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Feed intake, milk yield and variation in the reticulo-rumen pH with Norwegian Red dairy cows given access to concentrate feeds differing in the level of local ingredients

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Acknowledgment

This thesis has been written the spring of 2023, and by turning it in, I complete five years of study at the Department of Animal and Aquacultural Sciences (IHA), NMBU.

I wanted a subject for the master thesis that was oriented towards the future and focus on self-sufficiency and sustainable alternatives. Because of this, I was happy to be able to partake in this experiment. It has been an interesting topic and I have learnt a lot in the process of writing this thesis.

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Abstract

When trying to achieve a higher degree of self-sufficiency, there is a challenge with the high starch levels in grain and the Norwegian climate, which is not particularly well adapted to the cultivation of oil crops. To ensure high milk production, it is important to ensure that the cow has enough rumen-degradable and rumen-indegradable proteins. Because of its nine essential amino acids, soy has been much used as a raw material. The soy sector is highly concentrated and requires large landmasses. The environmental movement has contributed to increased demands for more local food. A high proportion of starch in the feed ration of ruminants can lead to metabolic disturbances, such as subacute rumen acidosis, also referred to as 'subacute rumen acidosis' (SARA). In dairy cows, SARA is characterized by daily episodes of low pH (5.2-6.0) over a longer period, which results in reduced fiber digestion and possibly reduced milk production. Alkaline concentrate has a higher pH, and thus an expected buffer capacity, and this is believed to counteract the negative consequences of feeding with high starch. Alkaline products also contain increased levels of non-protein nitrogen (NPN), and when the rumen microbes are in the presence of easily fermentable carbohydrates from grains and NPN it is expected to produce high quality microbial protein synthesis.

The aim of this study was to evaluate the effect of different concentrate mixtures with alkaline grain in diets for high-yielding dairy cows on feed intake, rumen environment and milk production. The experiment was conducted with a 4x4 Latin square design where 4 different formulations of concentrate (commercial feed (CON), alkaline concentrate with fine physical structure (AUF), alkaline concentrate with coarse physical structure (AUC), and ingredients as in AUC, except that the Alka-150 ingredient has been replaced with grain and urea inclusion (NUF)), tested over 4 periods. All the animals had free access to good quality grass silage. Daily milk production, milk composition, rumen fermentation products, rumen pH, body condition score, and body weight were recorded. No significant effect of the AUF concentrate compared

to CON when looking at milk yield, ECM, BCS, and protein yield. There were only numerical pH and VFA differences between the four diets. Substitution of soya with alkaline grains with fine physical structure showed promising results in this trial with high-yielding dairy cows given good quality grass silage.

Sammendrag

Når man forsøker å oppnå høyere selvforsyningsgrad, er det en utfordring med de høye stivelsesnivåene i korn og det norske klimaet som ikke er særlig godt tilpasset dyrking av oljevekster. For å sikre høy melkeproduksjon er det viktig å sørge for at kua har tilstrekkelige mengder vomnedbrytbare og vom-unedbrytbare proteiner. På grunn av sine ni essensielle aminosyrer har soya blitt mye brukt i kraftfôr til melkekyr. Soyasektoren er svært konsentrert og krever store landmasser. Miljøbevegelsen har bidratt til økte krav til mer lokal mat. En høy andel stivelse i fôrrasjonen til drøvtyggere kan føre til metabolske forstyrrelser, som subakutt vomacidose, også omtalt som 'subacute rumen acidosis' (SARA). Hos melkekyr er SARA preget av daglige episoder med lav pH (5,2-6,0) over en lengre periode, noe som resulterer i redusert fiberfordøyelse og muligens redusert melkeproduksjon. Alkalisk kraftfôr har høyere pH, og dermed en forventet bufferkapasitet, og dette antas å motvirke de negative konsekvensene av fôring med høy stivelse. Alkaliske produkter inneholder også økte nivåer av ikke-proteinnitrogen (NPN), og når vommikrobene er i nærvær av lettfermenterbare karbohydrater fra korn og NPN forventes det å gi en mikrobiell proteinsyntese av høy kvalitet.

Formålet med denne studien var å evaluere effekten av ulike kraftfôrblandinger med alkalisk korn i dietter til høytytende melkekyr på fôropptak, vommiljø og melkeproduksjon. Eksperimentet ble utført med en 4x4 latinsk kvadratisk design hvor 4 forskjellige kraftfôrblandinger (kommersielt fôr (CON), alkalisk kraftfôr med fin fysisk struktur (AUF), alkalisk kraftfôr med grov fysisk struktur (AUC), og ingredienser som i AUC, bortsett fra at Alka-150-ingrediensen er erstattet med korn og urea (NUF)), testet over 4 perioder. Alle dyrene fikk fri tilgang til grasensilasje av god kvalitet. Daglig melkeproduksjon, melkesammensetning, vomgjæringsprodukter, vom-pH, hold og kroppsvekt ble registrert. Ingen signifikant effekt av AUF kraftfôret sammenlignet med CON når man ser på melkemengde, EKM, hold og proteinytelse. Det var bare numeriske pH- og VFA-forskjeller mellom de fire diettene. Erstatning av soya med alkalisk korn med fin fysisk struktur viste lovende resultater i dette forsøket med høytytende melkekyr som ble tildelt surfôr av god kvalitet.

Abbreviations

AAT = amino acids absorbed in the small intestine

ADF = Acid detergent fiber

ATP = adenosine triphosphate

BCS = body condition score

BW = body weight

CP = crude protein

DIM = days in milk

DM = dry matter

DMI = dry matter intake

ECM = energy corrected milk

GMO = genetically modified organisms

MUN = milk urea nitrogen

MY = milk yield

NDF = neutral detergent fiber

NPN = non protein nitrogen

SARA = sub-acute ruminal acidosis

SE = standard error

VFA = volatile fatty acids

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1 Introduction

With population growth and climate change, as well as consumer demands for healthy, safe, and locally produced foods, the need for food security and self-sufficiency is increasing. Most of the protein ingredients in dairy cow feeds are imported in Norway (Landbruk, 2018a). When increasing the inclusion rate of locally produced ingredients in diets for dairy cows, it is challenged by the high level of starch and low level of protein in grains. Starch is an important component of cereal grain, and important in the cereals feed value for ruminants (Humer & Zebeli, 2017).

There have been many different methods to improve digestion and utilization of grain starch, which would also optimize the feed value. These methods were mainly physical (grinding, rolling, and crushing) or chemical (Humer & Zebeli, 2017). This have been done to enhance the degradation of starch in the rumen and increase the energy utilization as well as nutrient utilization (Humer & Zebeli, 2017). However, when feeding diets rich in starch there are negative effects such as incidences of metabolic disorders (like subacute rumen acidosis, SARA) and impaired degradation of fiber in the rumen. Subacute rumen acidosis also compromises both feed efficiency and animal health, as well as animal welfare, which have a large economic impact (Krause & Oetzel, 2006). Many attempts have therefore been made to develop technologies for grain processing that promote animal performance and feed utilization without impairing the animal's health or welfare (Humer & Zebeli, 2017).

To increase the food production and self-sufficiency, which is a national goal (Government, 2015), there is a need for local feed resources as well as new technologies and treatment methods. An emerging feed ingredient used for ruminants in Norway, is locally produced alkaline grain. The alkali treatment is believed to cleave hydrolysable linkages in lignin and glycosidic bonds of polysaccharides. This causes a reduction in the degree of polymerization and crystallinity, swelling of the fibers, and a disruption of the lignin structure (Chen et al., 2013). This is expected to enhance the fiber degradation.

The pH level of 8.0 to 9.0 in products treated with alkaline treated products is expected to serve as a buffer and give opportunity to increase the amount of local grains in concentrates for high yielding dairy cows, without adverse effects on the digestibility of the feed and animal health (Fjeldberg, 2022). Alkaline products contain increased levels of non-protein nitrogen (NPN), and when the rumen microbes are in the presence of readily fermentable carbohydrates from cereals and NPN it is expected to give a microbial crude protein synthesis of high quality (Sjaastad et al., 2016). Microbial crude protein and rumen undegraded dietary protein contributes to a large part of the amino acids absorbed in the small intestine (AAT). This is important for milk production in high yielding dairy cows (Sjaastad et al., 2016).

