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The use of *Saccharomyces cerevisiae CNCM I-1077* in feed for ruminants – effect on digestibility, rumen environment and methane emission

Bruk av *Saccharomyces cerevisiae CNCM I-1077* i fôr til drøvtyggere- effekt på fordøyelighet, vom-miljø og metanutslipp

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Abstract

The main objective of this experiment was to evaluate the effect of adding live yeast (*Saccharomyces cerevisiae* CNCM I-1077) in rations to dairy cows in early lactation on apparent total tract digestibility, rumen physiological parameters, methane emission, milk yield- and milk composition.

Six rumen cannulated dairy cows of the breed Norwegian Red (NRF) in ≥ 2. lactation (average 35 days in milk at start) were blocked by: 1) days in milk 2) milk yield 3) body weight and divided in two groups: yeast + (Y+) and control (Y-). The animals were held in tie stalls at the Animal Production Experimental Centre, NMBU. The experimental design was a crossover design with two periods of 28 days, with a 14 days washout period in between. All animals were fed a high fiber (NDF) grass silage (520 g NDF/kg dry matter) in combination with a commercial concentrate as a total mixed ration (TMR). The experimental animals were fed three times a day, either 21 or 22 kg dry matter daily as a fixed amount. Each day at 09:00, the Y+ group had 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 inserted into the rumen through the rumen cannula. The Y- group had their cannula opened, but with no adding of yeast. Methane emission were measured for five days in each period by the ERUCT method with SF_6 as marker. Daily milk yield, milk composition, rumen fermentation products, rumen pH, body condition score and body weight were registered. Apparent total tract digestibility was calculated from a 72-hour total collection of faeces and urine.

There was in general no effect of dietary treatment (Y+/Y-) on nutrient digestibility, milk yield, milk composition or methane emission (*P>0.05).* There was a numerically higher rumen pH in the Y+ group compared to the Y- (*P=0.11).* There was difference between the Y+ and the Y- group on rumen fermentation pattern, with a trend (*P=0.06*) towards a higher concentration of total acids (mmol/L) in the Y- group. There was a significant effect of dietary treatment on rumen propionate (*P=0.05*) and butyrate (*P=0.05*) concentrations (mmol/L) with the highest concentrations in the Y- group. The results showed a trend (*P=0.10*) towards higher acetate concentrations in the Y- group compared to the Y+ group. There was no effect of treatment on rumen propionate, acetate or butyrate when measured as molar percentage, and no effect on the acetate:propionate ratio.

This study found no effect of adding *Saccharomyces cerevisiae* CNCM I-1077 on nutrient digestibility, milk yield, milk composition or methane emission in dairy cows fed a high fiber grass silage and concentrate diet. However, *Saccharomyces cerevisiae* CNCM I-1077 may have an effect on rumen fermentation pattern and rumen pH.

Sammendrag

Formålet med dette forsøket var å evaluere effekten av å tilsette levende gjær (*Saccharomyces cerevisiae* CNCM I-1077) i rajonen til melkekyr i tidlig laktasjon på apparent total fordøyelighet, fysiologiske parameter i vom, metanutslipp, melkeytelse og kjemisk komposisjon i melk.

Seks vomfistulerte melkekyr av rasen norsk rødt fe (NRF) i ≥ 2. laktasjon (gjennomsnittlig 35 dager i melk ved forsøksstart) ble blokkert etter: 1) dager i melk 2) melkeytelse 3) kroppsvekt og delt mellom to grupper: gjær (Y+) eller kontroll (Y-). Kyrne ble holdt i båsfjøs ved Stoffskifteavdelingen, NMBU. Forsøksdesignet var et crossover design med to forsøksperioder av 28 dager, med en 14 dagers utvaskingsperiode imellom. Alle kyrne ble fôret med en grassilo med et høyt innhold av fiber (NDF) (520 g NDF/kg tørrstoff), i kombinasjon med en kommersiell kraftfôrblanding som en fullfôrblanding (TMR). Forsøksdyrene ble fôret tre ganger om dagen, enten 21 eller 22 kg tørrstoff daglig som en fiksert mengde. Hver dag kl. 09:00 fikk Y+ gruppen 1 x 10^{10} CFU/dag *Saccharomyces cerevisiae* CNCM I-1077 lagt inn i vom via vomfistelen. Y- gruppen fikk deres vomfistler åpnet men ikke innlagt gjær. Metanutslipp ble registrert i fem dager hver forsøksperiode ved ERUCT metoden, med SF_6 som markør. Daglig melkeytelse, kjemiske komponenter i melk, fermenteringsprodukter i vom, pH i vom, holdpoeng og kroppsvekt ble registrert. Apparent total fordøyelighet ble kalkulert ut fra en 72 timers total oppsamling av urin og gjødsel.

Det var generelt ingen effekt av behandling (Y+/Y-) på næringsstoff fordøyelighet, melkeytelse, kjemisk innhold i melk eller metanutslipp. Det var en numerisk høyere pH i vom i Y+ gruppa sammenlignet med Y- gruppa (*P=0.11*). Det var en forskjell mellom Y+ og Y- gruppene ved fermenteringsprodukter i vom, med en trend (*P=0.06*) mot høyere konsentrasjon av totale syrer (mmol/L) i Y- gruppa. Det var signifikant effekt av behandling på propionsyre (P=0.05) og smørsyre (*P=0.05*) konsentrasjoner (mmol/L) i vom med de høyeste konsentrasjonene i Y- gruppa. Det var en trend (*P=0,10*) til høyere konsentrasjon av eddiksyre i Y- gruppa sammenlignet med Y+ gruppa. Det var ikke effekt av behandling på propionsyre, eddiksyre eller smørsyre når tallene ble presentert i molar prosent, det var heller ikke effekt av behandling på forholdet eddiksyre:propionsyre.

Dette studiet fant ingen effekt av å tilsette *Saccharomyces cerevisiae* CNCM I-1077 på næringsstoff fordøyelighet, melkeytelse, kjemisk innhold i melk eller metanutslipp for melkekyr fôret med en grassilo med høyt innhold av fiber og kraftfôr. Men, *Saccharomyces cerevisiae* CNCM I-1077 kan ha en effekt på fermenteringsmønsteret i vom og pH i vom.

Content

1 Introduction

Ruminant animals have a central role in agriculture and food security as they have the unique ability to utilize fibrous feedstuffs to produce high-value food products. However, ruminant production systems are under increasing pressure because of methane emissions, claimed inefficiently use of arable land and use of feed resources that could more efficiently be utilized directly for human consume (Peyraud & Peeters, 2016). Consequently, increasing the feed utilization and reduce methane emission per produced unit food is of considerable interest in ruminant production systems.

Sustainable meat- and milk production is essential for future agricultural production in Norway. A primary goal for the government is food production based on national feed resources (St.meld.nr 11 (2016-2017)). In Norway, only 2.9 % of the total land area is arable land, where two thirds of this is only suitable for grass production. (Alvseike et al., 2017; Mathiesen, 2014). Diets for high-yielding dairy cows in Norway commonly consists of locally produced grass silage combined with concentrates, in ratio between 70:30 to 50:50. The concentrates are based on Norwegian grain, but often have a high and increasing level of imported feed materials. Because of these imported feedstuffs, roughage should be the main part in Norwegian dairy cattle diets if the goal of food production on national resources is to be met.

Different qualities of grass silage results in a wide variation of feed intake and production responses, where botanical composition (Thomas et al., 1981), fermentation quality (Krizsan & Randby, 2007) and stage of maturity (Steen, 1984) affects the production response. Grass produced for silage is recommended to be mown on an early stage of maturity, when there is a higher proportion of available energy for the animal and a higher protein value compared to that of a later harvest (Mo, 2005). Due to variable climate and weather conditions in Norway, not all grass for feed is harvested at the optimal stage of maturity. This can result in a suboptimal feed quality and grass silage that often have a high indigestible fiber (iNDF) and potential degradable fiber content. When plants mature, the ratio between stem:leaf increases in addition to a lignification of the structural carbohydrates in the stem, resulting in an increase in iNDF and fiber (NDF) content in the silage (Mo, 2005). NDF is important to maintain rumen function and milk yield (NRC, 2001). However, the digestibility of NDF in forages varies considerably. Factors in the feed and characteristics of the animal results in varying nutritional value of the NDF fraction. Although dairy cattle require NDF for

maximum production and health, excess dietary NDF will limit the voluntary feed intake because of physical fill in the rumen (Volden et al., 2011). NDF degradability in the rumen is following an important factor for feed- and energy intake.

Probiotics are becoming increasingly popular internationally as an alternative to antibiotics to promote production in ruminants. Preventative usage of antibiotics is illegal in Norway, but the effect of probiotics are of interest to e.g. improve roughage utilization. Probiotic supplements have reported enhanced effects on production by increased milk yield, induce better nutrient digestibility and enhance growth rate (Bajagai et al., 2016). Studies have indicated that live yeast supplements enhance fiber degradation in both high-fiber- and/or high concentrate diets (Chaucheyras-Durand et al., 2016) and that the effect is especially pronounced in high-fiber diets (Desnoyers et al., 2009). The most prominent effects of probiotic additives have been in periods or phases that the animals are exposed to stress, e.g. weaning, early lactation or drastic changes in diet.

In addition to effects on production traits, both *in vitro* and *in vivo* experiments have indicated that yeast additives can affect methane production, however the results are inconsistent (Carro et al., 1992b; Doreau & Jouany, 1998; Mutsvangwa et al., 1992; Sullivan & Martin, 1999). The reported effects probiotics have on rumen fermentation pathways, indicates that these kind of feed additives is an important field of study to lessen environmental impact of ruminants.

The main objective of this experiment was to evaluate the effect of adding live yeast (*Saccharomyces cerevisiae* CNCM I-1077) in rations to dairy cows in early lactation on total tract digestibility of nutrients, rumen physiological parameters, methane emission, milk yield and milk composition. The experiment is a part of the project FeedMilage, WP 3: “Improving the utilization of local feed recourses in ruminants”.

The following hypothesis were tested:

Addition of live yeast in rations to dairy cows in early lactation fed high-fiber grass silage will

I. Increase total tract digestibility of fiber (NDF) in the ration
II. Reduce methane emission per kg ECM produced
III. Stabilize pH in rumen
IV. Increase feed efficiency and thus increase milk yield
V. Influence the chemical composition of milk

2 Theory

2.1 Main nutrients in feed

McDonald et al. (2011) describes feed as "a material that after ingestion by animals is capable of digestion, absorption and utilization". Feedstuffs for dairy cattle can traditionally be divided between concentrates and roughage. This classification depends on energy density, fiber content, moisture content and particle length in the feed (Volden, 2011). However, more precisely can feed be separated into nutrients where the energy yielding categories are carbohydrates, protein and lipids.

2.1.1 Carbohydrates

Carbohydrates are the main source of energy for the dairy cow (NRC, 2001), and will usually constitute ~70 % of the organic matter (OM) in the feed (Weisbjerg et al., 2003). Carbohydrates are chemical compounds that consists of carbon, hydrogen and oxygen but may in addition contain phosphor, nitrogen or sulfur (McDonald et al., 2011). Carbohydrates can be divided into subgroups depending on chemical structure (e.g. monosaccharides) or into fractions as structural and non-structural carbohydrates depending on qualities and origin.

Monosaccharides are the most basic form of carbohydrates and is often called simple sugars. They have the empirical formula ($C_nH_{2n}O_2$) and are divided between subgroups dependent on number of carbon (C) atoms in the molecule: trioses (3C), tetroses (4C), pentoses (5C), hexoses (6C) or heptoses (7C) (McDonald et al., 2011). For dairy cattle, the nutritionally most important monosaccharides are glucose and fructose (Weisbjerg et al., 2003).

Oligosaccharides are polymers that consists of two or more (typically up to ten) monosaccharides. Oligosaccharides are separated between subgroups depending on the number of monosaccharides they contain. Disaccharides consists of two monosaccharides, trisaccharide have three, tetrasaccharide with four and pentosaccarides contain five monosaccharides (McDonald et al., 2011). For dairy cattle the nutritionally most important oligosaccharides are sucrose, maltose and lactose (Weisbjerg et al., 2003). Oligosaccharides are also referred to as sugars.

Polysaccharides are divided between homoglycans and heteroglycans. Homoglycans are polymers that consist of one single type of monosaccharide bound together with glyosidic linkage, while heteroglycans have two or more different monosaccharides in its structure. Starch, cellulose and hemi-cellulose are important polysaccharides in ruminant nutrition.

Starch is a homoglycan that consist of the polysaccharides amylose and amylopectin in a ratio of 30:70 (McDonald et al., 2011). Amylopectin consists of glucose molecules bound together with α1:4 - and α1:6 bindings, while amylose only have α1:4 bonds. Due to the α 1:6 bindings, amylopectin have frequent branching while amylose have no such structure. In feed, starch occurs in granules that alternate between semi-crystalline layers and amorphous layers due to the different characteristics of amylose and amylopectin (Svihus et al., 2005).

Cellulose is also a homoglycan, but differs from starch by that the glucose molecules are linked together by β1:4 bindings in contrast to α bindings This results in that cellulose have a linear structure. Cellulose is an important part of the plant cell wall, where it is a central part in the microfibrils. Hemicellulose is a heteroglycan with similarities to cellulose but compared to cellulose, it is a more complex molecule. Hemicellulose are composed of glucose, galactose, mannose, xylose or arabinose in different ratios bound together by glyosidic linkage (McDonald et al., 2011).

Carbohydrates can be categorized as soluble or insoluble cell-wall carbohydrates, starch or water-soluble carbohydrates. The insoluble cell-wall carbohydrates consist of cellulose, hemicellulose and lignin, which is usually referred to as NDF in a feed analysis. While lignin is not per definition a carbohydrate, it is included in the NDF fraction. Pectins is an example of soluble cell-wall carbohydrates. Water-soluble carbohydrates refers to different types of sugars. In a standard feed fraction analyses, some carbohydrates will fall outside this classification e.g. xylans, glucans and organic acids. These will constitute to a restCHO fraction (Nozière et al., 2010).

2.1.2 Proteins

Proteins are large organic molecules that are built from small organic compounds (amino acids) and contains the elements hydrogen, carbon, oxygen, nitrogen and usually sulfur. Dependent on their amino acid composition, proteins varies in size, shape and function (NRC, 2001). Amino acids consist of one amino group (NH_2) and an acidic carboxyl group (-COOH), in addition to a basic nitrogenous group and a side chain. This side chain is unique to the specific amino acid and gives the amino acids their different characteristics. Over 200 different amino acids have been recognized, but only 20 are generally found in proteins (McDonald et al., 2011). All cells contain proteins and proteins are art specific, which explains the great diversity found in the plant- and animal kingdom.

In feedstuffs the protein content is usually expressed as crude protein (CP). Crude protein is normally calculated as $nitrogen\ content * 6.25$ where the factor 6.25 is from the simplification that protein in feedstuff contains 16 % nitrogen (NRC, 2001). Crude protein, as it is calculated from nitrogen analysis, includes amino acids and non-protein nitrogen (NPN). NPN is normally made up of NH_3N, urea and nucleic acids (Volden, 2011).

2.1.3 Lipids

Lipids are a small part of a normal diet for dairy cows. But to increase the energy density in the feed, lipids may be added as they are energy dense compounds (Volden, 2011).

In diets for dairy cows' lipids are present as triglycerides in concentrates, and glycolipids and phospholipids in roughage. Triglycerides constitutes of one alcohol, glycerol and three fatty acids. Glycolipids and phospholipids are similar to triglycerides, but in the glycolipids one of the fatty acids are replaced by a galactose molecule while in the phospholipids phosphate replaces one of the fatty acids in the structure (McDonald et al., 2011).

The quality of lipids varies between feedstuffs and may be presented as a percentage of the lipids that is in the form of fatty acids. Lipids in concentrates have approximately 80-85 % of the crude fat as fatty acids, while typical forage lipids will have a proportion closer to 45 %. This phenomena is explained by that triglycerides have a higher percentage of fatty acids than glycolipids or phospholipids (Volden, 2011).

Lipid characteristics are dependent on the fatty acids in the complexes. Fatty acids are carboxylic acids that have one carboxyl group and one unbranched carbon chain, and the basic empirical formula of $C_nH_{2n+1}COOH$. The number of carbons in the carbon chain will vary between 2-20. Furthermore, fatty acids differentiate between saturated and unsaturated. Saturated fatty acids are bound by simple hydrogen bonds, while unsaturated fatty acids have one or more double bonds between the carbon atoms (McDonald et al., 2011). Fatty acids with more than one double binding are frequently described as polyunsaturated fatty acids (PUFA). The qualities and characteristics of the fatty acids depends both on the carbon chain length and whether the fatty acids are saturated, unsaturated or polyunsaturated.

2.2 Digestion in the ruminants

For a high-yielding dairy cow roughage will be the main source of structural carbohydrates, while the main portion of protein and easily fermentable carbohydrates will come from concentrates.

Digestion in the ruminant animal have three main stages: 1) physical breakdown of the feed by chewing and rumination 2) microbial digestion in the forestomaches and large intestine 3) chemical digestion by the animal's own digestive enzymes in the abomasum and small intestine.

Ruminants have an unique way to digest forages and fibrous roughage. This due to their symbiotic relationship with microorganisms in their forestomaches, and thus microbial fermentation of feed (McDonald et al., 2011). The main site of microbial fermentation is in the reticulorumen by the complex ecosystem of microorganism located here. The rumen microorganisms consists of different families of bacteria, fungi and protozoa (Kristensen et al., 2003; McDonald et al., 2011). The microbial ecosystem is influenced by interactions between factors in the feed, the animal and the microbial populations that consequently will affect the total digestion of a feed ration (Huhtanen et al., 2006). Fermentation is an anaerobic, energy-yielding redox reaction where both the initial electron donor and final electron acceptor are organic compounds. But the fermentation process is not an energy efficient pathway for microorganisms to produce energy-rich molecules (ATP, NADH, NADPH) (Owens & Basalan, 2016), and most of the energy is lost in by-products or heat. By-products from microbial fermentation is, among others, volatile fatty acids (VFA), carbon dioxide and methane. In ruminants, the VFAs are mainly absorbed over the ruminal wall and constitute to 70-80 % of the animal's daily energy requirements (Houtert M.F.J, 1993).

