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Effects of *Candida utilis* as a protein source and replacement of soybean meal and yeast with barley on feed utilization and nitrogen use efficiency in dairy cow diets

Effekter av *Candida utilis* som proteinkilde og erstatning av soyamel og gjær med bygg på fôrutnyttelse og nitrogeneffektivitet i rasjoner til melkekyr

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Acknowledgement

This thesis concludes my master's degree in Animal Science and was performed at the Faculty of Biosciences. These last five years have been incredible. I have met so many amazing people and gained memories that I would not be without, both from the university and the student community in general.

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Kristin Heggen

Abstract

An increasing global population requires increasing sustainable food production. Today, protein from soybeans is used in concentrates to dairy cows. Climatic conditions make it difficult to grow soybeans and other protein crops in Norway, and most protein ingredients are imported. New protein ingredients are developing from sustainable resources such as yeast from lignocellulosic biomass from forests.

The aim of this study was to evaluate *Candida utilis* as a protein source in diets of high-yielding dairy cows. In addition, the aim was to study the effects of reducing protein content in the diet by replacing soybean meal and yeast with barley. The effects evaluated were milk production, digestibility and nitrogen use efficiency. Forty-eight NRF dairy cows were fed one out of three treatments for a 56-days experimental period. Three diets differing in basic protein source were studied. The diets were soybean meal (SOYABP), *C. utilis* yeast (YEASTP) and a negative control with less protein (BARLEY). The two last weeks pre-experimental all cows were fed SOYABP and registrations were used as covariates for milk, feed intake and body weight in the statistical analysis. Acid-Insoluble Ash was used as an internal marker to determine digestibility based on faecal samples taken the last experimental week.

No significant effect of replacing soybean meal with *C. utilis* as a protein source was found on dry matter intake, milk production, digestibility or nitrogen use efficiency. Results indicated lower milk protein and milk urea in the barley-based diet compared with the average of soybean meal and yeast. Barley-based diet indicated a decrease in milk and energy corrected milk yield towards the end of the experiment. Urinary N output was lower in the barley-based diet, but no large difference in nitrogen use efficiency was observed. To conclude, replacing soybean meal with *C. utilis* showed promising results in dairy cows. Feeding diets with lower protein concentration can reduce urinary N emission to the environment.

Sammendrag

Stadig befolkningsvekst i verden krever økning i bærekraftig matproduksjon. I dag er protein fra soyabønner brukt i kraftfôr til melkekyr. Klimaet i Norge gjør det vanskelig å dyrke soya og andre proteinvekster og mesteparten av proteinråvarene til husdyr er importert. Nye proteinråvarer som for eksempel gjær fra ligno-cellulose er under utvikling. Vi har rikelig tilgang på ligno-cellulose fra skogen og dette vil kunne bidra til økt selvforsyning og mer bærekraftig matproduksjon.

Formålet med denne oppgaven var å evaluere effektene av å bruke *Candida utilis* som en proteinkilde i fôrrasjoner til høytytende melkekyr. I tillegg var formålet å undesøke effekten av å redusere proteininnholdet i rasjonen med å bytte ut soyamel og gjær med bygg. Effektene som ble undersøkt var på mjølkeytelse, fordøyelighet og nitrogenutnyttelse. I forsøket ble 48 kyr av rasen NRF fôra tre dietter over 56 dager. Diettene varierte i proteinkilde bestående av enten soyamel (SOYABP), gjær (YEASTP) eller bygg (BARLEY). De to siste ukene før forsøksstart ble alle kyr tildelt SOYABP og registreringer fra denne perioden ble brukt som kovariat i den statstiske analysen. Melkeytelse, fôrinntak og vekt ble registrert på individbasis. Tilsvarende ble syreuløselig aske brukt som intern markør på invidbasis for å bestemme fordøyelighet på tørrstoff og næringsstoffer. Stikkprøver av gjødsel fra siste uke i førsøket ble analysert og benyttet til dette.

Å skifte ut soyamel med gjær i kraftfôr ga ingen signifikante forskjeller mellom diettene på fôrinntak, melkeytelse, fordøyelighet eller nitrogeneffektivitet. Resultatene viste lavere innhold av protein og urea i melk hos dyr fôra på bygg sammenlignet med gjennomsnittet for dem som ble fôra på soyamel og gjær. Bygg viste også en tendens til avtagende ytelse i melk og energikorrigert melk mot slutten av forsøket. I dietten med bygg var N-utslippet i urin lavere enn for de to andre diettene, men det var ingen forskjell i N effektivitet. Konklusjonen fra forsøket er at bruk av *C. utilis* som proteinkilde fremfor soyamel viste lovende resultater hos melkekyr. Bruk av bygg i stedet for soya eller gjær i rasjoner til melkekyr ga lavere innhold av råprotein i fôret og kan gi redusert N-utslipp til miljøet via urin.

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Abbreviations

AIA = acid-insoluble ash

BW = body weight

CP = crude protein

DIM = days in milk

DM = dry matter

DMD = dry matter digestibility

DMI = dry matter intake

ECM = energy corrected milk

FFA = free fatty acids

GIT = gastrointestinal tract

MUN = milk urea nitrogen

MY = milk yield

NDF = neutral detergent fibre

iNDF = indigestible neutral detergent fibre

NPN = non-protein nitrogen

NUE = nitrogen use efficiency

OM = organic matter

RMSE = root mean square error

RUP = rumen undegraded protein

SEM = standard error LSmeans

TCM = total collection method

UN = urinary nitrogen

VFA = volatile fatty acids

YMP = yeast-derived microbial protein

1 Introduction

According to the Food and Agriculture Organization (FAO), food production will increase 60% by 2050 to cover the increasing demands of the growing global population (FAO, 2016). With increasing food production, food security is discussed along with self-sufficiency. Moreover, there is an increased focus around food production towards climate change and the emission of greenhouse gases. Thus, the increase in food production must focus on sustainable production and the use of locally produced food and feed ingredients. A higher self-sufficiency rate would contribute to increased food security and the distribution of resources around the world. Recent years the self-sufficiency rate of food in Norway has been just below 50%, and in 2019 self-sufficiency rate was at 45% (Budsjettnemnda for jordbruket, 2020; Helsedirektoratet, 2020).

Only about 3% of Norway's land area is arable land (Kjos et al., 2019), of which 2/3 is for grass production. In addition, there are large areas suitable for grazing. Therefore, ruminants are an important resource in food production. Ruminant production in Norway, as is the case with most Nordic countries, is dependent on roughage feeds like grass silage supplemented with concentrate feeds. In the concentrate feeds most of the cereal grains are produced in Norway and for carbohydrates, self-sufficiency rate is around 70% (Landbruksdirektoratet, 2019). However, protein and fat sources are imported and for protein ingredients only around 5% is produced in Norway (Landbruksdirektoratet, 2019). This is not sustainable in the long run and has necessitated the search for alternative protein ingredients in livestock diets. To this end, use of new feed ingredients based on sustainable resources from biomass such as seaweed (Makkar et al., 2016), wood from trees (Cruz et al., 2019; Øvrum Hansen et al., 2019), and insects (Makkar et al., 2014) have been studied. These are among new developing protein resources in feed production for animals. However, whereas insects may be used as protein ingredient directly, seaweeds and wood need to be converted to protein by the help of microorganisms like yeast.

With respect to sustainable food productions, low emission of greenhouse gases is not the only focus. Improved feed utilisation and reducing the excretion of nitrogen (N) in urine and faeces are also important factors. Nitrogen contributes to emissions through nitrous oxide (N_2O) produced from manure. The utilisation of N for milk production in dairy cows is on average 25% (Calsamiglia et al., 2010) with huge variation among studies. Dijkstra et al. (2013b) reported that in theory, N utilisation could be 43%, giving an average cow a potential

for improvement. The easiest way of improving N efficiency is to lower the dietary N, which will reduce the N excretion in manure (Dijkstra et al., 2013a).

The objectives of this thesis were to evaluate the effect of replacing soybean meal with *Candida utilis* yeast as a protein source in diets of dairy cows on milk production, nutrient digestibility and nitrogen use efficiency, and to evaluate the same response variables replacing soybean meal with barley.

The following hypotheses were studied:

H1: Replacing soybean meal with *Candida utilis* yeast will not affect milk yield, nutrient digestibility and nitrogen use efficiency in dairy cows.

H2: Replacing soybean meal with barley will not affect milk yield negatively, but improve nitrogen use efficiency in dairy cows.

2 Literature

Ruminants are special from monogastric animals in their ability to convert heavily digestible nutrients in grasses and other roughages into edible meat and milk for human consumption (Oltjen & Beckett, 1996). In addition to roughages, the diet of high-yielding dairy cows consists of concentrates where more easily digested nutrients dominate. Quantitatively, carbohydrates compose the main part of the diet, followed by protein, fat and minerals.

2.1 Nutrients in feed

Feedstuff is mainly divided into roughage and concentrates. Different feeds are composed of various proportions of nutrients. Usually, they are analysed for the chemical composition to know the feed value. Feeds consists of dry matter (DM) which is divided into organic matter (OM) and ash. The OM contains all the main nutrients in feeds namely carbohydrate, protein and fat. NorFor divides the OM into protein, neutral detergent fibre (NDF), starch, fermentation products, rest carbohydrates and crude fat (Volden, 2011). Chemical composition of some commonly used feed ingredients is given in Table 1. As the table shows, the chemical composition varies considerably between feed ingredients.

Table 1. Chemical composition (g/kg dry matter) of some commonly used feed ingredients for ruminants (NorFor Feed Table).

	Straw	Grass silage early	Grass silage late	Barley	Oats	Soybean meal	Rapeseeds
Dry matter, g/kg	900	224	260	883	896	885	936
Protein	38	168	121	113	113	516	218
NDF[1]	767	507	610	198	287	133	176
Starch	0	0	0	615	492	16	8
Fat	15	31	23	32	64	22	452
Ash	52	69	67	23	28	66	47

[1] NDF=neutral detergent fibre

2.1.1 Carbohydrates

Carbohydrates make up the most important energy source for ruminants. Carbohydrates are divided into sugars such as the mono- and disaccharides glucose, fructose, galactose, sucrose and lactose; and polysaccharides including fibre and starch (Asp, 1996). Glucose can be utilised as an energy source in all cells (Sjaastad et al., 2016). Fibres in the form of NDF dominates in roughages, and starch in concentrates like cereal grains. In addition, both

roughages and concentrates contain various carbohydrates in the form of simple sugars and soluble fibres such as fructans, pectin and β-glucans (Weisbjerg et al., 2003).

NDF is a structural carbohydrate found in plants and is commonly named lignocellulose in the bio-refining industry. It is the dominating nutrient in ruminants' diets and consists of cellulose, hemicellulose and lignin (Volynets et al., 2017). The content of NDF varies from almost nothing in some concentrate ingredients to more than 75% of DM in straw (Table 1). In grass silage, NDF typically varies between 40 and 65% (NorFor Feed Table). In Norway, the average NDF content of analysed grass silage samples in 2019 was around 52% (Schei, 2019). The most abundant structural component of the plant cell wall is cellulose (Madadi et al., 2017). Chemically, cellulose is a homoglycan of glucose bound in a β-1,4 glycosidic configuration with hydrogen bonds between laminar fibrils (Figure 1), and where the repeating unit is cellobiose (McDonald et al., 2011). Hemicellulose is a heteroglycan where hexoses (mannose, glucose, galactose) and pentoses (arabinose, xylose) linked mainly with β-1,4 and β-1,3 glycosidic bonds, are dominating sugars (Madadi et al., 2017; Van Soest, 1994). Lignin is not a carbohydrate, but a polyphenolic component closely bound to hemicellulose and cellulose, which both are polysaccharides. In the plant cell wall, cellulose fibrils are wrapped in hemicellulose and lignin polymers (Figure 1) (Volynets et al., 2017). As plant material mature, the bonds between lignin and carbohydrates develop, decreasing the digestibility of roughages. These bonds as well as the β-glycosidic bonds in cellulose and hemicellulose cannot be degraded by mammalian digestive enzymes, but they can be degraded by microbial enzymes in ruminants.

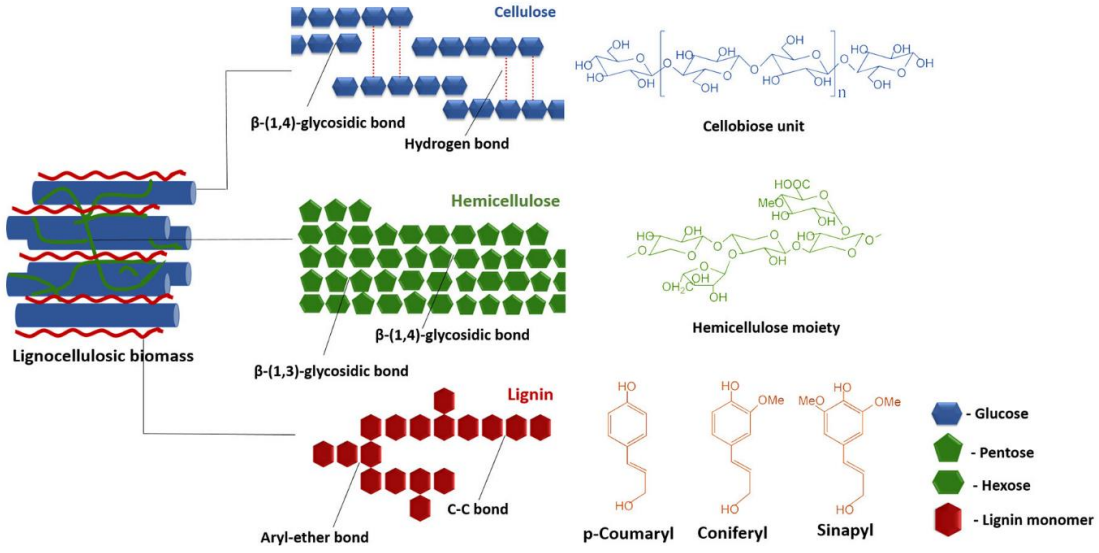

Figure 1. Chemical organization of lignocellulose (cellulose, hemicellulose and lignin) in plant cell walls (Baruah et al., 2018).

Starch is the most abundant component in cereal grains like barley and oats (Table 1). Starch is organized in spherical granules varying in size in the endosperm of the cereals (Figure 2). Starch consists of the polysaccharides amylose and amylopectin (Santana & Meireles, 2014), both homoglycans of glucose. Amylose contains α-1,4-glucose polymers in a linear structure with maltose as repeating units, whereas amylopectin, also, contains some α-1,6 bonds giving a branched structure (Figure 2) (Parker & Ring, 2001). Usually, the starch granule consists of 70 – 80% amylopectin and amylose is the remaining 20 – 30% (McDonald et al., 2011). In contrast to NDF, starch is usually easily digested in the small intestine of mammals.

