreover, the level of concentrates given was individually adapted to each cow's milk yield, making it likely that any amounts of concentrate leftovers were small. Feed preferences may however influence willingness to enter the AMS, affecting both concentrate DMI and MY.

5.1.2 Nedap sensors

Several times, Nedap sensors were discovered to be in the wrong position on a cow. Warning of a sensor in the wrong position was given on the connected Nedap website. The sensor then

had to be placed back into correct position by staff at SHF. In the time elapsed from detection to correct replacing of the sensor, data recorded is less reliable. It can nevertheless be assumed that any error caused by the sensors will be equal for all treatment groups.

There are 1.440 minutes in a twenty-four-hour day, but when adding up recorded minutes of different behaviours, they equate to more than 1.440. This is likely caused by the overlap of lying time with ruminating and inactive time. As Nedap sensors count behaviours separately also when occurring simultaneously, those minutes will have been “counted twice”. When adding up only chewing time and inactive time, total time reached around twenty-three hours/day for all treatments, leaving approximately one hour/day unaccounted for (figure 5). A cow could have spent this last hour walking, drinking, and performing social behaviours. Since measured behaviour adds up to approximately twenty-three hours for all treatment groups, any measurement errors like under- or overestimation, are considered similar or minor between groups.

Nedap data were automatically downloaded in different formats by laptops and an Android mobile phone. The format downloaded to the mobile phone was challenging to work with. Creating one uniform, functional format for all downloaded data was complex and time-consuming. Despite efforts to prevent any loss of data points in this process, the possibility can not be ignored.

5.2 Data analysis

As the current experiment used an adaptation period as baseline, the baseline values were included as a covariate in the statistical model, as suggested by Committee for Proprietary Medicinal Products (CPMP) (2003). A statistical model with a covariate included is known as ANCOVA (analysis of covariance), and recommended when group assignment is randomised like in this experiment. It is also recommended if group assignment is based on data from the adaptation period, but not for pre-existing groups (van Breukelen, 2013). In the ANCOVA mixed model a repeated measure (cow ID) was included since measurements were repeated on each cow. According to SAS Institute Inc. (2015) a mixed linear model is appropriate to use when handling repeated measures.

‘Change from baseline’ was discussed as an alternative model for considering the adaptation period, where the difference between the covariate and trial periods was included as a dependent variable in a linear model. This model yielded significant results in terms of

differences between treatments, and effects of treatments on response variables; it was however incomplete as it lacked repeated measurements of cow ID. The ANCOVA model used for statistical analysis gave less significant results than the ‘change from baseline’ model. Contradictory results between the models can happen if groups differ in the adaptation period, known as Lord’s paradox (van Breukelen, 2013). This could signify that the grouping of animals into different treatments was not optimal, and that the groups were intrinsically different from each other.

Alternatively, the lesser treatment effects seen from using ANCOVA could have been caused by the inclusion of both DMI and MY as fixed effects in the model. These factors were included to adjust for their effects on feeding behaviour, without regard to DMI and MY also being correlated with each other. Moderate to strong correlations ($r = 0.47-0.85$) between DMI and MY were summarised in the meta-analysis by Hristov et al. (2004), where they specified that DMI was the major influencing variable on MY. Although the interrelationship between DMI and MY differs in strength between trials, it is nonetheless present. Consequently, including both DMI and MY in the statistical model is surmised to have had an undesired effect on results, and possibly reduced any treatment effects. The inclusion of DMI and MY in the model seems a more probable explanation of different results than Lord’s paradox, especially considering the grouping strategy of animals pre-trial.

5.3 Feed composition and intake

5.3.1 Concentrate feed

The soy-based concentrate feed (‘Soy’) was designed to be iso-nitrogenous with the yeast-based concentrate feed (‘Yeast’), but as shown in table 3, Soy did have a slightly higher CP content than Yeast. A higher content of CP could improve rumen microbe growth, if energy levels are sufficient to ensure optimal nitrogen utilisation. Indeed, the WSC level is highest in Soy, providing easily available carbohydrates for rumen microbe energy supply.