Another specific characteristic of the ruminants is the mechanism of rumination. Rumination is the process where the animal regurgitates feed in boluses from the reticulorumen to the oral cavity for addition chewing. Rumination reduces the particle size of the feed and increases the surface of the particles (Sjaastad et al., 2010). During mastication in the oral cavity, the feed is mixed with salvia secreted from salivary glands. Saliva secretion is, among other factors, stimulated by chewing. Salvia contains water, sodium, potassium, chloride, calcium, magnesium, urea, phosphate and bicarbonate in addition to some mucoproteins (Nørgaard & Hveplund, 2003) For dairy cattle daily secretion of saliva is typically 120-150 liters (McDonald et al., 2011).

The digestive system of a ruminant animal consists of a muscular tube that goes from the oral cavity to anus with adjoining glands (Figure 1). The digestive tract consists of the pharynx, esophagus, the forestomaches (reticulum, rumen and omasum), the stomach (abomasum) and small and large intestine. Duodenum, jejunum and ileum make up the small intestine while the large intestine consists of cecum, colon and rectum (Membrive, 2016).

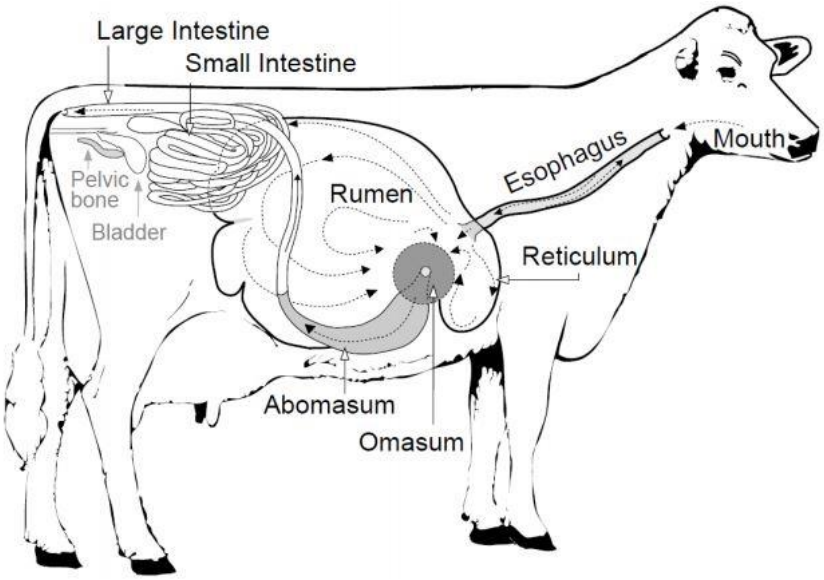


Figure 1. Overview of the cow's digestive system (Wattiaux & Howard, 2000).

When the cow eats and swallows, the feed enters the reticulorumen through the esophagus. The reticulorumen stretches from the diaphragm to the pelvis and fills the whole left side and part of the right side in the abdominal cavity, and it lies in contact with the left abdominal wall (Sjaastad et al., 2010). The rumen in an adult cow is approximately 6 % of the adult body weight and have a volume between 150-200 liter (Membrive, 2016). The rumen consists of three blind sacs; the dorsal, ventral and cranial sac. The different sacs are partly divided by muscle folds; the cranial and caudal pillar. The internal rumen wall is lined with mucosa and short, ruminal papillae. The ruminal papillae greatly increases the surface area of the rumen and contributes to transport of end-products from the microbial fermentation, in addition to transport of nutrients and trace elements from rumen to blood (Sjaastad et al., 2010).

The reticulum is in front of the rumen, directly down from the esophagus and lies close to the diaphragm. The rumen and reticulum have a constant flux of fluid because of an unobstructed, large opening between the two compartments. The reticulum is characterized by ridges that ranges from 5-20 mm high and 2-3 mm wide, which make up a crisscross pattern that

composes a “honeycomb” wall structure on the reticulum’s inner surface (Sjaastad et al., 2010).

With a feed ration that consists of concentrates and roughage, the size of feed particles entering the reticulorumen will have considerately variation, from particles originating from milled feedstuffs in concentrate pellets to uncut roughage. Concentrate particles in the Nordic feed evaluation system (NorFor) are defined as smaller than 6 mm (Volden, 2011), while forage particles will vary from 200 µm to well over 1200 µm (Martz & Belyea, 1986). Martz and Belyea (1986) claimed that the particles must be reduced to less than 1.2 mm before passage from the rumen can occur. McDonald et al. (2011) refers to that particle size must be reduced to similar size (1-2mm) before passage but that particles up to 3-4 mm have been found to escape the reticulorumen.

Consequently, in the rumen there is are a wide variation of particle size, which is distributed different throughout the rumen (Sjaastad et al., 2010). In the uppermost part of the rumen it is a layer of different gases. Under this gas layer, in the remaining part of the dorsal sac, size varies from the largest, newly swallowed forage particles to the smaller concentrate particles, or to almost fully digested ones. The coarser, larger particles will float in the top sheet of the rumen because of a lower density and small gas bubbles in their physical structure. When these particles are reduced in size and exposed to microbial digestion, they sink closer to the bottom of the rumen; either into the ventral sac, cranial sac or the reticulum (Sjaastad et al., 2010). This different distribution of particles in the rumen are a consequence of characteristics of the particles, but also mixing contractions in the ruminal wall.

The reticulorumen content is continuously mixed by contraction in the reticulum- and ruminal wall. The contractions start in the reticulum with a two-phase contraction. The first phase forces parts of the coarser content of the reticulum into the cranial sac, while in the second phase the reticulo-omasal orifice opens, and a part of the finest well-fermented content of the reticulum passes through to the omasum. This transition happens because of differences in hydrostatic pressure between the two compartments. Then the reticulum relaxes, and the contractions continues through to the ruminal wall. This by first contracting the cranial sac, and then the contractions spreads to the dorsally and caudally part and end in the upper rumen wall. When the contraction in the upper rumen are completed, similar contractions begin in the ventral part of the rumen. The contraction starts in the cranial part of the ventral rumen and continues toward the ventral blind sac. All of these contractions lead to that the reticulorumen content are thoroughly mixed in slow, circular motions. (Sjaastad et al., 2010).

Passage from the reticulorumen depends on the particle size, but also its chemical composition. It is recognized that particles will follow different passage rates, age-independent or age-independent passage. In addition will most particles follow the first order of kinetics for passage, but some will not (see later in the chapter).

After the digesta passes through the reticulo-omasum orifice it enters the omasum. The omasum is sometimes described as the third stomach even though this may be an imprecise description. It is located after the reticulum, at the right side of the cranial part of the rumen and has a round outer shape (Sjaastad et al., 2010). It is characterized with that its inner structure is built up from several leave-like folds that are covered in short, keratinized papillae. Because of this surface structure the absorptive capacity of the omasum is greatly advanced. Full knowledge of the functions of the omasum is not fully understood, but it appears to play a role in the absorption of water and nutrients from digesta, and prevent passage of larger particles to the abomasum (Membrive, 2016; Van Soest, 1994).

The abomasum follows the omasum and is the true stomach in ruminants. It has a pear-like shape and is located close to the abdominal floor. The internal structure of the abomasum is divided between the cardiac region, the fundus and the antrum. The cardiac region is the area closest to the omasal orifice, the fundus is the main body of the abomasum and the antrum is near the pylorus/sphincter to the small intestine. The antrum is the general site for acid secretion (Van Soest, 1994). Digesta that enters the abomasum is a mix of microbial protein, buy-pass protein, starch and lipids in addition to minerals and vitamins. In the abomasum there is a single layer of columnar epithelium forming glands that secrete a strong acid, hydrochloric acid (HCl) (McDonald et al., 2011; Sjaastad et al., 2010). Because of HCl secretion the pH in the rumen is between 2-3 (Sjaastad et al., 2010).

The small intestine is the main site for enzymatic digestion and absorption. When digesta enters the duodenum, it is mixed with secretions from the pancreas and bile from the gallbladder. These fluids neutralizes the acids, and consequently pH in digesta increases. Absorption of nutrients happens over the intestinal wall, when the digesta comes in contact with intestinal villi (Van Soest, 1994).

The colon and cecum of ruminants are additional sites for microbial fermentation if potentially fermentable carbohydrates reach this point. In the large intestine it is absorption of water, minerals, nitrogen and VFA (Van Soest, 1994).

2.2.1 Carbohydrate digestion

Several types of carbohydrates are present in feedstuffs, from cell-wall soluble and insoluble carbohydrates to starch and water-soluble fractions. These different carbohydrates will consequently have different digestion as they have distinct characteristics and qualities.

Mechanical digestion starts in the mount by chewing and rumination which exposes the feed particles to hydration, microbial colonization and microbial enzymes (Nozière et al., 2010). In the rumen, starch and simple sugars are hydrolyzed by microbial amylases, maltase or similar enzymes, which breaks the chemical bonds in these disaccharides or polysaccharides. This breakdown results in the monosaccharides glucose, maltose or fructose dependent on the original chemical composition. These monosaccharides are immediately taken up by ruminal microorganisms and utilized in their metabolism, where pyruvate from glycolysis is the main intercellularly intermediate (McDonald et al., 2011). Because of the microbes, one will seldom find residue of starch or sugar digestion in rumen liquid, and total tract digestion of starch and sugars are usually total.

NorFor, the Nordic feed evaluation system, operates with a rate of degradation of soluble starch with 150 %/h and that of sugar as 300 %/h (Volden & Larsen, 2011). In a study by Stensig et al. (1998) rate of degradation of simple monosaccharides in the rumen varied between 553-577 %/h. These numbers reflects on the rapid breakdown of these feed components.

Insoluble cell wall carbohydrates (NDF) have a total tract digestibility of 40-58 % for forages and 20-90 % for concentrates (Nozière et al., 2010). This variation is due to that fiber digestibility depends on both qualities and quantities of the feed, and factors affecting the rumen environment (Van Soest, 1994).

The different carbohydrate fractions of NDF are broken down by the following digestive pathways; cellulose is first broken down to oligosaccharides then to cellobiose and lastly glucose by cellulase complexes that break the β 1:4 and β 1:6 bonds. These cellulases are produced by ruminal cellulolytic bacteria and fungi. Hemicellulose are similarly chemically digested in the rumen by enzymes secreted from ruminal microorganisms, but the end-products of hemicellulose digestion is fructose and trioses (Houtert M.F.J, 1993). The monosaccharide products are immediately transported into the microbes and further metabolized in the glycolysis (Sjaastad et al., 2010).

Amounts of nutrients available for absorption from the gastro-intestinal tract are determined by the rate of digestion relative to the rate of passage. Most particles in the rumen are expected to follow 1. order kinetics which says that any particle, independent of age or particle size, will have the same probability of passage out of the rumen. This also indicates that if no new material enters the rumen, the rumen-pool will fall exponentially with time. This theory appears to be correct for most of the feed fractions but will be an incorrect description of passage of NDF. NDF have selective detention in the rumen, and passage are both dependent on particle size and age of the particle. Resultingly, NDF have an age-dependent passage where the NDF particles must be reduced in size and increased in density for passage out of the rumen. This occurs after the particles have been exposed to the rumen environment for a set time (Weisbjerg et al., 2003). When plotting the digestion of NDF in the rumen, a lag phase before onset of fermentation is often registered. This lag phase is assumed to represent hydration and microbial colonization before the following microbial digestion starts (Huhtanen et al., 2006).

By-products of microbial carbohydrate digestion are short volatile fatty acids (VFA), lactic acid, hydrogen gas and carbon dioxide. VFA are produced from pyruvate intercellularly in rumen bacteria by different biochemical pathways. Under normal conditions acetate will be produced in largest quantities, followed by propionate and butyrate These three acids constitute up to 95 % of the VFA produced. The remaining 5 % is iso-butyric acid, valeric acid and iso-valeric acid (McDonald et al., 2011).

In Sjaastad et al. (2010) the relative amount of VFA produced by different roughage:concentrate ratios presented, as showed in Table 1.

Table 1. Relative proportions of VFA produced in the rumen by different roughage:concentrate ratios (Sjaastad et al., 2010).

Ratio roughage:concentrate	**Acetate**	**Propionate**	**Butyrate**	**Other acids**
60:40	66	20	10	4
30:70	56	30	10	4
10:90	46	40	9	5

2.2.2 Protein digestion

Protein digestion in ruminants can be said to have two stages. First by microbial digestion in the reticulorumen, and then by chemical digestion in the abomasum and small intestine. In the abomasum and small intestine, the chemical digestion is driven by enzymes secreted from the animals owns glands.

In the rumen, feed protein are degraded to peptides, amino acids and simple nitrogenous compounds in processes which both bacteria and protozoa are involved. Proteins are first degraded to oligopeptides by proteolytic proteases secreted from the microorganisms. The oligopeptides are then broken down to dipeptides and lastly amino acids. The degradations from proteins to amino acids happens extracellularly. Then a large portion of these amino acids are transported into the cell and broken down to ammonia, carbon dioxide and the adjoining organic acid (Hveplund et al., 2003).

Following the protein breakdown, the microorganisms will utilize small peptides, free amino acids and/or ammonia to synthesize their own body protein. A nutritional characteristic of the ruminant is that, because of their relationship with the microbial ecosystem in the rumen, they are normally not dependent on supply of essential amino acids in their diet. The ruminal microorganisms can synthesize all the necessary amino acids, given that there is sufficiently access to energy, nitrogenous compounds and sulfur (McDonald et al., 2011).

Ammonia in the rumen pool are derived from the breakdown of proteins, but also from non-protein nitrogen (NPN). Up to 30 % of the nitrogen in ruminant feed may be NPN (McDonald et al., 2011). In the liver, nitrogenous compounds are converted to urea. Urea is a small molecule that are easily transported in the body, and ruminants have a mechanism to recycle nitrogen by transporting urea to the oral cavity and recycle nitrogen in salvia when chewing and ruminating (Hveplund et al., 2003; McDonald et al., 2011).

Not all protein that enters the rumen are digested at this site, some so-called buy-pass protein will pass undegraded from the reticulorumen. In the review by Chalupa (1975) the proportion of buy-pass protein varied from 20 % to 60 % of the total dietary protein. The amount of buy-pass protein is correlated with the degree of protein breakdown in the rumen. Factors as feed ration composition (by retention time and passage rate) and protein solubility affects the amount of rumen degraded and undegraded protein (Satter, 1986).

Proteins that enter the abomasum and small intestine will be a combination of buy-pass feed protein, microbial protein and endogenous protein from the digestive tract. The microbial

protein account for the largest quantities (Clark et al., 1992). In the abomasum there is secretion of HCl from the epithelia and the enzyme pepsinogen. Low pH activates pepsinogen to pepsin. Pepsin breaks the peptide bonds in proteins at specific sites resulting in that the products of protein digestion in the abomasum is a combination of peptides and free amino acids.

In the small intestine the peptides are further broken down to small peptides (e.g. dipeptides) and amino acids by the enzymes pepsin, trypsin, chymotrypsin and elastase secreted from the pancreas and aminopeptidases secreted from intestinal mucosa. Characteristic for the ruminant is that the secretes form the pancreas contains bicarbonate that neutralizes the acids from the abomasum. This happens first in the middle of the small intestine (jejunum), which leads pepsin still to be active in the duodenum (Hveplund et al., 2003).

The small peptides and amino acids are then transported across the intestinal wall by different transport systems, mostly active transport. Ben-Ghedalia et al. (1974) observed that the fastest rate of absorption was in the jejunum and proximal part of the ileum.

2.2.3 Lipid digestion

To increase the energy density in the diet for high yielding dairy cows, the adding of lipids or free fatty acids is a normal practice. However, a too high fat percentage in the diet can negatively affect the ruminal environment and decrease the carbohydrate digestion. McDonald et al. (2011) supposes that a ration with more than 100 g crude fat/feed DM will decrease the efficiency of ruminal microorganisms, and a general recommendation is to not exceed 3 % crude fat/kg DM (NRC, 2001).

Lipids are not digested in the rumen but through the process of lipolysis and biohydrogenation, some changes will occur. Lipolysis is a process where triglycerides, glycolipids and phospholipids are broken down to free fatty acids and glycerol, phosphate or galactose. Glycerol and galactose enter the ruminal carbohydrate digestion. Lipolysis is controlled by lipases, galactosidases and phospholipases secreted from the rumen bacteria (Børsting et al., 2001).

Free fatty acids from the lipolysis is a combination of saturated and unsaturated fatty acids, but the unsaturated fatty acids are rapidly hydrogenated to saturated fatty acids in the process of biohydrogenation. Biohydrogenation is a process where specific microbes in the rumen break the double bonds in the unsaturated fatty acids and make them saturated by adding

hydrogen from the rumen pool. The full process of biohydrogenation is sometimes incomplete (Hobson & Stewart, 2012).

Fatty acids disappear from the rumen either across the rumen wall by passive absorption or follows the digesta bound to feed particles. Fatty acids reaching the small intestine is a combination of fatty acids originating from feed and fatty acids of microbial origin, where the fatty acids of microbial origin are generated in *de novo* synthesis in the ruminal microorganisms (Hobson & Stewart, 2012). Because of this *de novo* synthesis, apparent fat digestion in the rumen is often negative (Jenkins, 1993). Some triglycerides will escape the processes in the rumen, and in the small intestine these triglycerides are broken down to glycerol and free fatty acids of by lipases secreted from the pancreas. This process is usually exhaustive but if large quantities of triglycerides reach the small intestine this process may be incomplete (NRC, 2001).

The short chained fatty acids (< C12 and to some degree C14) are absorbed across the intestinal wall to the blood and transported through the body. Components in bile secreted from the gallbladder emulgates the longer fatty acids (> C14) to smaller, water-soluble molecules. These make up micelles which are easily transportable. The micelles are re-esterified to triglycerides in the epithelium in jejunum and stored in chylomicrons. Chylomicrons are passive transported across the intestinal wall over into the lymph system (Børsting et al., 2001).

2.3 The rumen environment

Ruminants are adapted to a wide variation of diets. They can utilize feed that are high or low in moisture, high fibrous diets as well as diets that have a high starch content (Owens & Basalan, 2016). However, the nutrients and feed fractions in the diet will affect the ruminal environment which will have repercussions for the animal.

