Norwegian University of Life Sciences

**Master's Thesis 2021 30 ECTS**
Faculty of Biosciences

# Factors affecting urea levels in goat milk

*Faktorar som påverkar ureanivå i geitemjølk*

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Animal Science

# Forord

Studiet presentert i denne oppgåva er utført ved Fakultet for biovitskap, Noregs miljø- og biovitskapelege universitet på Ås, og markerer slutten på min mastergrad i husdyrvitskap, retning drøvtyggarernæring.

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*«Kom, studenter, dette er refrenget. La Studenterånden leve lenge! Kanskje verden er litt stri, men når det gråner skal du si at du har hatt en bra studentertid…»*

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# Abstract

The optimal *milk urea level* for Norwegian dairy goats is not known, and further study on the area is required if to be used as an indicator of efficiency in nitrogen metabolism. The aim of this study was to examine factors affecting milk urea levels in dairy goat production. Another aim was to investigate the suitability of *fourier-transform infrared spectroscopy (FTIR)* for analysis of milk urea levels in goats.

Data from two experiments, named *G110* and *D174*, were analysed. In G110, 48 multiparous goats of the Norwegian dairy goat breed were assigned to six different isonitrogenous concentrate types with different types and levels of lipid supplements. Measurements of milk urea was taken throughout lactation, including both indoor feeding and mountain grazing period. In D174, 9 rumen fistulated goats of the Norwegian dairy goat breed were assigned to three different isonitrogenous concentrate types. The experiment was performed as a 3x3 Latin square with three replicates, where the daily concentrate level was gradually increased from 1.5 to 2.55 kg dry matter (DM).

In G110, a significant effect of lactation stage on milk urea levels was found, while parity showed no effect on milk urea levels. A significant negative correlation between milk yield and milk urea levels was found at 185 and 225 days in milk. A significant negative correlation between milk urea levels and milk protein percentage was found at 55 and 85 days in milk. In early and late lactation of D174, milk urea levels were significant positively correlated to the level of dietary crude protein (CP), protein balance in the rumen (PBV), and amino acids absorbed in the small intestine per feed unit milk (AAT/FEm). A discrepancy between milk urea levels analysed by FTIR and milk urea levels analysed by chemical methods was observed in both experiments.

In conclusion, milk urea levels vary according to several factors related to both dietary factors and physiological status. In order to utilize FTIR for analysis of milk urea levels in Norwegian dairy goats, a better calibration of the FTIR-instrument is necessary. The results suggest that milk urea levels have a potential to be used as an indicator of efficiency in nitrogen metabolism of Norwegian dairy goats.

# Samandrag

Optimalt *ureanivå i mjølk* hos norske mjølkegeiter er ikkje kjent. Dersom ureanivå i mjølk skal bli innarbeidd som effektivitetsindikator på nitrogenmetabolismen til norske mjølkegeiter, er ytterlegare forsking på området derfor naudsynt. Formålet med studiet var å kartlegge faktorar som påverkar ureanivå i mjølk hos norske mjølkegeiter. Det var også eit mål å undersøke om *Fourier-transform infrared spectroscopy (FTIR)* er eigna til å analysere ureanivå i mjølk hos norske mjølkegeiter.

Det vart utført analyse av data frå to forsøk, høvesvis G110 og D174. I G110 vart 48 geiter av rasen Norsk mjølkegeit med laktasjonsnummer > 1, tildelt seks ulike isonitrogene kraftfôrtypar med ulikt feittinnhald. Ureanivå i mjølk vart målt gjennom heile laktasjonen, inkludert både innandørs fôring og beiteperiode. I det andre forsøket, D174, vart 9 vomfistulerte geiter av rasen Norsk mjølkegeit tildelt tre ulike isonitrogene kraftfôrtypar. Forsøket vart bygd opp som i eit 3x3 latinsk kvadrat med tre replikatar, der dagleg kraftfôrnivå gradvis vart auka frå 1.5 til 2.55 kg tørrstoff (TS).

I G110 viste laktasjonsstadium ein signifikant effekt på ureanivå i mjølk, medan laktasjonsnummer ikkje hadde effekt på ureanivå i mjølk. Ein signifikant negativ korrelasjon mellom mjølkeavdrått og ureanivå i mjølk vart observert på laktasjonsdag 185 og 225. Ein signifikant negativ korrelasjon mellom ureanivå i mjølk og proteinprosent i mjølk vart observert på laktasjonsdag 55 og 85. I det andre forsøket, D174, vart det i både tidleg- og seinlaktasjon observert ein positiv korrelasjon mellom ureanivå i mjølk og råprotein i fôrrasjon, proteinbalanse i vom (PBV) og amino syrer absorbert i tynntarm per fôreining mjølk (AAT/FEm). Eit avvik mellom urea i mjølk analysert med FTIR og urea i mjølk analysert med kjemisk metode vart observert i begge forsøka.

Ureanivå i mjølk frå norsk mjølkegeit blir påverka av fleire fôringsrelaterte og fysiologiske faktorar. Dersom FTIR skal bli nytta for analyse av ureanivå i mjølk hos norske geiter, må FTIR-instrumentet bli betre kalibrert med omsyn til ureanivå i mjølk hos geit. Resultata indikerer at ureanivå i mjølk har potensiale til å bli innarbeidd som effektivitetsindikator på norske geiter sin nitrogenmetabolisme.

# Abbreviation key

| | |
|---|---|
| AAT | Amino acids absorbed in the small intestine |
| AAT/FEm | Amino acids absorbed in the small intestine per feed unit milk |
| ADF | Acid detergent fibre |
| AMP | Adenosin-monophosphate |
| aNDF | Amylase-treated neutral detergent fibre |
| ATP | Adenosin-triphosphate |
| BU | Blood urea |
| BUN | Blood urea nitrogen |
| BW | Body weight |
| CFat | Crude fat |
| CP | Crude protein |
| DCP | Digestible crude protein |
| DIM | Days in milk |
| DM | Dry matter |
| ECM | Energy corrected milk |
| FEm | Feed unit milk |
| FTIR | Fourier-transform infrared spectroscopy |
| GP | Grazing period |
| GTP | Guanosine-triphosphate |
| iNDF | Indigestible neutral detergent fibre |
| MU (ch) | Milk urea analysed by wet-chemical method |
| MU (FTIR) | Milk urea analysed by fourier-transform infrared spectroscopy |
| MUL | Milk urea levels |
| MUN | Milk urea nitrogen |
| $NAD^+$ | Nicotinamide adenine dinucleotide |
| $NADP^+$ | Nicotinamide adenine dinucleotide phosphate |
| NDF | Neutral detergent fibre |
| $NE_l$ | Net energy lactation |
| NPN | Non protein nitrogen |
| PBV | Protein balance in the rumen |
| RDP | Rumen degradable protein |
| Total-N | Total nitrogen |
| TS | Tørrstoff (Norwegian) |
| VFA | Volatile fatty acids |
| $W^{0.75}$ | Metabolic weight |

# Content

# 1 Introduction

Like other animal husbandries, Norwegian dairy goat husbandry face demands in regard to increased efficiency in production (TINE Rådgiving og Medlem, 2020e). In a period lasting from year 2000 to 2016, the total number of dairy goats was reduced by 17 percentage, while the total volume of produced goat milk increased by 5 percentage – a development explained by the fact that the milk yield per Norwegian dairy goat increased by 27% in the same period (Hillestad et al., 2018). The increased efficiency in goat milk production is a result of many factors, such as breeding, better health, new technology in regard to feeding, grazing and milking system, as well as improved nutrition (Hillestad et al., 2018; TINE Rådgiving og Medlem, 2020f).

In regard to nutrition, efficient utilization of dietary nitrogen plays an important role in several issues related to husbandry of dairy goats. Dietary nitrogen affects both milk production, fertility, and at the same time constitute a large variable cost (Ahlstrøm & Anders, 2017; Bindari et al., 2013; Paulsen Rye, 2019). In addition, nitrogen used in feed for Norwegian dairy goats consist mainly of imported soya meal and rape seed meal, rising question in regard to Norway's self-sufficiency and sustainability in production (Ahlstrøm & Anders, 2017; TINE Rådgiving og Medlem, 2020e). Metabolization of dietary nitrogen might also cause problems in regard to waste endogenous nitrogen excreted in urine and manure, constituting a threat in regard to pollution either in the shape of nitrous oxide or run off leading to eutrophication (Khan & Mohammad, 2014; McDonald et al., 2011; Miljødirektoratet et al., 2020). An efficient utilization of dietary nitrogen is therefore of interest in many issues related to dairy goat production – both in regard to efficiency in production, self-sufficiency, and sustainability.