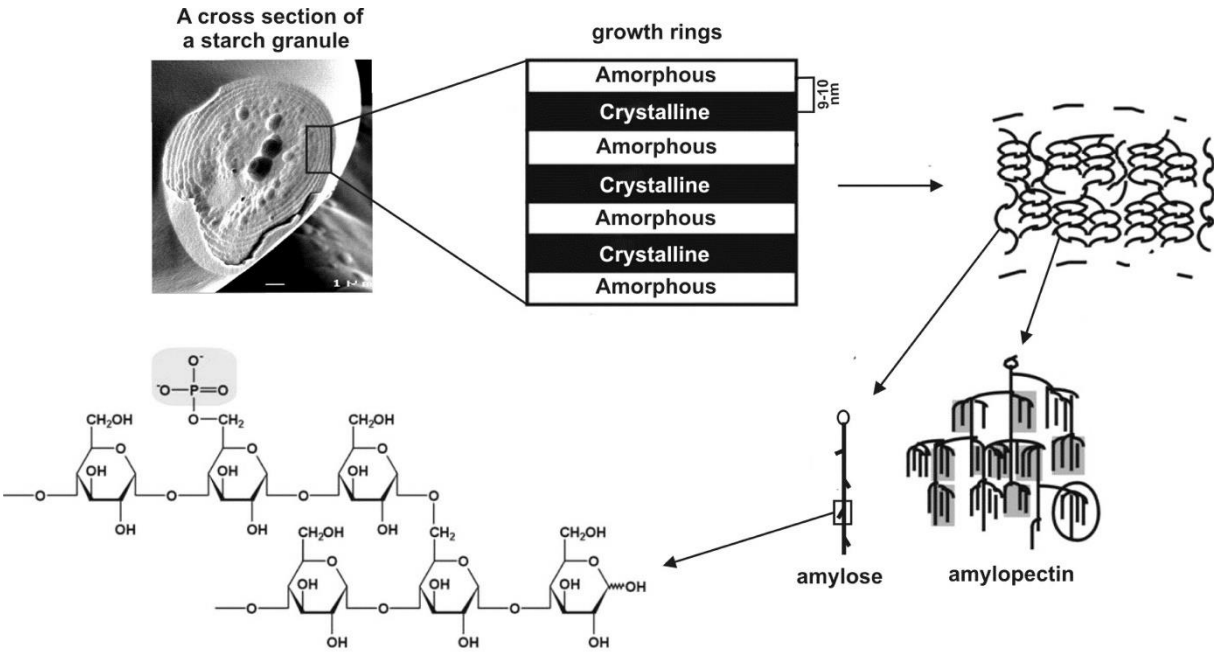

Figure 2. Structure and organization of starch (Nazarian-Firouzabadi & Visser, 2017).

2.1.2 Protein

Proteins are building blocks in all living cells and important in most functions of the body (Sadava et al., 2014). The protein content in grass silage decreases by increasing stage of maturity (Table 1). Protein ingredients, such as soybean meal and rapeseed cake, are added to concentrate feeds to cover protein requirements for high-yielding dairy cows and growing animals. Thereby, making concentrate feeds an important protein source for ruminants.

Proteins are complex molecules containing the elements carbon, hydrogen, oxygen, nitrogen and often sulphur. Proteins are composed of polypeptide chains made by amino acids linked together by peptide bonds, as shown in Figure 3 (McDonald et al., 2011). Amino acids consist of an amino group -NH, a carboxyl group -COOH and a side chain (R group) that vary among

amino acids (Figure 3). There exist many amino acids, but only 20 are found in proteins (Hvelplund et al., 2003). Dietary protein contains true protein N and non-protein nitrogen (NPN). True protein N is built up of amino acids, whereas NPN is N from amides, amines, peptides, free amino acids, N-containing bases in nucleic acids, urea, nitrates and ammonium ions (Sjaastad et al., 2016). The protein content is usually expressed as crude protein (CP) found by multiplying N by 6.25 based on analyses of N and the assumption that proteins contain 16% N on a molecular basis (Weisbjerg & Hvelplund, 2003). However, this does not apply for all proteins, whereas milk protein contains around 15.7% N, thus N in milk is multiplied by 6.38.

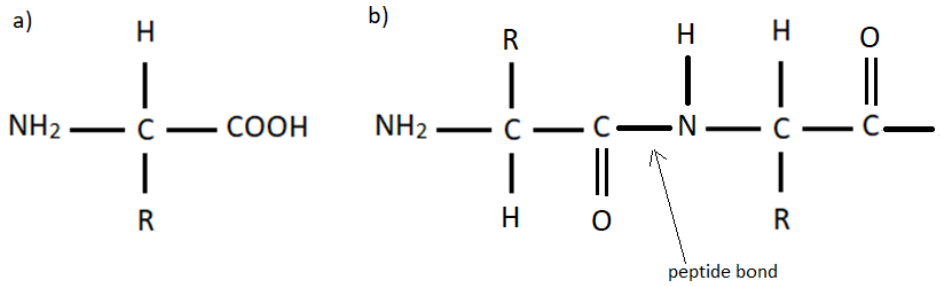

Figure 3. Structure of amino acids (a) and structure of the peptide bond between amino acids in proteins (b).

Amino acid composition of different protein ingredients varies considerably (Table 2). The amino acids can be divided into essential amino acid, that the body cannot synthesise, and non-essential amino acid that can be synthesised in the body. The essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Hvelplund et al., 2003). As Table 2 show, the composition of essential amino acids in cereal grains are lower compared to the protein supplements, soybean meal and rapeseed. These supplements, however, complements the cereal grains in the amino acid composition of feed rations. Soybean meal has an amino acid profile similar to fishmeal, except for the methionine content. Fishmeal is not allowed in ruminant feeds. Therefore, soybean meal is a good protein source in ruminant diets.

Table 2. Amino acid composition (g AA/100 g CP) of some feedstuffs (NorFor Feed Table).

	Barley	Oats	Grass silage	Straw	Soybean meal	Rapeseed	Fish meal[1]
Crude protein, g/kg DM	113	113	157	38	516	218	736
Alanine	4.30	4.70	6.42	4.42	4.20	4.39	6.19
Arginine	5.30	6.90	2.69	3.13	7.40	6.73	5.98
Aspartic acid	6.10	8.20	7.78	6.69	11.20	7.42	9.09
Cysteine	2.40	3.10	0.65	1.26	1.50	2.07	1.03
Glutamic acid	24.60	20.00	7.64	8.12	18.20	15.94	12.99
Glycine	4.20	5.10	3.97	3.85	4.20	6.55	6.63
Histidine	2.50	2.30	1.67	1.06	2.70	2.74	2.50
Isoleucine	3.80	4.10	3.84	2.82	4.70	4.19	4.35
Leucine	7.10	7.20	6.21	4.82	7.50	6.86	7.13
Lysine	3.80	4.30	3.57	3.09	6.10	5.52	7.48
Methionine	1.80	1.70	1.29	1.34	1.30	1.88	2.74
Phenylalanine	5.40	5.10	4.16	3.24	5.00	4.01	3.79
Proline	11.0	5.30	4.96	3.91	5.00	6.22	4.09
Serine	4.80	5.20	3.60	3.63	5.20	4.52	4.24
Threonine	3.60	3.50	3.34	3.42	3.90	4.37	4.14
Tryptophan	1.30	1.40	1.12	0.24	1.40	1.24	-
Tyrosine	3.20	3.20	2.91	2.03	3.80	3.12	3.20
Valine	5.30	5.40	4.97	3.78	4.80	5.39	5.05

[1](Øverland et al., 2009)

2.1.3 Lipids

Lipids are important constituents in cell membranes, in storages as adipose tissue and products like milk fat. Lipids, or fats, are substances that are insoluble in water, but soluble in non-polar solvents (Sjaastad et al., 2016). Lipids in ruminant feeds consists mainly of triglycerides and glycolipids. In addition, some feeds may contain phospholipids. Triglycerides consist of a glycerol molecule and three fatty acids and are the most abundant fat in oils and concentrates for ruminants (Shingfield & Garnsworthy, 2012). In glycolipids, a fatty acid is replaced by a sugar molecule. In roughages the sugar molecule is galactose, and thus galactolipids are the dominant lipid (McDonald et al., 2011). Phospholipid contains a phosphate group instead of a fatty acid. In addition to these natural lipid sources, various commercial fat products can be obtained. In ruminants, the most important products are free fatty acids (FFA), usually obtained from hydrolysation and refining of triglycerides into specialised feed products, and calcium soaps obtained by linking one or two fatty acids to a calcium molecule (Handojo et al., 2018).

The number of carbon atoms in the carbon chain of the fatty acids can vary from 4 – 24 (Gjefsen, 1995). Thus, the fatty acid composition in fat sources vary. Linoleic acid (C18:2) is a dominating fatty acid in soybean oil and barley, whereas oleic acid (C18:1) dominates in rapeseed oil and oats (Shingfield & Garnsworthy, 2012). The dominating fatty acids in grasses are linolenic acid (C18:3). Other important fatty acids are palmitic acid (C16:0) and stearic acid (C18:0). Palmitic acid dominates in palm oil together with oleic acid, whereas stearic acid rarely occurs in high concentration in natural vegetable feed products, but can be elevated in commercially modified fat products like FFA and Ca-soaps.

2.2 The ruminant animal

Ruminants have three fermentation chambers, an extension in the gastrointestinal tract (GIT) between the oesophagus and the true stomach. These three forestomachs are called the reticulum, the rumen and the omasum. The fourth chamber, the abomasum, has the same functions as the stomach of monogastric animals (Figure 4).

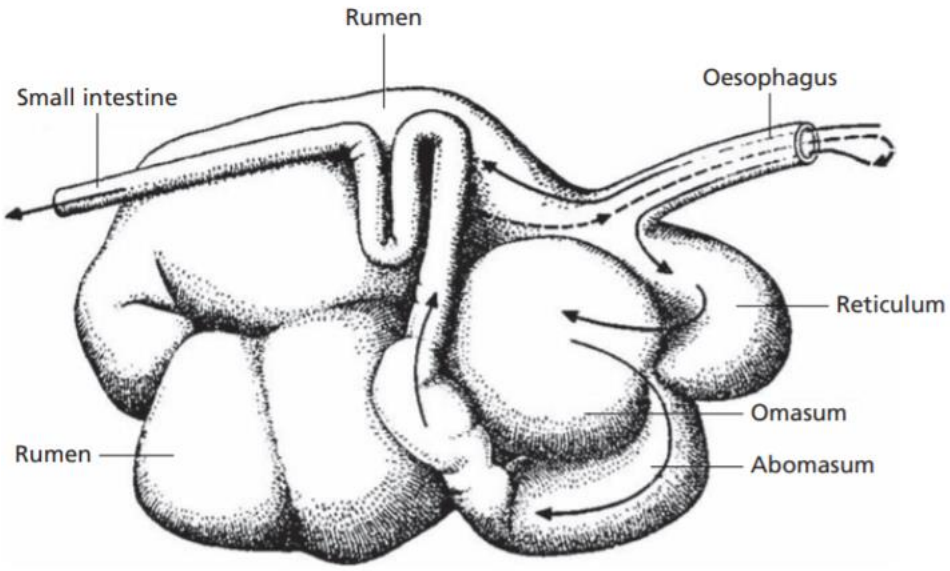

Figure 4. The stomachs of the ruminant animal (From Annison and Lewis (1959) cited in McDonald et al. (2011).

Ruminants live a symbiotic relationship with microorganisms in the reticulorumen, which is a common name of the main compartment consisting of the rumen and the reticulum (Wattiaux & Howard, 2000). The microbes can digest feed fractions the animals can not manage on its own. These rumen microbes are bacteria, protozoa and fungi and they ferment the feed in the

forestomachs (Sjaastad et al., 2016). Fermentation is anaerobic degradation of feed without the use of oxygen. The main products of microbial fermentation in the rumen are the volatile fatty acids (VFA) acetate, butyrate and propionate, which is the most important energy source for ruminants. Other fermentation products are ammonia (NH_3) and the gases methane (CH_4) and carbon dioxide (CO_2).

2.2.1 Digestion of carbohydrates

Carbohydrates are broken down in the forestomachs where microorganisms excrete enzymes capable of breaking the glycosidic bonds including the β-1,4 bonds in cellulose. Cellulose is degraded by cellulase to cellobiose, extracellularly. Cellobiose is further degraded to glucose monomers by cellubiase. Starch is degraded into maltose by the enzyme amylase, and by maltase to glucose (McDonald et al., 2011). Glucose enters the microbes and is fermented into VFA, intracellularly. This process involves over several steps and starts with glycolysis and production of pyruvate (Sjaastad et al., 2016). Thereafter, pyruvate is transformed into VFA through the process shown in Figure 5. The different VFAs have different pathways of production. Easily fermentable carbohydrates stimulate propionic acid production, whereas structural carbohydrates stimulate the production of acetic acid. The microbes use energy from the fermentation of carbohydrates for its microbial growth (Sjaastad et al., 2016). Starch is usually highly digested in the rumen, whereas NDF has longer degradation time and is less digested. NDF contains an indigestible fraction named iNDF (Weisbjerg et al., 2003), that influence the degradability of NDF.

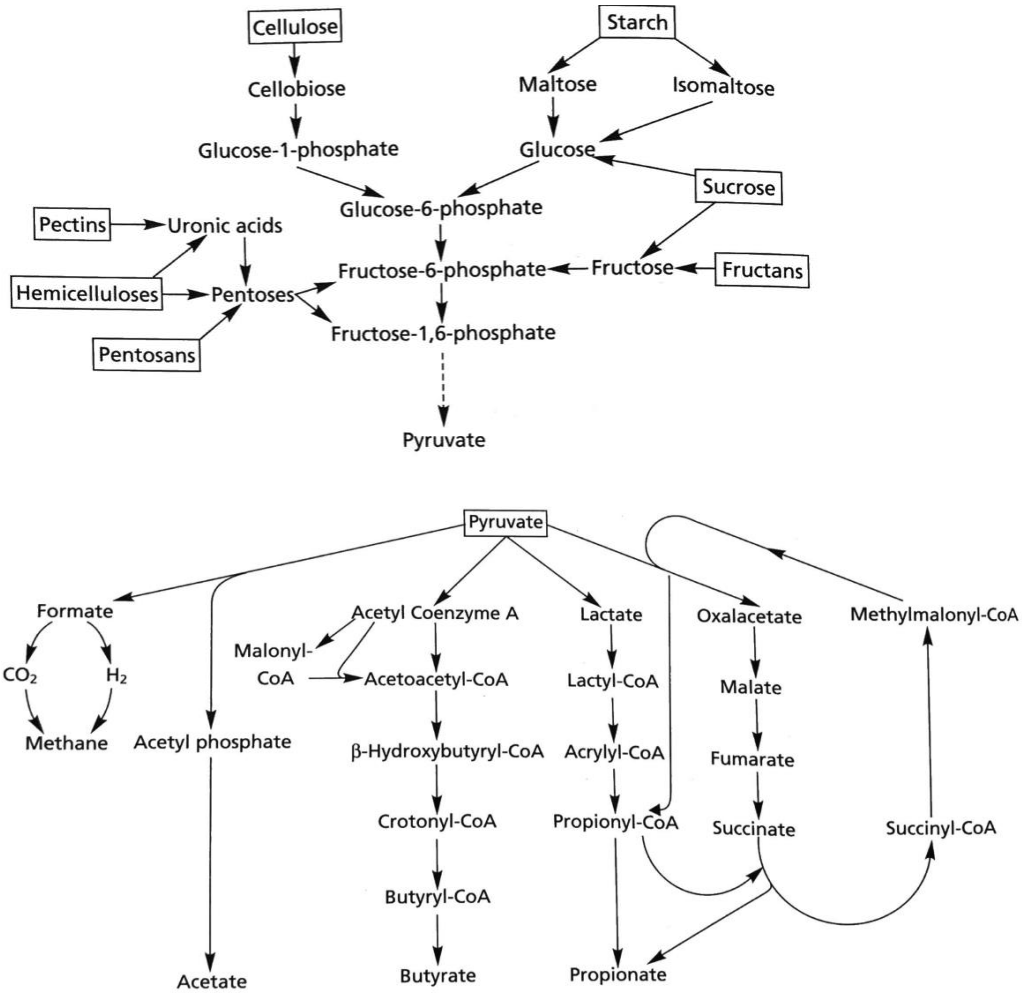

Figure 5. Conversion of carbohydrates into volatile fatty acids in the rumen (McDonald et al., 2011).