The ruminal ecosystem is complex. There are millions of different microorganisms that coexists in a steadily, intricate competition for nutrients and organic matter. Some microbes can and will utilize a wide variety of nutrients while others have a very small niche (Sjaastad et al., 2010). Microorganisms in the ruminants' gut is a mix between several different species of bacteria, protozoa and fungi. The numerically largest fraction is the bacteria with 10^9-10^{10} cells/mL rumen liquid. Protozoa are larger in size than bacteria and constitute to a bigger part of the ruminal biomass even though they count 10^6 cells/mL rumen liquid. The ruminal fungi

are normally less numerous than both the bacteria and protozoa, and their number are strongly influenced by feed composition. Fungi will vary between $10^3 – 10^7$/mL rumen liquid and they are most numerical in a high fiber diet, in which they can constitute up to 10 % of the ruminal biomass (Kristensen et al., 2003; McDonald et al., 2011).

Under a normal and unvarying feeding pattern, conditions and composition in the rumen will be relatively stable. The environment is strictly anaerobic with a "pH between 5.7-7.0, a temperature of 36-40^o C and with an osmotic pressure of 250-350 mosmole/kg" (Kristensen et al., 2003). These conditions represents the normal where the rumen ecosystem function at optimum, but changes occur that can shift this "ideal" in one or the other directions.

To lessen the extent of this chapter factors affecting pH in relation to acid production will only be described.

As mentioned VFA and lactate are products of microbial fermentation. The production of these acids can decrease the pH significantly if the rumen buffering capacity is lower than the production of these acids. The buffering in the rumen include absorption of VFA across the rumen wall, passage of the acids with rumen liquid and the buffering capacity of bicarbonate added to the rumen with salvia (McDonald et al., 2011).

pH follows the Henderson–Hasselbalch equilibrium $pH = pK_a - \log\left(\frac{[Acid^-]}{[HAcid]}\right)$

Concentrations of acids and pH is negatively correlated and an increase in acids results in a decrease in pH.

VFA are medium weak acids. Acetate has a pKa of 4.75, propionate an pKa of 4.87 and butyrate 4.82. Even though they are not the strongest acids they will lower the ruminal pH if they are present in a high concentration. Lactate is a stronger acid with pKa of 3.86 and an increase in concentration of lactate will decrease the pH more quickly than the VFA (Dijkstra et al., 2012).

After the intake of a meal, rapid fermentation of soluble carbohydrates by amylolytic bacteria and the following acid production decrease the pH. This will result in a shift in the VFA profile towards more propionate at the expense of acetate (Dijkstra et al., 2012). This will happen because if pH drops below 6.2-6, there is a change in the rumen microflora activity, with a decreased activity of the cellulolytic bacteria. The cellulolytic bacteria degrades fiber and produce acetate (Kristensen et al., 2003). The amylolytic bacteria are active at lower pH (5.8-5.5) than the cellulolytic bacteria (Sjaastad et al., 2010). However, if pH declines below

5.7 lactate will accumulate in the rumen and result in a drastic decrease in pH (Dijkstra et al., 2012).

VFA and lactate are products from metabolism of glucose (Figure 2). As the figure shows proinate and acetate can be produced by two different pathways; by lactate or not. Under most feeding situations, the pathway straight from acetyl co-enzyme A (Acetyl CoA) to VFA will be the primary route. But in cases with a high percentage of easily fermentable carbohydrates in the diet, an increase in bacteria (*Lactobacillus*) that produce lactate are observed. This results in a larger production of lactate in the rumen. Fortunately, most of this lactate will be converted to propionate. But in situations where the increase of lactate in rumen liquid decreases the ruminal pH to such an extent that bacterial growth is inhibited, lactate will accumulate in the rumen. Lactate accumulation and the consequently low pH will lead to negative production response and distress for the animal, e.g. subacute acidosis or acidosis (Sjaastad et al., 2010).

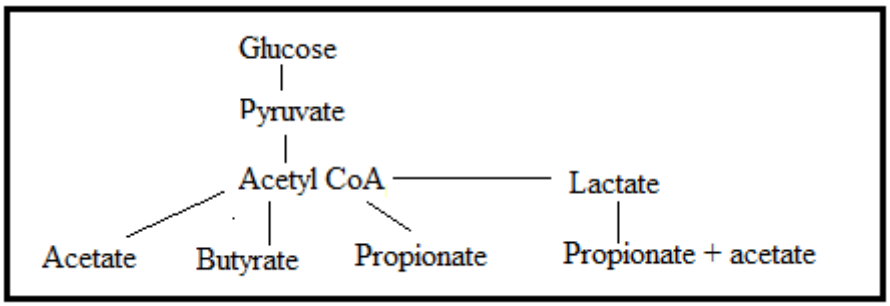


Figure 2. Simplified overview the pathways of VFA from glucose (Sjaastad et al., 2010).

When pH in the rumen drops below 5.6 and remains between 5.2-5.6 for minimum 3 hours per day it is referred to that the animal suffers from subacute ruminal acidosis (SARA) (Gozho et al., 2005). SARA is a health problem with substantial economic consequences as the animals will have a reduced milk yield due to "loss of appetite, diarrhea, dehydration, impaired rumen motility and reduced fiber digestibility" (Plaizier et al., 2008). Thus, optimizing diets to keep the rumen environment stable is important for both feed utilization and animal welfare. Feeding with a TMR compared to separate roughage and concentrate feeding will also theoretically result in a more stable pH, as the nutrient intake will be constant (Kristensen et al., 2003).

The VFA profile as molar percentage is of interest as the acetate, propionate and butyrate have different metabolic fates. Converted butyrate (β-hydroxy butyrate) functions as an energy substrate for ruminal epithelia and synthesis of microbial fatty acids while acetate is

important substrate in the *de novo* milk fat synthesis. Propionate is a substrate in the glycogenesis and the main source of glucose for the ruminant (Dijkstra et al., 2012).

2.4 Methane production

Methane emission from ruminants gains increasing focus as methane is a potent greenhouse gas. Gas produced in the rumen consists of carbon dioxide, methane, hydrogen, oxygen and nitrogen, distributed approximately 40 % carbon dioxide, 30-40 % methane, 5 % hydrogen and the rest is a mix of oxygen and nitrogen. The rate of gas production is most eminent after feeding and may exceed 30 l/hour (McDonald et al., 2011).

Methane produced and lost from the animal equals a loss of energy. This loss accounts for 2-12 % of dietary gross energy (GE) depending on diet (Johnson et al., 1996) but is typically ~ 7 % of GE intake (McDonald et al., 2011). Thus, the aim to reduce methane production is important both to increase the utilization of energy in feed and to reduce methane emission to the atmosphere.

Methane produced per cow per day varies considerately depending on feed level, feed quality and feed composition, energy consumption, size of the animal, level of production and genetics (Hegarty, 2004; Johnson & Johnson, 1995). Of the total methane produced in a ruminant animal, ~ 90 % is produced in the rumen and the majority excreted by eructation. Lower tract fermentation accounts for the remaining methane production, where most (~90%) is absorbed from the hindgut into the portal blood and excreted through the lungs. The methane not absorbed is lost through the anus (Murray et al., 1976).

Enteric methane is produced in the process of methanogenesis by methanogens, microorganisms that belong to the domain of *Archaea* (Broucek, 2014) and by some species of protozoa and fibrolytic microorganisms (Morgavi et al., 2010).

Production of methane is an adaptation to the anaerobic conditions in the rumen and necessary to keep the environment in the rumen stable by removal of H_2 (Kristensen et al., 2003). Formation of acetate and butyrate results in net production of H_2 (see equation below). Formation of propionate utilizes hydrogen, resulting in that the net sum of hydrogen is negative. Simple linear overview of this, adapted from Kristensen et al. (2003) is presented in the equations below.

1) $Glucose + 4\,H_2O \rightarrow 2\,\boldsymbol{acetate} + 2\,HCO_3^- + 4\,H^+ + 4H_2$

2) $Glucose + 2\ H_2 \rightarrow 2\ \boldsymbol{propionat} + 2\ H^+ + 2\ H_2O$

3) $Glucose + 2\ H_2O\ \rightarrow \boldsymbol{butyrate} + 2\ HCO_3^- + 3\ H^+ + 2\ H_2$

The removal of H_2 is necessary to keep other biochemical pathways functioning. During the microbial fermentation of glucose and other monosaccharides, NADH is oxidized from NAD+. To maintain the biochemical pathways dependent on these cofactors, it is crucial to regenerate NAD+. This process will not happen if the ruminal environment has a high concentration of H_2. In other animal cells, the excess of hydrogen is converted to water with oxygen. But as the ruminal environment is almost completely devoid of oxygen, hydrogen has to be removed by other means. Various anaerobic rumen bacteria can reduce CO_2 and hydrogen to methane and thus reduce the concentration of hydrogen gas (Sjaastad et al., 2010). This process follows the equation $CO_2 + 4H_2 \leftrightarrow CH_4 + 2H_2O$

Feed composition and feed level affects methane emission. The ratio between concentrates and roughage affects the methane produced as the VFA produced will differ by carbohydrate composition. Moe and Tyrrell (1979) showed that the digestion of the same amount cellulose compared to that of hemicellulose resulted in nearly three times as much methane produce, and five times that of soluble residue digestion. Increased feed level decreased CH_4 emission per dry matter intake (DMI) (Ramin & Huhtanen, 2013), but daily feed intake is positively correlated with daily methane emission (Hook et al., 2010). In a review article by Broucek (2014) it was referred to studies that showed that increased feed level, increased lipid content and higher concentrate:roughage ratio also reduced methane emission per DMI. The reduction of CH_4/DMI by increased feed level can be explained by higher rate of passage and thus reduced digestion (Ramin & Huhtanen, 2013). Increased lipid portion will decrease the fiber degradation in the rumen and affect the VFA profile and thus methane.

2.5 Probiotics

The Food and Agricultural Organization of the United Nations (FAO) defines probiotics as "live micro-organisms which when administered in adequate amounts confer a health benefit on the host" (Bajagai et al., 2016).

Probiotics gain increasing interest as an alternative to antibiotics as feed additives. Antibiotics, either as growth promoting feed additives or preventative usage, is illegal in Norway (Norsvin, 2018) and the European Union (Casewell et al., 2003). In countries where antibiotic additives still are legal, it is observed a gradually out-phasing of preventative use of

antibiotics following international concern regarding development of antibiotic resistant bacteria. To lessen the impact removal of antibiotics have on production response, probiotics is an alternative. In contrast to antibiotics which kills bacteria, probiotics are designed to encourage different strains of microbiota in the gastro-intestinal tract and give them an advantage over less favourable microorganism (McDonald et al., 2011).

In Norway use of probiotic feed additives are of interest to improve feed utilization and improve the animals' health. The most prominent effects of probiotics in animal productions have been reported in high stress situations or stressful life phases, e.g. weaning, start of lactation and dietary changes (Bajagai et al., 2016).

Probiotics are used in both monogastric- and ruminant production systems, but the effect and mechanism of probiotics differs between species and digestive systems. The mechanisms and mode of action in monogastric animals are unlike that in the ruminant animal because of differences in extent and sites of microbial digestion. Probiotics in simple-stomach animals and young pre-ruminants generally targets the small- and large intestine, while probiotics in adult ruminant animals have main site of action in the rumen. In young calves where the rumen is not yet fully developed, probiotics have reported effect of stabilizing the gut microbiota and limit the risk of pathogens (Chaucheyras-Durand & Durand, 2010). In other young ruminants, probiotic additives are expected to attribute to promoting optimal maturation of the rumen and rumen microbiota (Chaucheyras-Durand & Fonty, 2001). In adult ruminants' probiotics have reported effect on changing the rumen microbial ecosystem, this by initiating a greater density of culturable bacteria populations, as well as stimulating cellulolytic microorganisms and thus increasing the fiber digestibility (Chaucheyras-Durand & Durand, 2010).

To limit the extent of this chapter, the thought benefits and mechanisms related to ruminants will be closer described. Some factors affecting the effect of probiotics will also be mentioned.

In ruminant nutrition there is increasing interest to influence the ruminal ecosystem by manipulating the ruminal fermentative processes, to increase the animal's productivity or reduce unwanted by-products. Production responses varies between trials, but probiotic bacteria applied in a variety of cattle production systems have reported effect on increased milk yield (Desnoyers et al., 2009; Nocek & Kautz, 2006), increased growth weight gain/day (Ghazanfar et al., 2015) and a tendency toward better nutrient digestibility (Boyd et al., 2011). Probiotics have been shown to prevent or treat ruminal acidosis by stabilizing rumen pH, but the effect appears to differ with the severity of the acidosis (Lettat et al., 2012).

Multiple studies have reported effects of probiotic on ruminal pH (Bach et al., 2007; Marden et al., 2008; Thrune et al., 2009) by increasing the ruminal pH and/or stabilizing it, while other trials have showed no effect on rumen pH (Hristov et al., 2010). The theories on which mechanisms regulates the effect on pH may differ, but conclusions from different studies include that probiotics decrease lactate concentrations in the rumen by stimulate the activity of lactate utilizing bacteria (Chaucheyras et al., 1996) and/or lead to a decrease in activity of lactate producing bacteria (Guedes et al., 2008). Another theory is that probiotics stimulate certain rumen microorganisms that will compete with the amylolytic bacteria on the ruminal starch pool and thus lessen the rapid acid production (Brossard et al., 2006).

Probiotics have reported different effects on dry matter intake and nutrient digestibility (Desnoyers et al., 2009), but single studies have reported an effect of yeast by increased NDF digestibility (Durand et al., 2010; Harris et al., 1992). However, other studies have found no effect on NDF digestibility (Nocek & Kautz, 2006).

Increased fiber digestibility is thought to be a response of the stimulation of cellulolytic bacteria. A decrease in lactate concentration and following stabilized pH may result in a higher activity of rumen cellulolytic bacteria resulting in an improved total digestion of fiber (Guedes et al., 2008). Chaucheyras-Durand and Fonty (2001); (2002) reported an effect of *Saccharomyces cerevisiae* CNCM I-1077 on the activity of cellulolytic bacteria and fibrolytic activity in the rumen of young lambs. These two articles theorized that the reason for the numerical increase of cellulolytic bacteria when added *Saccharomyces cerevisiae* CNCM I-1077 could be explained by an increased oxygen scavenging and/or vitamin supply in the rumen. Similarly, Chaucheyras-Durand et al. (2016) found that supplementation of yeast, *Saccharomyces cerevisiae*, increased the number of three types of cellulolytic bacteria *in vivo*.

The usage of probiotics to manipulate the rumen environment with the goal to lessen the ruminal methane production is a field of study with increasing interest. *In vitro* trials have indicated that supplements of probiotics may decrease methane production (Chaucheyras-Durand et al., 2016), but the results are inconsistent (Chaucheyras-Durand, F et al., 2008). Hopes for probiotics is that they will manipulate the biochemical pathways and that probiotics can function as modulators for rumen metagenesis. The most promising theory is that probiotics will affect the H_2 production during fermentation of feed, either redirecting H_2 away from methanogenesis or stimulate fermentative pathways that produces the least amount of H_2. Redirecting H_2 from methanogenesis to homoacetogenesis is an interesting theory. But there have not been found an acetogen that survive in the rumen and are capable to compete

with rumen metagons for substrate. Stimulation of propionate production in the rumen may therefore be a preferred alternative, as it both will reduce the methane production in the animal and increase the energy retention from the feed (Jeyanathan et al., 2014).

The effect of probiotics are varying between different strains of the same bacteria species. Newbold et al. (1995) compared six different stains of *Saccharomyces cerevisiae* on their effect on stimulating the numbers of total bacteria and cellulolytic bacteria *in vitro*. Three of the stains types were also tested on effect to alter rumen fermentation *in vivo*. In the *in vitro* trial the effects of the different strain types varied considerably, from no effect to a solid increase of bacteria in the medium. In the *in vitro* trial all three types had a numerical effect on the bacteria numbers, but only one strain type gave significant effect on total bacteria count or count of cellulolytic bacteria.

The metabolic activity and the probiotic's ability to effectively colonize the rumen will also affect the effects probiotics have on production parameters. Some strains have been proven to remain metabolic active in rumen liquid up to 48 hours, while other have difficulties with viability (Chaucheyras-Durand, F. et al., 2008). Therefore, it important to know the stability of the probiotic when using it in feed and/or fabricate products to optimize the effects.

In conclusion, several hypothesis on how probiotics work and their effect are proposed in the literature. But as they are animal studies, it may not always be easy to conclude that effects shown are singularly because of the probiotics, and not results of other factors. The varying effect of probiotic effects on fiber degradation is thought to be explained by ruminal pH across trials, as the activity of cellulolytic bacteria varies with rumen pH (Russell & Wilson, 1996). The diet fed the experimental animals and rumen fermentation results in considerable variation between experiments, and further study with a wide selection of strain types and feedstuffs is needed to assess the benefits.

Yeast by different strains of *Saccharomyces cerevisiae* is a commonly used probiotic for ruminants (Chaucheyras-Durand & Durand, 2010), which mainly affects the microbial dynamics in the rumen and breakdown of nutrients (Bajagai et al., 2016). In their review Chaucheyras-Durand, F et al. (2008) named three effects of this type of yeast; improvement of rumen development by favoring microbial establishment, stabilizing the ruminal pH and increasing fiber degradation.

In this experiment Levucell ® SC *Saccharomyces cerevisiae* CNCM I-1077 produced at Lallemand (Lallemand Animal Nutrition) was used. Benefits of Levucell ® SC according to

Lallemand is that "LEVUCELL SC optimizes rumen digestion and function which leads to an improvement in income over feed cost" (LALLEMAND, 2019).

2.6 The collection method to determine digestibility

There are a wide number of methods to determine digestibility of feedstuffs and diets. These include different *in vivo* methods (e.g. *in sacco,* marker-method and total collection) and variations of *in vitro* procedures and mediums. Because of the wide range of methods, this chapter is limited to describing the method used in this experiment, the total collection method, in addition to one alternative to this method. Both described methods are used to determine apparent digestibility.

The following section is based on "Evaluation of food: digestibility" found in Animal nutrition McDonald et al. (2011) if not otherwise specified.