An understanding of the nitrogen metabolism of dairy goats is essential in order to improve efficiency of dietary nitrogen utilization. A product of nitrogen metabolism is urea produced in the liver (McDonald et al., 2011; Sjaastad et al., 2016). Low levels of produced urea indicate either low levels of dietary protein and/or efficient utilization of protein. On the other hand, high levels of produced urea indicate a less efficient utilization of proteins (Volden, 2012). As urea diffuses easily across cell membranes, the level of urea in milk increases along with increased levels of urea in blood (Sjaastad et al., 2016). *Milk urea level* is a much-used indicator of the efficiency of protein metabolism in dairy cows (Volden, 2012). However, this

indicator is not much used in practical Norwegian dairy goat industry - mainly due to time consuming methods (chemical analysis) or unreliable methods (*Fourier-transform infrared spectroscopy (FTIR)*) of urea analysis in goat milk, and due to lack of studies on the issue (FOSS, n.d; Schei, 2003).

Based on two experiments performed in 2016 and 2018, this thesis will focus on milk urea level as a key performance indicator in Norwegian dairy goat production. The objective of the thesis is to examine factors affecting milk urea levels in dairy goat production, and to investigate the suitability of FTIR for analysis of milk urea levels in goats. In order to understand the context between dietary protein and milk urea, the digestion and metabolization of proteins in a ruminant animal will be addressed in the literature part of the thesis. Then the experiments will be addressed in regard to accomplishment and results. The following hypothesis will be tested:

- Milk urea levels are affected by stage of lactation.
- Milk urea levels are negatively correlated to milk yield.
- Milk urea levels are negatively correlated to milk protein percentage.
- Milk urea levels are affected by energy and protein level in the diet.

# 2 Literature

## 2.1 Aspects of nitrogen in an animal body

In an animal body, nitrogen plays an important role as key component of proteins. The function of proteins varies greatly. Some proteins are important for transport and storage of small molecules, some have structural roles, and some function as enzymes catalysing important biochemical reactions (Mathews et al., 2013). Numbers from *TINE Rådgiving og Medlems*' annual statistical report, states that milk from Norwegian dairy goats in average contained 3,34% protein in year 2020 (TINE Rådgiving og Medlem, 2021). As much a 95% of all nitrogen found in milk is bound in these proteins (McDonald et al., 2011). According to physiological and chemical properties, milk proteins are classified into immunoglobulins, caseins, lactalbumin, lactoglobulin, as well as enzymes and other proteins with certain roles. Of the aforementioned protein types, casein constitutes 80% of the total protein content of milk from ruminants. Evolutionarily, milk proteins constitute a nutritive supply to the animal's offspring (Sjaastad et al., 2016). However, in modern dairy goat production, the content and quality of milk protein is valued in regard to humane nutrition and cheese properties (Greppi et al., 2008; Helsedirektoratet, 2016; TINE Rådgiving og Medlem, 2020f). In regard to economy in production, protein percentage, alongside with other milk properties, affects the price pr litre milk paid to Norwegian dairy goat producers (TINE Råvare, 2020). Due to the many important biological functions of proteins, and especially their economic role as components of milk, great focus is directed to the animal's synthesis of proteins in dairy goat production (Cannes et al., 2008).

The synthesis of proteins is dependent on the dietary supply of nitrogen (Strudsholm & Sejrsen, 2003). An animal's nitrogen requirement may be defined as the quantity of nitrogen needed in order to compensate for nitrogen losses and to ensure efficiency of feed utilization, without any negatively effect on reproduction and animal health (Cannes et al., 2008). For ruminants, this also involves feeding an amount of nitrogen that ensures activity and growth of the microbial population in the rumen. The importance of microbial protein metabolization is emphasized by the fact that rumen microbial protein make up the main protein source of ruminants and covers the ruminant animal's requirement of essential amino acids (Cappellozza, 2013; McDonald et al., 2011). Due to the complex connection between the rumen microbial population and the ruminant animal, many considerations must be taken when estimating the protein requirement of ruminants.

Dietary energy supply is an important consideration that must be taken when calculating the protein requirement of a ruminant animal. Energy is the main driving force of protein metabolization in all animals. Hence, lack of dietary energy will reduce the efficiency of protein metabolization. Therefore, the protein requirement of an animal must be put in context with its requirement for dietary energy (Miller, 2004). In respect of ruminant animals, supply of energy is important in regard to the protein metabolism in the animal, but also in regard to the protein metabolism of the rumen microbial population (Hvelpelund et al., 2003; McDonald et al., 2011). In addition to a comprehensive understanding of the symbiosis between the rumen microbes and the ruminant animal, the context between metabolization of different nutrients, is therefore necessary when estimating the protein and energy requirement of a ruminant animal.

## 2.2 Dietary nitrogen and energy

Normal feeding regime of Norwegian dairy goats throughout a year, may be divided into indoors feeding during winter months and pasture grazing during summer (TINE Rådgiving og Medlem, 2020e). During winter, the feed diet is based on a combination of concentrate and preserved roughage, constituting about 40% and 60% of the feed diet's total dry matter content, respectively. During grazing season, the dietary supply is normally ensured by grazed pasture and concentrate supplementation (TINE Rådgiving og Medlem, 2020b). Concentrate and roughage differs in chemical composition, especially in sight of nitrogen and energy content, where concentrate usually contain the highest concentration of both nitrogen and energy (Ahlstrøm & Anders, 2017; Søegaard et al., 2003; TINE Rådgiving og Medlem, 2020d). In a well-balanced diet, concentrates and roughage complements each other and constitutes a diet able to meet the nitrogen and energy requirement of dairy goats (TINE Rådgiving og Medlem, 2020b).

### 2.2.1 Dietary nitrogen

The main *dietary nitrogen* source of Norwegian dairy goats is proteins (TINE Rådgiving og Medlem, 2020d). Proteins are characterized by their composition of amino acids linked together with peptide bonds. Amino acids are molecules distinguished by one acidic carboxylic unit ($COO^-$), one basic nitrogenous group ($NH_3^+$), and one rest group (R) (Figure 2.1) (McDonald et al., 2011). In addition to proteins, feed for ruminants contain non-protein nitrogen compounds, such as amino acids, amines, amides, urea, as well as nitrate, and do,

together with proteins, constitute the dietary nitrogen sources of a ruminant animal (McDonald et al., 2011).

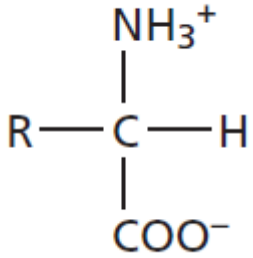


*Figure 2.1. The general structure of an amino acids. Figure from McDonald et al. (2011).*

In Norwegian feeding standards of dairy goats, calculation of parameters related to dietary nitrogen are based on the content of dietary *crude protein* and it's degradability (Madsen et al., 1995). Crude protein may be defined as nitrogen content (g/kg) multiplied with 6,25. The calculation of crude protein has two obvious shortcomings: firstly, one assumes that all protein contains 160g nitrogen per kg of proteins, and secondly, that all nitrogen originates from proteins. These assumptions are not necessarily correct: firstly, the nitrogen content varies between different proteins and secondly, nitrogen may originate from other nitrogenous compounds other than protein such as amides, alkaloids, and amino acids. However, the assumptions are justified in practice, because the animal's requirement is expressed indirectly as requirement for nitrogen and because the animal's nitrogen requirement, similarly to the dietary nitrogen content, is expressed based on crude proteins (McDonald et al., 2011).

Typically, the average crude protein content of Norwegian roughage varies between 140 g to 167 g per kg dry matter (g/kg DM), while the crude protein content of an often-used commercial concentrate mixture from Norgesfôr *(Drøv Geit)* is around 17% per kg DM (Eurofins, n.d.; Norgesfôr, n.d). Extracted soya meal and extracted rape seed meal are the most abundant protein sources used in concentrate for Norwegian dairy goats. The content of crude protein in extracted soya meal is about 50%, while the crude protein content of extracted rape seed meal is about 35% (TINE Rådgiving og Medlem, 2020d). The crude protein content of roughage depends on plant species, the plant's morphological stage, preservation, and nitrogen fertilization. In fresh gras, about 75-90% of all nitrogen exists as proteins. However, during preservation, some of the proteins will be degraded to simpler nitrogen compounds by proteolysis and deamination (Mo, 2005). The crude protein value of pasture varies greatly depending on altitude, plant species, the plant's morphological stage,

and the degree of cultivation (McDonald et al., 2011; TINE Rådgiving og Medlem, 2020b; Todnem & Lunnan, 2014). A continuously assessment of pasture quality during grazing period must therefore be performed, in order to determine the need of concentrate supplementation (TINE Rådgiving og Medlem, 2020b).