At the other end, the barley-based concentrate feed (‘Barley’) had the least CP, NDF and WSC. According to table 3, Barley had a higher DM content than Soy and Yeast, but also the most ash, which has no nutritional value and indicates that Barley is lowest in energy (McDonald et al., 2011).

The highest level of NDF and NDFOM is seen in Yeast (table 3), supplying the rumen microbes with material for VFA production. Acetate in particular is the result of rumen NDF fermentation. Acetate is considered the most important energy source for cows, increasing MY as well as milk fat content through its role in the *de novo* synthesis (Harstad, 2018).

Research has shown that cows may change their eating behaviour based on individual preferences of the feed given. Forbes and Kyriazakis (1995) stated that innate individual feed preferences are of less importance than the nutritional value of the feed. Over time animals can learn to associate different feed types with their respective nutritional contents, making learning experiences more important than sensory qualities of the feed. One experiment observed that cows do not discriminate between pelleted feed of different contents; the same preference was shown for pellets containing soybean meal as for pellets where soybean meal had been replaced with rapeseed or pea meal (Spörndly & Åsberg, 2006). On the other hand, Madsen et al. (2010) found that cows were more eager to eat some types of pelleted feed than others, affecting their behaviour in an AMS situation. A preference for soy-based concentrate feed was seen by Primdal (2013), where CP content was higher, and NDF lower, than control. In the current experiment Soy also stands out as the feed type with highest CP and lowest NDF contents, leading to speculation of whether true concentrate DMI was affected by preference.

5.3.2 Silage

As roughage constitutes the main part of ruminant diets, it plays a major role in feeding behaviour and chewing time. All cows were offered the same silage *ad libitum* throughout the trial. Hence, no differences in behaviour between treatments are attributed to silage qualities.

When comparing mean silage values from table 4 to reference values from Eurofins (Eurofins Agro Testing Norway AS, n.d.-a), the current silage was higher in NDF, CP, and CF, while DM content was slightly lower.

The ratio of silage to concentrate feed was designed to be 60:40 on a DM basis for all groups. The content of DM in Soy was lower than in Yeast and Barley, meaning the amount of concentrate feed given could have been affected. This is of interest as concentrate feed intake affects silage intake (the substitution effect, ref. chapter 2.2.2). Nonetheless, silage DMI was approximately equal for all groups, though slightly lower for the barley group (table 5).

According to Harstad (2016), there is no clear substitution effect seen in a ration with less than 60 % concentrate feed on a DM basis.

5.3 Treatment effects

5.3.1 Productivity

All groups decreased their MY (table 6) with increasing stage of lactation, as expected. The average MY for all groups was around 30 L per day, with the largest reduction seen in the barley group, followed by the yeast group. Meanwhile, the soy group had the smallest reduction of MY throughout the trial. As previously stated, roughage is of utmost importance to milk production. Nevertheless, a more stable MY in the soy group should not be attributed to a higher silage DMI, since all groups had a similar DMI. Alternative explanatory factors include protein and glucose intake, both vital for milk production (Harstad, 2018; Hristov et al., 2004). Protein and glucose intakes are explained by CP and WSC intakes, respectively, and the highest contents of these were seen in Soy.

As explained by table 9, MY was significantly correlated ($p \leq 0.05$) with eating and lying parameters. The soy group had the highest eating time, but the lowest lying time; the highest lying time was seen in the yeast group during both periods. Decreased MY for the yeast group was almost as large as for the barley group however, leading to doubt around the accuracy of MY and lying time correlation. Evidence is varied when explaining MY in terms of feeding behaviour.