Nutrients in the feed and what's available for the animal is not necessarily the same thing. By chemical analyses the quantities of nutrients in the different feedstuffs are quantified, but when the feed passes through the gastrointestinal tract not all is absorbed. There are also losses associated with absorption and metabolism in the animal.

McDonald et al. (2011) defines digestibility as "the proportion of the feed that is not secreted in the faeces and that is, therefore, assumed to be absorbed by the animal".

To determine the digestibility of different feedstuffs, digestibility trials by different designs can be used. In a total collection method apparent digestibility may be determined by a trial where the amount of feed the animals eats is known, and total amount of faeces and urine in a specific time period is collected. This type of trial is divided into two or three periods where each lasts 7 to 10 days. With e.g. three periods, the first period is an adaption period where the animal are gradually adapted to the experimental diet, then follows a preliminary period to make sure that the animal and the digestive system are adapted to the new feed and that there are not old residues of feed in the digestive system. The last period is the collection period where feed intake and faecal output are registered. For ruminants this last period is normally 72 hours. The digestibility is then calculated by:

$$Digestibility = \frac{nutrient\ consumed - nutrients\ in\ faeces}{nutrient\ consumed}$$

This type of trial can be used for total tract digestibility or digestibility in specific sections of the digestive tract if the animal has fistulas in specific region e.g. ruminal cannulas or cannulas in duodenum or ileum. The calculation is similar but changed from nutrients in faeces to nutrients in e.g. ileal sample.

The total collection method can be time consuming and expensive. An alternative is then to use a marker in an indicator method. The indicator method is also useful when total collection is impractical or impossible, for example when the animal is grazing outdoors where both registration of daily feed intake and fecal output is challenging.

In the indicator method a marker in the feed is used to calculate the digestibility, and one differentiates between an internal or external marker. Internal markers are compounds already present in the feed e.g. iNDF or lignin, while external markers are substances added to the feed. One example of commonly used external marker for ruminants is chromic oxide (Cr_2O_3). Important qualities for markers used in digestibility trials is that they do not affect the digestibility of the feed or themselves are digested. Owens and Hanson (1992) summarized that an ideal marker must have the following qualities "1) must not be absorbed 2) must not affect or be affected by the digestive tract or its microbe population 3) must flow parallel to or by physically similar to or intimately associated with the material it is to mark and 4) must have a specific and sensitive method of estimation". While no known marker fulfils all these criteria, those that are regularly used have known faults that may be corrected for.

Digestibility by the marker method is calculated by:

$$Digestibility = \frac{marker\ in\ feaces\ - marker\ in\ food}{marker\ in\ faeces}$$

3 Method

This experiment was performed at the Metabolism Unit at the Animal Production Experimental Center at Norwegian University of Life Sciences (NMBU). The experiment started on Thursday 11.10.2018 and ended Thursday 20.12.2018.

3.1 Experimental design and dietary treatment

3.1.1 Experimental animals

Six rumen cannulated lactating dairy cows of the breed Norwegian Red were used. The cows were ≥ 2. lactation, with an average of 35 days in milk (DIM) at the start of the experiment. The experimental animals were kept in the Metabolism unit in tied stalls.

An overview of the experimental animals with date of calving, yield and lactation number is presented in Table 2.

Table 2. Overview of the experimental animals (ID nr.) with date of calving, daily milk yield (kg milk) and lactation number.

Experimental animal ID	**Date of calving**	**Milk yield (02.10.18)**	**Lactation number**
6155	21.08.2018	35	4
6416	29.08.2018	38	2
6375	24.08.2018	30	2
6411	21.08.2018	33	2
6354	11.09.2018	33	2
6160	16.09.2018	35	4

3.1.2 Design

The experimental animals were blocked by 1) DIM 2) milk yield and 3) body weight and divided between two experimental dietary treatments groups: yeast (Y+) and control (Y-). The experimental design was a crossover design with two experimental periods of 28 days, with a 14 days washout period in-between. Period 1 started 11.10.2018 and ended 08.11.2018 while period 2 started 22.11.2018 and ended 20.12.2018. In each period one group of three

experimental animals were given a probiotic yeast additive *Saccharomyces cerevisiae CNCM I-1077* (Levucell ® SC), while the remaining three animals functioned as a control group (Table 3).

Table 3. Overview of experimental animals divided between dietary treatment group Y+[1] and Y-[2] in period 1 and period 2.

Experimental animal ID	**Period 1** **11.10.18-08.11.18**	**Period 2** **22.11.18-20.12.18**
6354	Y+	Y-
6411	Y+	Y-
6416	Y+	Y-
6155	Y-	Y+
6375	Y-	Y+
6160	Y-	Y+

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2] Y- = control, no additives

3.1.3 Treatment

From day 1 to day 28 at 09:00 in each period the Y+ group had 0.5 g Levucell ® SC *Saccharomyces cerevisiae CNCM I-1077* from Lallemand (Lallemand, Toulouse, France) inserted in the rumen through the rumen cannula. The dosage of *Saccharomyces cerevisiae CNCM I-1077* equaled $1*10^{10}$ colony-forming units (CFU) per cow per day. *Saccharomyces cerevisiae CNCM I-1077* was wrapped in thin layer of toilet paper to ensure that the complete amount was inserted into the rumen. The Y- group had their rumen cannula opened at 09:00 but no adding of yeast.

In the washout period no animals were given yeast additives.

3.1.4 Feed

All animals were fed a total mixed ratio (TMR) for the full duration of the experiment. The TMR was a mixture of grass silage and a commercial concentrate. The dry matter (DM) content of the grass silage was 330 g/kg, while crude protein content was 142 g/kg DM and

neutral detergent fiber (NDF) was 520 g/kg DM. The concentrates used based on a commercial mixture from Norgesfôr, Drøv Energirik låg, but altered to have a higher content of Norwegian feedstuff than the original. The concentrate was produced by Norgesfôr Mysen in one batch that was used for both period 1 and period 2.

The TMR was optimized in TINE Optifôr two days before the experiment started to meet the animals' requirements for maintenance and milk production. The TMR was planned for a fictitious cow representing the average of the experimental animals in the middle of the experiment: ≥ 2 lactation, 49 DIM and a daily milk yield of 38.3 kg and aiming a dry matter content of 38 %.

The TMR was made in a Lely Siloking in batches once a week. It contained 54 % concentrates and 46 % roughage on dry matter basis.

When producing the TMR, the concentrate and water (equal to 50% of concentrate weight) were added to the Siloking and then let to swell for two hours. Then three round bales were gradually added to the mix in addition more to water. Amount of water added varied as it was calculated to match the dry matter in the grass silage to make a TMR with ~38 % dry matter.

The TMR were kept frozen at -20^{o}C to keep the feed stable and keep it for fermenting. Daily feed rations was taken out to defrost the day before it was fed the experimental animals.

3.1.5 Feeding

The daily feed ration of TMR for each animal was divided in three equal portions that were fed three times a day at 06:30, 14:00 and at 19:00. Daily feed intake and remains of feed were registered daily. The amount of TMR (kg/DM) the animal was fed per day was fixed and thus similar for both periods. Before experiment started, the feed intake was measured for three days, and with basis on this and milk yield the experimental animals were either given 21 kg /DM or 22 kg/DM daily.

3.1.6 Water

Individual water intake was measured continuously with automatic registrations in each experimental animals' water trough.

3.1.7 Body weight and body condition score

Body weight (BW) was recorded at the start and at the end of each period, (day 0 and 1 and day 27 and 28.). The average body weight (BW) was then calculated.

Body condition score (BCS) was registered using the scale adapted by Geno (2018). The score was registered at day 28 in period 1, and at day 4 and 28 in period 2.

3.2 Total collection of faeces and urine

In each experimental period there was a 72 hours total collection of faeces and urine, from day 25 to day 28. Faeces and urine were continually collected in separate buckets for each individual experimental animal during this time period, and full buckets were stored in a cool room (+4^{o}C) until the end of collection.

Before collection started, the tie stalls were washed with water to remove bedding, remains of feed and manure. Buckets of 30 l was used which were marked with animal ID, tara weight without top and FAECES or URINE. To keep nitrogen from evaporating from the urine, 0.5-1 L 10 % sulfuric acid were added to keep pH in urine below 4. Faults and deviations were noted.

In the total collection period, daily amount of faeces (kg) and urine (l) were registered and representative 10 % of the amount were stored until end of the total collection period for samples.

3.3 Methane measurements and samples

Enteric methane emission was measured between days 14 and 19 in each experimental period.

The methane was measured using the SF_6 tracer technique, also called the ERUCT technique (Emissions from Ruminants using a Calibrated Tracer) as described in Johnson et al. (1994), Kidane et al. (2019) and McGinn et al. (2006). The ERUCT technique involves placing a permeation tube filled with ultra-pure SF_6 (sulfur hexafluoride) with a known release rate into the rumen. The release of the marker, SF_6, is regulated by a semipermeable Teflon membrane in the tube, resulting in a constant flow of SF_6 out of the permeating tube. The release rate of each individual permeation tube was determined by monitoring the weight changes by weekly measurements for about 6 weeks before experimental start. The permeation tubes were maintained in an incubator kept at 39^{o}C for this period.

The permeation tubes were placed in the rumen of the experimental animals seven days ahead of experiment start. Other equipment used for the measurements was eight cannisters

connected to head-halters with an in-line capillary tube. Each individual head halter were placed on the animal so that the in-line capillary tube ended over the nostrils of the experimental animal.

Before collection there was added negative pressure to the cannisters using a vacuum pump, 2 bars for 12 seconds, and psi and mmHg in the cannisters were noted.

The gas collection period started at 09:30 by placing the halters connected to the cannisters on the animals. Each cannister were placed above an individual experimental animal. Two extra sets of cannisters and halters were placed in the north- or south end of the room the animals were held, this to account for the background marker and methane gas in the barn.

After 24 hours the collecting cannister were changed with fresh, negative pressure (vacuum pump, 12 seconds, 2 bars) cannisters, and the pressure in the "used" canisters were registered and compared to the pressure when they were mounted. During the collection period the pressure inside the cannisters should normally be reduced by 50%.

After registration of the pressure, the cannisters were pressured with nitrogen gas for 15 seconds, 2 bars, with the new pressure of the cannisters noted. Then the cannisters were set aside for 45 minutes to allow gas mixing. After this time period three samples from each cannister were taken by a syringe, and filled in three 20 mL evacuated glass vials.

After sampling, the cannisters were emptied and thrice cleaned with nitrogen gas and added negative pressure so that they were ready for collection the following day.

The glass vials with the gas samples were later sent to analysis for methane and SF_6 at the Soil lab, NMBU, using gas chromatography.

3.4 Experimental samples

3.4.1 Feed

One sample of grass silage from each round bale were taken one or two days in advance of mixing the TMR. The samples were taken by drilling a hole in each round bale and extracting a set mass of grass silage. A part of these samples was used to determine dry matter content in the round bales to later optimize the TMR, while the remaining was stored frozen at -20^{o}C.

At the end of the experiment, the grass silage samples from each experimental period were mixed together and a representative sample were sent to Eurofins AS (Eurofins, Moss,

Norway) and Labtek (Labtek, IHA, Ås) for analysis. The samples sent to Eurofins were analysed by NIR and fermentation quality (NorFor pakke). Samples destined for LabTek were previously freeze-dried and ground (1,0mm) and then sent to LabTek for analysis of chemical components (dry matter, ash, Kjeldahl-N, crude fat, NDF and NDF/ash corrected).

One sample of the concentrate batch was taken at the beginning of the experiment. The sample was stored frozen at -20^{o}C until the end of the experiment. At the end of the experiment the concentrate sample was divided into two parallels and dried at 45^{o}C for >48 hours, and then equilibrated in room temperature until stable weight (minimum >24 h). The weight before and after equilibration of each parallel were noted. Following this, the parallels were mixed together and ground (0,5 mm). The sample were then sent to LabTek for chemical analysis (dry matter, ash, NDF, NDF w/ash corrected, Kjeldahl-N, starch w/sugar, starch, crude fat and water-soluble carbohydrates).

Ten batches of TMR were made during the experiment. In connection with morning feeding Monday through Friday a 200 gr representative sample was taken from the TMR fed that day. These samples were stored frozen at -20^{o}C until the end of the experiment.

At the end of the experiment the TMR samples from each experimental week were mixed together to a homogenous mass. The samples from week 3 and week 4 in each experimental period (in total four samples) were divided into five parallels. Two parallels of ~ 200 gr was dried at 103^{o}C > 24 h to determine dry matter content, two parallels of ~ 200 gr were freeze-dried then equilibrated in room temperature until stable weight (>24 h). The freeze-dried samples were then ground in 0,5 mm and 1,0 mm sieve size using a Retch cutting-mill SM200. The remaining parallel was a reserve, and stored frozen at -20^{o}C.

The ground samples were sent to LabTek for chemical analysis (dry matter, ash, NDF, NDF w/ash correction, Kjeldahl-N, starch, starch w/sugar, crude fat).

3.4.2 Rumen samples: pH and fermentation products

Samples for rumen fermentation pattern and pH was taken at day 25 in each period. In these samples pH was measured and then the samples were analyzed for NH_3N and volatile fatty acids (VFA). The samples were taken every hour from 5 minutes before feeding to eight hours post feeding.

When sampling rumen liquid the rumen cannulas were opened, and a metal probe connected to a syringe was inserted into the rumen. 50 mL samples were extracted from the lower part of the dorsal sac and straight into small containers. Immediately pH was measured using a pH-

probe. Then 10 mL of the rumen liquid was pipetted into smaller container with 0,5 mL concentrated formic acid and sent to LabTek for analyses.

3.4.3 Milk

The experimental animals were milked twice a day at 06:30 and 18:30. Daily milk yield was registered before the experiment started at day -8 to -6 in addition to day 1-28 each experimental period. Milk yield was measured using a DeLeval Milk Meter MM6.

Milk samples were taken two times a day from day 1 to day 3, and at day 25 to day 27 in both experimental periods. For each sampling, two samples were taken. One sample were sent to TINE for analysis of chemical composition while the other sample was frozen at -20^{o}C.

Experimental animals with a somatic cell count (SCC) above 200’ in the milk indicated subclinical mastitis (Res., 2003).

3.4.4 Faeces and urine

For each total collection period, the faecal sample was divided into seven parallels. Two of these parallels were used to determine dry matter content (drying at 103^{o}C > 24 h) while the remaining five parallels were used for other tests. Two parallels were used to determine particle size, with two parallels as reserve (outside of this theasis). One parallel was used for chemical analyses. This parallel was previously freeze-dried, equilibrated in room temperature >24 h and ground in a Retch chopping-mill SM200 on 0,5 mm and 1,0 mm before it was sent to LabTek. At Labtek this sample was for analyzed of dry matter content, ash, NDF, NDF w/ash correction, Kjeldahl-N, starch, crude fat.

From the total collected urine, three parallels was made, but only one parallel were sent to LabTek for chemical analysis of Kjeldahl-N. The two remaining parallels functioned as reserves.

3.5 Analyses

Analyses of the roughage, faeces, urine and rumen fermentation pattern were performed at Eurofins or LabTek while milk samples were sent to TINE for analysis. The methane samples were analyzed at the Soil lab at Norwegian university of Life Sciences (NMBU).

3.5.1 Dry matter

Dry matter content was determined following the procedure by Berg, B. M. (2018). This procedure includes drying the samples for minimum of four hours or overnight at 103^{o}C ± 2^{o}C. This is the standard procedure for other feed than roughage. Roughage is dried at 60^{o}C for minimum four hours to prevent loss of volatile components.

The dry matter content is calculated when the samples have reached constant weight.

3.5.2 Ash

Ash content in the samples were determined by burning the samples at 550^{o}C for minimum 4 hours but no longer than 20 hours, and what is left of the sample after this time is the ash fraction (Berg, B. M., 2018a).

3.5.3 Kjeldahl-N

Kjeldahl-N were analyzed as described by Berg, B. M. (2018b). The sample is added acid and heated to a high temperature that leads the amino acids to deaminate and nitrogen content is analyzed in a Kjeltec Auto TM 8400.

3.5.4 Crude fat

Crude fat is analyzed for in ASE® 350 Accelerated Solvent Extractor. It's an instrument that pumps petroleum ether and acetone into an extraction cell under a specific pressure and temperature and the fat is extracted. The solvents are then removed, and the remaining sample is dried and weighed (Tingstad, 2018).

3.5.5 aNDF with ash

aNDF were analyzed by LabTek following the procedure described in Berg, B. M. (2018c) which include that the sample is heated in a neutral detergent solution that leads the cell content to be solved, but the cell walls remains intact. NDF is then determined gravimetric. aNDF determined with this method will have a small fraction of unorganic matter.

3.5.6 aNDFom

The first steps of an aNDFom analysis is the same as for as an analysis of aNDF, but with an additional step for correction for inorganic matter. After aNDF analysis the sample is burned at 550^{o}C and the rest fraction is subtracted from the original result (Berg, B. M., 2018d).

3.5.7 Starch / Starch included sugar

The starch content is determined by adding α amylase to the sample which results in that the starch three-dimensional structure breaks down to shorter, water soluble units. An additional enzyme is then added which breaks these units further down to simple glucose molecules. Glucose is then analyzed for in a MaxMat PL II Multianalyzer (Svihus, 2018).

3.5.8 Water soluble carbohydrates (WSC)

The carbohydrates in the samples are extracted in an acetate buffer that hydrolysis the sucrose and fructose to glucose and fructose. These monosaccharides are then converted to glucose-6-phosfate and fructose-6-phosfate which reduces NADP to NADPH. The measurements of NADPH before and after the reaction is registered since it is positive proportional to the fructose and glucose concentrations in the sample. The absorbance of NADPH is measured using a spectrometer (Jørgensen, 2018).