### 2.2.2 Dietary energy

The main dietary energy source for ruminant animals is carbohydrates. Normally, carbohydrates constitute 70% or more of the total dry matter content in diets of goats. Carbohydrates are characterized by their content of carbon (C), hydrogen (H), and oxygen (O) in the composition ratio of $(CH_2O)_2$ (McDonald et al., 2011). When discussing carbohydrates in respect of the ruminants, carbohydrates are often divided into *fibre*, *starch*, and *soluble carbohydrates.* The term fibre embraces structural carbohydrates found in the cell wall, such as hemicellulose, cellulose, lignin, and small quantities of nitrogen containing material. Structural carbohydrates are characterized by being insoluble in neutral detergent, and are therefore often referred to as neutral detergent fibre (NDF) (Bannink & Tamminga, 2004). Starch is found as reserve carbohydrates in seed, tubers, roots, and fruits, and are known for high energy density (Bannink & Tamminga, 2004; McDonald et al., 2011). Soluble carbohydrates, defined as organic matter subtracted for crude fat, crude protein, starch, and fibre, are soluble in water and embraces a wide range of different carbohydrates, such as sugars and fermentative acids in silage (Bannink & Tamminga, 2004). In the diet of ruminants, fibre is mainly found in roughage and pasture, starch in concentrate, and soluble carbohydrates are found in both concentrate, roughage, and pasture. Of the aforementioned terms of carbohydrates, fibre is the most important in ruminant nutrition both in quantity and quality. However, starch constitutes an important role in supplying energy for high yielding animals (McDonald et al., 2011) Together, fibre and starch constitute important energy supplementation for ruminants (TINE Rådgiving og Medlem, 2020b).

## 2.3 Protein digestion in dairy goats

The digestive system of ruminants are characterized by a gastrointestinal enlargement, termed the forestomachs, and the ability to regurgitate and rechew swallowed ingesta - a process called rumination (McDonald et al., 2011; Sjaastad et al., 2016). The forestomachs consist of three compartments, named, rumen, reticulum, and omasum (Figure 2.2). Thereafter comes the abomasum, the small intestine, and the colon (McDonald et al., 2011). Compared to cows, the rumen and omasum of goats constitute a relatively smaller share of total body mass. However, the surface of the rumen papilla epithelium, as well as the size of the colon, is relatively larger in goats compared to the cow (TINE Rådgiving og Medlem, 2020a).

The degradation of proteins in a dairy goat happens mainly in the rumen, the abomasum, and the small intestine (McDonald et al., 2011).

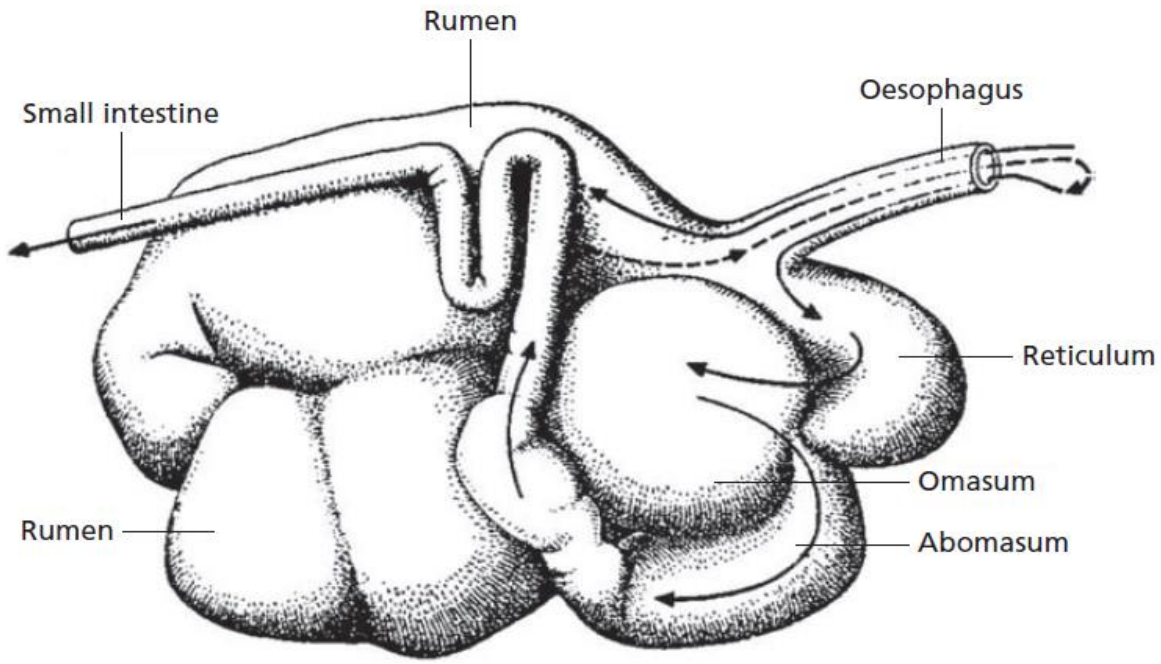


*Figure 2.2. The forestomachs, the abomasum, and the small intestine of a ruminant animal. Figure from McDonald et al. (2011).*

### 2.3.1 Microbial fermentation and digestion in the rumen

In the rumen, proteins are degraded by proteolytic microbes. The most important proteolytic microbes in the rumen are the two bacteria species, *Peptostreptococcus* and *Prevotella ruminicola*, as well as the protozoa (McDonald et al., 2011). Firstly, the degradation happens extracellularly on the surface of the microbe, where proteins are hydrolysed to peptides and amino acids by proteolytic microbial enzymes (Hvelpelund et al., 2003). Amino acids are then transported into the microbes, where they will either be utilized directly in synthesis of microbial proteins or deaminated to organic acids and ammonia (Hvelpelund et al., 2003; Sjaastad et al., 2016).

In principals, rumen microbes do not need supply of dietary protein as such in order to synthesize their own proteins (McDonald et al., 2011). Rumen microbes are able to synthesise microbial amino acids, both essential and non-essential, from all nitrogen sources. This include utilization of non-protein nitrogen from the diet, ammonium ($NH_4^+$) and ammonia ($NH_3$) originating from microbial protein degradation, as well as recirculated urea (see Chapter 2.4.6) (Bannink & Tamminga, 2004; McDonald et al., 2011).

As previous mentioned, the microbes are dependent on energy supply in order to utilize nitrogen compounds for growth (McDonald et al., 2011). Through microbial fermentation, carbohydrates, glycerides from lipids, and carbon skeleton originating from degraded protein, volatile fatty acids (VFA) are produced. The ruminal production of microbes and VFAs may chemically be presented as showed in Equation 2.1.

$$C_6H_{12}O_6 + NH_3 \leftrightarrow microbes + CH_4 + CO_2 + VFA \quad (2.1)$$

(Van Soest, 1994)

The most abundant VFAs produced by the rumen microbes are acetate, propionate, and butyrate. In regard to the rumen microbes, the aforementioned VFAs are waste products of their own carbohydrate metabolism. However, in regard to the ruminant animal, VFAs are absorbed over the rumen wall and constitutes the most important energy source of the animal (Sjaastad et al., 2016). Summarized, microbial utilization of carbohydrates is important for microbial protein synthesis and indirectly important for the energy supply of the ruminant animal.

In general, lack of energy supply will result in low utilization of feed protein. On the other hand, due to the acidic effect of VFAs, large amount of easily degraded dietary carbohydrates and hence large production of VFAs might lead to an acidic milieu unfavourable to the rumen microbes. The normal ruminal pH is considered to be 6.0-6.8. (Sjaastad et al., 2016). Mechanisms of pH regulation in the rumen, includes the buffer function of salivary bicarbonate ($HCO_3$) and phosphate, as well as the absorption of VFA over the rumen wall (McDonald et al., 2011; Sjaastad et al., 2016). The absorption of VFA over the rumen wall is driven by differences in concentration of $H^+$-ions between the rumen and the blood. The absorption happens either by cotransport of VFA anions ($Ac^-$) in exchange with $HCO_3^-$ or by simple diffusion of undissociated VFAs (HAc). The diffusion of undissociated VFAs happens faster, compared to cotransport of VFA anions. The ratio of undissociated VFAs and VFAs anions in the rumen, is dependent on the pH of the rumen liquid. A low pH stimulates for undissociated VFAs as shown in Equation 2.2.

$$H^+ + Ac^- \leftrightarrow HAc \tag{2.2}$$

(Sjaastad et al., 2016)

By high production of VFAs and consequently reduced pH, more VFAs exists in the form of undissociated VFAs. Consequently, the absorption of VFA over the rumen wall happens more rapidly at high concentration of ruminal VFAs. Even though regulatory mechanisms of the rumen pH are present, excessively feeding of carbohydrates, especially starch, are unfortunate and might lead to rumen acidosis (pH< 5.0) and consequently reduced microbial growth (Sjaastad et al., 2016).