Sustainability and self-sufficiency are of increasing importance and the need for changes in the agriculture leads to an increase in local ingredients in the animal feed. Norwegian grains have the potential to substitute imported ingredients like soybean meal when treated with alkaline solutions because of the ability of ruminants for converting non protein nitrogen into microbial protein in the rumen. The objective of this study was to investigate the how different formulations with alkaline grain affect feed intake, milk yield, milk composition and reticulo-rumen pH relative to commonly used commercial dairy cow concentrate where there is a high proportion of imported ingredients. It was hypothesized that when including alkaline grain with a high expected buffering capacity, it would counteract negative consequences of a high concentrate level in diets for dairy cows.

2 Background

2.1 Agriculture in Norway

There is a marginal production area for a lot of crops in Norway due to the climate. Fodder crops are often the only alternative, making grass-based livestock important in Norwegian agriculture (Kildahl, 2020). Agricultural land accounts for about 3% of the total land area in Norway, and of this only 31% can be used for cereal production and only 5% for fruits and vegetables, berries, oil crops, herbs, and potatoes (Bondelaget. n.d b; TINE n.d.).

Because of this, Norway cannot grow sugar crops, which most other European countries can (Kildahl, 2020). Livestock production has been a prerequisite for settlement in Norway and has had a central role in food production for hundreds of years. The agroclimatic conditions require ruminants to utilize the resources available (Animalia, 2021). This makes ruminant production an important part of agriculture, especially dairy production which is largely based on the Norwegian Red breed (NRF). NRF produces both meat and milk, which results in low emissions of greenhouse gases with a greater production per animal. They also have an efficient production with good health and fertility (Geno, 2020).

In March 2022 there were a total of approximately 211,400 dairy cows in Norway distributed on 6,700 dairy farms, with an average production of 8,000 kg milk per cow per year (Melk.no, n.d.). Because of the livestock production Norway is able to cover 80-90% of the national demand when it comes to beef and lamb meat, and mainly covers the demand for milk and milk products, pork, chicken, and eggs (TINE, n.d.b). Norway does however not cover the demand for grains, fruits, and vegetables, only about 60% of the grain demand and 25% for fruits and vegetables.

2.2 Self-sufficiency and sustainability

Self-sufficiency is defined as the percentage of food produced of the total food consumption. However, it often does not account for the concentrates that are used in meat production. Self-sufficiency in Norway varied between 45 to 55% from 1970 to 2018, and due to the extensive drought in 2018, the degree of self-sufficiency fell to 45% in 2018, from 50% in 2017 (Knutsen, 2020). When only including food produced on Norwegian feed, the self-sufficiency falls to 40% (Landbruk, 2018b). Increased self-sufficiency and food security require the use of as much food and feed produced based on Norwegian land and feed resources. When looking at sustainability, the definition is meeting the needs of the current generations without compromising the ability of the future generations to meet their own needs. (UN, n.d.). To be more self-sufficient and in a sustainable way, it is important to incorporate more local ingredients in animal feeds, which leads to less importation and decreases the risk of new diseases, as well as increased food security and a more predictable food supply.

Norwegian concentrate ingredients, which mainly is grains, lack the same nutritional properties as the imported ones, especially protein ingredients like soy (Kristensen & Fjeldberg, 2018). The concentrates therefore consist of a high portion of imported ingredients (Kristensen & Fjeldberg, 2018). In the Norwegian animal husbandry, 95% of the protein raw materials were imported in 2015. When increasing the degree of self-sufficiency, it is important to produce more carbohydrates and protein to the animal feed (i.e., through increased production of Norwegian grain and plant protein) with concomitant decrease in imported ingredients in animal feed. In the concentrate for cattle today, there is 63% Norwegian raw materials, and a goal is 76%, with 95% carbohydrates, 60% fat, and 50% protein (Bondelaget, n.d. a).

The 31% of land area that can be used for cereal grain, is used for barley, wheat, oats, and rye. In 2022, there was a production of 591,000 metric tons of barley of the total production of 1,325,000 metric tons, which makes barley 44.6% of the total cereal production in Norway

(SSB, 2023). Most of the grain grown in Norway are used in animal feed concentrates. Of the 1,325,000 tons, 80% are used in concentrates. Barely is the third most readily degradable cereal for ruminants, behind oats and wheat, and has greater protein content and is richer in several amino acids compared to maize. It also has the highest levels of NDF and ADF, but at the same time the lowest levels of starch and fat compared to other cereal grains. Being highly degradable and having more rapid rumen starch as well as nitrogen fermentation, barley gives the animal more synchronized energy and nitrogen release (Nikkhah, 2012). This can improve both the microbial and host nutrient digestion, and feeding barley properly may reduce expensive protein requirements. With unproper feeding management and processing, barley can easily be a shortcut to prolonged metabolic disorders like SARA. In dairy cows this SARA is characterized by daily episodes with low pH (5.2-6.0) over a longer period, which results in reduced fiber digestion and possibly reduced milk production (Abdela, 2016).

Norwegian barley contains sufficient energy to replace several of the imported carbohydrate raw materials. With the use of alkaline technology, where the grain is stored together with urea, and urea is converted into ammonium salts which are bound in the grain, ruminants can utilize the nitrogen in the ammonium salts as protein due to their unique digestive system. In addition, the buffer effect will ensure a good rumen environment even with a lot of Norwegian grain, it also reduces the need for adding other protein sources. This gives concentrate with 75-80 percent Norwegian ingredients for high yielding dairy cows, which is a significant increase compared to other concentrate for high yielding dairy cows with a Norwegian percentage of 45-60 (Fjeldberg, 2022).

When the demand for food and feeds have increased rapidly in the past, the agriculture had a period of intensification, which caused considerable harm to the environment. Agricultural systems became less efficient by degrading the ecosystems goods and services. It also imposed costs on economies (Pretty, 2018). Increased food production from the existing farmland, is one response to these challenges. Mueller et al. (2012) estimated that a lot of the world´s croplands, can attain higher yields, and that there are potential for a 45-70% increase. This would place less pressure on the environment and does not eat away at the capacity to keep producing food in the future (Garnett et al., 2013). It also prevents agricultural expansion into ecosystems rich in biodiversity (Phalan et al., 2011, Pretty, 2018).

The environmental movement has contributed to an increase in demands for more local food, and it is important to be more self-sufficient and to continue to increase the on-land food production in Norway, due to the population growth (Government, 2015). More people purchase varied and resource-intensive diets, and because of this, food security is highly prioritized on the global policy agenda (Garnett et al., 2013). In a world with increasing globalization, food insecurity in one part of the world can have extensive economic and political consequences (Godfray et al. 2010).

Both the war in Ukraine and the 2019 corona pandemic have shown how fragile the food supply is, and the importance of having national food security (Ingvaldsen, 2023). Increased self-sufficiency will reduce importation and the consequences of the ongoing war and trade restrictions (Retvedt, Swensen & Snellingen, 2022). Disease outbreaks in other countries can also pose a risk to Norwegian animals when being dependent on importing feed ingredients. Situations like the mad cow outbreak in England, when there are little that can be done to the food to destroy the prions (Acheson, 2002), pose a risk of new diseases being introduced, which is a threat to the healthy animals in Norway (Mattilsynet, 2016). Another potential challenge is the fact that a lot of feed ingredients today are genetically modified organisms (GMO), but Norway is one of the few countries that does not import any GMO food or feeds (Mattilsynet, 2021). Felleskjøpet imports only about half a percent per thousand of the global soy production, but when it comes to GMO-free soy, Felleskjøpet imports 17 percent (Landbruk, 2018a). Many people are skeptical to novel food technologies and when accepting a food, the important factors are the perceived risks and benefits, as well at the perceived naturalness (Siegrist, 2008). If the production of GMO-free protein ingredients continues to be so small, it could be difficult to meet the demand with imported ingredients in the future. Therefore, it is imperative to increase the level of inclusion of local or regional ingredients in the concentrates.