2.2.2 Digestion of protein

Amino acids and N are needed for maintenance, growth and milk production in the ruminant (Wattiaux, 1998). The protein metabolism in the ruminant is illustrated in Figure 6. Protein fermentation in the rumen is done by microorganisms. This process can be divided into two main steps: extracellular degradation of proteins to peptides and intracellular degradation of peptides to amino acids (Sjaastad et al., 2016). Proteolytic enzymes, produced by the ruminal microbes, separate the proteins into peptides extracellularly. The peptides are then actively transported into the microbes where they are broken down to amino acids by intracellular proteolysis and deamination. Inside the microbe, the amino acids are fermented to VFA, NH_3 and energy in the form of adenosine triphosphate (ATP). If energy is present, amino acids are used for microbial protein synthesis (Bach et al., 2005). Some feed protein escapes microbial fermentation and forms different pathways (Figure 6). Rumen undegraded protein (RUP), also

named bypass or escape protein, avoid the microbial fermentation and continue to the intestines undegraded. Here they are degraded to amino acids by enzymes secreted by the animal.

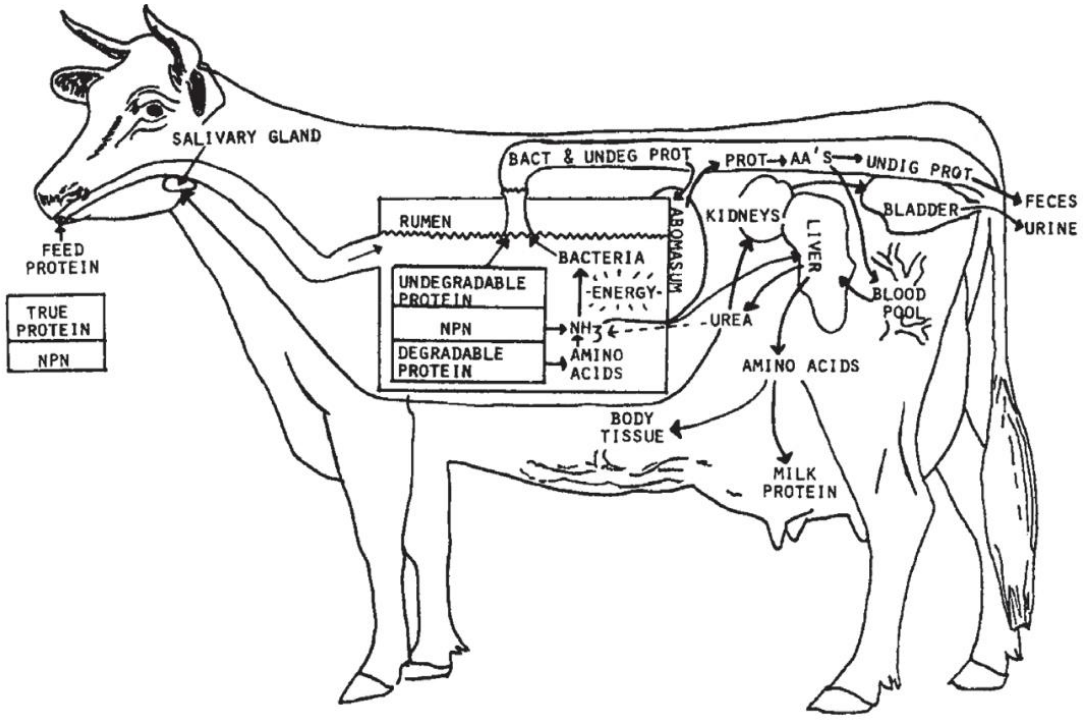

Figure 6. Protein metabolism in the ruminant and the pathways of amino acid absorption and nitrogen recycling (Stein et al., 2008).

The amino acids available to the ruminant are absorbed in the small intestine. Normally around 75% of absorbed amino acids derive from microbial protein (Nørgaard, 2003), whereas the rest derives from RUP and a minor fraction from endogenous protein. Undigested protein goes on to the large intestine, where they are broken down to NH_3 through microbial fermentation. There is no absorption of amino acids in the large intestine, only VFA and NH_3.

Microbial protein has a good amino acid composition (Table 3) (Clark et al., 1992). Thus, if the requirements for microbial growth are fulfilled, ruminants have access to all essential amino acids through the microbial synthesis. On average, the microbial mass contains 50% protein (Hvelplund et al., 2003), around 10% nucleic acids (Van Soest, 1994) as well as some fat, carbohydrates and ash. Rumen substrate balance can be given by the formula from Van Soest (1994): $C_6H_{12}O_6 + NH_3 \rightarrow$ microbes + CH_4 + CO_2 + VFA. In the rumen, microbial growth is dependent on the ruminal environment. Important growth factors for microbial synthesis are energy in the form of ATP, a source of N (e.g. NH_3), carbon skeleton and

minerals, such as sulphur and phosphorous (Hvelplund et al., 2003). The most important source of energy for microbial synthesis is carbohydrate fermentation.

Table 3. Essential amino acid composition of microbial protein in g/100 g of AA (Volden & Larsen, 2011).

Amino acid	Microbial protein
Arginine	5.1
Histidine	2.4
Isoleucine	5.9
Leucine	7.9
Lysine	7.4
Methionine	2.5
Phenylalanine	5.7
Threonine	5.3
Tryptophan	1.6
Valine	5.9

Like previously stated, microbial synthesis depends on the availability of N and energy, thus the amount of protein and carbohydrates degraded and available affects how much microbial protein that can be made (Bach et al., 2005). The microorganisms in the rumen receive N for protein synthesis from NPN in the feed, degraded feed protein, N from dead rumen microbes and recycled urea via saliva and the rumen wall. Usually, 50 – 70% of the N content in microbes derives from NH_3 (Sjaastad et al., 2016). There are around 70% of amino acids in microbial proteins. The digestibility of microbial amino acids is 85% (Hvelplund et al., 2003). When it comes to NPN, urea is an important source for ruminants. Urea recycles N and contributes to the NH_4^+-pool in the rumen. However, N from urea is less efficient than N from CP in the microbial protein synthesis (NRC, 2001).

The supply of proteins in the intestine often limits the milk production of the ruminant, and methionine and lysine are the most limiting amino acids in microbial proteins (NRC, 2001; Schwab et al., 1992). A low producing cow could manage with only an NPN source. In contrast, a high yielding dairy cow requires more protein than the microbes can synthesise and requires RUP for sufficient milk production (Sjaastad et al., 2016). An increased passage rate of the feed may increase the RUP fraction. This can be achieved by increasing feed intake. Production of new microbes and the turnover of microbes in the rumen affects the digestion, pH and passage rate of the ruminant. The microbes follow the digesta flow to the intestine. Half of the microbes continue from the rumen in the fluid phase, whilst the other half is

attached to particles. The microbes are then degraded by enzymes from the ruminant in the intestine.

When feed contains small amounts of protein, NPN is important for maintenance and production. In grains and fresh grass, 5 – 15% of total N content is NPN (Sjaastad et al., 2016). In silage, the NPN part has increased because of the breakdown of protein during the fermentation process. The NPN compose about 70% of total N content in silage.

2.2.3 Digestion of fat

Lipids are broken down to glycerol and free fatty acids in the rumen by hydrolysis extracellularly. The glycerol enters the rumen microbes and is fermented into VFA. Unsaturated fatty acids are saturated by ruminal microorganisms through biohydrogenation (NRC, 2001), removing the double bond in the fatty acid by adding hydrogen. Mostly, all fatty acids passing the rumen are hydrogenated. The oleic acid and linoleic acid, mostly found in roughages, are hydrogenated to stearic acid in the rumen. Too many lipids in the feed may implicate feed intake and digestion of other nutrients, as unsaturated fatty acids are toxic to microbes (Shingfield & Garnsworthy, 2012). This could be avoided by supplying the diet with rumen inert fat such as Ca-soaps (Jenkins & Palmquist, 1984). Dairy cows should not be fed more than 6 – 8% of fat in the diet (Wattiaux & Grummer, 2000), but this depends on the level of unsaturation of the fatty acids. In general, ruminant diets contain a low amount of lipids, due to low lipid content in many plants (Van Soest, 1994).

2.3 Methods for measurement of digestibility

Digestibility is defined as the proportion of feed not excreted in the faeces. In other words, it is determined by what disappears throughout the GIT. Usually, in a digestibility trial, the exact amount of feed ingested and faecal output is measured, and digestibility is calculated as the disappearance (McDonald et al., 2011). The total collection method (TCM) is the most accurate way to measure the digestibility of feeds, but it is laborious and requires extensive housing and adapted research facilities (Satter et al., 1986). Use of digestive markers is an alternative mean of determining digestibility and passage in different parts of the GIT without a total collection of digesta or faeces. The TCM may hold a comparable answer for the apparent digestibility and therefore important in research trials for the testing of marker methods.

Usually, digestive markers are divided into external and internal markers. External markers do not exist naturally as an integrated part in the feed and are added to the diet. Chromic oxide (CrO_2) is a common external marker used in digestibility studies (Sales & Janssens, 2003). Other external markers are ytterbium as Yb-acetate and chromium as Cr-EDTA. Internal markers are natural components of the feed. Common natural markers are indigestible neutral detergent fibre (iNDF) and acid insoluble ash (AIA).

The most important requirement for a marker in digestibility studies is a total faecal recovery (Sales & Janssens, 2003). To fulfil this, a marker must be non-absorbable, must not be affected by or affect the GIT or its microbes, must be physically equal or behave in the same way as to what it is marking, and it should also have an easy and secure analysis (Faichney, 1975). Methods of determining feed efficiency through digestibility have been frequently studied using different marker techniques (Guinguina et al., 2019; Huhtanen et al., 1994; Owens & Hanson, 1992), although, the ideal marker that fulfils all these requirements have not yet been found.

Acid-insoluble ash is a natural component of feeds and is the most used internal marker in digestibility studies (Sales & Janssens, 2003). The AIA fraction contains indigestible minerals, where silica is a main component (Sales & Janssens, 2003). By analysing for AIA in feed and faeces, the digestibility of feed can be calculated. The AIA method offers some advantages compared to the total collection method. There is no need for extensive housing by using the AIA method and faecal samples can be collected by only simple grab sampling, making the method more applicable for normal livestock housing. However, there is some disagreement in the accuracy of the AIA method. Also, collecting representative samples may be challenging when using only one grab sample.

An alternative to AIA is iNDF. Huhtanen et al. (1994) evaluated AIA to be the best suitable marker for measuring total dry matter digestibility (DMD), followed by iNDF. In another study, AIA tended to determine higher digestibility values than other marker methods and the total collection method (Lee & Hristov, 2013). Lee and Hristov (2013) said iNDF was a more reliable digestibility marker than AIA. The iNDF determination is time-consuming as it needs to be processed 288 hours in sacco (Åkerlind et al., 2011b), which requires rumen cannulated cows. Acid-insoluble ash, on the other hand, is based on chemical analysis only.

2.4 Nitrogen recycling

Nitrogen, or more precisely, amino acids are essential nutrients in animal and plant production. However, N also contributes to environmental pollution as NH_3 in the air and as nitrate (NO_3^-) in soil and groundwater (Tamminga, 1992). Thus, excess use of N and loss of N in urine and faeces to the environment should be avoided. The nitrogen recycling in the environment is illustrated in Figure 7.

In manure, part of the N is converted to nitrous oxide (N_2O), a potent greenhouse gas. Thus, the amount of dietary N intake and the utilization of N are factors affecting the release of N_2O gas. In the ruminant, excess N in the rumen is converted into NH_3 and is transported out of the rumen epithelium by simple diffusion to the liver. Here, the urea cycle takes place, transforming excess NH_3 to urea (Van Soest, 1994) and in urine, most of the N is in the form of urea (Dijkstra et al., 2013a). When overfeeding with protein, N will be lost as urea in the urine. This contributes to environmental pollution and metabolic cost for the animal. Urea in urine will quickly be transformed to NH_3 with the help of the enzyme urease. In addition, parts of N from undigested feed protein, undigested microbial protein and endogenous protein will be converted to NH_3. The ammonia in manure is transformed into nitrite (NO_{2-}), followed by nitrates (NO_{3-}) through the process nitrification. Thereby, NO_{3-} is available for denitrification into N_2 or N_2O, which is released into the atmosphere and contributing to greenhouse gas emissions (Figure 7). Denitrification is dependent on the availability of easily fermentable carbohydrates, NO_{3-} and anaerobic environment (Aaes et al., 2003).

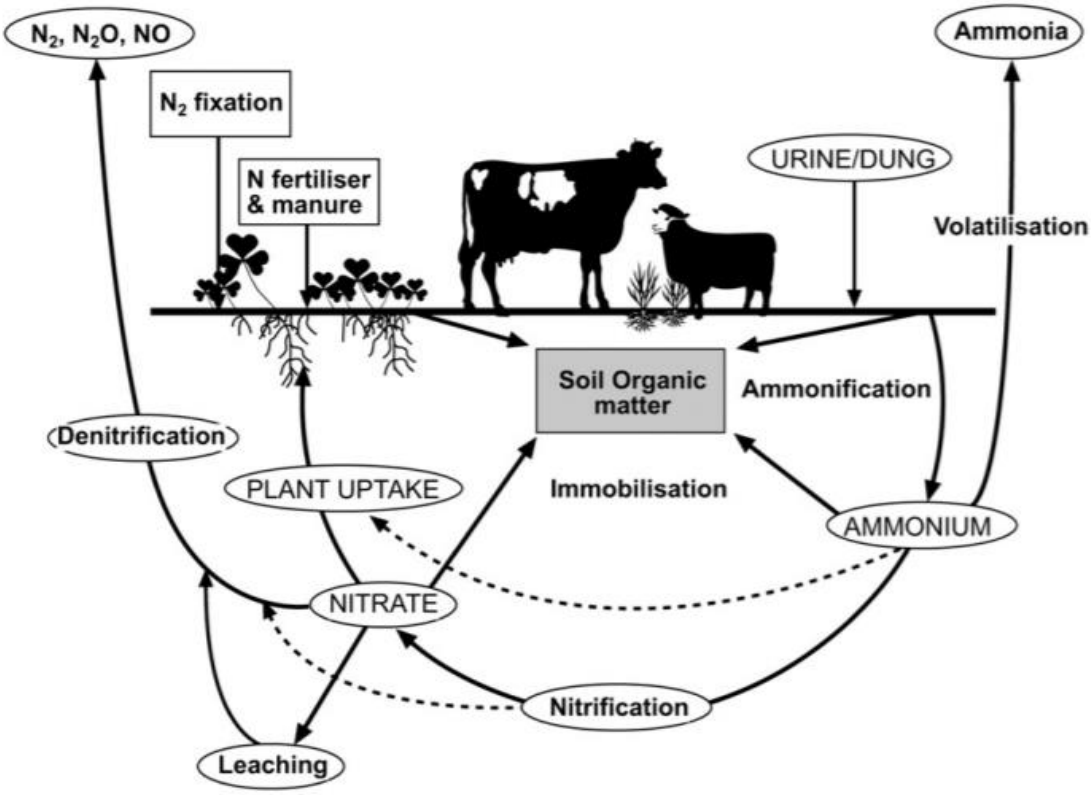

Figure 7. Nitrogen recycling in the environment by nitrification and denitrification (Saggar et al., 2012).