According to the results of Shabi et al. (2005), eating time had a positive effect on MY, whereas feed intake had a weaker effect. Meanwhile, Nielsen et al. (2000) saw that roughage level in the diet, and stage of lactation, had greater effect on time budget than individual milk yield. Nonetheless, Norring et al. (2012) observed effects of MY on ruminating time after adjusting for lactation stage: High-yielding cows had longer ruminating time than low-yielding cows. A positive relationship between MY and ruminating time is supported by Beauchemin (2018), although negative correlations have been found between MY and chewing- and ruminating times per kg DMI by Dado and Allen (1994). They saw that high-yielding dairy cows were able to consume and chew feed more effectively than low-yielding cows. A negative correlation between MY and ruminating time was also seen by Byskov et al. (2015). However, 48 % of the variation in ruminating time was explained by individual

variations between cows, emphasizing the uncertain connection between MY and feeding behaviour.

Seeing as the yeast group had the highest NDF- and total DMI intakes, one might attribute their relatively large decrease in MY to factors other than energy intake and stage of lactation. As mentioned, CP and WSC have a role to play in milk production; levels of both are lower in Yeast than in Soy and may explain how the yeast and soy groups could both gain BW, while the soy group maintained a more stable MY than the yeast group.

Body weight increased similarly for the soy and yeast groups in the trial period. Increased BW signifies a ration sufficient in energy, even though MY was decreased. Simultaneously, the barley group decreased both MY and BW in the trial period, signifying an energy-deficient ration (McDonald et al., 2011). Energy deficiency in the barley group can be explained by the higher ash content in Barley, and the slightly lower silage DMI of the barley group compared with the other groups.

5.3.2 Feeding behaviour

Using feeding behaviour as a measure of feed quality, all rations had healthy levels of energy and fibre (ref. chapter 2.2). The values in table 7 show that all treatment groups averaged just over four hours of eating time per day, and just below nine hours of ruminating time per day. This puts eating time on the lower end of the scale, and ruminating at the high end. Total chewing time of approximately thirteen hours per day signifies a ration with sufficient energy for a lactating cow, although we know this is most likely not the case for the barley group with its reduced BW. The barley group did have slightly lower chewing time than the soy and yeast groups, supporting the notion of lower energy supply for the barley group.

The current silage NDF content typically corresponds to a chewing time of 68-79 min/kg DMI (Eurofins Agro Testing Norway AS, n.d.-b), while the range as seen in figure 4 is at 35-38 min/kg DMI. Reference values are for silage only, while the measured chewing time is for the total ration including concentrate feed. Concentrate feed is high in DM yet low in NDF, and has a short rumen retention time which does not stimulate rumination. Consequently, the relatively low chewing time/kg DMI is attributed to the inclusion of concentrate DMI.

The current trial saw no significant differences in feeding behaviour between treatment groups (tables 10-14). Trends and smaller differences will nonetheless be discussed. The highest chewing time is found in the soy group, due to also having the highest eating time,

while the yeast and barley groups were similar. Ruminating time was lowest in the barley group, while the soy and yeast groups were similar. Although a relationship between eating- and ruminating time is unclear in the current trial, Schirmann et al. (2012) reported that longer ruminating time coincided with shorter eating time.

The yeast group had the highest NDF concentration and -intake, while the barley group had the lowest. As observed by Beauchemin (1991), increasing NDF concentration in rations to dairy cows gave increased total eating time and, consequently, chewing time; meanwhile, ruminating time decreased. This is contrary to a previous study where ruminating time increased with increasing NDF concentration (Beauchemin & Buchanan-Smith, 1989). Moreover, Krause et al. (2002) found no correlation between NDF intake and chewing activity, whereas Nørgaard et al. (2011) saw that ruminating time may have a higher correlation with NDF intake than NDF concentration in the ration. Increased ruminating time was related to increased starch and forage NDF intake in the trial by Byskov et al. (2015), while sugar and remaining CHO had a negative connection with ruminating time. De Mol et al. (2016) hypothesized that different results across studies might be due to differing methods in detecting ruminating time, or differences in the feed compositions. A note should be made of the accuracy of ruminating time measures, as the behaviour can be difficult to observe. Sensors may also classify ruminating behaviour differently from visual observers (Van Erp-Van der Kooij et al., 2016).