2.3 Contribution of dairy production for sustainable food production

2.3.1 Rumen energy and protein metabolism

Ruminants are equipped with an advanced digestive tract with various anaerobic microbes, which makes them great at converting feed resources that are of low value for other species to high nutritional foods and economic value (Owens & Basalan, 2016). The forestomach's microbial ecosystem gives adult ruminants the ability to break down complex fiber sources which thereafter produces nutrients essential for the ruminant (Owens & Basalan, 2016: Hvelplund & Nærgaard, 2003). The anaerobic microbes within the rumen can ferment most organic compounds, but the extent of digestibility is limited by either the microbial enzymatic activity, which will vary depending on the ruminal conditions, or accessibility of the different feed components to the microbes (Owens & Basalan, 2016).

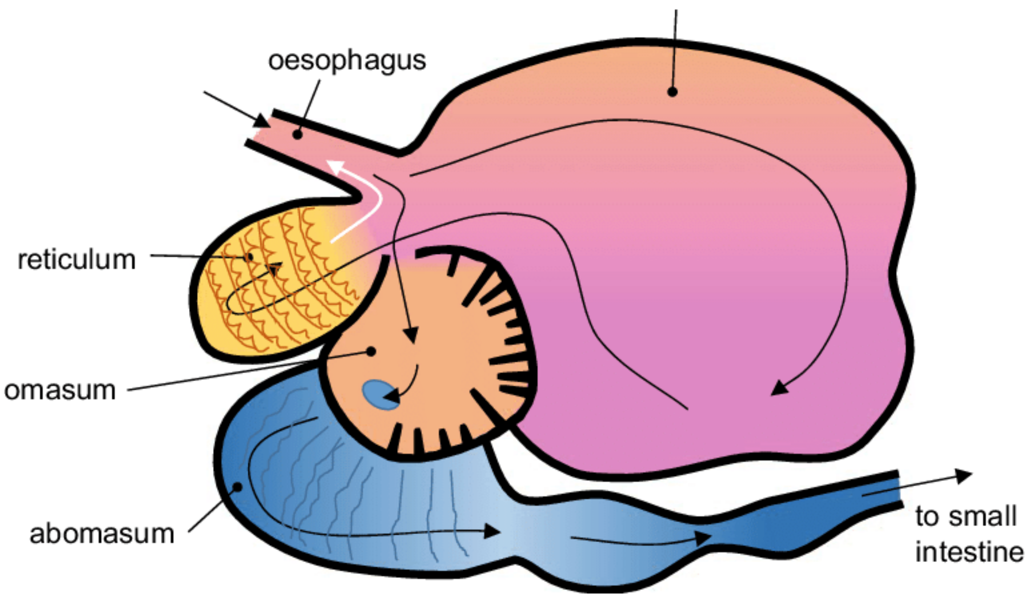

Figure 1: Ruminant stomach and the feed direction (Pérez-Barbería, 2020).

With the fermentation processes, protein and carbohydrates are converted to short chain volatile fatty acids (VFA; e.g. acetic acid, propionic acid, and butyric acid), and methane (CH_4), and carbon dioxide (CO_2) (Owens & Basalan, 2016: Hvelplund & Nærgaard, 2003). Butyric acid is metabolized into beta-hydroxybutyric acid before being oxidized in the tissues as an energy source, but acetate and propionate are not altered when passing through the rumen wall. Acetic acid is oxidized in the tissues to generate ATP. The liver captures propionic acid from the portal blood, and is then used as a substrate for gluconeogenesis, which is important for ruminants as most glucose cannot bypass the rumen fermentation, and therefore cannot reach the small intestine for absorption (Pérez-Barbería, 2020). When dietary protein enters the rumen, they are partially degraded to ammonia, VFA and subsequently gases. Lipids are cleaved to fatty acids and glycerol, and unsaturated fats are hydrogenated (Owens & Basalan, 2016). The VFA´s contributes to 50-70% of the total amount of nutrients absorbed from the digestive tract (Hvelplund & Nærgaard, 2003), and they also meet 70% of the ruminant's energy requirement (Gjefsen & Volden, 2018). The factors that most often limit microbial growth and thereby affect milk production are energy and nitrogen (Clark et al., 1992). Fermentation in the rumen and flow of protein, both microbial and dietary, to the small intestine are affected by the protein in the diet, and the source and amount of energy (Clark et al., 1992). With a high feed intake, more feed will pass undegraded to the small intestine because a high feed intake increases the passage of both fluids and solids to the small intestine. When dietary protein and carbohydrate pass not degraded, it may decrease the potential for microbial growth in the rumen.

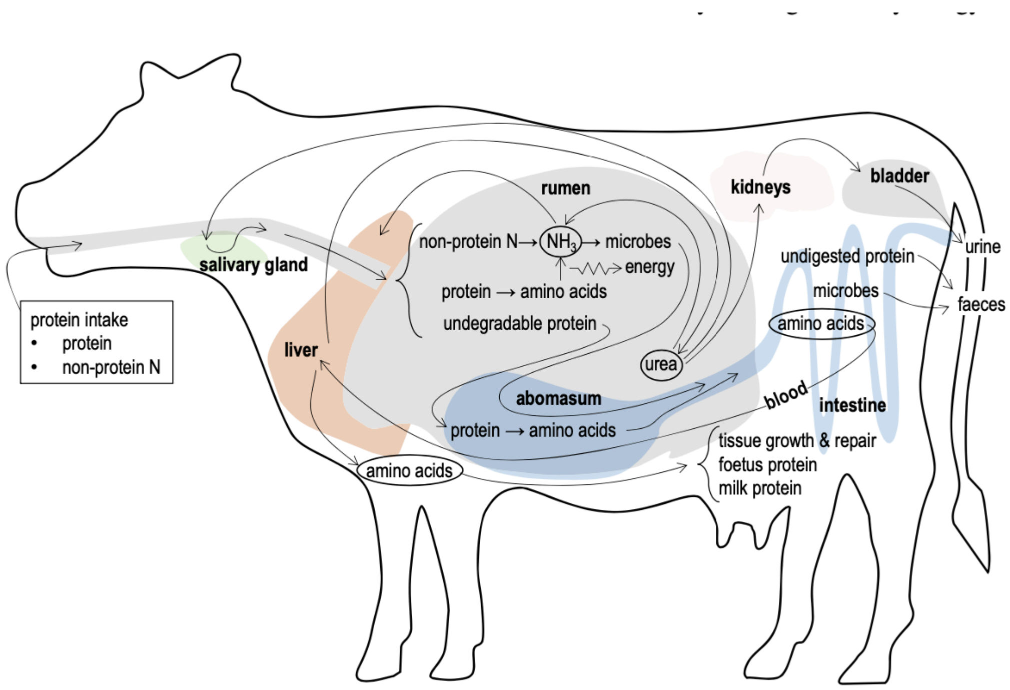

Figure 2: Carbohydrates and their use by the ruminant (Pérez-Barbería, 2020).

Carbohydrates give energy to the ruminal microbes and the host animal. It also contributes to creating a microbe friendly environment in the rumen. The ratio between soluble and structural carbohydrates is important for proper ruminal fermentation.

Ruminants get protein both from the feed (dietary protein) and from the microbial protein synthesis. Since 50-55% of the DM of microbial protein from yeast and fungi is protein, and over 80% for bacteria (Singh et al., 2020), microbes serve as a great protein source for the ruminant. Since both the host and the microbes need amino acids, the ruminants compete with its microbes for dietary protein (Pérez-Barbería, 2020).

The combination of microbes in the rumen influences the degradation rate of protein, as well as the level of nitrogen available. Somewhere between 20 to 100% of the dietary protein can be converted to ammonia, and the remaining fraction will escape and reach the small intestine for digestion and absorption. Like this, the microbial protein also passes through and get to the small intestine for enzymatic digestion (Pérez-Barbería, 2020).

Ruminants can also utilize non-protein dietary nitrogen (NPN) for microbial protein synthesis, but energy is a limiting factor. They also have nitrogen recycling, which is an effective mechanism where 40-80% urea-N are returned to the rumen from the liver and reused in the microbial protein synthesis. Because of this, ruminants can survive on diets with low nitrogen levels (Lapierre & Lobley, 2001). Urea can also be recycled through the saliva. Saliva production increases in diets with a lot of structural carbohydrates, and with little ammonia in the rumen (Pérez-Barbería, 2020).

There are 20 different amino acids that proteins consist of, and access to them are important for maintenance and production (Table 1). Most can be synthesized (non-essential) by the animal, but nine cannot (essential) and most animal species need a continuous supply of these, and they also need nitrogen for the synthesis of the other amino acids (Boisen et al., 2000).