Ruminants are less efficient in utilizing N than other animals. Pigs have an N utilisation of 38% (Aaes et al., 2003), while Kebreab et al. (2001) reported efficiency of N at 28% for ruminants. In average, N utilisation for ruminants has been 25% with variation from 10 – 40% (Calsamiglia et al., 2010). The main differences between monogastric animals and ruminants are the degradation and metabolism in the rumen.

2.5 Protein ingredients in feed

In Norway, around 50% of feed ingredients in concentrates are imported (Felleskjøpet Agri, 2015), most being protein and fat resources. The Norwegian cereals such as barley have a low protein content that is of low quality. The most used protein ingredients in concentrate feeds are soybean meal and rapeseed cake. Soybean is an important plant for animal and human nutrition, from which soybean oil and soybean meal are produced (Dei, 2011). Soybean meal is widely used in livestock feed due to its high protein content and its favourable composition of amino acids that complements cereal grains (Stein et al., 2008). Due to the climatic conditions, soybeans cannot be produced in Norway and are imported. Soybean production occupies huge areas and contributes to deforestation of the rainforest. Norway only imports

GMO-free soybeans and the import represents 0.33% of the world's total soybean production (Felleskjøpet, n.d). Of the imported soybeans, 80% goes into aquaculture, mainly salmon feed, whereas the rest is used for land-based animal feed. Soybeans make up about 10% of Norwegian concentrates (Felleskjøpet Agri, 2015). Thus, Norwegian agriculture, and salmon production particularly, are highly depending on imported soybean and alternative protein sources are needed.

Yeast protein produced from low-value biomass from forestry and agricultural industry may be a potential sustainable ingredient in feeds. Yeast is a good source of protein and vitamin B (Olvera-Novoa et al., 2002). Even better, it can be produced from the fermentation of sugar-rich feedstock such as sugar cane or lignocellulosic biomass (Øverland et al., 2013). The latter can be obtained in the form of wood from trees in Norway. Yeast has a protein content comparable to soybean meal (Table 4) and an amino acid composition similar to that of rumen microbial protein (Table 3) (Clark et al., 1992; Sabbia et al., 2012).

Table 4. Protein content (g/100 g DM) and amino acid composition (g/16 g N) of soy, yeast and barley (g/kg DM).

	Soybean meal[1]	*Candida utilis*[2]	Barley[1]
Protein, g/100 g DM	51.6	33.3	11.3
Essential amino acids, g/16 g N			
Arginine	7.40	5.20	5.30
Histidine	2.70	1.89	2.50
Isoleucine	4.70	5.23	3.80
Leucine	7.50	7.75	7.10
Lysine	6.10	6.85	3.80
Methionine	1.30	1.35	1.80
Phenylalanine	5.00	4.50	5.40
Threonine	3.90	6.19	3.60
Tryptophan	1.40	1.59	1.30
Valine	4.80	6.28	5.30

[1] (NorFor Feed Table).
[2] (Sharma et al., 2018).

Alternative protein sources are under development and *C. utilis* is a yeast product that can utilise sugar monomers in lignocellulosic biomass for growth (Nasseri et al., 2011). The production of yeast from lignocellulosic biomass occurs through four steps. These are pre-treatment, enzymatic hydrolysis, fermentation and downstream processing (Figure 8) (Øverland & Skrede, 2016). In the pre-treatment step, the hemicellulose-lignin complex is

broken, making cellulose and hemicellulose more available for enzymatic hydrolysis. They are broken down into pentose and hexose sugars, that can be used for growing of yeast. These sugars can be converted into yeast through a fermentation process, by access to yeast strains and nutrients such as nitrogen, inorganic phosphorous and sulphate. In the downstream processing, the yeast goes through washing, cell disruption and drying. Dried yeast can then be included in animal feeds.

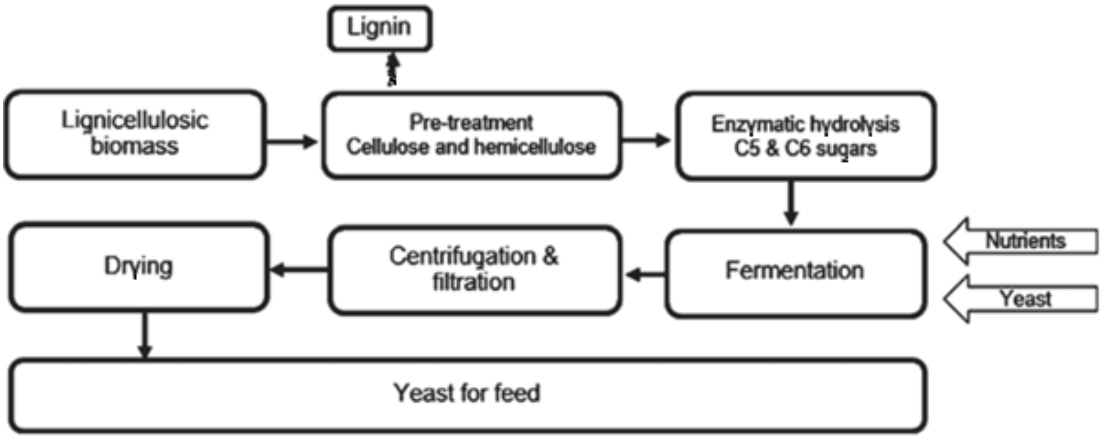

Figure 8. The steps in the production of yeast from lignocellulosic biomass, including pre-treatment, enzymatic hydrolysis, fermentation and downstream processing (Øverland & Skrede, 2016).

Until now, the use of yeast is most intensively studied in aquaculture (Olvera-Novoa et al., 2002; Reveco-Urzua et al., 2019; Øvrum Hansen et al., 2019), and Øverland et al. (2013) showed promising results in using *C. utilis* as a protein source in diets for Atlantic Salmon. In ruminants, the use of yeast like *C. utilis* is more scarcely studied and thus a subject for this experiment.

3 Method

The experiment was performed at the Animal Production Experimental Centre (SHF) at the Norwegian University of Life Sciences (NMBU). The experiment was part of the Foods of Norway project and took place from the 31st of January 2019 until the 11th of April 2019.

3.1 Experimental animals

Forty-eight Norwegian Red dairy cows in 1st or ≥ 2nd lactation and early lactation (50-150 days in milk (DIM)) were used in the experiment. The cows were blocked by DIM, lactation number, milk yield and genetic background and divided into three groups of 16 animals. Each group was given one of three experimental diets. The dairy cows were housed in a free-stall and milked by a DeLaval Robot milking system.

3.2 Experimental design

The experimental design is shown in Figure 9. The experiment lasted for ten weeks. The first 14 days was an adaptation period during which all animals were fed a control diet with grass silage and concentrate with soybean meal as the protein source. During the adaptation period, average milk yield and milk chemical composition, feed intake and body weight was registered for each cow. These averages were used as covariates to correct the starting point of the animal. The animals were fed one of the experimental diets for 56 days.

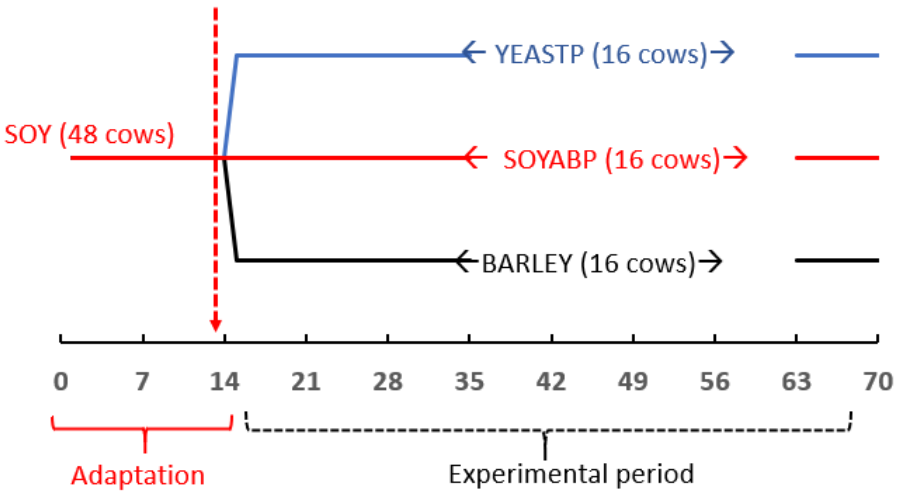

Figure 9. The experimental design.

3.3 Diets and feeding

The experiment had three experimental diets that varied in the concentrate fraction. All dairy cows were fed the same grass silage, ad libitum. The experiment contained three different concentrates differing in the basic protein source. The experimental treatments were:

1) SOYABP: containing 7% soybean meal per kg concentrate,

2) YEASTP: including 7% *Candida utilis*, which replaced 7% of the CP from soybean meal,

3) BARLEY: a negative control diet with lower CP, where the soybean meal was replaced by barley.

The rough ingredients composition of the concentrates is shown in Table 5. All three concentrates were iso-energetic. SOYABP and YEASTP were iso-nitrogenous. All other compounds were held as constant as possible. The concentrates were produced by Felleskjøpet Agri (FKA, Vestnes) using a normal expander process.

In addition to the feeds, some mineral supplement was added on top of the grass silage.

Table 5. Ingredients composition of the experimental concentrates.

Ingredients composition (%)	Soy	Yeast	Barley
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	-	-
Yeast	-	7.0	-
Vegetable oil	3.1	2.8	3.0
Others	3.4	3.3	4.2

3.4 Registrations and collection of samples

3.4.1 Feed intake

Animals were identified by individual electronic sensors for registration of feed intake. Silage intake was then measured by weight scales on the feeding trough, giving the silage intake in kilograms. The concentrate was fed in automatic feeding systems and divided into portions throughout the day based on calculated individual needs. Additionally, the cows were offered 1 kg of the soybean meal concentrate in the milking robot each day.

Weekly samples of the feed were collected. Both the silage and the concentrates were dried and ground at 1 mm, using a cutting mill (Retsch SM 200, Retsch GmbH, Germany). The samples were analysed for DM, NDF, CP, Ash and AIA.

3.4.2 Milk

Daily milk yield was registered by the milking robot. Milk samples were collected in week 2 (pre-experimental period), 4, 6, 7 and 10 of the experiment. The milk samples were sent to TINE for analysis of protein, fat, lactose, free fatty acids and milk urea using infrared milk analyser (TINE, milk laboratories).

3.4.3 Body weight

The animals' body weight was registered in the milking robot every time they were milked. The data used are based on a daily average for each cow. Body weights deviating too much from expected values were removed, and out of 2688 values, 115 were set as missing or removed.

3.4.4 Faeces collection and preparation of samples

Faecal samples were collected in week 2, 6 and 10 of the experiment. It was collected one grab sample from each cow, with a total of 48 faecal samples. These were either directly sampled from the cow through rectal palpation or grabbed when a cow was observed defecating. After sampling the samples were contained in aluminium foil trays and stored in a freezer. Only the faecal samples from week 10 were used for analysis, thus covariate was not used for these observations. The faecal samples were freeze-dried and ground at 1 mm using the cutting mill. The samples were split into two replicates, one for analysis and one reserve sample. The samples were analysed for DM, NDF, CP, ash and AIA.

3.5 Analyses

The chemical analysis of the feed and faeces were performed by Labtek at the Department of Animal and Aquacultural Sciences (IHA), NMBU. Also, a silage sample was sent to Eurofins for chemical analysis and analysis of fermentation products.

Dry matter in concentrate was determined by drying the samples at 103°C ± 2°C (Berg, 2018c). In silage, DM was determined by drying at 60°C until constant weight. The content of

ash in a sample was determined using the ISO 5984 method (ISO, 2002), by complete combustion at 550°C between 4 and 20 hours as modified by Berg (2018a). The inorganic matter remaining after combustion was the ash. Nitrogen content in a sample was determined by Kjeldahl-N using the method 2001.11 (AOAC, 2002) according to Thiex et al. (2002) as modified by Berg (2018b). Nitrogen content was measured using a Kjeltec TM 8400 instrument (Foss, Denmark) and CP was estimated as N x 6.25. The NDF fraction was determined using an ANKOM^{200} Fiber Analyzer (Ankom Technology). The method was according to Mertens (2002) using heat-stable α-amylase and correction for residual ash. Thus, NDF was referred to as aNDFom. In feeds and faeces, AIA was analysed using the same principles as recorded in Van Keulen and Young (1977) modified by Johnsen (2020).

3.6 Calculations

3.6.1 Feed intake and digestibility

Acid-Insoluble Ash was used as an internal marker to determine the digestibility of the feeds. By known AIA concentration in the feed and faeces the DMD was given by the formula from Kidane et al. (2018b)):

Equation 1

$$DMD_{AIA} = \frac{AIA\ in\ faeces - AIA\ in\ feeds}{AIA\ in\ faeces}\ x\ 100$$

where AIA concentration is given in g/kg DM and DMD_{AIA} is given in percent.

The mineral supplement was spread randomly over the grass silage and exact intake was not recorded. Scenarios for different intakes of mineral supplement at 50 g, 100 g and 150 g were plotted against intake of zero mineral supplement (data not shown). Assuming the intake of minerals was random within groups, zero intakes of the mineral supplement was used in the calculation of the digestibility.

Nutrient intake was calculated based on feed analysis and mean DMI of silage and concentrate:

Equation 2

$$Nutrient\ intake\ (g) = concentrate\ DMI\ (kg)\ x\ concentrate\ nutrient\ content\ \frac{g}{kg\ DM} + silage\ DMI\ (kg)\ x\ silage\ nutrient\ content\ \frac{g}{kg\ DM}$$

Nutrients in faeces were estimated based on the undigested DM fraction, calculated from DMI and DMD by AIA:

Equation 3

$$Nutrient\ in\ faeces\ (g) = undigested\ DM(kg)\ x\ nutrient\ in\ faeces\ (\frac{g}{kg\ DM})$$

The digestibility of NDF, OM, CP and ash were estimated by the following formula:

Equation 4

$$Digestibility\ of\ nutrient\ (\%) = \frac{nutrient\ in\ feeds - nutrient\ in\ faeces}{nutrient\ in\ feeds}\ x\ 100$$

where nutrient in feeds is nutrient intake in g/day and nutrient in faeces is in g/day.

3.6.2 Milk production and nitrogen balance

Energy corrected milk (ECM) was calculated by the formula from Åkerlind et al. (2011a):

Equation 5

$$ECM = MY\ x\ (0.01 + 0.122\ x\frac{f_{milk}}{10} + 0.077\ x\frac{p_{milk}}{10} + 0.053\ x\frac{l_{milk}}{10})$$

where ECM is energy corrected milk in kg/day, MY is milk yield in kg/day and f_{milk}, p_{milk} and l_{milk} is the content of fat, protein and lactose, respectively, in g/kg.

Nitrogen in milk is given by:

Equation 6

$$N\ in\ milk = \frac{MY\ x\ CP_{milk}}{6.38}$$

where MY is milk yield in kg/day and CP_{milk} is the crude protein content of milk in gram.

Dietary nitrogen use efficiency (NUE) is given by:

Equation 7

$$NUE = \frac{N\ in\ milk}{N\ intake} x\ 100$$

where values of N are given in g/day. The NUE is N in milk in percentage of daily N intake.

Residual N (dietary N intake that was not accounted for in milk and faeces) is given by:

Equation 8

$$Residual\ Nitrogen = N\ intake - N\ in\ milk - N\ in\ faeces.$$

Urinary nitrogen excretion (UN, g/day) was calculated using the formula from Kidane et al. (2018a):

Equation 9

$$UN = 0.2232\ x\ BW\ x\ MUN$$

where BW is average body weight in kg for the last week of the experiment, and MUN is average milk urea nitrogen (mg/dl) over the total experiment.