3.6 Calculations

The chemical content of nutrients was calculated by the following formulas:

$$Dry\ matter\ (g/kg) = \frac{weight\ after\ drying\ (g)}{weight\ before\ drying\ (g)} * 1000$$

$$Organic\ matter\ g/kg\ DM = dry\ matter\ (g/kg) - ash\ (g/kg\ DM)$$

$$Crude\ protein\ (g/kg\ DM) = KjeldahlN\ (g/kg\ DM) * 6.25$$
$$Crude\ protein\ in\ milk\ (g/kg\ DM) = KjeldahlN\ (g/kg\ DM) * 6.38$$

$$RestCHO\ (g/kg) = 1000 - ash\ (g/kg\ DM) - crude\ protein\ (g/kg\ DM) - crude\ fat\ (g/kg\ DM) - NDFom\ (g/kg\ DM) - starch\ (g/kg\ DM)$$

Apparent total tract digestibility was calculated by:

$$Apparent\ total\ tract\ digestibility\ (\%) = \frac{nurtient\ in\ feed - nutritent\ in\ feaces}{nutient\ in\ feed} * 100$$

Energy corrected milk (ECM) was calculated by:

$$ECM\ (kg) = milk\ yield\ (kg) * (0.01 + 0.122 * fat\% + 0.077 * protein\% + 0.053 * lactose\%)$$

Calculations of ECM was based on fat-, protein- and lactose percentage were a weighted average of analyses from TINE (corrected for variations between morning- and evening milking).

Amount of milk components (milk fat, -protein or lactose) produced daily was calculated by:

$$Milk\ component\ (g/day) = milk\ (kg) * milk\ component\ ^{1}(\%) * 10$$

1)milk fat, milk protein or lactose

Nitrogen efficiency in milk were calculated by:

$$Nitogen\ efficiency = \frac{Nitrogen\ in\ milk}{Nitrogen\ in\ feed} * 100$$

Daily methane emissions were calculated after McGinn et al. (2006):

$$Q_{CH_4} = \frac{C_{CH_4} - C^b_{CH_4}}{C_{SF_6} - C^b_{SF_6}} Q_{SF_6} \frac{MW_{CH_4}}{MW_{SF}}$$

Where Q_{CH_4} is the daily enteric emission (g/day), Q_{SF_6} is the SF_6 marker release rate (g/day), C_{CH_4} and C_{SF_6} are the methane and SF_6 mixing rations in the canisters (µmol/mol), $C^b_{CH_4}$ and $C^b_{SF_6}$ are the background collections of methane and SF_6 respectively and. $\frac{MW_{CH_4}}{MW_{SF}}$ is the molecular weight ratio used accountant for the differences in the density of the gases (Kidane et al., 2019).

3.7 Statistics

For calculation of statistical values, the statistical data program SAS 9.4 was used (Inc., 2019). Two models were used when calculating the statistics. Either Proc GML (Model 1), which calculates based on independence in the dataset or Proc mixed (Model 2 or Model 3), which calculates values based on repetitive observations and dependence in the dataset. In results calculated by the GLM model, root mean square (RMSE) are presented as the standard deviation to the residual. When the data was treated in Proc Mixed the standard error (SE) are

presented in the tables, which is a term of the accuracy in the dataset. When the SE values of the two dietary treatments were different, an average of the SE values were calculated and presented in the tables. The GLM model was used calculating statistics for digestibility and nitrogen balance, while the Proc Mixed model was used for the remaining calculations.

In this thesis, results with a *P-value* < 0.05 was said to be significant and a trend if *P-value* < 0.1.

Model 1:

$$y_{ijk} = \mu + a_i + b_j + c_k + \varepsilon_{ijk}$$

$$y_{ijk} = variable\ of\ interest$$

$$\mu = overall\ mean$$

$$a_i = random\ effect\ of\ cow\ i = 1,2,3,4,5,6$$

$$b_j = fixed\ effect\ of\ period\ j = 1,2$$

$$c_k = fixed\ effect\ of\ treatmet\ k = 1,2$$

$$\varepsilon_{ijk} = error\ term$$

Model 2:

$$y_{ijk} = \mu + a_i + b_j + d_l + c_k + \varepsilon_{ijkl}$$

$$y_{ijk} = variable\ of\ interest$$

$$\mu = overall\ mean$$

$$a_i = random\ effect\ of\ cow\ i = 1,2,3,4,5,6$$

$$b_j = fixed\ effect\ of\ period\ j = 1,2$$

$$c_k = fixed\ effect\ of\ treatment\ k = 1,2$$

$$d_l = fixed\ effect\ of\ time\ l = 1,2,3\ldots14$$

$$\varepsilon_{ijk} = error\ term$$

Model 3:

$$y_{ijk} = \mu + a_i + b_j + c_k + d_l + \varepsilon_{ijkl}$$

$$y_{ijk} = variable$$

$$\mu = population\ mean$$

$$a_i = random\ effect\ of\ cow\ i = 1,2,3,4,5,6$$

$$b_j = fixed\ effect\ of\ period\ j = 1,2$$

$$c_k = fixed\ effect\ of\ treatment\ k = 1,2$$

$$d_l = fixed\ effect\ of\ time\ l = 1,2,3,4,5,6,7,8$$

$$\varepsilon_{ijk} = varians\ not\ explained\ by\ the\ model$$

4 Results

All the results are calculated from data collected from day 14-27 in each experimental period if not otherwise specified.

4.1.1 Chemical composition of feed

Chemical composition of the grass silage and the concentrate used in this experiment are presented in Table 4, and the chemical composition of the TMR is shown in Table 5.

Table 4. Chemical composition of the grass silage and concentrate used in this experiment.

	Period	DM[1]	OM[2]	NDF[3]	Crude protein	Crude fat	Starch	Rest-CHO[4]	Ash
		g/kg			g/kg dry matter				
Silage	1	336		519	145	35			55
	2	331		520	140	36			58
Concentrate	Both	890	930.4	178.6	183.1	42.0	414.9	137.3	69.6

[1]DM = dry matter [2] OM = organic matter [3]NDF = aNDFom [4]RestCHO= 1000-ash -crude protein -crude fat -NDF -starch

Table 5. Chemical composition of the TMR used in this experiment with difference between the two experimental periods.

	Period	DM[1]	OM[2]	NDF[3]	Crude protein	Crude fat	Starch	RestCHO[4]	Ash
		g/kg			g/kg dry matter				
TMR[5]	1	387.3	937.8	398.2	169.3	53.3	195.8	131.9	62.2
	2	375.5	936.6	400.0	170.3	48.0	192.9	137.3	63.4
Difference P1-P2		11.8	14.3	0.3	1.0	5.3	4.2	14.3	0.7

[1]DM = dry matter [2] OM = organic matter [3]NDF = aNDFom [4]RestCHO= 1000-ash -crude protein -crude fat -NDF -starch [5] TMR=total mixed ration

4.1.2 Feed- and water intake

Figure 3 shows the average daily intake of TMR (kg DM) for each individual experimental animal in the two experimental periods. There was little variation of dry matter intake between the experimental periods, which is in accordance to the goal of minimizing this variation.

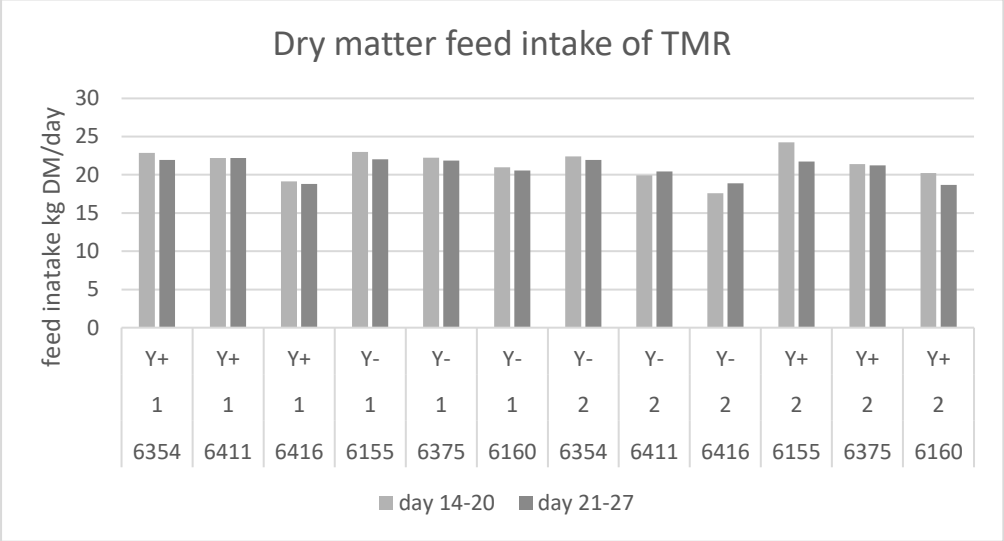


Figure 3. Average dry matter intake of TMR[3] for the different experimental animals during week 3 and week 4 in period 1(1) and period 2 (2) shown in kg DM/day.

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077(Levucell ® SC)
[2] Y- = control, no additives

Figure 4 shows the average daily water intake for each experimental animal in the two periods.

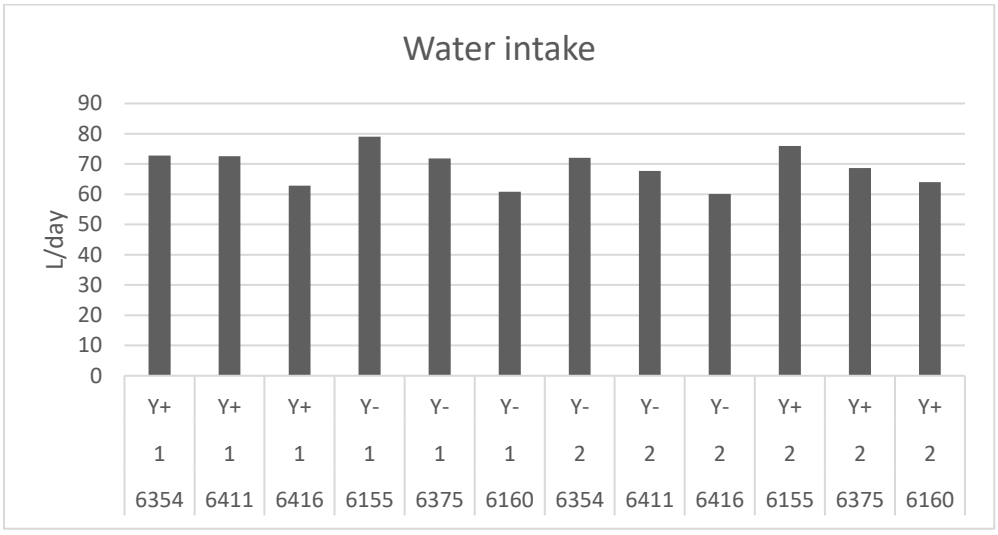


Figure 4. Average daily water intake for individual experimental animals for period 1(1) and period (2).

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2] Y- = control, no additives 1=period 1 2=period 2

The average daily intake of dry matter and nutrients from feed in each period are shown in Table 6. The values are presented in kg dry matter/day, except dry matter intake which is shown as kg/day. No differences was observed between the dietary treatments (*P≥0,05*) which were as expected because of the experimental design.

Table 6. Average daily feed intake of TMR (kg DM/day) and nutrients in the feed (kg DM/day) for the two different dietary treatments.

		Y+[1]	**Y-[2]**	**SE[3]**	***P-value***
		Average daily intake day 14-27			
Dry matter	kg/day	21.2	21.0	0.7	0.27
Organic matter	kg DM/day	19.8	19.6	0.6	0.28
NDF[4]		8.2	8.1	0.3	0.33
Crude protein		3.6	3.6	0.1	0.43
Crude fat		1.1	1.1	0.0	0.80
Starch		4.1	4.1	0.2	0.24
RestCHO[5]		2.8	2.8	0.1	0.29

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2] Y- = control, no additives [3] SE= standard error [4] NDF=aNDFom [5]RestCHO= 1000-ash -crude protein -crude fat -NDF -starch

In Table 7 the average daily water intake for each dietary treatment presented with standard error (SE) and *P-value*. There was no effect of dietary treatment in daily water intake, but there was variation between days (*P*-value < 0.*05*).

Table 7. Average daily water intake for the two dietary treatment groups presented with SE and *P*-value.

	Y+[1]	**Y-[2]**	**SE[3]**	***P-value***
Water intake l/day	70	70	2,29	0.71

[1]Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2]Y- = control, no additives [3]SE = standard error

4.1.3 Total collection of faeces and urine

The average daily amount of faeces and urine produced per day from each dietary treatment group is presented in Table 8. There were no effect of dietary treatment.

Table 8. Average amount of urine (L/day), nitrogen in urine (g/day) and faeces (kg/day and kg DM/day) for the two dietary treatment groups.

	Y+[1]	**Y-[2]**	**SE[3]**	***P-value***
Urine L/day	21.8	21.6	0.75	0.77
Nitrogen in urine g/day	193.0	186.2	36.84	0.34
Faeces kg/day	46.9	47.0	2.15	0.99
Faeces kg DM[4]/day	6.1	6.1	0.22	0.60

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2] Y- = control, no additives [3] SE= standard error [4]DM=dry matter

4.1.4 Digestibility

Apparent total tract digestibility of dry matter and nutrients in the feed is presented in Table 9, as a percentage of feed intake. There was effect of dietary treatment on apparent total tract digestibility of crude fat (*P≤0,05*).

Table 9. Effect of dietary treatment on apparent total tract digestibility of dry matter and the main nutrients, shown in percentage digested of total feed intake.

	Y+[1]	**Y- [2]**	**RMSE[3]**	***P-value***
	% of feed intake day 14-27			
Dry matter	71.4	70.9	1.22	0.48
Organic matter	72.3	71.8	1.19	0.46
NDF[4]	59.1	57.7	2.22	0.35
Crude protein	71.8	71.2	1.22	0.40
Crude fat	73.6	77.6	2.36	0.042*
Starch	99.2	99.2	0.11	0.47
RestCHO[5]	71.8	71.4	1.42	0.68

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2] Y- = control, no additives [3] RMSE=root mean square error [4] NDF=aNDFom [5]RestCHO= 1000-ash -crude protein -crude fat -NDF -starch

4.1.5 Milk

Presentation of milk data from each experimental animal from both periods is presented in Table 10. The values are calculated from milk analyses from day 25-27 in each experimental period.

6160 and 6155 had somatic cell count (SCC) above 200' in period 2, indicating subclinical mastitis (Res., 2003). Milk samples from the start (day 1-4) and end (day 25-27) of period 2 showed that 6160 had high SCC the whole period, while 6155 had normal SCC in the beginning of period 2 (average of 33') but had a drastic increase evening milking day 26 (510') and an average SCC of 480' day 27.

Table 10. Overview of average milk yield, ECM, fat, protein and lactose, urea (mmol/L), FFA (micromol/mL) and SCC for each experimental animal in both the experimental periods.

Animal ID	Treatment	Period	Kg milk	Kg ECM[3]	Fat%	Protein%	Lactose%	Urea	FFA[4]	SCC[5]
6354	Y+[1]	1	32.9	34.5	4.12	3.58	4.81	4.8	0.4	13.3
6411	Y+	1	30.8	32.5	4.64	3.07	4.58	5.9	1.0	13.3
6416	Y+	1	33.9	33.8	4.23	2.93	4.60	7.0	0.6	86.7
6155	Y-[2]	1	37.5	35.9	3.62	3.25	4.81	5.3	0.3	23.3
6375	Y-	1	28.8	31.4	4.69	3.31	4.83	5.5	0.9	85.0
6160	Y-	1	30.7	30.6	3.91	3.52	4.62	4.4	0.3	88.3
6354	Y-	2	33.9	36.3	4.30	3.61	4.89	5.1	0.2	15.0
6411	Y-	2	31.0	31.2	4.22	3.16	4.47	5.3	0.6	10.0
6416	Y-	2	33.2	32.8	4.15	3.07	4.47	5.7	0.4	11.7
6155	Y+	2	34.2	32.6	3.59	3.30	4.70	5.1	0.2	251.7
6375	Y+	2	27.3	29.0	4.30	3.58	4.68	5.0	0.3	10.0
6160	Y+	2	22.7	23.8	4.31	3.74	4.21	5.1	0.2	221.7

Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2]Y- = control, no additives [3] ECM = energy corrected milk [4]FFA = free fatty acids [5]SCC = somatic cell count in 100'000

Effect of dietary treatment on milk yield, ECM, fat- protein- and lactose percentage, free fatty acids and urea is reported in Table 11. Daily production of milk components are also presented. There was a tendency towards a trend to more lactose (gr lactose produced per day) in the Y- group (*P=0,13*) compared to the Y+ group.

6160 was excluded from the calculation because of drop in yield and increase in SCC in the washout period, as the drop continued in period 2. 6416 was included in the calculation despite high SCC because of no obvious effect on milk yield and milk composition.

Table 11. Milk yield, ECM and milk composition sorted by dietary treatment with SE and *P-value*s, in addition to daily production of milk fat, -protein and lactose. The data are calculated from milk yield and milk analysis from day 25-27 in each period.

	Y+[1]	**Y-[2]**	**SE[3]**	***P-value***
Milk kg	31.2	31.8	1.25	0.20
ECM[4] kg	31.8	32.3	0.89	0.46
Fat %	4.1	4.2	0.18	0.56
Protein %	3.3	3.3	0.09	0.63
Lactose %	4.7	4.7	0.07	0.31
Urea mmol/L	5.1	5.4	0.29	0.27
FFA[5] micromol/L	0.4	0.4	0.12	0.61
Milk fat produced g/day	1304.2	1309.2	29.11	0.90
Milk protein produced g/day	1041.7	1053.9	46.02	0.54
Lactose produced g/day	1457.0	1488.1	67.13	0.13

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2]Y- = control, no additives [3]SE = standard error [4] ECM = energy corrected milk [5]FFA= free fatty acids

4.1.6 pH and fermentation products

Effect of dietary treatment on pH, ammonia and VFA in rumen liquid is presented in Table 12. There was numerical higher pH in the Y+ group compared with the Y- group (*P=0.11*). There was an effect of dietary treatment on propionate and butyrate concentration (*P=0.05*), with the highest concentrations in the control group. There was a trend (*P=0.06*) towards higher concentrations of total acids in the Y- group compared to the Y+ group.

Table 12. Average of fermentation products and pH for the two different treatments with SE and *P-value*. Ammonia is presented as mg/L while the remaining products are presented in mmol/L.