Table 1: Essential and non-essential amino acids.

Amino acids	
Essential	**Non-essential**
Lysin	Alanine
Methionine	Aspartate
Threonine	Cysteine
Tryptophan	Glutamate
Isoleucine	Serine
Leucine	Tyrosine
Histidin	Proline
Phenylalanin	Glycine
Valine	Glutamine
	Asparagine
[1]Arginin	

[1]Arginin can be synthesized by ruminants in the urea cycle.

High yielding dairy cows require a large amount of rumen escape protein. The profile of the rumen escape protein could influence the profile of the amino acids that enters the small intestine, and possibly lead to individual amino acids becoming limiting. (Boisen et al., 2000).

Dairy cattle can get metabolizable protein, which is the total amino acids available for the intestinal digestion to meet growth, production, maintenance, and fetal growth, from both microbial protein and rumen-undegraded protein that passes to the small intestine (St-Pierre & VandeHaar, 2006), therefore the dietary protein may not be as important for ruminants (Boisen et al., 2000).

To ensure a high milk yield, it is important to make sure the cow has adequate amounts of rumen-degradable and rumen-undegradable proteins (St-Pierre & VandeHaar, 2006).

The naturally occurring proteins are usually highly digestible, but the feed digestibility can be impaired by anti-nutritional constituents like lectins or be impaired by compact protein structure (Boisen et al., 2000). These effects might be hindered by controlled heating or chemical treatments, but unsuitable treatment of feeds can reduce the digestibility by adversely altering the physical structure. Giving animals a diet that consist of forage, grains and protein supplements also increases the digestibility of the diet, and the daily dry matter intake, the energy available for the cow and the milk production per cow (St-Pierre & VandeHaar, 2006). Animals that are fed correctly have less metabolic diseases and a better immune function, and it can change the fatty acid profile of the milk fat (St-Pierre & VandeHaar, 2006).

When underfeeding protein there is an economic risk that is a greater economic risk than overfeeding protein, therefore protein efficiency has not been maximized prior, and it is not likely to happen soon either (St-Pierre & VandeHaar, 2006). Since the feed accounts for up to 50% of the costs on a dairy farm, many are tempted to reduce the feed costs, especially when the prices are high.

2.3.2 Local feed resources

As mentioned earlier Norway produces grains that are used in concentrates, but when it comes to protein and fat there is not enough production to meet the demand. The degree of self-sufficiency when looking at concentrate is only 57% for fat ingredients and 6% for protein (Ahlstrøm & Skrede, 2017). Since much of the cultivated land only is suitable for growing grass, the silage quality is important, especially when increasing the Norwegian share of the feed ration. Norwegian dairy cows get most of their energy from silage, approximately 45% (Gjefsen & Volden, 2018). High yielding dairy cows need more and faster energy than roughage alone can give, due to its large volume and slow fermentation, and they are therefore given concentrates as well which contains higher levels of starch that are broken down quickly (Hvelplund & Nærgaard, 2003). Different feed protein has different amino acid composition and digestibility, and they therefore give another potential for supplying amino acids to the cow. Lysine is the first limiting amino acid when looking at grains like barley, oats, and maize. Soybean meal have methionine as the first limiting amino acid (Boisen et al., 2000).

Imported carbohydrates like maize, beets and molasses are important ingredients in feed rations for high yielding dairy cows given a large portion of concentrate. This is unfortunate in situations with limited opportunities for importation. Norwegian grain has a starch quality that serves as a good energy source for the rumen microbes (Stene & Lostuen, 2018). Norwegian grain has a rate of degradation of 50-60% per hour, while maize has a rate of degradation of under 10%, so Norwegian grain supplies the rumen microbes with easily degradable starch. This can in many feeding situations give a higher production of microbial protein (Stene & Lostuen, 2018). The amount of grains used for food depends on the quality, including gluten quality which depends on the climate. (Brød & korn, 2022). Grains that do not achieve this quality is used for animal feed (Valberg & Kjeldsen, 2021). Of the four different cereals Norway produces, it is mainly wheat that is imported as Norway has been more of less self-sufficient in barley and oat since the 1970s (Opplysningskontoret for brød og korn, 2020).

2.3.3 Imported feed resources

Due to the low self-sufficiency for fat and protein ingredients, as mentioned above, these concentrate feed ingredients are imported. Because of its nine essential amino acids, the soybean has become an important protein source for animals and humans. About 85% of the soybean cultivation is destined for animal feed, and the remaining for human consumption (Bermudez et al., 2020). Europe consumed approximately 12% of the global soybean production in 2017 and is therefore the second largest marked for soybeans (Bermudez et al., 2020).

There is a need for a reduction in greenhouse gas emission, and an aim to use more local feeds and less imported soybeans (Kildahl, 2020). In 2017 Norway imported almost 500 000 tons of soybeans, where 300,000 tons were from Brazil and ca. 160,000 tons from Canada (Ridgway, 2019). When using soy in concentrate it gives opportunity to use more Norwegian grain also, since soy contains a high amount of protein and a small amount of carbohydrates.

In Norway, the proportion of soy in concentrate in total is approximately 8.4%. Importation of rapeseeds have decreased the need for soy the last couple of years (Animalia, 2022). The soybean sector is very concentrated and requires large landmasses. Cultivation is mainly in Brazil, Argentina and the United States, and India (Bermudez et al., 2020). The production of soybeans grew from 0.26 million to about 56 million hectares in South America from 1961 to 2014 because of the population growth the world experienced, and the increased demand for meat, which led to a greater production of animal feed based on soya (Bermudez et al., 2020). Demand for soybeans seams to continue growing due to several factors. Both consumption of meat and soy-based health products, make the demand for soybeans grow, especially when the population figures are slated to increase (Bermudez et al., 2020).

Molasses is also added to concentrates and gives better taste as well as better pellet quality. There are imported 70,000 tons from Latin-America, Asia, and Central America each year (Nordbø, n.d.). However, there have been experiments with molasse made with Norwegian spruce and pine included in concentrates for dairy cows (Nordbø, n.d.).

Germany and Eastern Europe produces larger amounts of oil crops, which are imported to Norway, especially rapeseeds (Melk.no, 2023). Rapeseeds can be cultivated in Scandinavia, but the quantity is not large enough for it to be profitable to have its own factory for processing. Rapeseeds are added as a protein ingredient (Gjefsen & Volden, 2018). Increased cultivation of rapeseeds is one relevant measure to limit the importation of protein raw materials.

Another concentrate ingredient in Norway is maize which is imported from EU (Landbruksdirektoratet, 2021). For the reasons discussed before, i.e., self-sufficiency and sustainability, the level of these imported ingredients in animal diets should be decreased through the use of alternative locally produced feed resources.

2.3.4 Alternatives for imported feed resources.

Because of increasing demand for protein feed for animals, the prices rise, and it becomes expensive and unsustainable. There are different opportunities that have been investigated, for example several insect species give an opportunity to have a viable addition to animal feeds and can provide high-quality protein and energy (Souza-Vilela et al., 2019). Animal feeds containing soy poses environmental issues due to different reasons including deforestation. Different studies have investigated the possibility for including insects as a protein source in concentrates (Sánchez-Muros et al., 2014; Makkar et al., 2014). When compared to soy and fishmeal, some insect species have an adequate profile of amino acids, which makes them a sustainable protein rich ingredient for feed and gives a new perspective when it comes to animal feeding without reducing production or give health risks (Sánchez-Muros et al., 2014).

Another option is yeast protein from renewable biomass, like wood biomass which gives high-quality protein. There have been found that yeast protein can replace soybean meal in feeds for dairy cows. When cows are given free access to grass silage of good quality, there is no unfavorable consequences on milk production or composition (Kidane et al., 2022). However, commercial availability of such products is a limiting factor now.

One way of utilizing the Norwegian resources is alkaline treatment of grains, which gives NPN available for the animals. With the use of urea and enzymes to the grain, it can be created NPN, by creating small crystals that bind NPN (Fjeldberg, 2022). The grain is mixed with the promoters and water and are stored under plastic for three weeks.

Because of its high pH, it works as a buffer for the rumen environment and can be used as a raw material in concentrate for ruminants (Fjeldberg, 2022). It allows a lower inclusion of protein supplements and therefore a more cost-effective production, with using other, and more sustainable, raw materials as a protein source (Fjeldberg, 2022).