3.7 Statistics

Statistical analyses were performed using the SAS 9.4 software (SAS, 2012). Three days moving average was used for daily registrations of milk yield, feed intake and body weight. For these data the following mixed model (Proc MIXED) was used:

$$Y_{ijkl} = \mu + A_i + B_j + C_k + D_l + DIM + (BC)_{jk} + \varepsilon_{it}$$

where Y_{ijkl} = response variable, μ = overall mean, A_i = random effect of cow (i = 1,…,16), B_j = fixed effect of treatment (j = 1, 2, 3), C_k = fixed effect of day (k = 1,…, 56), D_l = covariate, DIM = effect of days in milk (only included for milk yield and body weight), $(BC)_{jk}$ = interaction between treatment and day, and ε_{it} = residual error. Day was considered a repeated measurement and TOEP(1) covariance structure was used. Results are presented as Least Square Mean (LSmeans) and multiple comparison adjustment of the P-value. Standard error of LSmeans (SEM) is used.

For digestibility, nutrient and N balance variables the following general linear model (Proc GLM) was used:

$$Y_{it} = \mu + T_i + \varepsilon_{it}$$

where Y_{it} = response variable, μ = overall mean, T_i = effect of treatment (i = 1,2,3) and ε_{it} = residual error. Results are presented as Least Square Mean (LSmeans) and differences among treatments were evaluated using the PDIFF statement. Variation is given as root means square error (RMSE).

In both models the following contrasts were used:
Contrast 1: SOYABP and YEASTP versus the negative control, BARLEY.
Contrast 2: SOYABP versus YEASTP.

Differences were considered as statistically significant if P-value ≤ 0.05. P-values between 0.05 - 0.10 were considered as a tendency.

4 Results

The data used in this study are from day 49 – 56 of the experiment (week 10) unless stated otherwise.

4.1 Feed

Chemical composition of the feed is given in Table 6. The CP content was lower for the concentrate based on barley, hence a negative control. Grass silage shows to have high CP content and a good fermentation quality.

Table 6. Chemical composition of the grass silage and the different concentrates (g/kg DM if not stated otherwise).

	Soy	Yeast	Barley	Silage	Mineral supplement
Dry matter, g/kg	875.2	881.3	875.3	300.0	969
Organic matter	931.9	931.5	927.9	921.2	125.9
Ash	68.2	68.5	72.1	78.8	874.1
NDF (aNDFom)[1]	172.7	161.4	173.7	520.9	
Crude protein	160.3	157.1	132.9	180.0	
Acid-Insoluble Ash	2.75	3.14	2.57	3.83	56.3
Fermentation products					
Lactic acid				67.7	
Acetic acid				1.9	
Propionic acid				14.3	
Formic acid				10.3	
Butyric acid				0.9	
Ethanol				4.8	
Ammonium-N, g/kg N				71.7	
Nitrate, g/kg N				4.9	
pH				3.9	

[1] aNDFom = neutral detergent fibre

4.2 Feed intake and digestibility

Mean feed intakes corrected with covariate are given in Table 7. No significant effect of dietary treatment was found in DMI or nutrient intake, except for in CP intake ($P < 0.0001$). This was significantly lower for the negative control diet, BARLEY, than the other treatments for average over the experimental period of 56 days ($P < 0.0001$). The contrast of SOYABP versus YEASTP showed a tendency for different concentrate intake between the treatments.

Table 7. Mean feed intake over the total experiment (56 days) including covariate (kg/day).

	SOYABP	YEASTP	BARLEY	SEM[2]	P	Contrast[3]	
Intake						1	2
Concentrate	7.73	7.60	7.68	0.05	0.209	0.828	0.081
Silage	14.4	14.5	14.1	0.19	0.443	0.206	0.914
Total dry matter	22.1	22.0	21.9	0.18	0.866	0.622	0.847
Nutrients							
Organic matter	20.5	20.4	20.2	0.18	0.640	0.424	0.629
Ash	1.66	1.66	1.68	0.02	0.667	0.382	0.831
NDF[1]	8.84	8.76	8.72	0.10	0.658	0.518	0.530
Crude protein	3.83[a]	3.79[a]	3.57[b]	0.03	<0.0001	<0.0001	0.378

[1] NDF = neutral detergent fiber (aNDFom).
[2] SEM = standard error LSmeans.
[3] Contrast 1 = SOYABP and YEASTP versus BARLEY (negative control). Contrast 2 = SOYABP versus YEASTP. Significant at P < 0.05.
[a-b] Different uppercase letters within a row indicate significant differences (P < 0.05).

Overall, the DMI over the experiment varied in a similar fashion for all groups (Figure 10). In Figure 10a feed intake shows the lowest for BARLEY and the highest for YEASTP, but with covariate adjustment in Figure 10b, intakes were approximately the same.

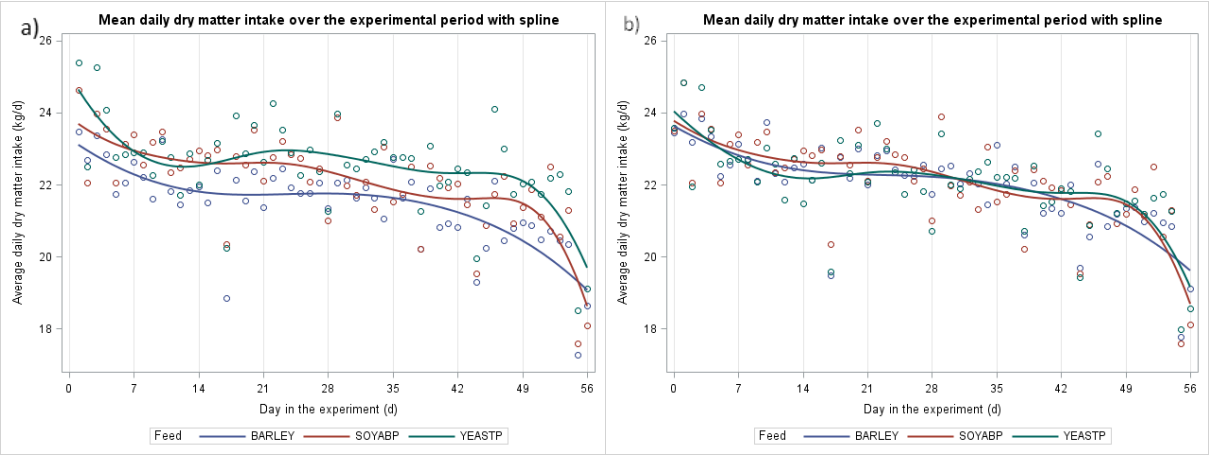

Figure 10. Mean daily dry matter intake over the experiment a) observed values without covariate and b) adjusted by covariate.

Table 8 presents the registered average feed intake in DM of the three treatments, along with the intake of each nutrient and nutrient excreted in faeces. There was no significant difference between observed dietary treatment on DMI. However, a tendency (P = 0.073) was shown for lower silage intake in BARLEY than YEASTP, and contrast showed a significant difference for BARLEY (P = 0.031) than the average of others. The observed CP intake was

significantly lower for BARLEY than both SOYABP and YEASTP (P = 0.034, P = 0.006, respectively). YEASTP and BARLEY also differed (P = 0.041) in observed NDF intake. The contrast of SOYABP and YEASTP versus negative control, BARLEY, showed a tendency for lower NDF intake in BARLEY. Treatment had a significant effect on the AIA intake, where YEASTP was significantly higher than BARLEY (P = 0.003) and tended to be higher than SOYABP (P = 0.087). A 100% recovery of ingested AIA was assumed. No significant effect of treatment was observed for nutrients excreted in faeces. Table 8 also shows the digestibility values calculated based on AIA analysis. No significant differences in digestibility were found.

Table 8. Dry matter intake, nutrient intake, nutrients in faeces and digestibility values of the nutrients for the three treatments, calculated by the AIA method.

	SOYABP	YEASTP	BARLEY	RMSE[3]	P	Contrast[4]	
Intake, kg/day						1	2
Silage intake	14.1[ab]	14.6[a]	13.1[b]	1.83	0.073	0.031	0.437
Concentrate intake	6.44	6.61	6.84	1.23	0.649	0.398	0.706
Nutrients intake, kg/day							
Dry matter	20.5	21.2	19.9	2.29	0.306	0.193	0.411
Organic matter	19.0	19.6	18.4	2.12	0.295	0.183	0.413
Ash	1.55	1.60	1.53	0.17	0.440	0.346	0.387
NDF[1]	8.45[ab]	8.74[a]	8.00[b]	1.00	0.118	0.058	0.410
Crude protein	3.57[a]	3.66[a]	3.27[b]	0.39	0.016	0.005	0.489
Acid-Insoluble Ash, g/day	71.7[ab]	76.7[a]	67.8[b]	8.08	0.012	0.013	0.087
Nutrients in faeces, kg/day [2]							
Dry matter	3.54	3.83	3.61	0.75	0.525	0.725	0.283
Organic matter	3.20	3.45	3.25	0.69	0.557	0.706	0.313
Ash	0.34	0.38	0.36	0.07	0.255	0.952	0.100
NDF[1]	1.78	1.92	1.82	0.45	0.668	0.815	0.388
Crude protein	0.64	0.68	0.64	0.13	0.517	0.513	0.346
Digestibility, % [2]							
Dry matter	82.9	82.0	81.9	2.56	0.486	0.476	0.335
Organic matter	83.3	82.5	82.3	2.55	0.537	0.496	0.379
Ash	78.1	76.3	76.4	3.14	0.190	0.409	0.104
NDF[1]	79.2	78.1	77.3	4.19	0.429	0.283	0.464
Crude protein	82.2	81.4	80.5	2.75	0.218	0.119	0.437

[1] NDF = neutral detergent fiber (aNDFom).

[2] Calculated using acid-insoluble ash (AIA) as a marker.

[3] RMSE = root mean square error.

[4] Contrast 1 = SOYABP and YEASTP versus BARLEY (negative control). Contrast 2 = SOYABP versus YEASTP. Significant at P < 0.05.

[a-b] Different uppercase letter within a row indicates significant differences (P < 0.05).

4.3 Milk production and chemical composition

Mean milk yield and ECM corrected for the pre-experimental period are shown in Table 9. Dietary treatment did not have any significant effect on average milk yield or ECM over the 56 days of the experiment. A tendency (P = 0.100) was shown for lower milk yield in BARLEY compared to SOYABP in the last week of the experiment.

Milk composition is given as an average value over the total experiment. Treatment showed no significant effect on milk production variables or chemical composition (Table 9). However, the analysis showed a tendency for effect of dietary treatment on protein content and milk urea. A contrast of negative control diet, BARLEY versus the average of SOYABP and YEASTP was significant for both milk protein content and milk urea.

Table 9. Milk production and chemical composition over total experiment adjusted by covariate.

	SOYABP	YEASTP	BARLEY	SEM[5]	P	Contrast[6]	
Production 56 d average, kg/day[1]						1	2
Milk yield	30.9	30.0	29.7	0.45	0.317	0.692	0.155
ECM[2]	32.8	32.9	31.8	0.60	0.917	0.783	0.775
Composition of milk, g/kg[3]							
Protein	36.1	36.2	34.9	0.47	0.094	0.031	0.853
Fat	43.8	45.2	44.2	0.80	0.464	0.821	0.229
Lactose	47.9	48.0	47.8	0.19	0.766	0.554	0.676
Milk urea, mmol/L	5.27	5.28	5.08	0.07	0.090	0.030	0.976
Free fatty acids, meq/L	0.86	0.75	0.98	0.14	0.480	0.291	0.568
Milk component yields, g/kg[3]							
Protein	1.09	1.09	1.02	0.02	0.099	0.033	0.980
Fat	1.32	1.35	1.29	0.03	0.411	0.281	0.430
Lactose	1.47	1.44	1.40	0.03	0.248	0.132	0.456
Production week 10, kg/day[4]							
Milk yield	29.0	28.4	26.9	0.67	0.796	0.909	0.515
ECM[2]	31.4	31.0	29.4	0.74	0.137	0.058	0.654

[1] Calculated with 3 days moving average over 56 d.
[2] ECM = energy corrected milk.
[3] Milk composition and component yields based on 4 milk sampling days and corresponding yields.
[4] Milk yield and ECM based on week 10 (corresponding to the faecal collection week).
[5] SEM = standard error LSmeans.
[6] Contrast 1 = SOYABP and YEASTP versus BARLEY (negative control). Contrast 2 = SOYABP versus YEASTP. Significant at P < 0.05.

Figure 11 presents the average daily milk yield throughout the experiment, from the first day of treatment diet. Although the treatments had no significant difference in milking yield (Table 9), Figure 11b shows a greater drop in milk yield for BARLEY than the other diets.

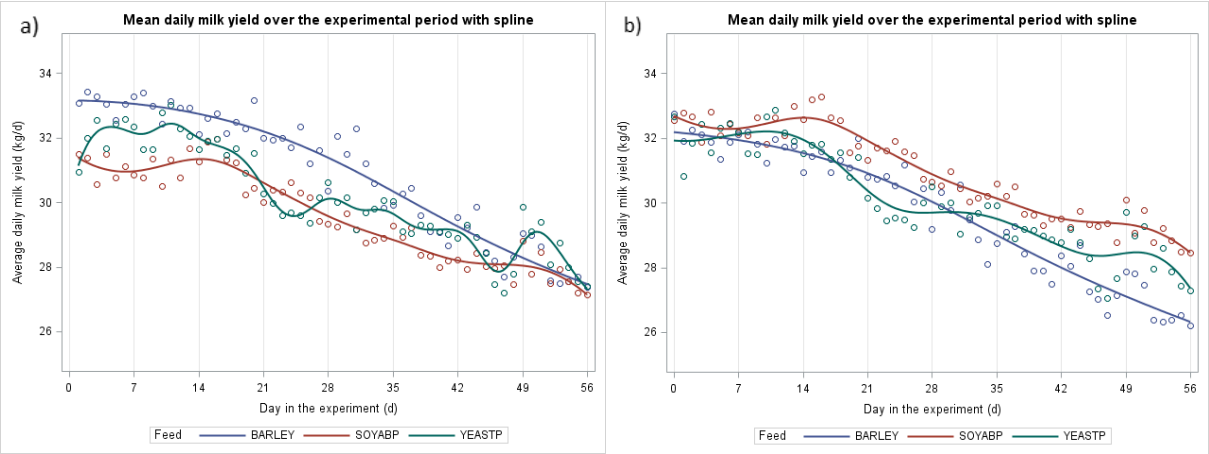

Figure 11. Mean daily milk yield (kg/day) over the experiment with spline, a) observed values and b) adjusted by covariate.

The graphs in Figure 12 shows the average ECM throughout the experiment estimated by milk analysis from corresponding periods. Corrected with a covariate, BARLEY showed lower ECM than the other treatments.