	Y+[1]	**Y-[2]**	**SE[3]**	***P-value***
pH	6.11	6.0	0.05	0.11
Ammonia	138.9	141.1	11.48	0.89
Acetate	66.3	70.5	2.03	0.10
Propionate	22.1	24.0	0.87	0.0542*
Iso-butyric acid	0.8	0.9	0.05	0.23
Butyrate	13.2	14.6	0.74	0.0528*
Iso-valerian acid	1.2	1.2	0.07	0.52
Valerian acid	1.6	1.7	0.06	0.18
Sum acids	105.2	112.8	2.97	0.06

[1]Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2]Y- = control, no additives [3]SE = standard error

In Table 13 the relationship between concentrations of acetate and propionate in rumen liquid for the two different dietary treatments presented. There was no effect of *Saccharomyces cerevisiae* CNCM I-1077 on this ratio.

Table 13. The relationship between acetic- and propionate by the two different dietary treatments presented with SE and *P-value*.

	Y+[1]	**Y-[2]**	**SE[3]**	***P-value***
Acetate:propionate	3.1	3.0	0.15	0.38
mmol/L				

[1] Y+ = added 1 x 10^{10} CFU/day (Levucell ® SC) [2]Y- = control, no additives [3]SE = standard error

Figure 5 shows the variation in pH and concentrations of acids in rumen liquid measured at hourly from 06:30 to 13:30. The values are an average of measurements from day 25 in each experimental period. The figure shows that the total acid content and pH are inverted reflections of each other.

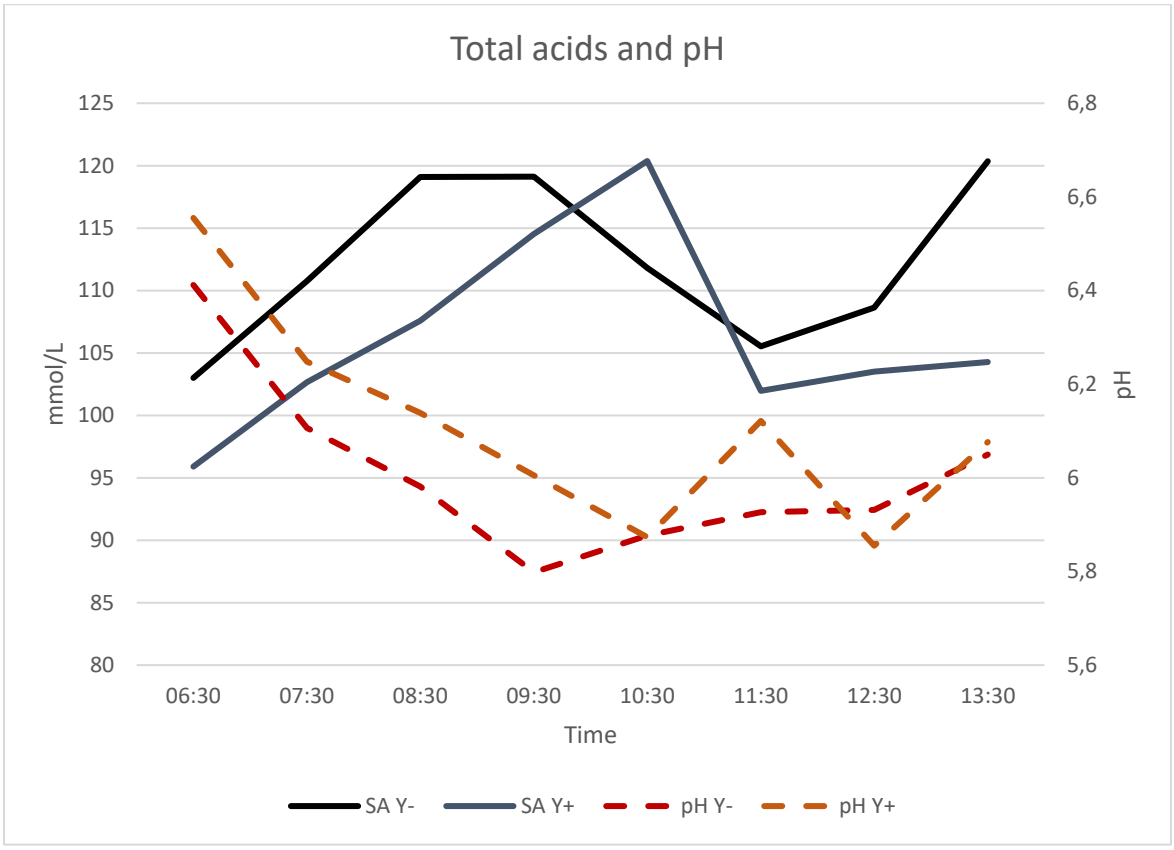


Figure 5. pH and concentrations of acids (mmol/L) variations measured at day 25 each period sorted by different dietary treatment.

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2]Y- = control, no additives SA=sum of acids (mmol/L)

Figure 6, Figure 7 and Figure 8 are graphic presentations of molar percentage for acetate, propionate and butyrate. The numbers are shown as an average of concentrations measured each hour from 06:30-13:30, day 25 in each experimental period. There was no effect of treatment in molar percentage of acetate (*P=0.30*), propionate (P=0.49) or butyrate (*P=0.51*), but molar percentage of butyrate had a trend towards an effect of treatment*time (*P=0.08*).

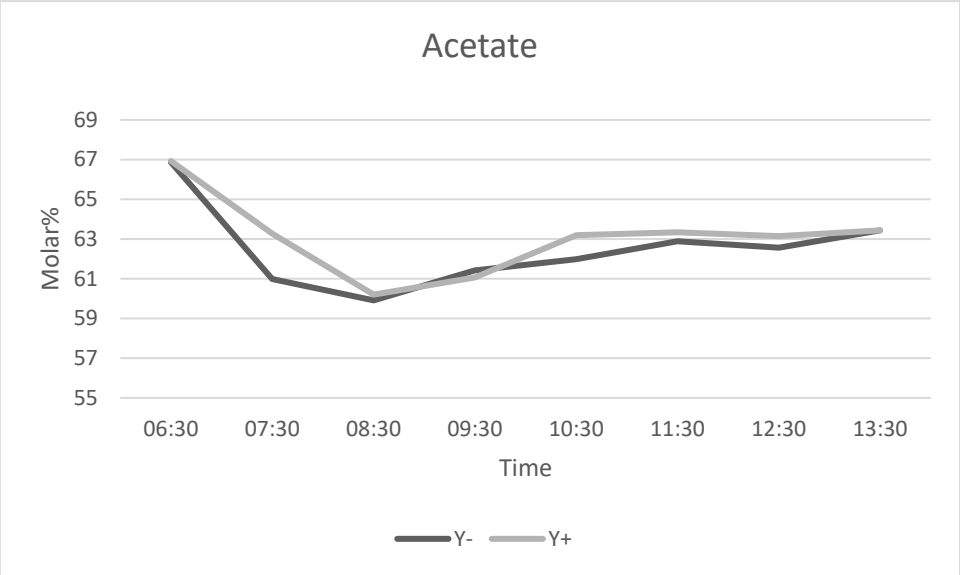


Figure 6. Molar percentage of acetate as an average of measurements from day 25 in each experimental period for the two different dietary treatments.

Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC)

Y- = control, no additives

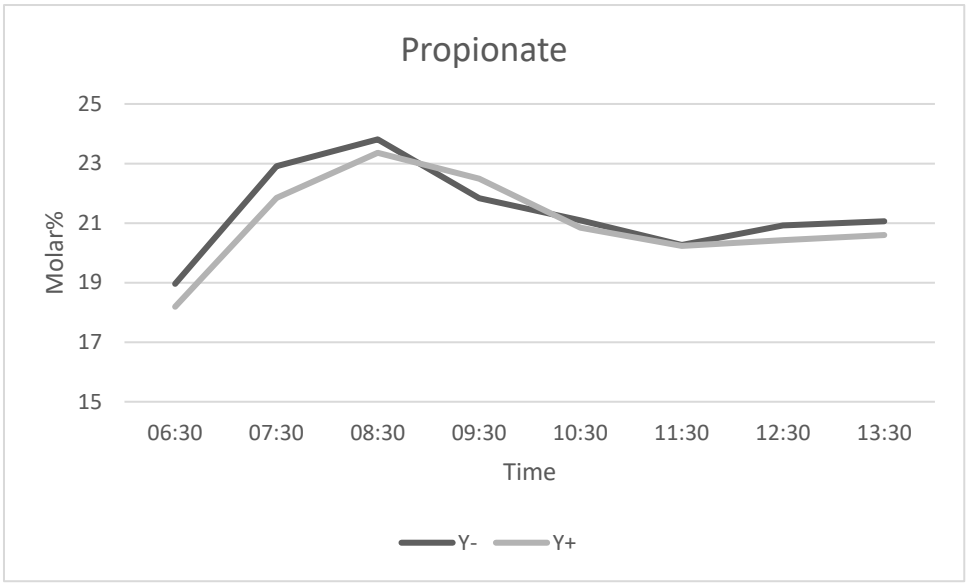


Figure 7. Molar percentage of propionate as an average of measurements taken day 25 in each experimental period for the two different dietary treatments (Y+/Y-).

Y+ = added 1 x 10^{10} CFU/day Saccharomyces cerevisiae CNCM I-1077 (Levucell ® SC) Y- = control, no additives

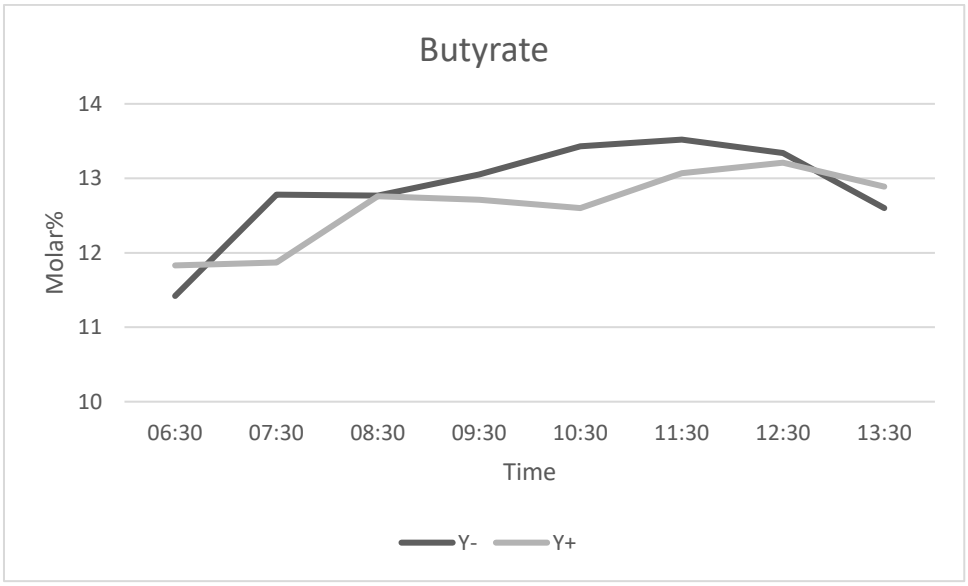


Figure 8. Molar percentage of butyrate as an average of measurements taken day 25 in each experimental period for the two different dietary treatments (Y+/Y-).

Y+ = added 1 x 10^{10} CFU/day Saccharomyces cerevisiae CNCM I-1077 (Levucell ® SC) Y- = control, no additives

4.1.7 Nitrogen balance

Values for nitrogen content in feed, milk, faeces and urine for the two different dietary treatment groups are presented in Table 14. The numbers are shown as g/day or as a percentage of recovered nitrogen from feed in milk, faeces or urine. Nitrogen efficiency in milk are also presented. The recovered percentage of nitrogen form feed was high, which indicates that the total collection of faeces and urine was close to total.

Table 14. Effect of dietary treatment on nitrogen recovered in faeces, milk and nitrogen as a percentage of feed. Nitrogen balance, RMSE and *P*-values are also shown.

		Y+[1]	**Y-[2]**	**RMSE[3]**	***P-value***
Nitrogen in feed g/day		574.8	574.4	23.75	0.98
Nitrogen in g/day	Milk	172.9	166.7	6.74	0.19
	Feces	161.8	165.3	3.86	0.18
	Urine	191.0	186.3	15.29	0.65
Recovered nitrogen of feed %		91.9	90.6	13.45	0.42
Proportion of nitrogen recovered in %	Milk	32.9	32.2	4.24	0.62
	Feces	30.8	31.9	1.42	0.45
	Urine	36.4	35.9	1.02	0.13
Nitrogen efficiency in milk		30.3	29.3	2.27	0.76

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2]Y- = control, no additives [3]RMSE = root mean square

4.1.8 Body condition score and body weight

Table 15 and Table 16 shows an overview of body weight (BW) and body condition score (BCS) for the experimental animals during the experiment.

6160 had no registered weight in period 1 and 6375 were not weighed day 28 in period 1. Four animals gained points on the body condition score scale.

Table 15. Bodyweight of the experimental animals for the duration of the experiment. The bodyweight are presented as an average of two observations on following days.

Bodyweight kg	**P1D0/1[3]**	**P1D28/29[4]**	**P2D0/1[5]**	**P2D28[6]**
6354	593	603	610	602
6411	523	548	548	559
6416	523	532	540	535
6155	636	633	627	638
6375[2]	596	556	619	626
6160[1]		662	668	670

P=period D=day [1)] 6160 had only one registered weigh in period 1 from day 29 [2)]6375 had missing registered weight for day 28 period 1, and weight presented for P1D28/29 are from weight registered at P1D29. [3] weight periode 1 day 0 and day 1 [4] weight period 1 day 28 and day 29 [5] weight period 2 day 0 and day 1 [6] weights period 2 day 28 and day 29

Table 16. BCS of the experimental animals on day 2 period 1, and BCS day 4 and day 28 period 2, in addition to the difference of BCS between periods.

BCS[1]	**Day 28 P1[2]**	**Day 4 P2[3]**	**Day 28 P2**	**Difference from P1 to P2**
6354	2.9	3	3.4	0.5
6411	2.8	3	3.1	0.3
6416	2.9	3	3.3	0.4
6155	3	3	3.5	0.5
6375	3.8	3.8	3.8	0
6160	3.8	3.75	3.6	-0.2

[1]BCS=body condition score [2]P1=period 1 [3]P2=period 2

4.1.9 Methane

Daily methane emission from each experimental animal in each experimental periods is shown in Figure 9. The numbers are an average of the daily methane emission from each experimental animal.

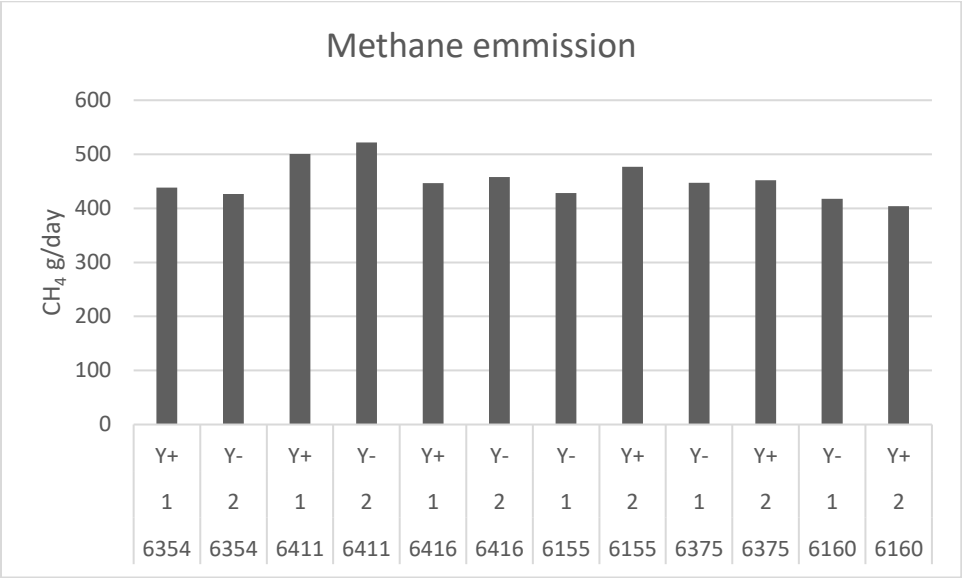


Figure 9. Overview of the average daily methane emission for each experimental animal in period 1 and period 2.

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae CNCM I-1077* (Levucell ® SC) [1] Y- = control, no additives.

Statistics connected to methane and methane parameters is presented in Table 17. The experimental animal 6160 was taken out of calculations for CH4_perECM in period 2 because of mastitis. There was effect of day (P<0,001) on daily methane production (g/day). Similar effect of day (P=0,005) and period (P=0,0005) was observed on methane produced per kilo ECM.

Table 17. Average daily methane production (g/day) and parameters on methane emission, with standard error and *P-values*.

	Y+[1]	**Y-[2]**	**SE[3]**	***P-value***
CH_4 g/day	445.1	437.5	14.73	0.52
energyCH_4 MJ/day	24.6	24.3	0.83	0.63
CH_4_perECM[4]	14.4	13.6	0.97	0.14
CH_4_perkgDMI[5]	20.5	21.0	1.07	0.40
Part GE[6] lost as CH_4 %	6.2	6.3	0.32	0.47

[1] Y+ = added 1 x 10^{10} CFU/day *Saccharomyces cerevisiae* CNCM I-1077 (Levucell ® SC) [2] Y- = control, no additives [3] SE = standard error [4] ECM = energy corrected milk [5] DMI=dry matter intake [6] GE = gross energy

4.2 Discussion

The total tract digestibility of dry matter, organic matter, NDF, protein and starch was not affected by *Saccharomyces cerevisiae* CNCM I-1077 supplementation. This is in accordance to the study by Doreau and Jouany (1998) where *Saccharomyces cerevisiae* CNCM I-1077 did not alter the total- or ruminal digestion in dairy cows in early lactation. Similarly, Harrison et al. (1988) found no effect on apparent total tract digestibility by yeast supplementation in standard cows. Contradictory to these reported results, single studies have reported an increased organic matter- and crude protein digestibility when adding *Saccharomyces cerevisiae* to diets in lactating dairy cows (Yoon & Stern, 1996), or trend towards improved NDF digestibility when supplementing lactating Holstein cows with yeast (Harris et al., 1992). Desnoyers et al. (2009) reported an effect of *Saccharomyces cerevisiae* on total tract digestibility of organic matter with an increase of 0.8 % but no effects of *Saccharomyces cerevisiae* supplements on digestibility of other nutrients. This meta-analysis by Desnoyers et al. (2009) concluded that the effects on organic matter digestibility were too small to warrant exclusive effect of *Saccharomyces cerevisiae* and that the results of organic matter digestibility were a result of other factors as feed or metabolic state of animals.