Table 9 shows that total DMI was significantly correlated with all behaviour parameters except for Chewing min/kg DM and Inactive min/kg DM. However, significant correlations with total DMI are seen for both Ruminating min/kg DM and Eating min/kg DM. Seeing as chewing time is the sum of eating- and ruminating time, the lack of significance for Chewing min/kg DM seems unlikely. This could be due to the statistical model and procedure, where total DMI is used both as a factor in response variables (min/kg DMI), and as a fixed effect in the model. Contrary to current results, Clément et al. (2014) found no significant interaction between DMI and ruminating time, nor did Schirmann et al. (2012). De Mol et al. (2016) did however find correlations between eating time and total feed intake ($r > 0.53 < 0.59$).

While the grouping of cows in the current trial was adjusted for MY, parity and lactation stage to reduce noise, feeding behaviour is more complex than simply recording eating- and ruminating time. Researchers have reported large variations between the feeding behaviours of individual cows (Beauchemin, 1991; Friggens et al., 1998), and others have looked at

social and herd effects on feeding behaviour. Maekawa et al. (2002) saw that multiparous cows spent more time eating and ruminating per day than primiparous cows, after correcting for DMI. Shorter eating time in primiparous cows was also found by Theije (2017), in herds using an automatic milking system. Another trial showed that placing multiparous and primiparous cows in two separate groups was advantageous in terms of increased feed intake and MY for primiparous cows (Grant & Albright, 2001). The varying results of eating time in dairy cows found across studies could be due to differences in how eating time was defined in the studies, or differences in feed management and animals (Beauchemin, 2018). For instance, eating rate (kg DM/min) can increase in a competitive feeding environment, decreasing total eating time per day while maintaining DMI. This was seen by Proudfoot et al. (2009) in multiparous dairy cows, and supported by the results of Crossley et al. (2017). In an AMS situation however, like in the current trial, feeding competition should be less than when feed is delivered to all cows simultaneously (Theije, 2017). Another contributing factor to eating time is age, as older cows generally eat faster than younger cows (Dado & Allen, 1994).

5.3.3 Lying and inactive behaviour

The motivation for lying has been observed to be stronger than the motivation for eating after a short time of being deprived of both (Metz, 1985). Cows can spend around 50 % of their time lying down in a drowsy state, and approximately a third of this in a sleep state (Ruckebusch, 1972). Cows in the current trial spent twelve to fourteen hours per day lying down (table 7), and were thus within this range. Lying behaviour recorded by Nedap Smarttag Leg has been found to match visual observations with great accuracy (Van Erp-Van der Kooij et al., 2016), making it likely that these cows truly did spend over half their time lying down. The current trial found significant correlations between lying time and MY (table 9), although no significant differences in lying time were seen between groups (table 13). From figure 3 we saw that total lying time increased for all groups from the covariate to the trial period, i.e. with advancing lactation stage, in line with previous research.

Norrington et al. (2012) saw that multiparous cows did more of their ruminating lying down than primiparous cows, increasing their total lying time. Deming et al. (2013) observed that when MY decreased with advancing lactation stage, total lying time increased. Norring et al. (2012) also saw that high-yielding cows had shorter lying time than low-yielding cows, and fell asleep faster. Lying bout frequency however, decreased with advancing lactation stage. This

could indicate that high-yielding cows have a higher motivation for lying down than low-yielding cows, a hypothesis later strengthened (Norrington & Valros, 2016).

While the motivation of high-yielding cows to lie down appears high, their shorter total lying time might be due to a strong motivation to eat in order to sustain milk production. High-yielding dairy cows generally have higher eating time to achieve sufficient feed intake for their milk production, leaving less time for other activities including lying; ultimately, high-yielding cows may have trouble fulfilling their needs for both eating and lying (Botheras, 2007).