### 2.3.2 Protein digestion in the abomasum

Microbial proteins and proteins not degraded in the rumen, termed rumen bypass proteins, will be transported together with rumen liquid to the abomasum. In the abomasum, proteins are digested in the same way as in the stomach of monogastric animals. The gastric gland region of the abomasum contains oxyntic cells which produce hydrochloric acids. In addition, production of the inactive enzyme, pepsinogen, also happens in the gastric gland region of the abomasum. Hydrochloric acid converts pepsinogen to the active enzyme pepsin, which degrades proteins enzymatically to peptides and some amino acids. Pepsin mainly attacks peptide bonds adjacent to aromatic amino acids like tyrosine, tryptophan and phenylalanine. It also attacks peptide bonds connected to glutamate and cysteine (McDonald et al., 2011). The

result of the digestion in the abomasum is the creation of polypeptide and amino acids, which are sent to the small intestine.

### 2.3.3 Protein digestion in the small intestine

Once the polypeptide from the abomasum is transported to the small intestine, the enzymatic degradation process continues. The small intestine may be divided into three sections: duodenum, jejunum, and ileum. The duodenum is the main section where degradation of proteins and polypeptides in the small intestine occurs. Once nutrients arrive the small intestine, secretion of digestive enzymes is stimulated by the hormone cholecystokinin. Enzymes involved in intestinal degradation of proteins are trypsinogen, chymotrypsinogen, procarboxypeptidases A og B, proelastase, and nuclases (McDonald et al., 2011). The products from peptide degradation in the small intestine are short-chained peptides and a small amount of amino acids, which are absorbed from the small intestine, mainly the jejunum, into the portal vein and transported to the liver (McDonald et al., 2011; Sjaastad et al., 2016).

## 2.4 Protein metabolism

Amino acids absorbed in the small intestine will either go through transamination, be oxidized to energy by deamination, or utilized directly for synthesis of proteins (McDonald et al., 2011). Alongside, body proteins are degraded into amino acids. These amino acids, in the same way as amino acids absorbed from the small intestine, can be reutilized for synthesis of proteins, go through transamination, or be deaminated (Mathews et al., 2013). The balance between protein synthesis and deamination highly affects the urea concentration in blood and milk (Sjaastad et al., 2016).

### 2.4.1 Protein turnover

A continuous biosynthesis and degradation of proteins happens in all tissues. This process is called *protein turnover*. Since the biosynthesis and degradation complement each other, the protein turnover ensures a constant concentration of several intracellular proteins over time (Mathews et al., 2013). The protein synthesis happens in four stages: activation of amino acids, initiation of peptide chain, chain elongation, and chain termination (McDonald et al., 2011) The order and composition of amino acids in a protein are genetically determined (Mathews et al., 2013). In all stages of proteins biosynthesis, energy is required. The energy for protein biosynthesis is provided by hydrolysis of guanosine-triphosphate (GTP) and adenosin-triphosphate (ATP) (McDonald et al., 2011). The degradation of proteins, also called proteolysis, are driven enzymatically. Associated enzymes are called proteases and exist both extracellularly, such as calpains and proteasome, and intracellularly, such as cathepsins. The amino acids used are either directly reutilized for biosynthesis of proteins or exposed for transamination and deamination (Mathews et al., 2013).

The speed of the protein turnover varies between different types of proteins. Some proteins last for months, while others only exist for a few minutes. For instance, proteins secreted into the extracellular fluid, such as digestive hormones and antibodies, are turned over relatively rapidly, while structural proteins, such as collagen, are turned over at a slower pace (Mathews et al., 2013). There is also a difference in the balance between biosynthesis and degradation of proteins between tissues. For instance, the degree of biosynthesis of proteins in muscular and mammary tissue are relatively similar, while the degree of protein degradation in muscular tissue is higher compared to in mammary tissue. This lower rate of protein degradation in mammary tissue is naturally explained by the fact that milk proteins are kept in secretory vesicles and milked out continuously during lactation (Madsen & Nielsen, 2003).

### 2.4.2 Transamination

In mammals, amino acids may be exposed to transamination in order to generate intermediates to the *citric acids cycle* or if to be used in synthesis of non-essential amino acids. Transamination is a reversible biochemical reaction where an amino group is transferred from an amino acid to an α-keto acid (Figure 2.3). The reaction is catalysed by enzymes called aminotransferases. In animal cells, aminotransferases are able to produce all amino acids, except lysine and threonine. However, since animal cells cannot synthesize the carbon skeleton of α-keto acids, a dependency on the supply of α-keto acids exists. The inability of animal cells to synthesize the carbon skeleton of α-keto acids explains why animal cells cannot synthesize essential amino acids (Mathews et al., 2013).

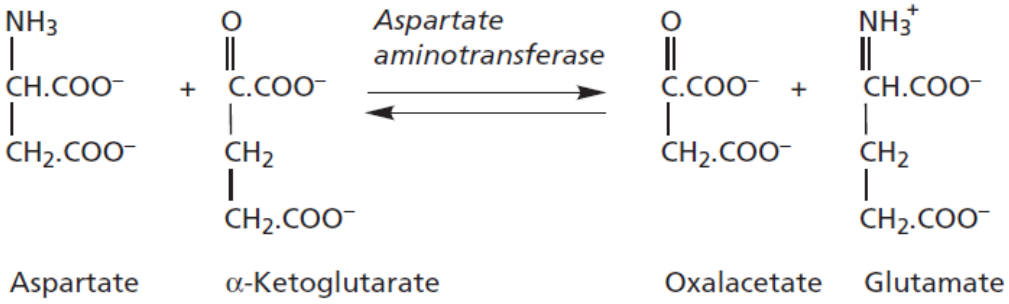


*Figure 2.3. Transamination of aspartate to glutamate. Figure from McDonald et al. (2011).*

The amino acid, glutamate, plays an important role in the process of transamination. Toxic ammonia is assimilated and detoxified in glutamate by reductive amination of α-ketoglutarate (Figure 2.4). Through transamination, glutamate, can further on be converted to other non-essential amino acids as shown in Figure 2.5 (Mathews et al., 2013; McDonald et al., 2011). In general, transamination constitutes an important part in the amino acid metabolism, both in regard to redistribution of nitrogen compounds and in regard to synthesis of different non-essential amino acids (Mathews et al., 2013)

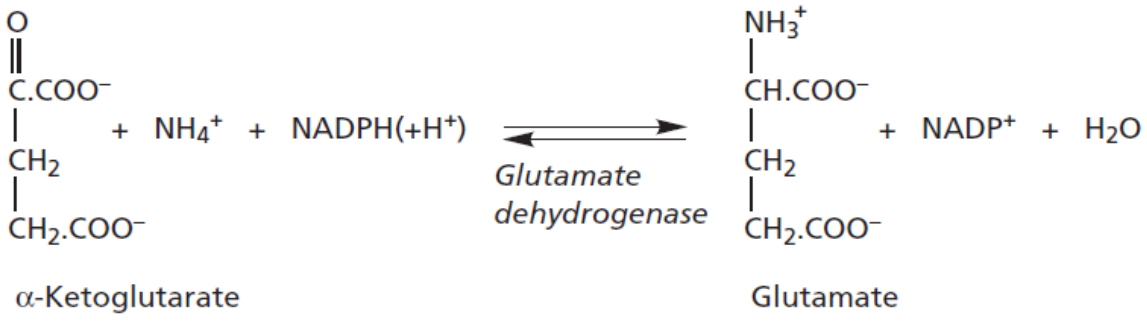


*Figure 2.4. Reductive amination of an α-ketoglutarate. Figure from McDonald et al. (2011).*