Earlier experiments have shown that use of alkaline concentrates affects the feed intake in a positive way, and it gives a new possibility for using more Norwegian grains in the concentrate (Kristensen & Fjeldberg, 2018). It was found that the rate of digestion increases when barley has had an alkaline treatment. Different buffers can also be added to improve the rumen environment (Ørskov & Greenhalgh, 1977).

Individual concentrate feeds are optimized for different use, both when it comes to the different ingredients and the quality and structure of the raw materials (Fjeldberg, 2022). Concentrate mixtures can therefore be optimized to influence production e.g., milk yield, fat percentage or growth. Alkaline treatment to grains gives an opportunity to give dairy cows a diet with local ingredients without the need to add fiber-rich raw materials to the concentrate (Fjeldberg, 2022). The rumen microbes are most efficient when the pH is around 6.2, and when most of the feed for ruminants is acidic the rumen environment is dependent on buffering from, for example, the saliva (Home n'dry, 2021). When adding alkaline feed to the diet, the pH increases which can lead to an increase in the feed intake and the performance and health of the animals given it (Home n'dry, 2021). Alkaline feeds open possibilities for feeding higher levels of starch without increasing the risk of SARA (Home n'dry, 2021), and enhances the protein content in the feed and can increase the share of local ingredients in ruminant diets.

3 Materials and Methods

3.1 Animals and experimental design

This animal experiment was conducted from 05.01.22 and till 27.05.22 at the Metabolism Unit of the Norwegian University of Life Sciences with all animal procedures approved by the national animal research authority of the Norwegian Food Safety Authority (FOTS ID 28729). Eight rumen cannulated NRF cows in their mid-lactation were used in the experiment (days in milk at start, 65±30). The experimental cows are described in Table 2. The experiment was conducted using a 4x4 Latin square design where 4 different formulations of concentrate feeds tested over 4 periods, each period lasting 35 days. The cows were housed in a tie-stall accommodation.

Table 2: Start Body condition score (BCS) and body weight for the eight rumen cannulated cows.

Cow ID	**BCS (start, robot)**	**Body weight (Period 1 start, kg)**
6354	3.4	661
6650	3.1	
6788	3.3	551
6556	2.6	700
6791	3.5	620
6405	3.4	675
6790	3.3	528
6640	2.9	579
6786	3.4	612
Average	3.2	612

Table 3: The different concentrate feeds, and their properties.

Number	Name	Type of feed	Imported/local ingredients	Abbreviation
1	Drøv Energirik as positive control	Concentrate feed, fine physical pre-pelleting structure	Relatively high proportion of imported ingredients	CON
2	Alka Ultramjølk Coarse	An alkaline concentrate feed, coarse physical pre-pelleting structure	High level of local ingredients	AUC
3	Alka Ultramjølk Fine	An alkaline concentrate feed, fine physical pre-pelleting structure	High level of local ingredients	AUF
4	Alka ingredient with nitrogen from feed grade ureal inclusion as negative control	A concentrate feed, fine physical pre-pelleting structure	Ingredients as in AUC, except the Alka-150 ingredient is replaced with cereal grains and urea inclusion	NUF

Information of the different concentrate feeds are presented in Table 3. The amount of these feeds (CON, AUC, AUF and NUF) were calculated for individual cows to meet their requirements by using the Nordic feed evaluation system, Norfor (Volden, 2011). The calculations assumed an *ad libitum* access to one quality grass silage and the CON used as the only available concentrate feed. The feed troughs were filled three times per day with grass silage, at 0700 before milking, 1300, and 1900 before milking. The feed was given in portions of 40%, 30% and 30% of the daily allowance. Adaptation period intake of grass silage was used to assess the daily allowance, and to avoid feed restrictions, there were aimed to be a silage refusal of 5-10%. For concentrate feeds, AUC, AUF and NUF quantitatively replaced the calculated amount for CON in their respective treatments.

The concentrate feeds were fed separately from the silage to see the effects of the alkaline diets on improving DMI, and especially the roughage intake of the cows. The concentrate was offered in separate bins in three portions, given at 0800, 1400 and 2000, like the silage. To give the cows a 1.0-hour delay after silage delivery. There were taken feed samples once every week. For concentrate it was taken approximately 400 g of each of the four concentrate feeds, and 500 g samples of grass silage. These were stored at -20°C until further processing and analysis.

3.2 Water intake

The cows had free access to fresh drinking water, and the individual water intake was monitored electronically throughout the experiment.

3.3 Milking and milk samples

The cows were milked twice per day. The AM milking was between 0700 and 0800, and the PM milking between 1900 and 2000. Milk yield was recorded at each milking. Milk samples were taken separately for the AM and PM milkings on all the sampling days. Bronopol (2-bromo-2-nitro-1,3-propaediol, Broad spectrum Microtabs® II) tablets were added the milk samples, which were then stored in a cold room (4°C) until it was sent for analysis.

3.4 pH monitoring

From day 1 to day 140 there was continuous rumen pH monitoring using indwelling Smaxtec pH boluses (smaXtec animal care GmbH, Graz, Austria). This was done to evaluate the effects the different concentrate feeds had on feed-induced SARA. There was electronical monitoring and collection of data using an application system for Smaxtec. To support and evaluate the individual boluses functionality, ruminal fluid pH measurements was done by using pH probes on the days of ruminal fluid sampling. The pH data was summarized as mean pH, and time spent beneath pH 5.6 over 24h period.

Time spent beneath pH 5.6 was done to evaluate any expected occurrence of SARA relatively to their high inclusion level and as modulated by the different concentrate feeds. If the rumen pH remained beneath 5.6 for more than 3 hours during the 24h cycle, the cow was considered as under SARA condition (Abdela, 2016).

3.5 Ruminal and reticulum samples for short chain fatty acids and pH

Samples of ruminal and reticular fluid was collected the day before the experiment started, and day 35 for the last three periods. There were 3 sampling points for each sampling day. The samples contained 250 ml of mixed phase rumen content which was filtered through strainer blender bags (0.50 mm pore size; Stomacher® 400 Seward BA 6041, Worthing, UK). There was made 2 samples of 9.5 ml, which was conserved with 0.5 ml of formic acid. Before the samples were taken, the pH was measured. There were only included samples of ruminal samples from the same timeslots as there were taken reticular samples, to be able to compare the data. This gave a limited sampling, which may affect the results.

3.6 BCS and bodyweight

The body weight was measured on day -1 and day 35 in the first period, and day 35 in the remaining 3 periods. To avoid that the expected variation in rumen-fill during the day could affect BW, all weighings was done between 1200 and 1300. Body condition score was evaluated the same days on a scale from 1.00 to 5.00, where 1.00 is very emaciated and 5.00 is very fat. The scale has increments of 0.25 and were simultaneously scored by two trained staff members.

3.7 Analysis

3.7.1 Milk samples

The milk samples were analyzed for fat, protein, lactose, urea, free fatty acids, and somatic cell count at TINE milk laboratories in Brumunddal, using infrared milk analyzer (TINE, milk laboratories).

3.7.2 Feed samples

The samples were then dried at 45°C and milled using a cutting mill (SM 200, Retsch GmbH). There were used different sieve sized for the analyses planned. Concentrate feed samples for starch analysis were milled through a 0.5-mm sieve. The other samples, both concentrate and silage, were milled through a 1.0 mm sieve. To find the DM content, the samples were dried at 103°C overnight, and ash content was found by incinerating the samples at 550°C. The samples were analyzed for starch, Kjeldahl-N, NDF, and crude fat as described by Kidane et al. (2022).

3.7.3 Ruminal- and reticular fluid samples

The total VFA and the individual VFA were analyzed using a TRACE 1300 Gas Chromatograph from Thermo Fischer Scientific S.p.A (Milan, Italy) equipped with a Stabilwax-DA column (Johnsen, 2016).

3.8 Calculations and statistical analysis

3.8.1 Calculations

Calculations and statistics on the recorded data were done in Microsoft excel (Microsoft Office 365 version 16.69.1) and RStudio-2022.12.0-353.