Apparent crude fat digestibility was different between the dietary treatment groups, where the Y- group had highest percentage of apparent crude fat digestion. When searching the literature, no similar results were found. There was no difference in crude fat intake between the dietary treatment groups, and the effect on apparent fat digestibility is a result of a higher amount of crude fat in the faeces in the Y+ group compared to Y- group. Crude fat in faeces originates from either fat in feed or fat of microbial origin (Hobson & Stewart, 2012), but no measurements performed in this trail can assess that the difference in crude fat in faeces originated from microbial *de novo* synthesis or poorer true digestibility of fat.

In contrast to other findings (de Ondarza et al., 2010; Desnoyers et al., 2009; Poppy et al., 2012) *Saccharomyces cerevisiae* supplements had no effect on milk yield in this experiment. In the study by de Ondarza et al. (2010) *Saccharomyces cerevisiae* CNCM I-1077 significantly increased fat corrected milk production in dairy cows in both early- and late lactation. However, the reported effects of yeast supplementation on milk yield is variable (Bagheri et al., 2009; Schingoethe et al., 2004), and single studies have reported no effect of *Saccharomyces cerevisiae* supplementation on milk yield (Dann et al., 2000). FAO refers to Desnoyers et al. (2009) on the effect of probiotics on milk yield, where the meta-analysis of 157 experiments with *Saccharomyces cerevisiae* additives showed that these type of yeast

supplements increased daily milk yield by 1.2 g/kg body weight. Why no similar results were found in this experiment is not easy to predict, but in a review by Robinson and Erasmus (2009) they suggested that milk production response was negatively correlated with NDF levels in the diet, and this TMR had a somewhat high level of fiber. But this effect should have been similar for both groups, and not just the Y+ group. Other reasons could be that this study was designed as a metabolic trail with few experimental animals (cannulated dairy cows). Therefore, the experimental design was not meant to focus on milk yield.

There was a numerical difference of daily lactose production (g/day) between the dietary treatments, with the highest quantities in the Y- group. However, results in the meta-analyses by Poppy et al. (2012) and Desnoyers et al. (2009) who reported no effect of *Saccharomyces cerevisiae* on daily lactose production. In addition, result from present study is in contrast to findings by Yalcin et al. (2011) where the dairy cows given supplemented with Rumisacc (*Saccharomyces cerevisiae)* had a numerical higher daily lactose production (g/day) compared to the control.

In the present study, there was a numerical higher average rumen pH in the Y+ group compared to Y- group. This is accordance with results from Thrune et al. (2009) who studied the effect of *Saccharomyces cerevisiae* on non-lactating dairy cows, and reported significantly higher average pH in the treatment group compared to the control group. Similar results of increased pH with *Saccharomyces cerevisiae* additives have been observed in lactating dairy cows (Bach et al., 2007; Marden et al., 2008). However, Hristov et al. (2010) found no significant effect of *Saccharomyces cerevisiae* on pH (*P=0.18*). In the study by Marden et al. (2008) *Saccharomyces cerevisiae* supplements were thought to have a decreasing effect on lactate concentrations in the rumen and hence higher ruminal pH. This is similar to findings in other studies (Guedes et al., 2008; Newbold et al., 1991). In the study by Bach et al. (2007) rumen fermentation products were not measured and cannot explain the effect on pH stabilization. In present experiment, lactate was not measured, but the numerically higher pH observed in the Y+ group might be due to the lower concentration of total acids in rumen liquid. The Y- group had a trend towards a total higher acid concentration in rumen, and with significant higher concentrations of butyrate and propionate.

The results of rumen acid concentrations reported in our study is in contrast to the results reported in Guedes et al. (2008), who observed higher VFA concentrations with supplementation of *Saccharomyces cerevisiae* additives. Other studies have not observed any changers in VFA concentrations (Carro et al., 1992a; Dawson et al., 1990).

In this experiment there was no effect of *Saccharomyces cerevisiae* CNCM I-1077 on molar percentage between the dietary treatment groups, which is in contrast to the results by Guedes et al. (2008). A study by Erasmus et al. (1992) showed a tendency that *Saccharomyces cerevisiae* additives increases the glucogenic potential of the diet by lowering the proportions of acetate:propionate. However, the results in present trial showed no such effect which is in accordance to results found in Desnoyers et al. (2009).

Saccharomyces cerevisiae CNCM I-1077 did not affect methane emission (g/day), energy lost as methane, methane produced per kg DMI or percentage of gross energy lost as methane in this experiment. Most of these results are consistent with reported results from similar studies (Bayat et al., 2015; Chung et al., 2011). Our results reflects upon that *Saccharomyces cerevisiae* supplements previously have shown effect on changing rumen environment towards a less methane production in *in vitro* trials (Chaucheyras-Durand et al., 2016), but consistent results have been difficult to prove in *in vivo* experiments (Chaucheyras-Durand, F. et al., 2008). Methane emission per kg ECM were numerically lower in the Y- group. This is the reverse of one of the initial hypotheses, where the theory was that *Saccharomyces cerevisiae* CNCM I-1077 supplements would decrease methane per ECM produced. These results of ECM and methane can be set in context with that the Y- group had a numerical higher ECM than the Y+ group.

Four of the six animals increased in body condition score (BCS), one was stable, and one animal had a drop in BCS. At start of lactation, dairy cows will be in negative energy balance due to increased energy requirements for milk production and inadequate energy intake. NorFor operates with that dairy cows mobilize energy from body reserves the first 80 days after calving (Volden & Nielsen, 2011), but there will be individual variation. As 6160 were less than 80 DIM for most of the trial, the drop in BCS can be explained by mobilization of fat. The animals that gained BCS were the animals that had the longest period from calving. It is then reasonable to say that the experimental animals might have been fed somewhat above their energy requirements. However, BCS is a subjective measure with human faults and variation, but these results are in accordance with that the weigh development of the animals either was stable or increased.

To set how many animals/experimental units is always challenging. We used power test to evaluate the number of animals necessary and considered that six animals in a cross over design were sufficient. The cost of the experiment is often the limiting factor when discussing increasing the number of animals. The cost of each experimental unit in this study was high

because it was a metabolic experiments using large, lactating animals. However, a large number of animals are not a guarantee of reliable results, similarly it is possible to prove effect on just a few animals.

One source of error in the present study may have been the duration of the experiment. By personal communication with Lallemand, the animal should at least be supplemented with yeast for 3 weeks to ensure that effects of live yeast will be visible, and effects may be more eminently after > 3 weeks. However, effects on rumen pH when adding yeast has also been reported after only one week (Bach et al., 2007). Another recommendation from Lallemand was that the animals should be given the *Saccharomyces cerevisiae* supplements before calving resulting that the yeast strain is well established in the rumen when the animal experiences the stress of calving and milk production. The lack of effect of *Saccharomyces cerevisiae* CNCM I-1077 on methane production observed in this trial may be due to the short period from start of supplementing yeast and the days of measure, as methane was measured when the animals had received yeast for only two weeks.

Using lactation dairy cows in experimental crossover designs is challenging. The animals will be in different days in milk in each period, hence in different metabolic states. This may be of particular concern when the cows are in the beginning of the lactation and not yet reached their peak production. However, when designing this kind of *in vivo* trials, similar concerns will always be present. One will always try to optimize design in animal trials, but it is only possible to correct and manage details so far. Biology is not one of them.

5 Conclusion

In this trial, supplements of *Saccharomyces cerevisiae* CNCM I-1077 did not affect nutrient digestibility, methane production, milk yield or milk components. There was an effect of treatment with a change in rumen fermentation products and pH, where addition of *Saccharomyces cerevisiae* CNCM I-1077 resulted in a numerically higher pH. The control group (Y-) had significant higher concentrations of propionate and butyrate ($P<0.05$), and tendency towards a higher sum of acids (mmol/L) in rumen liquid ($P=0.06$). Molar percentage of the three main VFA, however, was not different between treatments.

6 Literature

Alvseike, A. O., Kjos, A. K., Nafstad, O., Odden, H., Ruud, T. A., Saltnes, T. & Ytterdahl, M. (2017). *KJØTTETS TILSTAND - Status i norsk kjøtt og eggproduksjon*. Oslo: Animalia. Available at: https://www.animalia.no/globalassets/publikasjoner/kjottets-tilstand/kjottets-tilstand-2018.pdf (accessed: 17.04.2019).

Bach, A., Iglesias, C. & Devant, M. (2007). Daily rumen pH pattern of loose-housed dairy cattle as affected by feeding pattern and live yeast supplementation. *Animal Feed Science and Technology*, 136 (1): 146-153. doi: https://doi.org/10.1016/j.anifeedsci.2006.09.011.

Bagheri, M., Ghorbani, G. R., Rahmani, H. R., Khorvash, M., Nili, N. & S체dekum, K. H. (2009). Effect of Live Yeast and Mannan-oligosaccharides on Performance of Early-lactation Holstein Dairy Cows. *Asian-Australas J Anim Sci*, 22 (6): 812-818. doi: 10.5713/ajas.2009.80561.

Bajagai, Y. S., Klieve, A. V., Dart, P. J. & Bryden, L. W. (2016). *Probiotics in animal nutrition – Production, impact and regulation*. In Makkar, H. (ed.). FAO Animal Production and Health Paper No. 179. Rome: FAO. Available at: http://www.fao.org/3/a-i5933e.pdf.

Bayat, A. R., Kairenius, P., Stefański, T., Leskinen, H., Comtet-Marre, S., Forano, E., Chaucheyras-Durand, F. & Shingfield, K. J. (2015). Effect of camelina oil or live yeasts (Saccharomyces cerevisiae) on ruminal methane production, rumen fermentation, and milk fatty acid composition in lactating cows fed grass silage diets. *Journal of Dairy Science*, 98 (5): 3166-3181. doi: https://doi.org/10.3168/jds.2014-7976.

Ben-Ghedalia, D., Tagari, H., Bondi, A. & Tadmor, A. J. B. J. o. N. (1974). Protein digestion in the intestine of sheep. 31 (2): 125-142.

Berg, B. M. (2018a). *1038m Aske*. Ås: Institute for Animal science (IHA) Available at: https://www.nmbu.no/download/file/fid/17052 (accessed: 26.02.2019).

Berg, B. M. (2018b). *Msp 1040 Kjeldahl-N*. Ås: Institute for Animal science (IHA). Available at: https://www.nmbu.no/download/file/fid/17054 (accessed: 26.02.2019).

Berg, B. M. (2018c). *Msp 1041 aNDF (Neutral Detergent Fiber)*. Ås: Intitute for Animal science Available at: https://www.nmbu.no/download/file/fid/17056 (accessed: 26.02.2019).

Berg, B. M. (2018d). *Msp 1042 aNDFom (askekorrigert)*. Ås Intitute for Animal science Available at: https://www.nmbu.no/download/file/fid/17058.

Berg, B. M. (2018). *Msp 1044 Tørrstoff*. Ås Intitute for Animal science (IHA) Available at: https://www.nmbu.no/download/file/fid/17062 (accessed: 26.02.2019).

Boyd, J., West, J. W. & Bernard, J. K. (2011). Effects of the addition of direct-fed microbials and glycerol to the diet of lactating dairy cows on milk yield and apparent efficiency of yield. *J Dairy Sci*, 94 (9): 4616-22. doi: 10.3168/jds.2010-3984.

Brossard, L., Chaucheyras-Durand, F., Michalet-Doreau, B. & Martin, C. (2006). Dose effect of live yeasts on rumen microbial communities and fermentations during butyric latent acidosis in sheep: new type of interaction. *Animal Science*, 82 (6): 829-836.

Broucek, J. (2014). *Production of Methane Emissions from Ruminant Husbandry: A Review*, vol. 05.

Børsting, C., Weisbjerg, M. & Hermanse, J. E. (2001). Fedtomsætningen i mave-tarmkanalen. In Hveplund, T. & Nørgaard, N. (eds) *Kvægets ernæring og fysiologi. Bind 1 - Næringsstofomsætningen og fodervurdering*, p. 642. Denmark: Danmarks JordbrukForsining

Carro, M. D., Lebzien, P. & Rohr, K. (1992a). Effects of yeast culture on rumen fermentation, digestibility and duodenal flow in dairy cows fed a silage based diet. *Livestock Production Science*, 32 (3): 219-229. doi: https://doi.org/10.1016/S0301-6226(12)80003-0.

Carro, M. D., Lebzien, P. & Rohr, K. (1992b). Influence of yeast culture on the in vitro fermentation (Rusitec) of diets containing variable portions of concentrates. *Animal Feed Science and Technology*, 37 (3): 209-220. doi: https://doi.org/10.1016/0377-8401(92)90005-Q.

Casewell, M., Friis, C., Marco, E., McMullin, P. & Phillips, I. (2003). The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *Journal of antimicrobial chemotherapy*, 52 (2): 159-161.

Chalupa, W. J. J. o. d. s. (1975). Rumen bypass and protection of proteins and amino acids. 58 (8): 1198-1218.

Chaucheyras-Durand, F. & Fonty, G. (2001). Establishment of cellulolytic bacteria and development of fermentative activities in the rumen of gnotobiotically-reared lambs receiving the microbial additive Saccharomyces cerevisiae CNCM I-1077. *Reprod Nutr Dev*, 41 (1): 57-68.

Chaucheyras-Durand, F. & Fonty, G. (2002). Influence of a Probiotic Yeast (Saccharomyces cerevisiae CNCM I-1077) on Microbial Colonization and Fermentations in the Rumen of Newborn Lambs. *Microbial Ecology in Health and Disease*, 14 (1): 30-36. doi: 10.1080/089106002760002739.

Chaucheyras-Durand, F., Walker, N. & Bach, A. (2008). Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. *Animal Feed Science and Technology*, 145 (1-4): 5-26.

Chaucheyras-Durand, F., Walker, N. D. & Bach, A. (2008). Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. *Animal Feed Science and Technology*, 145 (1): 5-26. doi: https://doi.org/10.1016/j.anifeedsci.2007.04.019.

Chaucheyras-Durand, F. & Durand, H. (2010). Probiotics in animal nutrition and health. 1 (1): 3-9. doi: 10.3920/bm2008.1002.

Chaucheyras-Durand, F., Ameilbonne, A., Bichat, A., Mosoni, P., Ossa, F. & Forano, E. (2016). Live yeasts enhance fibre degradation in the cow rumen through an increase in plant substrate colonization by fibrolytic bacteria and fungi. *Journal of Applied Microbiology*, 120 (3): 560-570. doi: 10.1111/jam.13005.

Chaucheyras, F., Fonty, G., Gouet, P., Bertin, G. & Salmon, J.-M. (1996). Effects of a strain of Saccharomyces cerevisiae (Levucell® SC), a microbial additive for ruminants, on lactate metabolism in vitro. *Canadian journal of microbiology*, 42 (9): 927-933.

Chung, Y. H., Walker, N. D., McGinn, S. M. & Beauchemin, K. A. (2011). Differing effects of 2 active dried yeast (Saccharomyces cerevisiae) strains on ruminal acidosis and

methane production in nonlactating dairy cows. *Journal of Dairy Science*, 94 (5): 2431-2439. doi: https://doi.org/10.3168/jds.2010-3277.

Clark, J., Klusmeyer, T. & Cameron, M. J. J. o. d. s. (1992). Microbial protein synthesis and flows of nitrogen fractions to the duodenum of dairy cows. 75 (8): 2304-2323.

Dann, H. M., Drackley, J. K., McCoy, G. C., Hutjens, M. F. & Garrett, J. E. (2000). Effects of Yeast Culture (Saccharomyces cerevisiae) on Prepartum Intake and Postpartum Intake and Milk Production of Jersey Cows1. *Journal of Dairy Science*, 83 (1): 123-127. doi: https://doi.org/10.3168/jds.S0022-0302(00)74863-6.

Dawson, K., Newman, K. & Boling, J. J. J. o. A. S. (1990). Effects of microbial supplements containing yeast and lactobacilli on roughage-fed ruminal microbial activities. 68 (10): 3392-3398.

de Ondarza, M. B., Sniffen, C. J., Dussert, L., Chevaux, E., Sullivan, J. & Walker, N. (2010). CASE STUDY: Multiple-Study Analysis of the Effect of Live Yeast on Milk Yield, Milk Component Content and Yield, and Feed Efficiency. *The Professional Animal Scientist*, 26 (6): 661-666. doi: https://doi.org/10.15232/S1080-7446(15)30664-1.

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. *J Dairy Sci*, 92 (4): 1620-32. doi: 10.3168/jds.2008-1414.

Dijkstra, J., Ellis, J. L., Kebreab, E., Strathe, A. B., López, S., France, J. & Bannink, A. (2012). Ruminal pH regulation and nutritional consequences of low pH. *Animal Feed Science and Technology*, 172 (1): 22-33. doi: https://doi.org/10.1016/j.anifeedsci.2011.12.005.

Doreau, M. & Jouany, J. P. (1998). Effect of a Saccharomyces cerevisiae Culture on Nutrient Digestion in Lactating Dairy Cows. *Journal of Dairy Science*, 81 (12): 3214-3221. doi: https://doi.org/10.3168/jds.S0022-0302(98)75885-0.

Durand, F. C., Ameilbonne, A., Walker, N., Mosoni, P. & Forano, E. (2010). *Effect of a live yeast, Saccharomyces cerevisiae I-1077 on in situ ruminal degradation of alfalfa hay and fiber-associated microbes*. JOURNAL OF DAIRY SCIENCE: ELSEVIER SCIENCE INC 360 PARK AVE SOUTH, NEW YORK, NY 10010-1710 USA.

Erasmus, L. J., Botha, P. M. & Kistner, A. (1992). Effect of Yeast Culture Supplement on Production, Rumen Fermentation, and Duodenal Nitrogen Flow in Dairy Cows1. *Journal of Dairy Science*, 75 (11): 3056-3065. doi: https://doi.org/10.3168/jds.S0022-0302(92)78069-2.