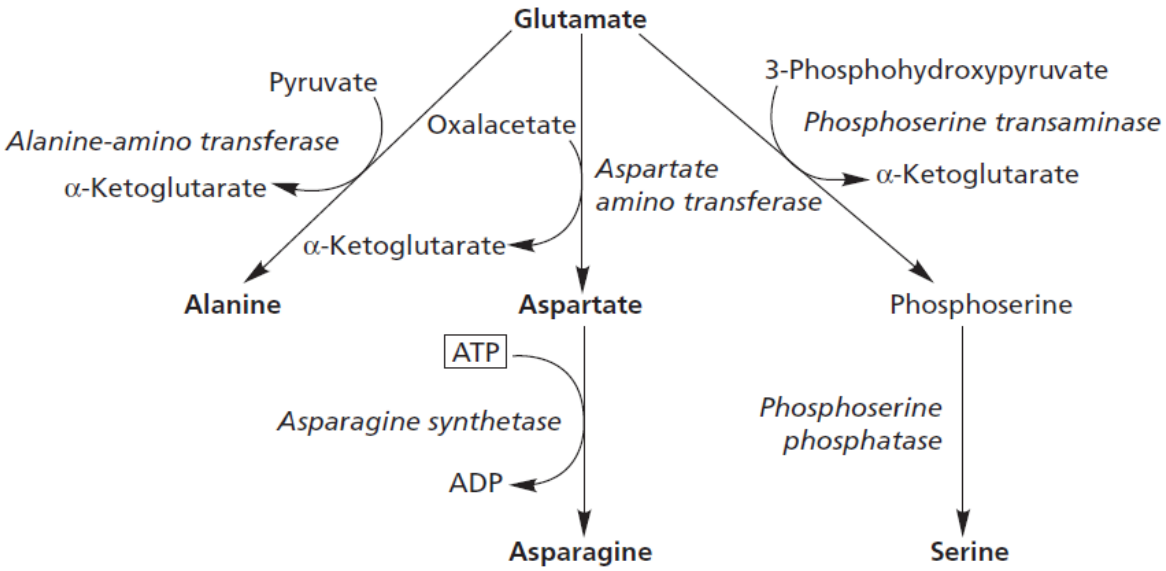


*Figure 2.5. Synthesis of different amino acids from glutamate. Figure from McDonald et al. (2011).*

### 2.4.3 Amino acids as a source of energy

Amino acids may be used as a source of energy in two cases: if the amount of consumed amino acids excides the animal requirement or when animals lack energy and is forced to degrade body tissues. In order to utilize amino acids as a source of energy, the amino acids must be oxidized. This oxidation takes place predominantly in the liver (McDonald et al., 2011). The degradation of amino acids starts with removal of the amino group through either deamination or transamination. Deamination is a biochemical reaction where the amino group of an amino acids is removed (Figure 2.6). The result of amino acid degradation is the production of keto acids, ammonia ($NH_3$), and ammonium ($NH_4^+$). The keto acids will enter the carbohydrate metabolism, while the ammonia and the ammonium will be converted to urea (Sjaastad et al., 2016).

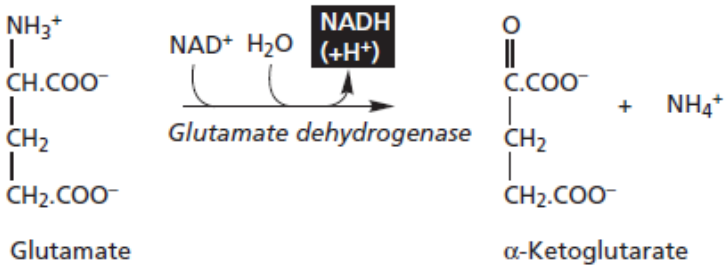


*Figure 2.6. Deamination of glutamate. Figure from McDonald et al. (2011).*

### 2.4.4 Synthesis of urea

Ammonia produced by deamination is toxic. As mentioned in Chapter 2.4.2, ammonia can be assimilated and detoxified in glutamate by reductive amination of α-ketoglutarate. However, most of the ammonia will be detoxified by transformation to urea in the liver. The transformation of ammonia to urea happens in two stages. Firstly, ammonia reacts with $H_2O$ and $CO_2$, resulting in production of carbamoyl phosphate. The carbamoyl phosphate molecule will then enter the urea cycle as illustrated in Figure 2.7, resulting in production of urea. (McDonald et al., 2011).

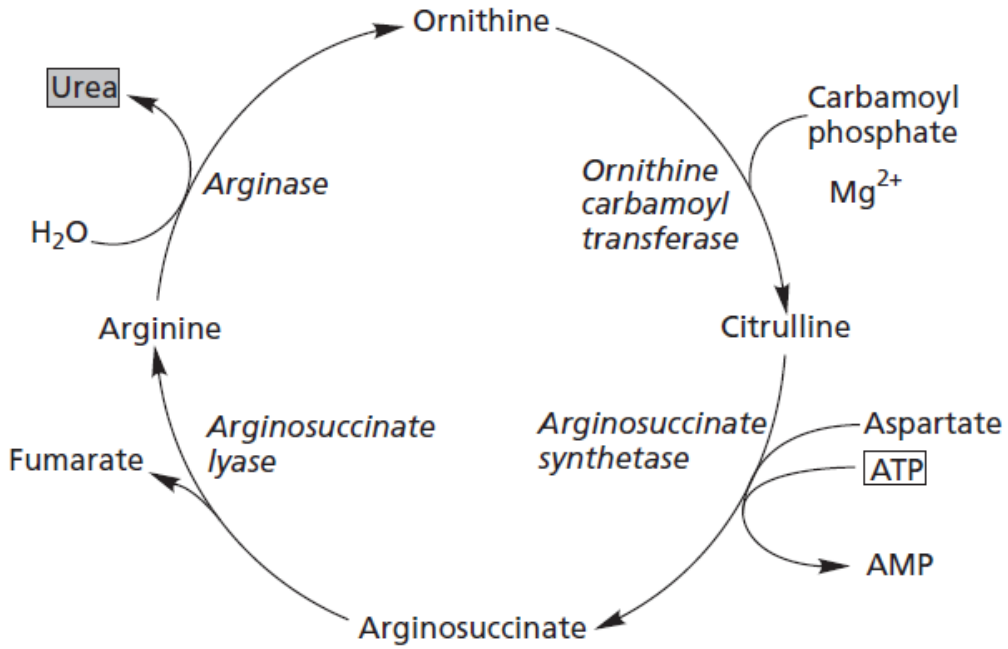


*Figure 2.7. The urea cycle. Figure from McDonald et al. (2011).*

### 2.4.5 Excretion of urea

After synthesized in the liver, urea is taken up by the blood. Urea diffuses easily across cell membranes, and some of the urea will therefore diffuse from blood to milk (Sjaastad et al., 2016). This results in a proportional relationship between concentration of urea in milk and blood (Pulina et al., 2008; Sjaastad et al., 2016). Few studies on urea levels in goat milk have been done (Pulina et al., 2008). Table 2.1 presents some of the variation in urea levels of goat milk found in previous studies.

*Table 2.1 Urea levels of goat milk reported by different authors.*

| **Authors (year)** | **Milk urea levels (mmol/l)** |
|---|---|
| Bonanno et al. (2008) | 1.62 - 5.90 |
| Rapetti et al. (2014) | 1.98 - 11.24 |
| Pazzola et al. (2011) | 2.91 - 4.63 |
| Min et al. (2005) | 2.93 - 3.60 |
| Superchi et al. (2007) | 7.01 - 8.26 |

Even though some of the urea is excreted in milk, most of the urea in blood will be excreted as urine produced by the nephrons of the kidneys. The nephrons consist of a capillary network called glomerulus. Each glomerulus receives blood from the arteriole, called afferent arteriole, which ensure supply of endogenous waste products to the nephrons, such as urea. After production, the urine is collected in the renal pelvis, passed through the ureter and temporarily stored in the urinary bladder (Sjaastad et al., 2016). As production of urea in the urea cycle requires energy in the form of ATP, excretion of urea represent a waste of both nitrogen and energy (McDonald et al., 2011).

### 2.4.6 Ruminal ammonia and recirculation of urea

The ammonia produced in the rumen is mainly present in the rumen liquid as ammonium ($NH_4^+$). The ratio of ammonia and ammonium in the rumen depends on the ruminal pH. With a ruminal pH on 7, the concentration of ammonium is normally 300 times higher than the concentration of ammonia in the rumen. This is explained by a pKa-value similar to 9.3 for the reaction presented in Equation 2.3 (Sjaastad et al., 2016).

$$NH_3 + H^+ \leftrightarrow NH_4^+ \quad (2.3)$$

The ruminal ammonia and ammonium can be reutilized for synthesis of microbial amino acids by reductive amination. As an indicator of the microbe's nitrogen supply, the ruminal concentration of ammonia may be used. For optimal microbial growth, the minimum concentration of ammonia is considered to 5 mmol/l (Sjaastad et al., 2016). However, if the amount of ammonia produced exceeds the requirement of the microbes, the ammonia is absorbed into the blood over the rumen wall and transformed to urea (McDonald et al., 2011). In general, the ammonia has greater potential to be absorbed, compared to ammonium. The amount of absorbed ammonium is therefore small (Sjaastad et al., 2016).