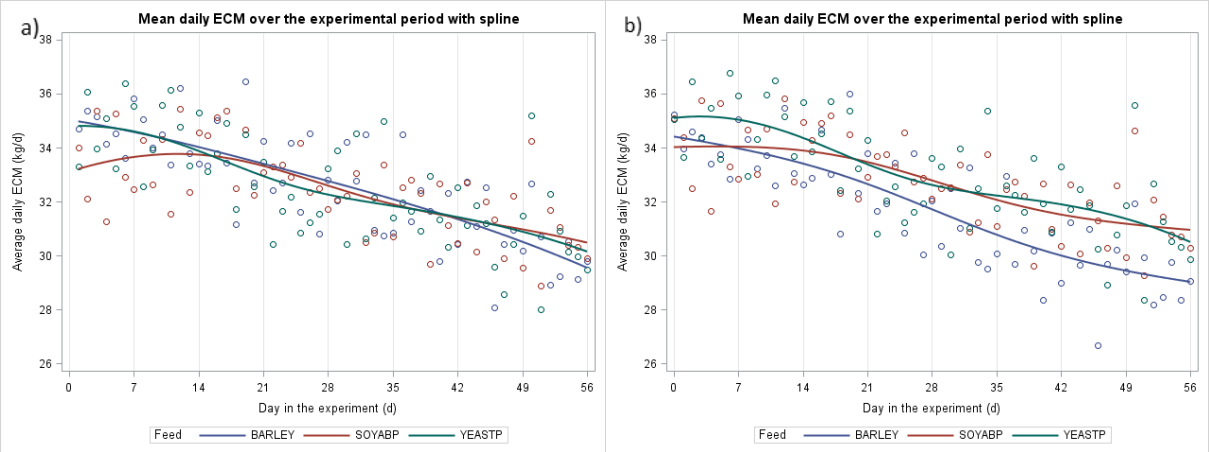

Figure 12. Mean daily energy corrected milk (ECM, kg/day) over the experiment with spline, a) observed values and b) adjusted by covariate.

4.4 Nitrogen balance

The N balance is shown in Table 10. All values are observed averages, no covariates included. For the calculation of N in milk, observed average milk yield for week 10 of the experiment (i.e. 27.7, 28.5, 28.0 kg/day) and average milk protein contents of all analysis of 36.3, 36.0, 34.8 g/kg for SOYABP, YEASTP and BARLEY treatments, in the respective order, were used according to Equation 6. Observed average of milk urea nitrogen (MUN) analysis for each cow throughout the experiment were used for the calculation of UN, which

were in average 15.1, 14.7 and 14.0 mg/dl for SOYABP, YEASTP and BARLEY, respectively.

Nitrogen in milk and faeces showed no significant effect of dietary treatment (Table 10). Estimated residual N showed an effect of treatment (P = 0.002), where BARLEY was significantly lower than the two other treatments. The residual N in percentage of N intake showed a tendency to be lower for BARLEY than SOYABP and further analysis showed a significant difference between the two (P = 0.041). A contrast of the negative control, BARLEY versus the average of the two other treatments was significant for residual N and the residual N in percentage of N intake.

Table 10. Nitrogen balance, with observed values in milk and faeces, and predicted residual nitrogen and urinary nitrogen.

	SOYABP	YEASTP	BARLEY	RMSE[2]	P	Contrast[3]	
						1	2
N intake, g/day	570.9[a]	586.4[a]	522.6[b]	62.5	0.016	0.005	0.489
N recovered, g/day							
Milk	157.1	160.1	151.3	25.3	0.611	0.351	0.743
Faeces	102.4	109.2	101.8	20.1	0.517	0.513	0.346
Residual Nitrogen	311.4[a]	317.1[a]	269.5[b]	38.8	0.002	0.001	0.679
N in urine calculated[1]	220.3[a]	217.9[a]	194.8[b]	25.7	0.013	0.004	0.794
N recovered	479.8[ab]	487.1[a]	447.8[b]	49.5	0.068	0.023	0.678
N not recovered	91.1[ab]	99.2[a]	74.8[b]	32.4	0.106	0.046	0.483
N recovered in % of N intake							
Milk (N use efficiency)	27.5	27.3	28.9	2.99	0.271	0.110	0.896
Faeces	17.8	18.6	19.5	2.75	0.219	0.119	0.437
Residual Nitrogen	54.7[a]	54.1[ab]	51.6[b]	4.22	0.094	0.033	0.678
Urine calculated (UN)	38.8	37.5	37.5	4.73	0.684	0.659	0.455
N recovered	84.0	83.4	85.9	4.85	0.324	0.147	0.712
N not recovered	16.0	16.6	14.1	4.85	0.324	0.147	0.712

[1] Calculated N in urine = 0.02232 x body weight (BW) x milk urea nitrogen (MUN) (Kidane et al., 2018a).
[2] RMSE = root mean square error.
[3] Contrast 1 = SOYABP and YEASTP versus BARLEY (negative control). Contrast 2 = SOYABP versus YEASTP. Significant at P < 0.05.
[a-b] Different uppercase letters within a row indicate a significant difference between the groups (P < 0.05).

Calculated UN showed a significant effect of treatment (Table 10). BARLEY was lower than the two other treatments and showed significant contrast to SOYABP and YEASTP. There was a tendency for N recovered in milk and faeces to be lower in BARLEY compared to YEASTP, whereas further analysis showed a significant difference (P = 0.030). For the N that

was not recovered, the analysis showed a difference between YEASTP and BARLEY (P = 0.038). A contrast of the negative control, BARLEY, showed lower recovered N in milk and faeces than for the average of the other treatments (P < 0.023), and the same for the N not recovered (P = 0.046). No significant differences between SOYABP and YEASTP were found in N balance or estimates of the UN (Table 10).

As shown in Table 10, the UN is estimated lower than the residual N for all treatments. Figure 13 compares the residual N and the calculated UN value, showing some linear relationship (R^2 = 0.42), although low.

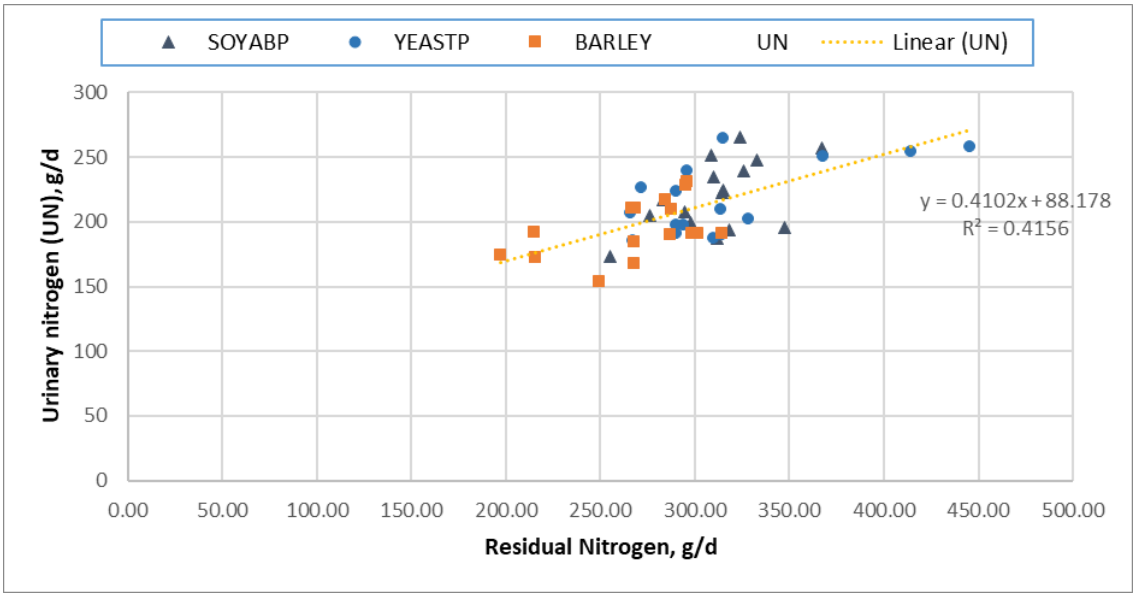

Figure 13. The linear relationship between the estimates of residual nitrogen and calculated urinary nitrogen.

4.5 Body weight

The average body weight of the animals for each treatment is shown in Table 11. Mean body weight for the last week of the experiment adjusted with covariate showed a significant difference between SOYABP and YEASTP (P = 0.040). As shown in Figure 14, YEASTP increased more in body weight towards the end of the experiment compared to both SOYABP and BARLEY. Overall, BARLEY had lower body weight, but when adjusted with the covariate, there were no large differences.

Table 11. Body weight (BW) in the different treatments.

	SOYABP	YEASTP	BARLEY	RMSE[1]	P	Contrast[3]	
						1	2
BW observed week 10	653.8	664.8	625.0	70.2	0.264	0.118	0.658
Adjusted for covariate				SEM[2]			
BW start	625.9	628.7	629.0	1.41	0.985	0.864	0.988
BW end	645.2[a]	653.5[b]	648.9[ab]	2.31	0.403	0.353	0.338
BW average	638.4	642.2	641.4	1.64	0.823	0.547	0.904

[1] RMSE = root mean square error.

[2] SEM = Standard Error of Least Square Means

[3] Contrast 1 = SOYABP and YEASTP versus BARLEY (negative control). Contrast 2 = SOYABP versus YEASTP. Significant at $P < 0.05$.

[a-b] Different uppercase letters within a row indicate a significant difference between the groups ($P < 0.05$).

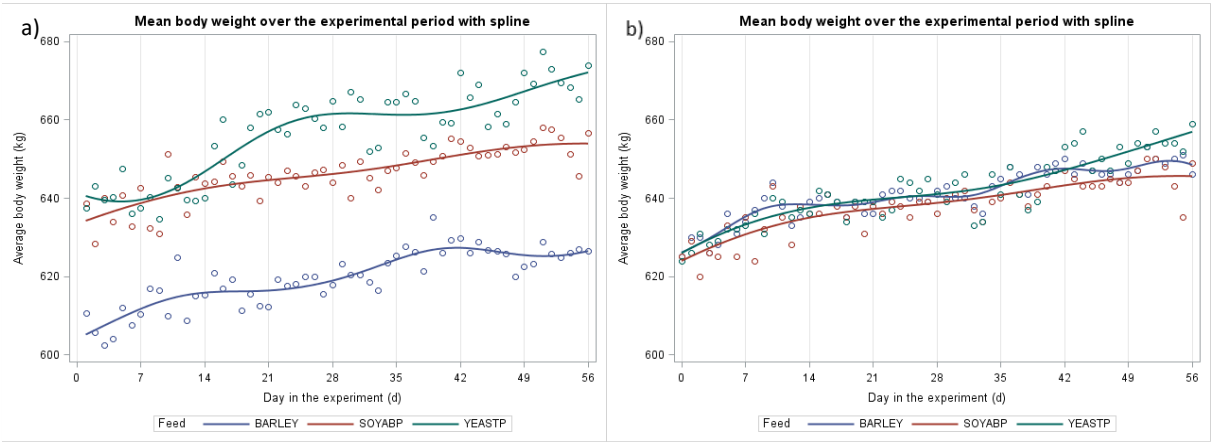

Figure 14. Mean body weight (kg) over the experiment with spline, a) observed values and b) adjusted by covariate.

5 Discussion

In the present study, soybean meal was replaced by *Candida utilis* yeast as a protein source in concentrate for dairy cows. Furthermore, barley was used as a replacement for both soybean meal and yeast. In addition to animal performance, faecal digestibility using AIA as an internal marker and calculation of urinary output was an important part of the study. The discussion will start with some methodological consideration on the use of AIA as a marker and calculation of urine using literature equations.

5.1 Use of AIA as an internal marker

The use of AIA as an internal marker allows predicting feed utilisation without the need for extensive housing for a total collection of faeces and urine. The concentration of AIA may vary considerably between feeds. In soybean meal, the AIA concentration has been reported to be around 1 g/kg DM, whereas 46 g/kg DM has been reported in fishmeal (Sales & Janssens, 2003). The AIA concentration in feed and faeces affects the precision in the calculation of digestibility. In this study, the AIA concentration was around 3.5 g/kg DM. Thonney et al. (1985) claimed that the AIA analysis may be inaccurate if the AIA content in feeds is below 7.5 g/kg DM, more than twice the concentration found in the current study. However, Sales and Janssens (2003) indicated that the analytical error could be described as the most common reason for failure when using AIA as a marker. Still, they concluded that AIA presents a reliable marker with several advantages that could be successfully utilised to determine faecal digestibility in animal species under certain circumstances. Moreover, in the current study AIA concentrations did not vary much between treatments. Thus, although AIA concentrations are low compared to the recommendations from Thonney et al. (1985), the results obtained should reflect treatment effects.

In this study, cows had access to a mineral supplement with an expected intake of 100 to 150 g/cow/day. Unfortunately, this supplement was provided as a top dressing on the silage and not recorded at cow level. Therefore, the digestibility estimates presented here are calculated assuming no intake of the supplement. To test for a possible influence of intake, a hypothetical model was made accounting for a range in intakes of mineral supplement from 50 to 150 g/cow/day, namely 50, 100 and 150 g mineral supplement intake per cow per day. If cows had eaten 150 g of the mineral supplement daily (which was hypothetical maximum), digestibility values decreased with a maximum of 2.3% for BARLEY and a maximum of

2.0% for the other dietary treatments. Therefore, the DMD and digestibility of other nutrients are most likely lower than our reported values. However, assuming intake did not differ and that this applied to all treatment groups, this would not have affected treatment differences although variation between animals may have increased.

In addition to the aspects mentioned, other factors may also contribute to incorrect values. Contamination of feed or faecal samples with sand, bedding material (e.g. sawdust) or soil is a possible source of errors. However, due care was taken when faecal samples were collected from the cows by moving them to a clean holding area, taking fresh samples from the rectum or from faeces samples from dropping when a cow was noticed defecating. But, admittedly faeces were sampled only once from each cow in this study, increasing the risk of not giving representative samples. Other studies (Kidane et al., 2018b; Mehtiö et al., 2015) have made representative samples out of several faecal samples to counteract this risk and increase the analytical accuracy.

Lee and Hristov (2013) reported underestimation of digestibility when using AIA and iNDF as an internal marker, iNDF being a more reliable marker than AIA. The estimated digestibility figures produced here, as stated earlier, appear to be inflated when compared to recent studies with NRF dairy cows whilst using total faecal collection (Naadland et al., 2017), using iNDF as a marker (Kidane et al., 2018b) or using C_{32} n-alkane as external marker under grazing conditions (Kidane et al., 2019). Digestibility values may have been overestimated by not accounting for the mineral supplement in this study. If this is the case, the N content in faeces is higher than the given values. This would consequently affect the residual and urinary N calculations, but again, this might not have been biased towards any of the dietary treatments.

5.2 Urinary nitrogen

Urinary N output was calculated as residual N not accounted for in milk or faeces, and by using the equation given by Kidane et al. (2018a). The amount of N retention in the body varies among cow individuals, depending on pregnancy, growth and maintenance needs.

The calculation of residual N and UN showed a difference at 91.1, 99.2 and 74.8 g/day for SOYABP, YEASTP and BARLEY, respectively. Residual N accounts for all N excreted in urine and body reserves, for maintenance, growth and for skin and hair. Some cows would require extra proteins due to pregnancy. The protein need for maintenance in gram per day is

given by $BW^{0.75}$x 2.2 (Madsen et al., 2003), implying a need of approximately 280 g protein per day, which includes nitrogen both released in faeces and urine. For skin and hair alone, protein need is given by (0.2 x $BW^{0.6}$)/0.67 (Nielsen & Volden, 2011), which equals to around 14 – 15 g/day. Therefore, the difference between residual N and calculated UN includes the N retained in the body for growth or pregnancy among others. Dijkstra et al. (2013b) reported the lowest N excretion in faeces and urine to be 89 and 174 g/day, respectively. Thus, the faecal recovery of N (average 18%) seems to be low in this study compared to other studies (Hristov et al., 2004), which recovered around 30% of dietary N intake in faeces. This corresponds with the possible underestimation of N in faeces by the marker method in the current study, which again might have influenced the estimates of urinary N in this study.