Energy corrected milk (ECM) was calculated using the equation according to Sjaunja (1990) as described in Eq 1.

$$Kg\ ECM = MY * \frac{\left(\frac{38.3}{100}*fat\ contnent\ (\%)\left(\frac{g}{kg}\right)+\frac{24.2}{100}*protein\ content\ (\%)\left(\frac{g}{kg}\right)+\frac{16.54}{100}*lactose\ content\ (\%)\left(\frac{g}{kg}\right)+\frac{20.7}{100}\right)}{3.14} \quad (1)$$

Where MY is the week average for milk yield.

Given the daily DMI and calculated ECM, milk production efficiency was calculated using Eq 2.

$$Milk\ production\ efficiency: \frac{ECM\ (\frac{kg}{d})}{DMI\ (\frac{kg}{d})} \quad (2)$$

Daily yield of fat, protein and lactose was calculated from the daily milk yield as indicated in Eq 3:

$$MY_{day} * \frac{fat\ or\ protein\ or\ lactose\ content\ (\%)}{100} \quad (3)$$

Dry matter intake per kg body weight (BW) and metabolic weight ($BW^{0.75}$) was calculated as indicated in Eq. 4 and 5 respectively:

$$DMI\ per\ kg\ bodyweight: \frac{DMI\ (g)}{BW\ (kg)} \quad (4)$$

$$DMI\ per\ metabolic\ BW\ (BW^{0.75}): \frac{DMI\ (g)}{BW^{0.75}(kg)} \quad (5)$$

3.8.2 Statistics

Feed intake, milk yield, and milk composition parameters were averaged per cow per week within a period and subjected to statistical analysis using a mixed model in R (R Core Team 2023 edition 4.2.3). Individual cow was treated as a random effect, while treatment, period, and week within a period were included as fixed effects. BW data, with a limited number of measurements per cow, were averaged per cow per period, and there were used a restricted model where the effect of week within period was excluded. An autoregressive (AR(1) or CAR(1)) covariance structure was selected based on Bayesian information criterion (BIC).

The full model used was as follows:

$$Y_{ijk} = \mu + Treatment_i + Period_j + \text{Week } (Period)_k + Cow_l + e_{ijkl}$$

Where Y_{ijkl} = response variable (e.g., DMI); μ= overall mean; $Treatment_i$ = fixed effect of concentrate type i (i.e., CON, AUF, AUC, NUF); $Period_j$ fixed effect of period where j= 1-4; Week $(Period)_{kj}$ = fixed effect of week (k = 1-4) within a period; Cow_l = random effect of cow where l = 1-8; and e_{ijkl} = residual error. Data are presented as estimated marginal means and the Tukey method was used for comparison of treatment means at P<0.05.

4 Results

4.1 Feed composition

The chemical composition for the grass silage and the four different concentrate feeds are shown in Table 4. The AUC and AUF feeds have the same chemical composition given that the only difference is the structure of the feed, AUF being fine and AUC being coarse.

Table 4: Chemical composition of the basal feed (grass silage) and concentrate feeds.

Dietary treatments					
	Grass silage	**CON[1]**	**AUC[2]**	**AUF[2]**	**NUF[3]**
Chemical composition					
DM%	31.0	87.6	87.1	87.1	87.6
Crude ash %	7.2	6.1	6.2	6.2	6.3
Crude protein %	15.6	15.8	15.7	15.7	15.8
Crude fat %		3.5	3.5	3.5	3.5
Starch (g/kg DM)		333.8	421.5	421.5	434.2
NDF (g/kg DM)	521.0	189.7	159.0	159.0	159.5
AAT20, (g/kg DM)	6.0	108.0	98.0	98.0	99.0
PbV20 (g/kg DM)	80.0	6.0	17.0	17.0	17.0
NEl20 MJ		6.2.	6.2	6.2	6.2
FEm		0.96	0.96	0.96	0.97

[1]Commercial concentrate composed (g/kg DM basis) of barley (342.9), oats (120), wheat bran (132.5), grinded maize (100), soybean meal (101.50), maize gluten meal (35), sugar beet pulp (80), molasses (50), and mineral and vitamin premixes (38.1).

[2]Alkaline concentrate composed of Alka grain barley (200), rolled barely (427.4), grinded barley (100), wheat (50), wheat bran (69.7), maize gluten meal (29.1), rapeseed expeller (30), molasses (50), and some mineral and vitamin premixes (43.8)

[3]Concentrate composed of barley (717), wheat (50), wheat bran (63.2), maize gluten meal (27.7), rapeseed expeller (33), molasses (50), urea (12.6), and mineral and vitamin premixes (46.5).

4.2 Feed intake, water intake, BCS and bodyweight

The feed intake, water intake, body condition score (BCS) and bodyweight are presented in table 5. There was no significant difference between the different concentrate feeds when looking at the total DMI and silage DMI, but the drinking water intake as well as the amount of water per kg DM were higher ($P < 0.0001$) for the cows fed NUF compared to the other three concentrates.

4.3 Milk parameters

The milk yield, milk production efficiency, ECM and milk composition are shown in Table 6.

The NUF concentrate gave a lower ($P<0.05$) ECM yield and efficiency than CON and AUF (Table 6), and NUF gave also lower ($P<0.1$) milk yield compared to both CON and AUF (Table 6). There was no significant difference amongst the different treatments for lactose and fat percentage, but the protein percentage were significantly higher for AUF and NUF compared to CON. The urea content in the milk did not differ amongst the different treatments (Table 6). There was also found a significant higher protein yield with CON compared to both AUC and NUF ($P = 0.01$; Table 6).

Table 5: Feed intake, water intake, BCS and bodyweight

	Treatment					P-value	
Parameters	CON	AUC	AUF	NUF	SE	Treatment	Period
Total DMI[1], kg/day	23.60	23.50	23.60	23.90	0.728	0.0686	< 0.0001
Silage DMI kg/day	14.40	14.40	14.50	14.70	0.598	0.3596	0.0001
Silage DMI /total DMI, g/g	0.615	0.615	0.617	0.618	0.024	0.5463	< 0.0001
Drinking water intake, L/day	90.10[b]	91.30[b]	89.70[b]	95.80[a]	3.62	< 0.0001	< 0.0001
Water intake per kg DMI,L	3.83[b]	3.88[b]	3.80[b]	4.01[a]	0.106	< 0.0001	0.0001
$BW^{0.75}$ [2]	127.00	128.00	127.00	127.00	3.08	0.8059	0.0002
BCS[3]	3.13	3.18	3.13	3.17	0.126	0.6096	0.0009
DMI per kg BW, g	37.00	36.70	37.00	37.30	1.06	0.391	< 0.0001
DMI per kg metabolic $BW^{0.75}$, g	186.00	185.00	186.00	187.00	4.76	0.3594	< 0.0001

[1]DMI = Dry matter intake

[2]$BW^{0.75}$= metabolic body weight

[3]BCS = Body condition score

[a] and [b] indicate significant difference within the row ($P < 0.05$)

Table 6: Milk yield and composition

	Treatment					P-values	
	CON	AUC	AUF	NUF	SE	Treatment	Period
Milk yield							
Milk yield, kg/day[2]	30.20[a]	29.20[ab]	29.70[a]	28.40[b]	1.70	0.0042	< 0.0001
ECM[1], kg/day	32.20[a]	31.30[ab]	32.30[a]	30.50[b]	1.77	0.0208	< 0.0001
ECM efficiency[3]	1.36[a]	1.32[ab]	1.36[a]	1.27[b]	0.049	0.0045	<0.0001
Milk composition							
Fat, %	4.43	4.58	4.65	4.49	0.098	0.1046	0.0078
Protein, %	3.67[ab]	3.62[b]	3.69[a]	3.69[a]	0.0709	0.0066	< 0.0001
Lactose, %	4.55	4.53	4.51	4.50	0.060	0.2657	0.0006
Fat yield (kg/d)	1.33	1.33	1.36	1.27	0.074	0.1817	< 0.0001
Protein yield (kg/d)	1.11[a]	1.06[b]	1.09[ab]	1.05[b]	0.061	0.0088	< 0.0001
Lactose yield (kg/d)	4.55	4.53	4.51	4.50	0.060	0.2657	
MUN[3], mg/dL	12.40	12.90	12.90	12.80	0.605	0.6906	0.6369
Free fatty acids, mmol/L	0.42	0.44	0.43	0.42	0.0354	0.4342	< 0.0001
Somatic cell count, (log(n)+1)	5.44	5.33	5.60	5.28	0.483	0.2916	0.0901

[1]ECM = energy corrected milk

[2]MUN = Milk urea nitrogen

[a] and [b] indicate significant difference within the row (P < 0.05)

4.4 Rumen fermentation products

The analysis of fermentation products and pH in the rumen fluid are shown in Table 7. There was not observed any significant effect of treatments on pH or the VFA production in the rumen.