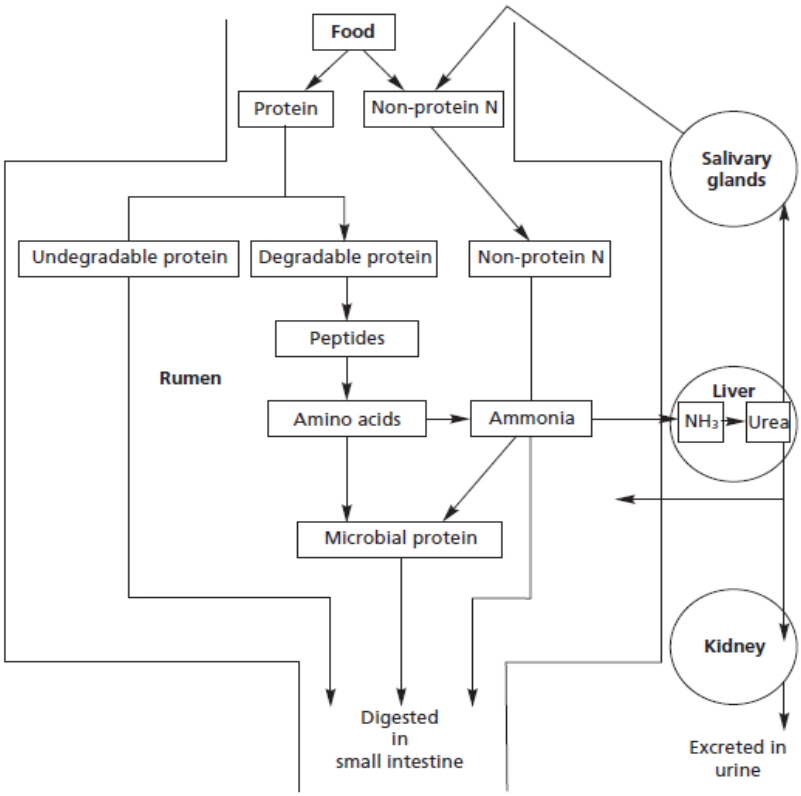


*Figure 2.8. The context between digestion and metabolism of nitrogenous compounds in the rumen. Figure from McDonald et al. (2011).*

After absorption over the rumen wall, ammonia will be transported to the hepatocytes in liver and converted to urea in the same manner as explained in Chapter 2.4.4. The produced urea will either be recycled back to the rumen - via salvia or directly over the rumen wall - or excreted in milk and urine (Figure 2.8) (McDonald et al., 2011). The recycled urea can be reused as a nitrogen source for microbial growth. Compared to cows, the amount of urea recycled back to the rumen is more considerable, entailing a relatively more efficient nitrogen utilization in goats. However, the importance of this difference is unclear (TINE Rådgiving og Medlem, 2020c).

## 2.5 Overall physiological aspects of protein synthesis

### 2.5.1 Synthesis of proteins

Amino acids used for synthesis of proteins derives either from processes within the body, or directly as end products of digestion (McDonald et al., 2011). The synthesis of proteins happens largely in the liver. However, protein synthesis also takes place in extrahepatic tissues such as mammary glands and muscular tissues. The distribution of amino acids to extrahepatic tissues depends on the tissues uptake and use of amino acids. As an example, the supply of ammino acids from mammary glands increases considerably at the beginning of lactation. As much as 50% of the oxygen in the arterial blood are transported to the mammary glands during lactation (Sjaastad et al., 2016). Naturally, the use of amino acids also varies between tissues. In muscle tissues amino acids are used for building proteins like actin and myosin, while in the mammary glands amino acids are used for synthesis of different types of milk protein (Madsen & Nielsen, 2003; Sjaastad et al., 2016).

*Synthesis of proteins in the liver*

Amino acids absorbed from the small intestine are transported to the liver by the portal vein. In the liver both venous blood and arterial blood will be taken up by capillaries called sinusoids. Amino acids from blood can easily be taken up by liver cells arranged adjacent to the sinusoids, due to the sinusoids incomplete cell walls. These liver cells are called hepatocytes. Hepatocytes are able to synthesize proteins from intestinal absorbed amino acids. Proteins synthesized by hepatocytes may be used in the liver or used in other body tissues. Examples of proteins produced by hepatocytes are enzymes, fibrinogen, coagulation factors, hormone-transporting globulins and albumins (Sjaastad et al., 2016).

*Synthesis of proteins in mammary glands*

The synthesis of milk proteins happens in the mammary epithelial cells. The precursors for synthesis of milk proteins are transported by the portal vein to the mammary glands, where they are taken up (Sjaastad et al., 2016). These precursors consist mainly of amino acids. However, a study performed by Backwell et al. (1996) indicates that mammary glands also take up and utilize small peptides for production of milk proteins. The mammary epithelial cells are dependent on supply of essential amino acids from the blood. They are, on the other hand, able to synthesise non-essential amino acid themselves by transamination (Sjaastad et al., 2016).

The synthesis of proteins takes place on ribosomes. After synthesised, proteins are transported into the endoplasmic reticulum and further into the Golgi apparatus. Some of the proteins are altered in the Golgi apparatus, before they are packed into secretory vesicles. The secretory vesicles are then transported to the apical cell membrane, where the proteins are released to the alveolar lumen by exocytosis (Sjaastad et al., 2016). The milk and its proteins are kept in the alveolar lumen until milked out (Madsen & Nielsen, 2003).

## 2.6 Feeding standard of Norwegian dairy goats

Different systems, so called feeding standards, have been developed in order to calculate the energy and protein requirement of ruminant animals (McDonald et al., 2011). In Norway, dairy goats are fed according to the AAT/PBV-system. PBV is an abbreviation for *protein balance in the rumen* and is calculated on the basis of dietary degradable crude protein and the amount of microbial protein produced (Equation 2.4) AAT is an abbreviation for *amino acids absorbed in the small intestine* and is calculated on the basis of dietary bye pass protein and microbial protein transported to the small intestine (Equation 2.5) (Madsen et al., 1995). The AAT/PBV-system refers to energy as *feed unit milk* (FEm). The terms FEm is based on the dietary content of nett energy to lactation ($NE_l$). 1 FEm corresponds to 6900 kJ $NE_l$. In more practical terms, 1 FEm corresponds to 1 kg barley with 87% dry matter content (Ekern & associates, 1991). By using the AAT/PBV-system one accounts for the microbial utilization of nitrogen in the rumen and the absorption of amino acids from microbial protein in the small intestine (Madsen et al., 1995).

*PBV g/kg DM = (g crude protein / kg DM x degradability in the rumen) – g microbial protein produced / kg DM* (2.4)

(Hvelplund & Madsen, 1993)

*AAT g/kg DM = (g crude protein / kg DM) x (1 – degradability in the rumen) x (proportion of amino acids in undegraded feed protein) x (digestibility in the small intestine of undegraded amino acids) + (g microbial protein produced / kg DM) x (proportion of amino acids in microbial protein) x (digestibility in the small intestine of microbial amino acids)* (2.5)

(Hvelplund & Madsen, 1993)

The energy and protein requirement of goats varies according to their physiological state. In order to meet the goat's protein requirement, one must therefore account for requirements linked to both activity levels, maintenance, lactation, pregnancy, and growth (Cannes et al.,

2008). This is accounted for in the AAT/PBV-system by dividing the requirement for AAT and energy into requirement for maintenance, requirement for pregnancy, requirement for lactation, and requirement for growth (Ekern & associates, 1991; Madsen et al., 1995) The PBV value should in general be around zero to slightly positive (TINE Rådgiving og Medlem, 2020b). By distinguishing the requirement for animals in different physiological states, one make it possible to feed an animal approximate to their requirement.