Urinary N calculation was based on average animal body weight in the last week of the experiment and average MUN for all milk analysis throughout the experimental period. The MUN concentration in milk change throughout the lactation period, as well as the other milk compounds. Kidane et al. (2018a) reported lower UN values than what is obtained in the present study. When using the same formula in this study, results can alter due to higher MUN values than the formula is calculated from. However, although both methods for estimating urinary N is hampered with some errors, they were not expected to bias treatment effects. Overall, the cows gained weight throughout the experiment, expecting some N retained in the body. Therefore, including growth and other factors probably would have improved urine output calculated as residual N, and thereby calculated UN values. However, as these factors were not expected to vary between treatments, they probably have not influenced treatment effects, except for maybe a tendency towards a higher weight gain in YEASTP.

5.3 Yeast as a protein source for dairy cows

Using *C. utilis* yeast as a replacement of soybean meal in diets of dairy cows did not affect the total feed intake, nutrient intake and estimated digestibility coefficients. However, other studies with yeast replacing soybean meal have shown various results on DMI. When replacing soybean meal with yeast Sabbia et al. (2012) reported increased DMI, whereas Neal et al. (2014) found lower DMI when feeding yeast-derived microbial protein (YMP). The meta-analysis done by Desnoyers et al. (2009) claimed that yeast supplementation in ruminants would increase DMI, milk yield and milk fat content. As a result of no difference in

DMI, nutrient intake or digestibility, the use of yeast as a replacement of soybean meal in diets of dairy cows made no large differences in milking yield or milk chemical composition.

Works with YMP on lactating dairy cows indicated that replacing soybean meal with YMP did not show any adverse effect on milk production (Neal et al., 2014; Sabbia et al., 2012). Sabbia et al. (2012) stated improved milk fat content and increased ECM when feeding YMP in high-yielding dairy cows on high forage diets.

Candida utilis has been studied in several animal species and the utilisation varies among species. Cruz et al. (2019) studied the effect of using *C. utilis* as a protein source in diets of piglets on growth and digestion and stated that *C. utilis* could replace 40% of the CP in the diet. Also, Cruz et al. (2019) recorded higher digestibility of CP and ash in piglets when feeding with yeast, but NDF digestibility was lower for piglets where 40% of the CP was from yeast. Similarly, a study with Atlantic salmon showed promising results of replacing 40% of fishmeal with *C. utilis*, resulting in similar digestibility, but higher N retention (Øverland et al., 2013). However, the current study involves ruminants, in which microbial fermentation and microbial protein synthesis in the rumen plays an important role in the quality and quantity of amino acids supplied to the host animal in contrast to monogastric animals such as pigs and salmon. Moreover, Sharma et al. (2018) noticed that some of the yeast fed to fish was not digested, probably due to cell wall components in the yeast. For ruminants, this may not be a problem due to its symbiotic ruminal environment. Also, yeast is said to contain high amounts of nucleic acids (Cruz et al., 2019), in which ruminants can utilise.

Yeast-based concentrate had no large influence on performance parameters when replacing soybean meal. As diets were iso-nitrogenous and no difference in dry matter intake or milk yield were found, the NUE showed no effect of *C. utilis* substituting soybean meal. On the contrary, Neal et al. (2014) reported improved nutrient utilisation efficiency when using YMP compared to soybean meal.

In the present study, the cows fed *C. utilis* yeast gained marginally more weight than the other animals, although feed intake did not vary. All cows increased in body weight throughout the trial. Given the iso-energetic nature of the diets, their similar estimated digestibility values, and their similar achieved milk yield, it is not clear why the yeast-based diet increased body weight gain.

5.4 Replacing soybean meal and yeast with barley

Replacing soybean meal and yeast in concentrates with barley lowered the CP content of the diet. Thus, CP intake was lower for the group on the barley-based diet, as expected. On the other hand, silage CP content (i.e. 180 g/kg DM) was high compared to normal average values for early harvested grass silage at 167 g/kg DM (NorFor Feed Table). As a result, the total CP content of the barley-based diet in this study was perhaps higher than planned. Nevertheless, weak tendencies were observed for milk chemical composition when BARLEY was compared to the yeast-based and soybean meal-based diets.

Milk production in dairy cows depends on the supply of substrates. For milk protein, the amino acid supply to the intestines is especially important. Reducing the protein content in the diet by replacing soybean meal and yeast with barley decreased the milk protein content and the milk urea concentration. However, comparing with the average values in TINE (2020), the milk protein content was normal for all diets and milk urea concentrations were within the 3 to 6 mmol/L limit considered normal (Geno, 2014). This suggested that replacing soybean meal with barley may be acceptable in the presence of good quality silage with high crude protein and soluble crude protein content, as used here. However, the barley-based diet seemed to decrease more in milk production and ECM towards the last part of the experiment. This is expected due to lower protein intake and therefore lower amino acid supply for milk protein synthesis. However, the lack of strong differences among the dietary treatments might have been due to the very good quality grass silage used, as stated earlier, which comprised about two-thirds of the total DMI of cows. Furthermore, replacement of soybean or yeast with barley resulted in increased starch intake in the group and this might have also increased microbial crude protein synthesis in BARLEY. Lower CP intake promotes higher N recycling of urea for the cow to maintain microbial synthesis (Mutsvangwa et al., 2016). As milk urea mirrors the blood urea concentration, the lower milk urea in barley-based diet indicates that there is more N being recycled, thus more N utilised, and less N excreted through urine.

The N content in milk as the proportion of N intake is wanted towards the highest possible value, theoretically estimated to be 43% (Dijkstra et al., 2013b). Kidane et al. (2018b) reported NUE of 33 and 30% for low CP diet and optimal CP diets, respectively. However, compared to the present study, N intake both in the low CP (12.8%) and optimal CP diets (14.8%) was lower in the reference study. It is expected that NUE would increase with decreasing dietary CP in dairy cow diets as observed in other studies (Broderick, 2003; Kidane et al., 2018a). As a result, the NUE observed in the current study could be lower due

to achieved higher dietary CP intake. Hristov et al. (2004) reported similar N intake for their adequate rumen degradable protein diet (595 g/kg DM) as in the optimal diets in this study, showing lower N content in milk and lower NUE values (18.4%). Higher dietary N intake resulted in lower values in NUE, but the N excretion in urine was higher, as indicated in the present study. The release of N through urine and faeces contributes to environmental emissions. Optimizing N utilisation has been studied (Calsamiglia et al., 2010; Kebreab et al., 2001; Mutsvangwa et al., 2016), and reducing the dietary N intake is one method towards this. In this study, although the NUE in BARLEY did not differ from the other treatments, urinary N output was lower. Thus, lower N emission to the environment was achieved.

6 Conclusion

No effect of replacing soybean meal with *Candida utilis* was found on DMI, milk production, DMD or NUE. Thus, *Candida utilis* as a protein source shows promising results in diets of dairy cows. This suggested that given adequate production of *C. utilis* based on local resources (with biomass from the sea, forest by-products, etc.), import of soybean meal as a protein ingredient for ruminants could be reduced. This is deemed to be beneficial both in the sense of reduced carbon footprint and reduced reliance on imported ingredients. However, a reduced protein content with barley replacing soybean meal and yeast in the feed suggested marginally decreasing milk production over time and decreased urinary N output. The latter is wanted, but the effects on milk production warrant long-term study (e.g. whole lactation) to be conclusive.

7 References

Aaes, O., Børsting, C. F., Sehested, J. & Hvelplund, T. (2003). Kvægets miljøpåvirkning, med fokus på kvælstof, fosfor og metan. In Strudsholm, F. & Sejrsen, K. (eds) *Kvægets ernæring og fysiologi. Bind 2 - Fodring og produktion, DJF rapport Husdyrbrug 54*, pp. 323-340. Denmark: Danmarks JordbrugsForskning.

AOAC. (2002). AOAC Official Method 2001.11: Protein (crude) in animal feed, forage (plant tissue), grain, and oilseeds. *Journal of AOAC International*.

Asp, N.-G. (1996). Dietary carbohydrates: classification by chemistry and physiology. *Food Chemistry*, 57 (1): 9-14. doi: 10.1016/0308-8146(96)00055-6.

Bach, A., Calsamiglia, S. & Stern, M. D. (2005). Nitrogen metabolism in the rumen. *Journal of dairy science*, 88: E9-E21.

Baruah, J., Nath, B. K., Sharma, R., Kumar, S., Deka, R. C., Baruah, D. C. & Kalita, E. (2018). Recent Trends in the Pretreatment of Lignocellulosic Biomass for Value-Added Products. *Frontiers in Energy Research*, 6 (141). doi: 10.3389/fenrg.2018.00141.

Berg, M. B. (2018a). *1038m Aske*. Ås: Institutt for Husdyr og akvakulturvitenskap, NMBU. Available at: https://www.nmbu.no/fakultet/biovit/forskning/tekniske-forskningssentre/node/10055 (accessed: 02.03).

Berg, M. B. (2018b). *Msp 1040 Kjeldahl-N*. Ås: Institutt for Husdyr og akvakulturvitenskap, NMBU. Available at: https://www.nmbu.no/fakultet/biovit/forskning/tekniske-forskningssentre/node/10055 (accessed: 27.02).

Berg, M. B. (2018c). *Msp 1044 Tørrstoff*. Ås: Institutt for husdyr- og akvakulturvitenskap, NMBU. Available at: https://www.nmbu.no/fakultet/biovit/forskning/tekniske-forskningssentre/node/10055 (accessed: 02.03).

Broderick, G. (2003). Effects of varying dietary protein and energy levels on the production of lactating dairy cows. *Journal of dairy science*, 86 (4): 1370-1381.

Budsjettnemnda for jordbruket. (2020). *Resultatkontroll for gjennomføringen av landbrukspolitikken avgitt April 2020*. Resultatkontroll for gjennomføringen av landbrukspolitikken: NIBIO. Available at: https://nibio.no/tema/landbruksokonomi/grunnlagsmateriale-til-jordbruksforhandlingene/_/attachment/inline/af4e716f-bcc8-4ee5-93f1-a1d6431f2160:826996bca959e79a41cfc2213f6da23ff327a9a6/UT-3-2020%20Resultatkontrollen%20Oppdatert%2011.5.pdf (accessed: 07.07).

Calsamiglia, S., Ferret, A., Reynolds, C., Kristensen, N. B. & Van Vuuren, A. (2010). Strategies for optimizing nitrogen use by ruminants. *Animal*, 4 (7): 1184-1196.

Clark, J. H., Klusmeyer, T. H. & Cameron, M. R. (1992). Microbial Protein Synthesis and Flows of Nitrogen Fractions to the Duodenum of Dairy Cows. *Journal of Dairy Science*, 75 (8): 2304-2323. doi: 10.3168/jds.s0022-0302(92)77992-2.

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.

Dei, H. K. (2011). Soybean as a Feed Ingredient for Livestock and Poultry. In: InTech.

Desnoyers, M., Giger-Reverdin, S., Bertin, G., Duvaux-Ponter, C. & Sauvant, D. (2009). Meta-analysis of the influence of Saccharomyces cerevisiae supplementation on ruminal parameters and milk production of ruminants. *Journal of Dairy Science*, 92 (4): 1620-1632. doi: 10.3168/jds.2008-1414.

Dijkstra, J., Oenema, O., Van Groenigen, J. W., Spek, J. W., Van Vuuren, A. M. & Bannink, A. (2013a). Diet effects on urine composition of cattle and N2O emissions. *animal*, 7 (s2): 292-302. doi: 10.1017/s1751731113000578.

Dijkstra, J., Reynolds, C., Kebreab, E., Bannink, A., Ellis, J., France, J. & Van Vuuren, A. (2013b). Challenges in ruminant nutrition: towards minimal nitrogen losses in cattle. In *Energy and protein metabolism and nutrition in sustainable animal production*, pp. 47-58: Springer.

Faichney, G. J. (1975). The use of markers to partition digestion within the gastro-intestinal tract of ruminants. In McDonald, I. W. & Warner, A. C. T. (eds) *Digestion and Metabolism in the Ruminant*, pp. 277-291. Armidale, Australia: New England Publ. Unit.

FAO. (2016). *The state of food and agriculture - Climate change, agriculture and food security*. Rome, Italy.

Felleskjøpet. (n.d). *Fakta om soya*. Felleskjøpet. Available at: https://www.felleskjopet.no/om-felleskjopet/barekraftig-landbruk-soya-og-palmeolje/fakta-om-soya/ (accessed: 13.04).

Felleskjøpet Agri. (2015). *Årsrapport 2015 Bondens partner*. Available at: https://www.felleskjopet.no/aarsrapport2015/fk_aarsrapport_2015.pdf.

Geno. (2014). *Urea og protein*: Geno. Available at: https://www.geno.no/Start/Brunst/Fagstoff/Foring-og-fruktbarhet/Urea-og-protein/ (accessed: 09.08).

Gjefsen, T. (1995). *Foringslære*. 2nd ed. Oslo: Landbruksforlaget.

Guinguina, A., Ahvenjärvi, S., Prestløkken, E., Lund, P. & Huhtanen, P. (2019). Predicting feed intake and feed efficiency in lactating dairy cows using digesta marker techniques. *animal*, 13 (10): 2277-2288. doi: 10.1017/s1751731119000247.

Handojo, L. A., Indarto, A., Shofinita, D., Meitha, A., Nabila, R. & Triharyogi, H. (2018). *Calcium soap from palm fatty acid distillate (PFAD) for ruminant feed: quality of calcium source*. MATEC Web of Conferences: EDP Sciences.

Helsedirektoratet. (2020). *UTVIKLINGEN I NORSK KOSTHOLD 2019 Matforsyningsstatistikk og forbruksundersøkelser*: Helsedirektoratet. Available at: https://www.helsedirektoratet.no/rapporter/utviklingen-i-norsk-kosthold/Utviklingen%20i%20norsk%20kosthold%202019%20%E2%80%93%20Fullversjon.pdf/ (accessed: 30.03).

Hristov, A. N., Etter, R. P., Ropp, J. K. & Grandeen, K. L. (2004). Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows1. *Journal of Animal Science*, 82 (11): 3219-3229. doi: 10.2527/2004.82113219x.

Huhtanen, P., Kaustell, K. & Jaakkola, S. (1994). The use of internal markers to predict total digestibility and duodenal flow of nutrients in cattle given six different diets. *Animal Feed Science and Technology*, 48 (3-4): 211-227. doi: 10.1016/0377-8401(94)90173-2.

Hvelplund, T., Madsen, J., Misciattelli, L. & Weisbjerg, M. (2003). Proteinomsætningen i mave-tarmkanalen og dens kvantificering. In Hveplund, T. & Nørgaard, P. (eds) *Kvægets ernæring og fysiologi. Bind 1-Næringsstofomsætning og fodervurdering, DJF rapport Husdyrbrug 53*, pp. 281-311. Denmark: Danmarks JordbrugsForskning.

ISO. (2002). *Animal feeding stuffs—Determination of crude ash. Standard 5984*: Organ. Standardisation, Geneva Switzerland.

Jenkins, T. C. & Palmquist, D. L. (1984). Effect of Fatty Acids or Calcium Soaps on Rumen and Total Nutrient Digestibility of Dairy Rations. *Journal of Dairy Science*, 67 (5): 978-986. doi: 10.3168/jds.s0022-0302(84)81396-x.

Johnsen, E. F. (2020). *MSP 1034 AIA*. Ås: Institutt for husdyr- og akvakulturvitenskap, NMBU. Available at: https://www.nmbu.no/fakultet/biovit/forskning/tekniske-forskningssentre/node/10055 (accessed: 28.02).