Table 7: pH and fermentation products in the rumen fluid samples.

	Treatment					**P-values**	
	CON	**AUC**	**AUF**	**NUF**	**SE**	**Treatment**	**Period**
pH	6.00	5.99	6.03	6.00	0.0692	0.8941	< 0.0001
Total VFA[1] (mmol/L)	109	109	108	107	3.19	0.9317	0.1027
VFA proportion (molar % of total VFA)							
Acetate	65.10	65.00	64.40	64.70	0.551	0.7693	0.0143
Propionate	17.60	17.40	17.80	17.40	0.403	0.8333	0.1964
Iso-butyrate	0.83	0.80	0.80	0.82	0.0349	0.8344	0.7651
Butyrate	14.30	14.50	14.40	14.50	0.345	0.7279	< 0.0001
Iso-valerate	1.10	1.04	1.07	1.12	0.0591	0.7331	0.6724
Valerate	1.31	1.29	1.35	1.34	0.055	0.8795	0.0457

[1]VFA = volatile fatty acids

4.5 Reticular and ruminal pH

Figure 3 shows the different pH measurements in the reticulum and rumen at different times relative to feeding. There is a slight positive correlation between the pH measured in the reticulum and the pH measured in the rumen.

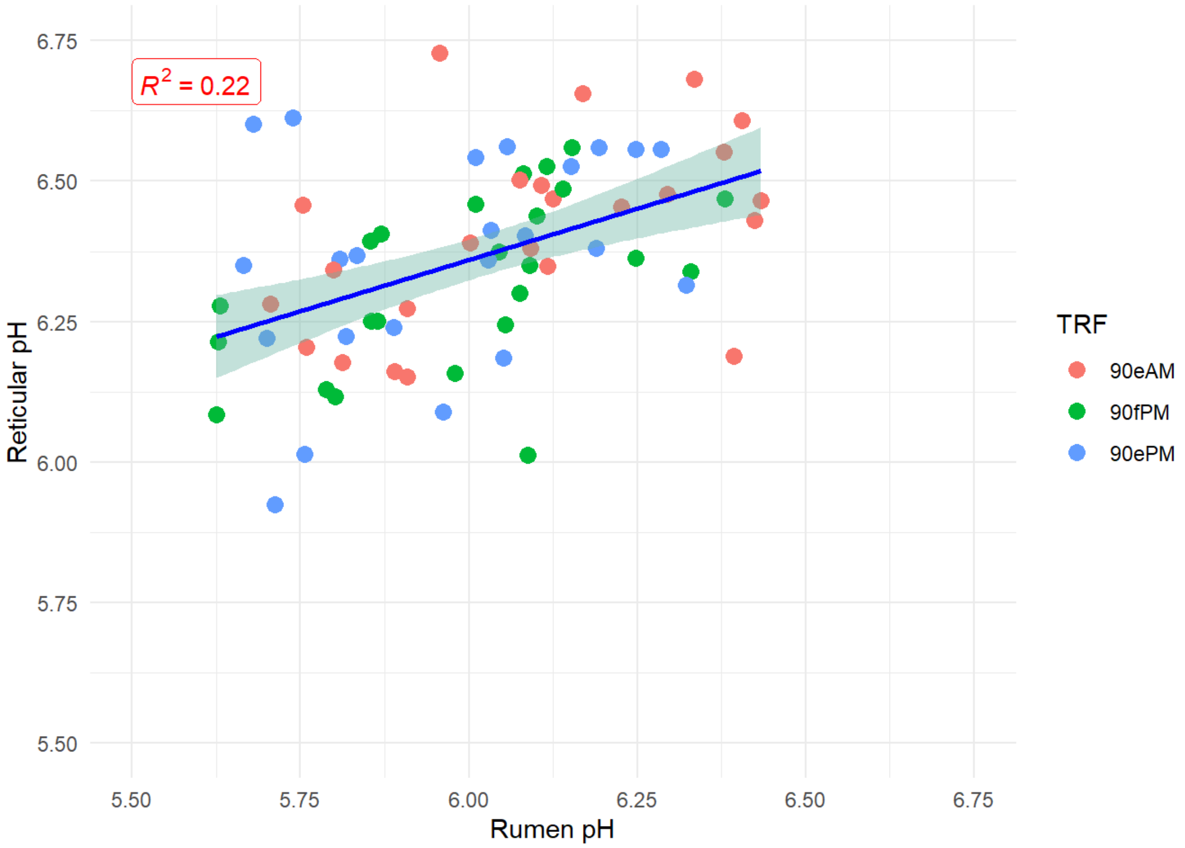

Figure 3: Reticular and ruminal pH at different times relative to feeding.
TRF: Time Relative to Feeding
90eAM: 90 minutes after morning feeding.
90fPM: 90 minutes before mid-day feeding.
90ePM: 90 minutes after mid-day feeding.

5 Discussion

This study was conducted to investigate the effects of replacing imported protein ingredients like soybean meal, with alkali treated barley in the concentrate feed on feed and water intake, ruminal pH, and VFA production, milk production and composition. The observed results are discussed below.

5.1 Effect on water intake

The urea inclusion in the NUF concentrate gave a significantly higher water intake than the other concentrates. Furthermore, the amount of water per kg DMI was also higher for NUF compared to the three other treatments. Research on effects of dietary protein sources on water intake of dairy cows is scarce. Existing reports with domestic animals, e.g., cattle and buffaloes (Razdan et al., 1971) and camel (Emmanuel et al., 2015) showed no effects of dietary CP levels or incremental urea levels in the ration of animals on water intake. On the other hand, drinking water intake has been shown to increase with high CP levels, indicating that additional water is necessary to excrete excess nitrogen (Holter et al., 1982). However, in the current trial, the diets were iso-nitrogenous and such effects of level of CP on water intake were not expected. Another factor influencing water intake in dairy cows is the mineral and vitamin content (Singh et al., 2022), but the feeds used here were not divergent in mineral and vitamin content, expect for a small numerical difference in the crude ash content as reported in Table 4, which was highest for NUF. Therefore, it could only be speculated that the extra water consumed by the cows fed the urea diet might have been caused by increased urea recycling in that group. Assessment of water excretion through urine (data not presented) has suggested that part of this extra water intake was excreted through the urinary route.

5.2 Effects on feed intake, BCS and BW

The DMI was not significantly different amongst the treatments which agrees with the findings of Robinson and Kennelly (1988), where they tested different ammoniation levels of high moisture barely. Furthermore, Santiago et al. (2015) did not observe any differences in DMI when comparing slow-release urea with soybean meal in concentrates fed to dairy cows, but McNiven et al. (1995) found a tendency for lower DMI for the ammoniated barley. When including more barley in the feed the starch content increases. In the present experiment, the CON concentrate had a starch content of 334 g/kg DM AUF and AUC 422 g/kg DM, and NUF contained. 434 g starch per kg DM. Diets with high levels of starch increases the risk of SARA due to the accumulation of VFA and insufficient buffering in the rumen.

Decreased feed intake can be a sign of SARA, which have negative consequences on production and animal welfare. Giving excessive amounts of urea may reduce DMI in urea treated feed due to ammonia poisoning because of rapid hydrolysis of urea in the rumen and absorption by the rumen epithelium cells. Keeping the ammonia concentrations below toxic levels can improve both he rumen degradation and voluntary feed intake (Hallaijan et al., 2021). In the present experiment, there was no noticeable effect of the diets on the total DMI of the animals. There was no significant difference on DMI per unit of body weight. It has been shown that a higher DMI/BW results in a higher passage rate, and it has been speculated that the starch utilization is lower when animals have a higher DMI (Anderson et al., 1981). In earlier experiments it has been found that barley supplements increase the silage intake by 16% (Kassem et al., 1987), but in this experiment the silage DMI did differ among the dietary treatments. The AUC diet gave a reduced starch digestibility compared to the three other diets (results not presented). The coarse AUC concentrate was less processed, which might have influenced the results given that the starch was possibly less available to the microbes and/or digestive enzymes compared to the other concentrates. When maximizing the utilization of barley grain, it can lead to a decreased feed intake (Allen, 2000). However, this was not observed in the presented study.