### 2.6.1 Energy and protein requirement for maintenance

When an animal is in a state of maintenance, it does not use any nutrients for production, work, or to cope with the environment (McDonald et al., 2011). The maintenance requirement make up the main part of the total nutrient requirement, and is therefore important to determine in order to optimize production (Cannes et al., 2008). The nitrogen requirement for maintenance corresponds the amount of nitrogen excreted in urine, faeces, skin, hair and hoofs, when the goat is fed a nitrogen free diet (McDonald et al., 2011). In the AAT/PBV-system, requirement for AAT is considered to be proportional to the animal's metabolic weight ($W^{0,75}$), and is calculated as shown in Equation 2.6. The energy requirement for maintenance in is calculated as shown in Equation 2.7.

$$AAT_{maintenance}\ (g/day) = 3.25 \times W^{0.75} \quad (2.6)$$

(Madsen et al., 1995)

$$FEm_{maintenance} = 0.0371 \times W^{0.75} \quad (2.7)$$

(TINE Rådgiving og Medlem, 2020b)

### 2.6.2 Requirement for lactation

Requirement for lactation depends on the milk yield and milk composition (McDonald et al., 2011). In the AAT/PBV-system, the milk yield is expressed as energy corrected milk (ECM) (Eq. 2.8). The AAT-requirement for lactation is calculated as shown in in Equation 2.9, while the energy requirement for lactation is calculated as shown in Equation 2.10.

$$kg\ ECM = ((Milk\ yield\ (kg) \times 0.01) + (0.122 \times fat\ \%) + (0.077 \times protein\%) + (0.053 \times lactose\%)) \quad (2.8)$$

(Ekern & associates, 1991)

$$AAT_{lactation}\ (g/kg\ ECM) = (40 \times kg\ ECM + 0.2 \times kg\ ECM^2)/kg\ ECM. \quad (2.9)$$

(Madsen et al., 1995)

$$FEm_{\ lactation} = 0.44 \times ECM\ (kg/day) + 0.0007293 \times ECM^2 \quad (2.10)$$

(Ekern & associates, 1991)

### 2.6.3 Requirement for pregnancy

Reproduction increases an animal's requirement for proteins, due to the growth of the foetus, as well as the growth of organs and tissues related to pregnancy (McDonald et al., 2011). In Norwegian dairy goat industry, a goat normally has one parturition a year (TINE Rådgiving og Medlem, 2020f). In the AAT/PBV-system, the AAT requirement for dairy goats is determined to be 20 g/day in fourth month of pregnancy and 40g/day in the fifth month of pregnancy (Madsen et al., 1995). In regard to energy, one calculates a goat's requirement for pregnancy equivalent to 0.15 FEm/day eight to three weeks before parturition and 0.35 FEm/day three to zero weeks before parturition (Ekern & associates, 1991).

### 2.6.4 Requirement for growth

Protein requirement for growth reflects the requirement for increased body weight and size. The requirement for growth varies with age. In general, the requirement for growth is highest in young animals. The variation in requirement for growth may be presented as a sigmoid curve, where the requirement for growth is high in young animals but evens out as the animal get older (McDonald et al., 2011). The requirement of energy and AAT for growing goats is presented in Table 2.2.

*Table 2.2. Requirement of feed unit milk (FEm) and amino acids absorbed in the small intestine (AAT) for growing goats. Values obtained from TINE Rådgiving og Medlem (2020b).*

| **Body weight** | **FEm[1]/ kg weight gain** | **g AAT[2]/ day** |
|---|---|---|
| Parturition- 10 kg | 1.9 | - |
| 10-20 kg | 2.0 | 40 |
| 20-30 kg | 2.3 | 50 |
| 30-40 kg | 2.6 | 55 |
| 40-45 kg | 3.0 | 55 |
| 45-50 kg | 3.5 | 55 |
| 50-55 kg | 4.0 | 55 |

### 2.6.5 Requirement for activity

In year 2020 Norwegian dairy goat spent in average 136 days grazing (TINE Rådgiving og Medlem, 2021). This entails a higher activity level during summer months, compared to periods where the goats are fed indoors. Goats are browsers, characterized by their ability to utilize many different types of forage and select for the highest nutritive value. It is therefore likely to assume that goats are willing to leave behind a longer distance in order to find pasture of best nutritive value (Morand-Fehr & Sauvant, 1991). In regard to energy, one calculates a goat's requirement for activity on pasture equivalent to 0.1-0.4 FEm per day. On flat pasture, the goat's maintenance requirement for energy increases with 20-25%. On ordinary Norwegian pasture, the goat's maintenance requirement for energy increases with 25-30%. In especially steep and varied terrain, the goat's maintenance requirement for energy increases with 30-40% (TINE Rådgiving og Medlem, 2020b).

### 2.6.6 The associations between PBV, AAT, and FEm

As discussed in Chapter 2.1, the utilization of dietary protein is dependent of dietary energy supply. This means that the amount of energy and the amount of protein supplied is not essential important in itself, but rather the ratio between the two feed parameters (McDonald et al., 2011). The AAT/PBV- system account for this optimal ratio by calculating different parameters. The parameter *protein and energy balance in the rumen* (PVB) accounts for production of microbial protein in the rumen. By calculating parameters such as *AAT* for weight gain and  AAT/kg ECM, the system accounts for the efficiency of amino acids utilization for weight gain and production Another parameter, however, not frequently used in current Norwegian dairy production, is AAT/FEm, where the system accounts for the energy needed in order to metabolize the amino acids absorbed in the small intestine (Madsen et al., 1995).

# 3 Material and Methods

## 3.1 G-110

The experiment "G110" was originally designed to study the effect of increased use of Norwegian plant lipid (rape seed) in concentrate for dairy goats in regard to milk production and milk fat composition (Breiland, 2017). In this thesis, the experiment has been used to examine the associations between milk urea levels and lactation stage, milk yield, parity, milk protein percentage, and blood urea, as well as the suitability of FTIR-analysis as a tool for measuring urea levels in goat milk. The coming research description will therefore focus on aspects related to milk urea. See the master thesis of Breiland (2017) here for additional research description.

### 3.1.1 Experimental design, test animals and treatment

The experiment was divided into three periods performed from the beginning of lactation in mid-February to late lactation in mid-October in 2016. Period 1 and period 3 were performed indoors at the Livestock Production Research Centre within the Norwegian University of Life Sciences (NMBU) at Ås (59° 39' N, 10° 46' Ø), 90 m.a.s.l., while period 2 was performed at Meløya Seter in Einundalen in Folldal (62° 19' N, 10° 1' Ø), 900 – 1000 m.a.s.l, where the goats were grazing mountain pasture.

The experiment was performed with 48 goats of the Norwegian dairy goat breed. The goats involved in the experiment were in their second to sixth lactation. The goats were divided into two batches depending on their date of parturition. Batch 1 consisted of 21 goats with average parturition date on the 16th of February 2016, while batch 2 consisted of 27 goats with average parturition date on the 3rd of March 2016. Hence, two weeks separated batch 1 and batch 2 in regard to average days in milk (DIM) through the experiment (Table 3.1).

*Table 3.1. Overview of experiment periods divided into two batches depending on their average days in milk (DIM)*

| | **Batch 1** | **Batch 2** |
|---|---|---|
| **Period** | **DIM** | |
| 1 | 1-130 | 1-115 |
| 2[a] | 130-200 | 115-185 |
| 3 | 200-240 | 185-225 |

[a] Average lactation stage for start and end of grazing period was 123 days in milk (DIM) and 193 DIM, respectively.

Each batch was divided into three groups, where each group consisted of eight goats. When dividing goats into groups, one aimed to make every group similar to each other in regard to average body weight, average lactation number, average parturition date, average milk yield, and the goat's genetical status in regard to casein.

The goats were assigned to six different treatments consisting of concentrate based on different content of lipid. Four of the concentrate types were based on rapeseed as a source of lipid, containing 2%, 4%, 6%, or 8% lipid. Two of the concentrate types were based on *Akofeed Gigant 60* as a source of lipid, containing 2% or 8% lipid. The chemical composition of the experiment concentrate is presented in and Table 3.2. The concentrate used in the experiment were produced by Centre of Feed Technology, NMBU.

*Table 3.2. Chemical composition of the experiment concentrates.*

| | **Akofeed (2% lipid)** | **Akofeed (8% lipid)** | **Rapeseed (2% lipid)** | **Rapeseed (4% lipid)** | **Rapeseed (6% lipid)** | **Rapeseed (8% lipid)** |
|---|---|---|---|---|---|---|
| **DM[1] (%)** | 88 | 90 | 89 | 89 | 89 | 89 |
| **CP [2](g/kg DM)** | 200 | 192 | 201 | 193 | 189 | 176 |
| **CFat[3] (g/kg DM)** | 50 | 117 | 55 | 78 | 102 | 123 |
| **Starch (g/kg DM)** | 355 | 329 | 367 | 426 | 251 | 329 |
| **ADF[4] (g/kg TS)** | 98 | 101 | 95 | 102 | 104 | 104 |
| **Ash (g /kg TS)** | 76 | 71 | 73 | 72 | 71 | 69 |

[1]*Dry matter*
[2]Crude protein
[3]Crude fat
[4]Acid detergent fibre

The concentrate was at all times distributed manually. During period 1 and period 3, the concentrate was distributed four times a day, while during period 2, the concentrate was distributed twice a day in connection with milking. The procedure of concentrate distribution is presented in Table 3.3.