‘Inactivity’ is here classified as time not spent on feeding behaviours nor on physical activities like standing up/lying down, walking, or social interactions. Succinctly, inactive behaviour excludes all activities besides vital movements like breathing. If inactive time is longer than normal, something could be wrong with the cow (Lammers & Harbers, 2019). Results from a trial by Stevenson et al. (2020), using the CowManager SensOor ear tag (Agis, Harmelen, Netherlands), showed that inactive time increased in sick cows compared with healthy cows.

All treatment groups averaged at approximately ten hours of inactive time per day (table 7). The yeast group had a slightly higher inactive time than the other groups. However, no significant differences were found, indicating that health status was similar for all groups.

According to Van Erp-Van der Kooij et al. (2016), recordings of ‘resting time’ by the Nedap SmartTag Neck were inaccurate. When comparing sensors with visual observations, discrepancies were noticed: The sensors seemed to be more particular than observers in what they classified as rest, insofar as any head movements while resting were recorded as ‘remaining behaviour’. Observers however, still recorded it as rest. The sensors also included short bouts of resting in-between ruminating as resting time, where observers classified it as ruminating time. As such, a distinction should be made between the classifications ‘inactivity’ and ‘rest’ when considering this behaviour in previous studies of time budgets.

5.4 Yeast in livestock diets

Using yeast-based protein in concentrate feed showed no significant effects on the behaviour parameters measured compared with conventional concentrate feeds containing soy protein and barley. No harmful effects were seen from replacing soy protein with yeast protein, but

the yeast and barley groups both had steeper declines in MY with advancing lactation stage than the soy group.

As the most widely commercially available yeast strain, *S. cerevisiae* is supplemented to livestock feed to a larger extent than *C. utilis*. Past publications have demonstrated that additions of *S. cerevisiae* to dairy cow diets can affect feeding behaviour and productivity. Diets supplemented with *S. cerevisiae* tended to give longer ruminating times (DeVries & Chevau, 2014), increased MY (Ramsing et al., 2009), increased DMI, and less BW drop during early lactation (Dann et al., 2000). In a meta-analysis, Desnoyers et al. (2009) looked at supplementation of *S. cerevisiae* in diets for ruminants including cattle, goats, sheep, and buffaloes. Collective results across 157 experiments showed increased DMI and rumen pH in animals fed the yeast-supplemented diet. Another study saw effects of live *S. cerevisiae* on meal patterning, in that cows ate smaller, more frequent meals. This feeding strategy helps to promote a stable rumen pH (DeVries & Chevau, 2014; DeVries, 2018). Supporting these results are those of Bach et al. (2007), where meals were also more frequent, and rumen pH more stable after meals, when supplementing with live *S. cerevisiae*.

The amount of research on *S. cerevisiae* is substantial, and there are fewer studies with *C. utilis* supplementation in dairy cow diets. Several animal species have been given diets with *C. utilis*, including fish, poultry, and pigs.

Fishmeal is a valuable ingredient in diets for Atlantic salmon, but a limited resource. When partially replacing fishmeal with protein from different yeast strains, *C. utilis* and *K. marxianus* both rivaled the control fishmeal diet in nutrient digestibility. In contrast, *S. cerevisiae* gave lower nutrient digestibility and growth than the other diets, and was ranked the least suitable yeast strain for Atlantic salmon (Øverland et al., 2013). Replacement of soybean meal with fishmeal and *C. utilis* in diets for Atlantic salmon gave higher growth rate than with the control soy-based diet. When *C. utilis* was added to a soy-based diet however, it was unable to counteract the negative effects of soybean meal on salmon intestinal health (Hansen et al., 2019).

Trials with pigs have shown that *C. utilis* might replace conventional protein sources. One trial concluded that *C. utilis* can replace fishmeal in pig diets if balanced for amino acid composition (Lagos & Stein, 2020). More interesting perhaps, was their observation of higher digestibility of CP and amino acids in the yeast diet than expected based on past research of

C. utilis, a difference attributed to the use of lignocellulosic biomass as yeast growth medium. Lignocellulosic biomass was also the growth medium used to produce *C. utilis* in the trial by Håkenåsen (2017). When up to 40 % of CP was yeast-derived, improved intestinal health in piglets was seen.