BCS is an assessment of the proportion of body fat and is an important factor in dairy cattle management. There are many factors influencing the BCS, including management, feeding level and diet type (Roche et al., 2009). The scale used for BCS ranges from 1.00 to 5.00, where 1.00 is very emaciated and 5.00 is very fat. With a healthy BCS being from 3.00 to 3.50. Cows offered diets with greater concentrations of non-structural carbohydrates can experience an increase in BCS after a period of energy deficit most likely due to the lipogenic and antilipolytic effects of insulin (Roche et al., 2009). The insulin concentration is expected to be greater with an increased production of propionate in the rumen (Roche et al., 2009). Throughout this experiment the BCS was not significantly different between the different dietary treatments. Given the results regarding feed and nutrient intake it is not surprising that the BCS results are quite similar between groups. However, NUF had a numerically higher BCS compared with the other groups, and a significantly lower milk yield.

Tye et al. (2017) investigated the lactational performance and energy partitioning in response to different corn grain types. They reported that the slow-release urea treatment decreased the amount of net energy that was partitioned into BW gain. Others have found that there were no significant differences in average daily BW gain when replacing soybean meal with slow-release urea (Hallajian et al., 2021). In the present experiment, there was no significant difference in BW change, or average BW between the groups. Since the DMI was unaffected by the different treatments, and the BW can be used as an indicator of the energy input and output (Mäntysaari & Mäntysaari, 2015), these results indicate that all treatments met the requirements for maintenance and milk production.

5.3 Effect on milk parameters

In this study, higher ECM yield for the CON and AUF treatments compared to NUF were found. In addition, NUF also gave lower milk yield than CON and AUF. This aligns with the results by Robinson and Kennelly (1989), who found that alkali treated barley did not influence milk yield. In another study, there was not found any effect of slow-release urea on milk yield when tested in high yielding dairy cows (Sinclair et al., 2012). The lack of difference between CON and AUF when looking at milk yield indicates that the inclusion of alkali treated local ingredients with fine structure does not reduce the productive performance of lactating dairy cows. It is believed that alkaline grain will improve the conditions for fermentation in the rumen and thereby the synthesis of microbial crude protein which increases the AAT supply to the small intestine giving a good basis for increased milk yield. In this study, it was found that the AUF treatment gave a quantitatively higher starch digestibility (results not presented), which also increases the energy supply for milk production.

Gonçalves et al. (2014) showed that feeding conventional urea can cause lowered milk production, due to a lower intake of non-fibrous carbohydrates, which has an impact on the proportion of VFA produced in the rumen, thus influencing the milk production. The NUF treatment with a significantly lower milk yield could also be related to an impaired absorption of amino acids in the small intestine, compared to the other treatments. In a study where soybean meal was replaced with different levels of slow-release urea and conventional urea, Gonçalves et al. (2014) reported that partial replacement had no influence on the protein percentage in the milk. However, in the present experiment CON had a significantly higher protein yield than both AUC and NUF with AUF not being significantly different from the other treatments.

The difference may be due to the microbes not being able to capture and transform nitrogen into microbial protein for all protein sources (Gonçalves et al., 2014). In an experiment conducted by McNiven et al. (1995), different dietary treatments lowered the milk fat by 8%, when comparing sodium hydroxide (NaOH) treated barley with roasted barley. Hallajian et al. (2021) also found that there was an increase in the milk fat percentage when partially replacing soybean meal with slow-release urea. In the present study, there was only found numerical differences in milk fat percentage between the groups, with the highest percentage in the AUF group (4.65) and lowest in CON (4.43).

When ruminants are given feed with a high starch level, it can cause low milk fat levels because of a shift in rumen fermentation towards relatively higher proportions of propionic acid compared to acetic acid (Sjaastad et al., 2016). The fat percentage were numerically higher for both AUF and AUC compared to NUF and CON, suggesting that the alkaline treatment gave an improved rumen environment, which increased the rumen digestion giving acetic acid for de novo synthesis giving increased milk fat. The AUF treatment had results similar to CON for milk composition, indicating that the alkaline concentrate with fine structure met the animals demands for energy, acetic acid, and nitrogen for milk production and synthesis of milk fat and protein.

5.4 Effect on ruminal pH and rumen fermentation products

When looking at the pH and major VFA production between the different treatments, there is no significant effect of dietary treatments. However, these results here are from a limited number of samplings and should be taken with care. This is because, the data on pH and VFA included in this thesis included those time points with concurrent data on these parameters from the reticulum. With this in mind, the lack of difference between the different dietary treatment groups indicated that the concentrate based on local ingredients all met the animals dietary requirements for a favorable VFA production and ratio, giving the animals a good basis for further production, i.e., milk fat and protein.

However, Robinson and Kennelly (1988) found that the mean pH declined with the higher level of ammoniation of high moisture barley, and that the hours below 6.0 increased with increased level. They also found that acetate and butyrate concentrations increased with higher ammoniation levels, but the other VFA ranges were not influenced by the dietary treatments. In the present study, the alkali diets contained more barley than the control diet which influence the results compared to only increasing the level of ammoniation on the same base diet. In an experiment by Hallajian et al. (2021) where they investigated the effects of partially replacing soybean meal with slow-release urea, they found that the acetic acid and propionic acid were different between the different treatments. The differences they observed were only observed in the first ten days, and thereafter the VFA concentrations were similar between treatments. Unlike acetic acid and propionic acid concentrations, they observed that butyric acid was affected by treatment throughout the whole experimental period, with the highest values in the control group. There was no significant difference in valerate concentrations amongst the different treatment groups (Hallajian et al., 2021). Degradation of structural carbohydrates is normally requiring more time than the degradation of non-structural carbohydrates. When having urea inclusion, it is common to expect that inclusion of urea in the diet will affect the rumen bacteria to access more nitrogen to the fiber-degrading microbes (i.e., bacteria, fungus, and protozoa), and thereby increase the fermentation products, like acetic acid and propionic acid (Hallajian et al., 2021). However, this effect was not observed in the present study.

When feeding high starch diets there is a risk of the animals developing SARA. By use of alkaline treatment, the pH of the feed increases and thereby work as a buffer, decreasing the risk of SARA (Home n'dry, 2021). With a result of no difference in pH between different dietary treatments with different inclusion rate of local ingredients, the feeds high in starch does not cause harmful decreases in pH over longer periods. McNiven et al. (1995) found a tendency for higher pH when feeding alkali treated barley, and less pH fluctuations. There was also found lowered isovalerate concentrations, which was not found in this experiment. Sudden changes in ruminal pH can cease the microbial activity, as well as low levels of rumen NH_3-N may limit fiber and organic matter fermentation and the protein degradability.

There was measured pH in both the rumen and reticulum to compare these two methods. The pH measurement in the reticulum is measured using an electronic pH device which is placed in the reticulum over a longer period to detect SARA. Sato et al. (2012) identified a significant positive correlation between ruminal and reticular pH in both healthy and SARA induced dairy cows, however in this study, there was only found a slight positive correlation, and R^2=0.22, between ruminal and reticular pH. Bryant (1964) found that sampling in the reticulum and ventral regions of the rumen gave false indications of the pH and VFA. The results in this study suggest that the rumen pH measurement is a better predictor for SARA than reticulum pH, and the observed weak correlation between ruminal fluid and reticular fluid pH could also be due to the limited data points available for this work.

6 Conclusion

Results from this study indicate that the inclusion of more local ingredients for dairy cows has potential for replacing soybean when treated with urea and enzymes. There was no difference in milk yield as well as BCS between the AUF and CON treatment. The NUF diet gave significantly lower production of both kg milk, kg ECM, and protein yield. The major VFAs were not significantly different between any of the diets, the ruminal pH had only numerical differences, and neither of the diets reduced rumen pH to SARA level. These results indicate that alkali treated barley, at the levels used here, can be added to the feed without adverse effects on health and production of high yielding dairy cows given *ad libitum* access to good quality grass silage.

7 References

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