Geno. (2018). *Holdvurderingsskjema for NRF-kyr* Available at: https://www.geno.no/Start/Brunst/Fagstoff/Hold-og-holdvurdering/Holdplansje/ (accessed: 12.05.2019).

Ghazanfar, S., Iqbal Anjum, M., Azim, A. & Ahmed, I. (2015). *Effects of dietary supplementation of yeast (Saccharomyces cerevisiae) culture on growth performance, blood parameters, nutrient digestibility and fecal flora of dairy heifers*, vol. 25.

Gozho, G., Plaizier, J., Krause, D., Kennedy, A. & Wittenberg, K. J. J. o. d. s. (2005). Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. 88 (4): 1399-1403.

Guedes, C. M., Gonçalves, D., Rodrigues, M. A. M. & Dias-da-Silva, A. (2008). Effects of a Saccharomyces cerevisiae yeast on ruminal fermentation and fibre degradation of

maize silages in cows. *Animal Feed Science and Technology*, 145 (1): 27-40. doi: https://doi.org/10.1016/j.anifeedsci.2007.06.037.

Harris, B., Jr., Dorminey, D. E., Smith, W. A., Van Horn, H. H. & Wilcox, C. J. (1992). Effects of Feather Meal at Two Protein Concentrations and Yeast Culture on Production Parameters in Lactating Dairy Cows¹. *Journal of Dairy Science*, 75 (12): 3524-3530. doi: 10.3168/jds.S0022-0302(92)78128-4.

Harrison, G. A., Hemken, R. W., Dawson, K. A., Harmon, R. J. & Barker, K. B. (1988). Influence of Addition of Yeast Culture Supplement to Diets of Lactating Cows on Ruminal Fermentation and Microbial Populations 1. *Journal of Dairy Science*, 71 (11): 2967-2975. doi: https://doi.org/10.3168/jds.S0022-0302(88)79894-X.

Hegarty, R. S. (2004). Genotype differences and their impact on digestive tract function of ruminants: a review %J Australian Journal of Experimental Agriculture. 44 (5): 459-467. doi: https://doi.org/10.1071/EA02148.

Hobson, P. N. & Stewart, C. S. (2012). *The rumen microbial ecosystem*: Springer Science & Business Media.

Hook, S. E., Wright, A.-D. G. & McBride, B. W. J. A. (2010). Methanogens: methane producers of the rumen and mitigation strategies. 2010.

Houtert M.F.J, V. (1993). *The production and metabolism of volatile fatty acids by ruminants fed roughages: A review*, vol. 43.

Hristov, A. N., Varga, G., Cassidy, T., Long, M., Heyler, K., Karnati, S. K., Corl, B., Hovde, C. J. & Yoon, I. (2010). Effect of Saccharomyces cerevisiae fermentation product on ruminal fermentation and nutrient utilization in dairy cows. *J Dairy Sci*, 93 (2): 682-92. doi: 10.3168/jds.2009-2379.

Huhtanen, P., Ahvenjârvi, S., Weisbjerg, M. R. & Nørgaard, N. (2006). Digetsion and passage of fiber in ruminants In Serjesen, K., Hveplund, T. & Nielsen, M. O. (eds) *Ruminant Physiology: Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress*, pp. 87-135. Wageningen, the Netherlands: Wageningen Press.

Hveplund, T., Madsen, J., Misciattelli, L. & Weisbjerg, M. (2003). Proteinomsætning i mave-tarmkanalen og dens kvantifisering. In Hveplund, T. & Nørgaard, N. (eds) *Kvægets ernæring og fysiologi. Bind 1- Næringsstofomsætning og fodervurdering*, p. 642. Denmark: Danmarks JordbbrugsForskning.

Inc., S. I. (2019). *What's new in SAS (R) 9.4*. SAS Institute Inc., Cary, NC, USA. Available at: https://documentation.sas.com/api/docsets/basewn/9.4/content/basewn.pdf?locale=en.

Jenkins, T. C. (1993). Lipid Metabolism in the Rumen. *Journal of Dairy Science*, 76 (12): 3851-3863. doi: https://doi.org/10.3168/jds.S0022-0302(93)77727-9.

Jeyanathan, J., Martin, C. & Morgavi, D. (2014). The use of direct-fed microbials for mitigation of ruminant methane emissions: a review. *Animal*, 8 (2): 250-261.

Johnson, D. E. & Johnson, K. A. (1995). Methane emissions from cattle. *Journal of Animal Science*, 73 (8): 2483-2492. doi: 10.2527/1995.7382483x %J Journal of Animal Science.

Johnson, D. E., Ward, G. M. J. E. M. & Assessment. (1996). Estimates of animal methane emissions. 42 (1): 133-141. doi: 10.1007/bf00394046.

Johnson, K., Huyler, M., Westberg, H., Lamb, B. & Zimmerman, P. (1994). Measurement of methane emissions from ruminant livestock using a sulfur hexafluoride tracer

technique. *Environmental Science & Technology*, 28 (2): 359-362. doi: 10.1021/es00051a025.

Jørgensen, I. J. (2018). *Msp 10144 WSC.docx*|. Ås: Institute for Animal sceince (IHA). Available at: https://www.nmbu.no/download/file/fid/17036.

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.

Kristensen, N. B., Hveplund, T., Weisbjerg, M. & Nørgaard, P. (2003). Mikrobiel omsætning i formaverne In Hveplund, T. & Nørgaard, N. (eds) *Kvægets ernæring og fysiologi. Bind 1- Næringsstofomsætning og fodervurdering*, p. 642. Denmark: DJF rapport Husdyrbrug 53.

Krizsan, S. & Randby, A. T. J. J. o. a. s. (2007). The effect of fermentation quality on the voluntary intake of grass silage by growing cattle fed silage as the sole feed. 85 (4): 984-996.

LALLEMAND. (2019). *LEVUCELL SC Rumen Specific Yeast*: LALLEMAND ANIMAL NUTRITION. Available at: https://lallemandanimalnutrition.com/en/asia/our-products/product-details/levucell-sc/dairy/ (accessed: 23.04.2019).

Lettat, A., Noziere, P., Silberberg, M., Morgavi, D. P., Berger, C. & Martin, C. (2012). Rumen microbial and fermentation characteristics are affected differently by bacterial probiotic supplementation during induced lactic and subacute acidosis in sheep. *BMC Microbiol*, 12: 142. doi: 10.1186/1471-2180-12-142.

Marden, J. P., Julien, C., Monteils, V., Auclair, E., Moncoulon, R. & Bayourthe, C. (2008). How Does Live Yeast Differ from Sodium Bicarbonate to Stabilize Ruminal pH in High-Yielding Dairy Cows? *Journal of Dairy Science*, 91 (9): 3528-3535. doi: https://doi.org/10.3168/jds.2007-0889.

Martz, F. A. & Belyea, R. L. (1986). Role of Particle Size and Forage Quality in Digestion and Passage by Cattle and Sheep1, 2. *Journal of Dairy Science*, 69 (7): 1996-2008. doi: https://doi.org/10.3168/jds.S0022-0302(86)80626-9.

Mathiesen, H. F. (2014). *Fulldyra jord og dyrkbar jord - en landsoversikt*. Ås: Norsk institutt for skog og landskap. Available at: https://brage.bibsys.no/xmlui/bitstream/handle/11250/2444163/SOL-Fakta-2014-14.pdf?sequence=1&isAllowed=y.

McDonald, P., Edwards, R. A., Greenhalg, J. F. D., Morgan, C. A., Sinclair, L. A. & Wilkinson, R. G. (2011). *Animal Nutrition* 7th ed. Great Britain Prentis Hall

McGinn, S. M., Beauchemin, K. A., Iwaasa, A. D. & McAllister, T. A. (2006). Assessment of the Sulfur Hexafluoride (SF6) Tracer Technique for Measuring Enteric Methane Emissions from Cattle. 35 (5): 1686-1691. doi: 10.2134/jeq2006.0054.

Membrive, C. M. B. (2016). Anatomy and Physiology of the Rumen. In Millen, D. D., De Beni Arrigoni, M. & Lauritano Pacheco, R. D. (eds) *Rumenology*, pp. 1-38. Cham: Springer International Publishing.

Mo, M. (2005). *Surfôrboka*. Oslo: Landbruksforlaget.

Moe, P. W. & Tyrrell, H. F. (1979). Methane Production in Dairy Cows. *Journal of Dairy Science*, 62 (10): 1583-1586. doi: 10.3168/jds.S0022-0302(79)83465-7.

Morgavi, D., Forano, E., Martin, C. & Newbold, C. J. A. (2010). Microbial ecosystem and methanogenesis in ruminants. 4 (7): 1024-1036.

Murray, R. M., Bryant, A. M. & Leng, R. (1976). *Rates of production of methane in the rumen and large intestine of sheep*, vol. 36.

Mutsvangwa, T., E. Edwards, I., H. Topps, J. & F. M. Paterson, G. (1992). *The effect of dietary inclusion of yeast culture (Yea-Sacc) on patterns of rumen fermentation, food intake and growth of intensively fed bulls*, vol. 55.

Newbold, C. J., Tait, C. A. G., Innes, G. M. & Williams, P. E. (1991). Effects of the inclusion of yeast culture (Saccharomyces cerevisiae plus growth medium) in the diet of dairy cows on milk yield and forage degradation and fermentation patterns in the rumen of steers. *Journal of Animal Science*, 69 (7): 3016-3026. doi: 10.2527/1991.6973016x %J Journal of Animal Science.

Newbold, C. J., Wallace, R. J., Chen, X. B. & McIntosh, F. M. (1995). Different strains of Saccharomyces cerevisiae differ in their effects on ruminal bacterial numbers in vitro and in sheep. *Journal of Animal Science*, 73 (6): 1811-1818. doi: 10.2527/1995.7361811x.

Nocek, J. E. & Kautz, W. P. (2006). Direct-Fed Microbial Supplementation on Ruminal Digestion, Health, and Performance of Pre- and Postpartum Dairy Cattle. *Journal of Dairy Science*, 89 (1): 260-266. doi: 10.3168/jds.S0022-0302(06)72090-2.

Norsvin. (2018). *Norge bruker mindre anitibiotika i husdyrholdet enn noe annet land i Europa* Available at: https://norsvin.no/norge-bruker-lite-antibiotika/ (accessed: 14.05.2019).

Nozière, P., Ortigues-Marty, I., Loncke, C. & Sauvant, D. J. A. (2010). Carbohydrate quantitative digestion and absorption in ruminants: from feed starch and fibre to nutrients available for tissues. 4 (7): 1057-1074.

NRC (ed.) (2001). *Nutrient requirements of dairy cattle*. 7th ed. ed. Washington DC: National Academy Press.

Nørgaard, N. & Hveplund, T. (eds). (2003). *Drøvtyggerens karakteristika* Kvægets ernæring og fysiologi. Bind 1 - Næringsstofomsetning og fodervurdering

Danmark Danmarks jordbrugsForskning

Owens, F. N. & Hanson, C. F. J. J. o. D. S. (1992). External and internal markers for appraising site and extent of digestion in ruminants. 75 (9): 2605-2617.

Owens, F. N. & Basalan, M. (2016). Ruminal Fermentation. In Millen, D. D., De Beni Arrigoni, M. & Lauritano Pacheco, R. D. (eds) *Rumenology*, pp. 63-102. Cham: Springer International Publishing.

Peyraud, J. L. & Peeters, A. (2016). *The role of grassland based production system in the protein security*, Ås, pp. 29-43: NIBIO.

Plaizier, J., Krause, D., Gozho, G. & McBride, B. J. T. V. J. (2008). Subacute ruminal acidosis in dairy cows: the physiological causes, incidence and consequences. 176 (1): 21-31.

Poppy, G. D., Rabiee, A. R., Lean, I. J., Sanchez, W. K., Dorton, K. L. & Morley, P. S. (2012). A meta-analysis of the effects of feeding yeast culture produced by anaerobic fermentation of Saccharomyces cerevisiae on milk production of lactating dairy cows. *J Dairy Sci*, 95 (10): 6027-41. doi: 10.3168/jds.2012-5577.

Ramin, M. & Huhtanen, P. (2013). Development of equations for predicting methane emissions from ruminants. *Journal of Dairy Science*, 96 (4): 2476-2493. doi: https://doi.org/10.3168/jds.2012-6095.

Res., S. P. J. V. (2003). Indicators of inflammation in the diagnosis of mastitis. 34 (5): 565-578.

Robinson, P. H. & Erasmus, L. J. (2009). Effects of analyzable diet components on responses of lactating dairy cows to Saccharomyces cerevisiae based yeast products: A systematic review of the literature. *Animal Feed Science and Technology*, 149 (3): 185-198. doi: https://doi.org/10.1016/j.anifeedsci.2008.10.003.

Russell, J. B. & Wilson, D. B. (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *J Dairy Sci*, 79 (8): 1503-9. doi: 10.3168/jds.S0022-0302(96)76510-4.

Satter, L. D. (1986). Protein Supply from Undegraded Dietary Protein. *Journal of Dairy Science*, 69 (10): 2734-2749. doi: https://doi.org/10.3168/jds.S0022-0302(86)80722-6.

Schingoethe, D. J., Linke, K. N., Kalscheur, K. F., Hippen, A. R., Rennich, D. R. & Yoon, I. (2004). Feed Efficiency of Mid-Lactation Dairy Cows Fed Yeast Culture During Summer*. *Journal of Dairy Science*, 87 (12): 4178-4181. doi: https://doi.org/10.3168/jds.S0022-0302(04)73561-4.

Sjaastad, Ø., Sand, O. & Hove, K. (2010). *Physiology of Domestic Animals* 2nd ed. Oslo: Scandinavian Veterinary Press.

St.meld.nr 11 (2016-2017). *Endring og utvikling— En fremtidsrettet jordbruksproduksjon*. Oslo Landbruks- og matdepartementet Available at: https://www.regjeringen.no/no/dokumenter/meld.-st.-11-20162017/id2523121/sec1.

Steen, R. J. A. S. (1984). A comparison of two-cut and three-cut systems of silage making for beef cattle using two cultivars of perennial ryegrass. 38 (2): 171-179.

Stensig, T., Weisbjerg, M. R. & Hvelplund, T. (1998). Digestion and passage kinetics of fibre in dairy cows as affected by the proportion of wheat starch or sucrose in the diet. *Acta Agriculturae Scandinavica, Section A — Animal Science*, 48 (3): 129-140. doi: 10.1080/09064709809362413.

Sullivan, H. M. & Martin, S. A. (1999). Effects of a Saccharomyces cerevisiae Culture on In Vitro Mixed Ruminal Microorganism Fermentation. *Journal of Dairy Science*, 82 (9): 2011-2016. doi: 10.3168/jds.S0022-0302(99)75438-X.

Svihus, B., Uhlen, A. K. & Harstad, O. M. (2005). Effect of starch granule structure, associated components and processing on nutritive value of cereal starch: A review. *Animal Feed Science and Technology*, 122 (3): 303-320. doi: https://doi.org/10.1016/j.anifeedsci.2005.02.025.

Svihus, B. (2018). *Msp 1159 Stivelse.docx* Ås: Institute for Animal science (IHA). Available at: https://www.nmbu.no/download/file/fid/17078.

Thomas, C., Gibbs, B. G. & Tayler, J. C. (1981). BEEF-PRODUCTION FROM SILAGE .2. THE PERFORMANCE OF BEEF-CATTLE GIVEN SILAGES OF EITHER PERENNIAL RYEGRASS OR RED-CLOVER. *Animal Production*, 32 (APR): 149-153. doi: 10.1017/s0003356100024934.

Thrune, M., Bach, A., Ruiz-Moreno, M., Stern, M. D. & Linn, J. G. (2009). Effects of Saccharomyces cerevisiae on ruminal pH and microbial fermentation in dairy cows:

Yeast supplementation on rumen fermentation. *Livestock Science*, 124 (1): 261-265. doi: https://doi.org/10.1016/j.livsci.2009.02.007.

Tingstad, H. (2018). *Met 1045 Råfett (ASE).docx* Ås: Institute for Animal science Available at: https://www.nmbu.no/download/file/fid/17064 (accessed: 26.02.2019).

Van Soest, P. (1994). *Nutritional ecology in the ruminant* 2nd ed. United States of America Cornell University Press.

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

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

Volden, H. & Nielsen, N. I. (2011). Energy and metabolizable protein supply. In Volden, H. (ed.) *NorFor- The Nordic feed evaluation system*. The Netherlands: Wageningen Academic Press.

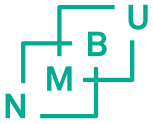
Volden, H., Nielsen, N. I., Åkerlind, M., Larsen, M., Havrevoll, Ø. & Rygh, A. J. (2011). Prediction of volunatry feed intake. In Volden, H. (ed.) *NorFor- The Nordic feed evaluation system*, pp. 113-126. The Netherlands: Wageningen Academic Publishers.

Wattiaux, M. A. & Howard, W. T. (2000). *Digestion in the dairy cow*. Wisconsin: Babcock Institute for International Dairy Research and Deveopment Available at: ftp://s173-183-201-52.ab.hsia.telus.net/Inetpub/wwwroot/DairyWeb/Resources/Babcock_orig/CowDigestion.pdf (accessed: 13.03.2019).

Weisbjerg, M., Lund, P. & Hveplund, T. (2003). Kulhydratomsætningen i mave-tarmkanalen. In Hveplund, T. & Nørgaard, N. (eds) vol. Bind 1 *Kvægets ernæring og fysiologi. Næringsstofomsætning og fordervurdering*, pp. 239-276. Denmark: Danmarks JordbrugsForskning.

Yalcin, S., Yalcin, S., Can, P., Gurdal, A. O., Bagci, C. & Eltan, O. J. A.-A. J. o. A. S. (2011). The nutritive value of live yeast culture (Saccharomyces cerevisiae) and its effect on milk yield, milk composition and some blood parameters of dairy cows. 24 (10): 1377-1385.

Yoon, I. K. & Stern, M. D. (1996). Effects of Saccharomyces cerevisiae and Aspergillus oryzae cultures on ruminal fermentation in dairy cows. *J Dairy Sci*, 79 (3): 411-7. doi: 10.3168/jds.S0022-0302(96)76380-4.



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