When adding torula yeast to dairy cow diets, Bush (1960) reported no significant differences in MY, milk fat content or rumen VFA production. Even so, reduced palatability was observed for the yeast-enhanced diets when fed to high-yielding cows. Meanwhile, Habeeb (2017) claims that yeast culture is both palatable and appetite stimulating in ruminants, through the action of improving rumen fibre fermentation. Alas, no assessment of palatability was done in the current trial due to the lack of values for true concentrate feed intake. An *in vitro* experiment by Wang et al. (2016) simulated the effects of *C. utilis*, *C. tropicalis*, and *S. cerevisiae* on rumen fermentation of cereal straws. Methane production was lower when adding *C. utilis* than other yeasts, which is interesting from an environmental perspective. Still, *C. utilis* gave lower VFA production and disappearance of DM and NDF, signifying a negative effect on ruminant energy supply. As such, *C. utilis* was rated the least effective yeast on rumen fermentation properties. Whether *in vivo* trials would show the same, is unknown. Yet, using BW as an indicator, the current trial saw no negative effects of yeast on energy supply.

5.5 Production of yeast protein

A report by Skogli et al. (2019) made on assignment from NMBU and Foods of Norway looked at market prospects for single-cell yeast-based protein. Europe holds the largest market share globally (37 %) for yeast-based feed ingredients, where Great Britain is the biggest producer followed by France, Germany, and the Netherlands. Meanwhile, the biggest consumer of yeast-based protein is the U.S. followed by China. Although uncertain, the researchers expected the market to grow during a five-year period (2019-2024). Several threats to a market growth for Norwegian yeast-based protein were identified: 1) The competition of alternative feed proteins which only require sunlight and methane or CO₂ for growth; 2) the globalisation of raw material markets; 3) an increase in demand and competition for wood used in yeast production; 4) economic pressure on farmers, as soy protein is affordable; 5) the rising popularity of vegetarianism and veganism, reducing total meat- and milk production. Meanwhile, factors expected to strengthen market growth include: 1) Increased sustainability by reduction of soy import; 2) reduction of antibiotics use

by exploiting the health-promoting properties of yeast; 3) consumer interest for locally produced food on Norwegian resources; 4) increased national food security as a governmental strategy.

It is possible to produce protein derived from *C. utilis* on Norwegian renewable resources, without using food crops. A growth medium based on national spruce trees, brown macroalgae and ammonium sulphate has given satisfactory yeast growth (Sharma et al., 2018). Currently, yeast-based protein production is not profitable, and very large production quantities are needed to make it cost-effective. Further technology developments may help lower processing costs. Production will also be more competitive if raw materials (wood and nutrients for yeast growth) are cheap and easily available. Popularity of the yeast product will increase if it helps lower feed costs and improve animal health. Finally, the process and product should satisfy any environmental concerns of authorities and consumers to elevate its ethical value, thus increasing demand (Skogli et al., 2019).

6.0 Conclusion

Comparing diets containing soy-protein, yeast-protein from *C. utilis*, and barley, no significant effects of treatment were found. Using yeast-protein in concentrate feed for dairy cows had no significant effects on feeding behaviour. Nor was productivity affected by treatment, although dry matter intake and milk yield were significantly correlated with behaviours, and the soy group had a gentler drop in milk yield throughout the experiment than the other groups.

In addition to effects of yeast on feeding behaviour, further research may want to explore any effects on nutrient digestibility when using *C. utilis* as protein source, and palatability of feed containing *C. utilis* for dairy cows. Moreover, possible differences between soy protein and *C. utilis* on the rate of milk yield decline through a lactation merits inquiry. Finally, *C. utilis* protein composition when using lignocellulosic biomass as growth medium should be closely investigated.

A Norwegian production of yeast-derived protein based on national resources is possible, but not yet profitable. While current market outlooks create room for optimism, lowering production costs is vital. Advancing technology may one day, hopefully, make production more cost-effective and economically feasible.

7.0 Literature

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