Kebreab, E., France, J., Beever, D. & Castillo, A. (2001). Nitrogen pollution by dairy cows and its mitigation by dietary manipulation. *Nutrient cycling in agroecosystems*, 60 (1-3): 275-285.

Kidane, A., Øverland, M., Mydland, L. T. & Prestløkken, E. (2018a). 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. *Journal of Animal Science*, 96 (9): 3967-3982.

Kidane, A., Øverland, M., Mydland, L. T. & Prestløkken, E. (2018b). Milk production of Norwegian Red dairy cows on silages presumed either low or optimal in dietary crude protein content. *Livestock science*, 214: 42-50.

Kidane, A., Prestløkken, E., Zaralis, K. & Steinshamn, H. (2019). Effects of three short-term pasture allocation methods on milk production, methane emission and grazing behaviour by dairy cows. *Acta Agriculturae Scandinavica, Section A — Animal Science*: 1-16. doi: 10.1080/09064702.2019.1577912.

Kjos, A.-K., Nafstad, O., Odden, H., Ruud, T. A., Saltnes, T. & Ytterdahl, M. (2019). *Kjøttets tilstand 2019 - Status i norsk kjøtt- og eggproduksjon*. Oslo: Animalia.

Landbruksdirektoratet. (2019). *Råvareforbruk til norsk kraftfôr til husdyr i Norge 2018*: Landbruksdirektoratet. Available at: https://www.landbruksdirektoratet.no/no/produksjon-og-marked/korn-og-kraftfor/marked-og-pris/statistikk?index=0&metaKey=11220 (accessed: 12.07).

Lee, C. & Hristov, A. N. (2013). Short communication: Evaluation of acid-insoluble ash and indigestible neutral detergent fiber as total-tract digestibility markers in dairy cows fed corn silage-based diets. *J Dairy Sci*, 96 (8): 5295-9. doi: 10.3168/jds.2012-6442.

Madadi, M., Penga, C. & Abbas, A. (2017). Advances in Genetic Manipulation of Lignocellulose to Reduce Biomass Recalcitrance and Enhance Biofuel Production in Bioenergy Crops. *Journal of Plant Biochemistry & Physiology*, 05 (02). doi: 10.4172/2329-9029.1000182.

Madsen, J., Misciattelli, L., Kristensen, V. F. & Hvelplund, T. (2003). Proteinforsyning til malkekøer. In Strudsholm, F. & Sejrsen, K. (eds) *Kvægets ernæring og fysiologi. Bind 2 - Fodring og produktion, DJF rapport Husdyrbrug 54*, pp. 113-131. Denmark: Danmarks JordbrugsForskning.

Makkar, H. P. S., Tran, G., Heuzé, V. & Ankers, P. (2014). State-of-the-art on use of insects as animal feed. *Animal Feed Science and Technology*, 197: 1-33. doi: 10.1016/j.anifeedsci.2014.07.008.

Makkar, H. P. S., Tran, G., Heuzé, V., Giger-Reverdin, S., Lessire, M., Lebas, F. & Ankers, P. (2016). Seaweeds for livestock diets: A review. *Animal Feed Science and Technology*, 212: 1-17. doi: 10.1016/j.anifeedsci.2015.09.018.

McDonald, P., Edwards, R. A., Greenhalgh, J. F. D., Morgan, C. A., Sinclair, L. A. & Wilkinson, R. G. (2011). *Animal Nutrition*. Harlow, England: Prentice Hall.

Mehtiö, T., Rinne, M., Nyholm, L., Mäntysaari, P., Sairanen, A., Mäntysaari, E., Pitkänen, T. & Lidauer, M. (2015). Cow-specific diet digestibility predictions based on near-infrared reflectance spectroscopy scans of faecal samples. *Journal of Animal Breeding and Genetics*, 133 (2): 115-125.

Mertens, D. R. (2002). Gravimetric determination of amylase-treated neutral detergent fiber in feeds with refluxing in beakers or crucibles: collaborative study. *Journal of AOAC international*, 85 (6): 1217-1240.

Mutsvangwa, T., Davies, K. L., McKinnon, J. J. & Christensen, D. A. (2016). Effects of dietary crude protein and rumen-degradable protein concentrations on urea recycling, nitrogen balance, omasal nutrient flow, and milk production in dairy cows. *Journal of Dairy Science*, 99 (8): 6298-6310. doi: 10.3168/jds.2016-10917.

Naadland, S. S., Steinshamn, H., Krizsan, S. J. & Randby, Å. T. (2017). Effect of organic grass-clover silage on fiber digestion in dairy cows. *animal*, 11 (06): 1000-1007. doi: 10.1017/s1751731116002421.

Nasseri, A., Rasoul-Amini, S., Morowvat, M. & Ghasemi, Y. (2011). Single cell protein: production and process.

Nazarian-Firouzabadi, F. & Visser, R. G. F. (2017). Potato starch synthases: Functions and relationships. *Biochemistry and Biophysics Reports*, 10: 7-16. doi: 10.1016/j.bbrep.2017.02.004.

Neal, K., Eun, J.-S., Young, A., Mjoun, K. & Hall, J. (2014). Feeding protein supplements in alfalfa hay-based lactation diets improves nutrient utilization, lactational performance, and feed efficiency of dairy cows. *Journal of dairy science*, 97 (12): 7716-7728.

Nielsen, N. & Volden, H. (2011). Animal requirements and recommendations. In *NorFor-The Nordic feed evaluation system*, pp. 85-111. Wageningen, The Netherlands: Wageningen Academic Publishers.

NorFor Feed Table. NorFor. Available at: http://www.norfor.info/feed-table/ (accessed: 11.07.2020).
NRC. (2001). *Nutrient requirements of dairy cattle*. 7th rev. ed. Washington, D.C: National Academy Press.
Nørgaard, P. (2003). Drøvtyggerens karakteristika. In Hvelplund, T. & Nørgaard, P. (eds) *Kvægets ernæring og fysiologi. Bind 1-Næringsstofomsætning og fodervurdering, DJF rapport Husdyrbrug 53*, pp. 11-38. Denmark: Danmarks JordbrugsForskning.
Oltjen, J. W. & Beckett, J. L. (1996). Role of ruminant livestock in sustainable agricultural systems. *Journal of Animal Science*, 74 (6): 1406. doi: 10.2527/1996.7461406x.
Olvera-Novoa, M. A., Martinez-Palacios, C. A. & Olivera-Castillo, L. (2002). Utilization of torula yeast (Candida utilis) as a protein source in diets for tilapia (Oreochromis mossambicus Peters) fry. *Aquaculture Nutrition*, 8 (4): 257-264. doi: 10.1046/j.1365-2095.2002.00215.x.
Owens, F. N. & Hanson, C. F. (1992). External and internal markers for appraising site and extent of digestion in ruminants. *Journal of dairy science*, 75 (9): 2605-2617.
Parker, R. & Ring, S. G. (2001). Aspects of the Physical Chemistry of Starch. *Journal of Cereal Science*, 34 (1): 1-17. doi: 10.1006/jcrs.2000.0402.
Reveco-Urzua, F. E., Hofossæter, M., Kovi, M. R., Mydland, L. T., Ånestad, R., Sørby, R., Press, C. M., Lagos, L. & Øverland, M. (2019). Candida utilis yeast as a functional protein source for Atlantic salmon (Salmo salar L.): Local intestinal tissue and plasma proteome responses. *PloS one*, 14 (12).
Sabbia, J., Kalscheur, K., Garcia, A., Gehman, A. & Tricarico, J. (2012). Soybean meal substitution with a yeast-derived microbial protein source in dairy cow diets. *Journal of dairy science*, 95 (10): 5888-5900.
Sadava, D., Hillis, D. M., Heller, H. C. & Berenbaum, M. R. (2014). Protein, Carbohydrates and Lipids. In vol. 1 The Cell and Heredity *Life: the science of biology*, pp. 39-61. USA: Macmillan.
Saggar, S., Jha, N., Deslippe, J., Bolan, N., Luo, J., Giltrap, D., Kim, D.-G., Zaman, M. & Tillman, R. (2012). Denitrification and N2O: N2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Science of the Total Environment*, 465: 173-195.
Sales, J. & Janssens, G. P. J. (2003). Acid-insoluble ash as a marker in digestibility studies: a review. *Journal of Animal and Feed Sciences*, 12 (3): 383-401. doi: 10.22358/jafs/67718/2003.
Santana, Á. L. & Meireles, M. A. A. (2014). New starches are the trend for industry applications: a review. *Food and public health*, 4 (5): 229-241.
SAS. (2012). SAS 9.4 for Windows. *SAS Institue Inc., Cary, NC, USA*.
Satter, L., Combs, D., Lopez-Guisa, J. & Nelson, W. (1986). Use of markers for measurement of feed digestibility in ruminants. In *Nuclear and related techniques in animal production and health*.
Schei, I. (2019). Gode avlingar, men fôrverdien er låg. *Buskap*, 71 (7): 78-80.
Schwab, C. G., Bozak, C. K., Whitehouse, N. L. & Mesbah, M. M. A. (1992). Amino Acid Limitation and Flow to Duodenum at Four Stages of Lactation. 1. Sequence of Lysine and Methionine Limitation. *Journal of Dairy Science*, 75 (12): 3486-3502. doi: 10.3168/jds.s0022-0302(92)78125-9.
Sharma, S., Hansen, L. D., Hansen, J. Ø., Mydland, L. T., Horn, S. J., Øverland, M., Eijsink, V. G. H. & Vuoristo, K. S. (2018). Microbial Protein Produced from Brown Seaweed and Spruce Wood as a Feed Ingredient. *Journal of Agricultural and Food Chemistry*, 66 (31): 8328-8335. doi: 10.1021/acs.jafc.8b01835.
Shingfield, K. & Garnsworthy, P. (2012). Rumen lipid metabolism and its impacts on milk production and quality. *Recent Advances in Animal Nutrition 2012/Garnsworthy, PC, Wiseman, J.*
Sjaastad, Ø. V., Sand, O. & Hove, K. (2016). *Physiology of Domestic Animals*. Oslo: Scandinavian Veterinary Press.
Stein, H. H., Berger, L. L., Drackley, J. K., Fahey, G. C., Hernot, D. C. & Parsons, C. M. (2008). Nutritional Properties and Feeding Values of Soybeans and Their Coproducts. In, pp. 613-660: Elsevier.

Tamminga, S. (1992). Nutrition Management of Dairy Cows as a Contribution to Pollution Control. *Journal of Dairy Science*, 75 (1): 345-357. doi: 10.3168/jds.s0022-0302(92)77770-4.

Thiex, N. J., Manson, H., Anderson, S., Persson, J.-Å. & M, C. A. S. B. E. B. G. B. D. E. C. E. S. F. G. F. E. H. C. H. C. K. M. L. L. M. H. M. J. R. (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. *Journal of AOAC International*, 85 (2): 309-317.

Thonney, M. L., Palhof, B. A., Decarlo, M. R., Ross, D. A., Firth, N. L., Quaas, R. L., Perosio, D. J., Duhaime, D. J., Rollins, S. R. & Nour, A. Y. M. (1985). Sources of Variation of Dry Matter Digestibility Measured by the Acid Insoluble Ash Marker. *Journal of Dairy Science*, 68 (3): 661-668. doi: 10.3168/jds.S0022-0302(85)80872-9.

TINE. (2020). *TINE Årsapport 2019 - Sammen skaper vi et levende Norge*. Available at: https://medlem.tine.no/praktisk-informasjon/eierdemokrati/aarsrapport/_attachment/498861?_ts=17042effb6a (accessed: 31.03).

Van Keulen, J. & Young, B. (1977). Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science*, 44 (2): 282-287.

Van Soest, P. J. (1994). *Nutritional ecology of the ruminant*. 2nd ed. Ithaca, NY: Cornell University Press.

Volden, H. (2011). Feed fraction characteristics. In *NorFor-The Nordic feed evaluation system*, pp. 33-40. Wageningen, The Netherlands: Wageningen Academic Publishers.

Volden, H. & Larsen, M. (2011). Digestion and metabolism in the gastrointestinal tract. In *NorFor-The Nordic feed evaluation system*, pp. 59-80. Wageningen, The Netherlands: Wageningen Academic Publishers.

Volynets, B., Ein-Mozaffari, F. & Dahman, Y. (2017). Biomass processing into ethanol: pretreatment, enzymatic hydrolysis, fermentation, rheology, and mixing. *Green Processing and Synthesis*, 6 (1): 1. doi: https://doi.org/10.1515/gps-2016-0017.

Wattiaux, M. A. (1998). Protein metabolism in dairy cows. *Technical Dairy Guide-Nutrition. 2nd edition. The Babcock Institute for International Dairy Research and Development, The University of Wisconsin, Madison*.

Wattiaux, M. A. & Grummer, R. R. (2000). Lipid metabolism in dairy cows. *Web site of Babcock Institute for International Dairy Research and Development. University of Wisconsin, USA*.

Wattiaux, M. A. & Howard, W. T. (2000). *Digestion in the dairy cow*. Wisconsin: Babcock Institute for International Dairy Research and Development.

Weisbjerg, M. R. & Hvelplund, T. (2003). Metoder til bestemmelse af kemisk sammensætning og tilgængelighed. In Hvelplund, T. & Nørgaard, P. (eds) *Kvægets ernæring og fysiologi. Bind 1 - Næringsstofomsætning og fodervurdering, DJF rapport Husdyrbrug 53*, pp. 69-86. Denmark: Danmarks JordbrugsForskning

Weisbjerg, M. R., Lund, P. & Hvelplund, T. (2003). Kulhydratomsætningen i mave-tarmkanalen. In Hvelplund, T. & Nørgaard, P. (eds) *Kvægets ernæring og fysiologi. Bind 1 - Næringsstofomsætning og fodervurdering, DJF rapport Husdyrbrug 53*, pp. 239-280. Denmark: Danmarks JordbrugsForskning.

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å. & Skrede, A. (2009). Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (Salmo salar)—Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. *Aquaculture*, 288 (3-4): 305-311. doi: 10.1016/j.aquaculture.2008.12.012.

Øverland, M., Karlsson, A., Mydland, L. T., Romarheim, O. H. & Skrede, A. (2013). Evaluation of Candida utilis, Kluyveromyces marxianus and Saccharomyces cerevisiae yeasts as protein sources in diets for Atlantic salmon (Salmo salar). *Aquaculture*, 402-403: 1-7. doi: 10.1016/j.aquaculture.2013.03.016.

Øverland, M. & Skrede, A. (2016). Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture. *Journal of the Science of Food and Agriculture*, 97 (3): 733-742.

Øvrum Hansen, J., Hofossæter, M., Sahlmann, C., Ånestad, R., Reveco-Urzua, F. E., Press, C. M., Mydland, L. T. & Øverland, M. (2019). Effect of Candida utilis on growth and intestinal health of Atlantic salmon (Salmo salar) parr. *Aquaculture*, 511: 734239. doi: 10.1016/j.aquaculture.2019.734239.

Åkerlind, M., Nielsen, N. & Volden, H. (2011a). Animal input characteristics. In *NorFor-The Nordic feed evaluation system*, pp. 27-32. Wageningen, The Netherlands: Wageningen Academic Publishers.

Åkerlind, M., Weisbjerg, M., Eriksson, T., Tøgersen, R., Udén, P., Ólafsson, B., Harstad, O. & Volden, H. (2011b). Feed analyses and digestion methods. In *NorFor-The Nordic feed evaluation system*, pp. 41-54. Wageningen, The Netherlands: Wageningen Academic Publishers.

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