*Table 3.3: Distribution of concentrate prior to and during the experiment.*

| **Period** | **Concentrate (kg/day)** | **Comment** |
|---|---|---|
| At parturition | 0,6 | Experiment concentrate |
| Lactation day 1-120 | 0,9 | + 0,1 kg experiment concentrate every 2. day |
| Lactation day > 120 | 0,7 | Experiment concentrate |

### 3.1.2 Roughage

The roughage used in period 1 and period 3 was first-cut, harvested on the 15th of June 2015. The gras was treated with preservatives (2,5 litre/ ton) and pressed into round balls. The preservative used was *Kofasil LP*, produced by Felleskjøpet Agri SA. The chemical composition of the roughage can be seen in Table 3.4.

*Table 3.4. Nutritional content of roughage used in period 1 and period 3.*

| DM[1] (%) | FEm[2]/kg DM | AAT[3] (g/kg DM) | PBV[4] (g/kg DM) | CP[5] (g/kg DM) | NDF[6] (g/kg DM) | iNDF[7] (g/kg NDF) | Feed intake (%) |
|---|---|---|---|---|---|---|---|
| 25,3 | 0,88 | 75 | -8 | 121 | 521 | 138 | 101 |

[1]Dry matter
[2]Feed unit milk
[3]Amino acids absorbed in the small intestine
[4]Protein balance in the rumen
[5]Crude protein
[6]Neutral detergent fibre
[7]Indigestible neutral detergent fibre

In order to obtain a homogenous mixture with limited possibilities for feed selection, the roughage was cut to a median particle length of 5 mm in a feed mixer (Siloking, Kverneland). In order to prevent heat production, acid was added to the roughage (2,0-2,5 litre/ton) from week 21 and onwards in connection with feeding. The acid used was named *Ensil Fullfôr*, produced by Felleskjøpet Agri SA.

The goats were given roughage *ad libitum* through the entire experiment. If the goats, nevertheless, had consumed the entire amount of roughage distributed, 1 kg extra roughage was given to the goat of interest.

The pasture utilized in period 2 consisted of different types of vegetation: marshlands with sedges (mainly *Carex nigraand* and *Carex rostrata*) and dry areas with grasses (mainly *Deschampsioa cespitosaand* and *Deschampsia flexuosa*), downy birch *(Betula pubescens),* dwarf birch *(Betula nana),* willow thickets *(Salix ssp.),* and different herbs. The goats had free access to the pasture both day and night during period 2, except during milking.

### 3.1.3 Milking

During period 1 and period 3, the goats were milked morning and evening in a milking stable (*DeLaval parallel parlour SG)* with 12 milking units and 24 milking boxes. During period 2, the goats were milked in an older milking stable with four milking units and 12 milking boxes

### 3.1.4 Registration and sampling

**i. Feed uptake**

Each goat's feed uptake of roughage was registered Monday morning, Tuesday morning, Wednesday morning, and Thursday morning every week in period 1 and period 3. Any residue of concentrate, both in the milking stable and the animal building, was registered every day during the entire experiment.

**ii. Collection and analysis of feed samples**

Feed samples from each round ball were collected on Monday morning, Tuesday morning, Wednesday morning, and Thursday morning every week in period 1 and period 3. Samples of roughage from the same week were merged into one sample. The feed samples from each week were sent to Eurofins for chemical analysis of dry matter (DM), ash, feed unit milk (FEm), crude protein (CP), total nitrogen (total-N), crude fat (CFat), amylase-treated neutral detergent fibre (aNDF), ammonia nitrogen ($NH_3$-N), ethanol, fermentation acids, and pH..

In order to detect feed selection against dry matter content and content of aNDF, any residue of roughage was collected Monday morning, Tuesday morning, Wednesday morning, and Thursday morning every week in period 1 and period 3. Samples of roughage residue were collected from each goat, separately. The samples of roughage residue belonging to each goat from the same week, were merged into one sample. These samples were analysed for DM and aNDF.

Samples of concentrate à 1 dl were collected Monday morning, Tuesday morning, Wednesday morning, and Thursday morning every week in period 1, period 2, and period 3. The samples of concentrate were analysed for DM, total- N, CP, CFat, fatty acids composition, starch, and aNDF.

**iii. Milk yield measurement, collection of milk samples and analysis of milk urea**

Milk yield measurements were performed on lactation day 30, 55, 85, 115, 185, and 225. In addition, milk was sampled from individual goats morning and evening on lactation day 30, 55, 85, 115, 185, and 225. The morning and evening samples were combined and transferred into a 40 ml container where one tablet of Bronopol were added in order to avoid microbial growth. One aliquot was analysed for urea and protein by f*ourier transformed infrared spectroscopy* (FTIR) by using a MilkoScan Combifoss 6500 (Foss, Hillerød, Denmark) at TINE Råmelkslaboratoriet in Bergen. Another aliquot was analysed for milk urea by a

kinetic, enzymatic UV method, performed on a MaxMat spectrophotometer on the laboratory of Faculty of Chemistry, Biotechnology and Food Sciences, NMBU. The analysis was performed by adding the enzymes *urease* and *glutamate dehydrogenase* to the milk. The enzymes were added in order to initiate a chemical reaction where NADH is produced (Eq. 1). The concentration of NADH was then measured by photometry. Because the concentration of NADH is equivalent to the concentration of milk urea in the original milk sample, the NADH content is used to determine the milk urea content (Equation 3.1)

$$Urea + 2H_2O \xrightarrow{Urease} 2NH_4 + CO3^{2-}$$
$$NH_4^+ + \alpha\text{-ketoglutarate} + NADH \xrightarrow{glutamate\ dehydrogenase} L\text{-}glutamate + NAD + H_2O \quad (3.1)$$

The milk urea levels analysed by using the chemical reaction presented in Equation 3.1, will hereby be referred to as *milk urea*. A specification will be made if the milk urea levels are analysed by FTIR-analysis.

**iv. Collection and analysis of blood samples**

On lactation day 10, 30, 55, 85, 115, 185, and 225, blood samples were taken from each goat. The blood samples were taken in the morning, prior distribution of concentrate and milking. Blood was collected form *vena jugularis* in 5 ml vacutainer tubes containing heparin. The blood samples were thereafter put on ice, before centrifugation of the blood samples were performed. The centrifugation was performed 20 minutes after the blood samples were taken. The blood samples were centrifuged in 15 minutes on 2000g. The samples were thereafter stored frozen at -80 °C before analysis of urea by chemical methods (Equation.3.1) at the laboratory belonging to the Department of Animal and Aquaculture Sciences, NMBU.

### 3.1.5 Calculations

The calculations of AAT, PBV, and FEm were performed according to the AAT/PBV-system, based on values from silage samples analysed by Eurofins and estimated values for the different concentrate ingredients. See calculation used in the AAT/PBV-system in Chapter 2.6.

### 3.1.6 Statistical analysis

The data was statistical analysed in SAS 9.4 (2016). The analysis of variance was done by *mixed procedure* (Littell et al., 1998), where each measurement was repeated several times for each goat. The measurements were assumed to be correlated, something that was taken into account when choosing a statistical model. Both the Akaike information criterion (AIC) and the Schwarz' Bayesian criterion (SBIC) (Wolfinger, 1996) were used in order to choose a suitable covariance structure within the statistical model. Both AIC and SBIC showed that *spatial power covariance structure* fitted the current data set well.

The statistical model used was the following: $Y_{ijkl} = \mu + A_i + B_j + A \times B_{(ij)} + C_k + \varepsilon_{ijkl}$, where μ represented the mean value, A*i* represented the fixed effect of concentrate types, *i*=1, 2,…,6 (Akofeed 2%, Akofeed 8%, Rape seed 2%, Rape seed 4%, Rape seed 6%, Rape seed 8%), B*j* represented the fixed effect of DIM, *j* =1,2,…,6 (DIM 30, 55, 85, 115, 185, 225), A x B*(ij)* was the effect of interaction between concentrate types *i* and lactation day *j*, *Ck* represented the fixed effect of parity, *k*=1,2,…,5 (parity 2, 3, 4, 5, 6), and *εijkl* represented the residuals.

In order to find potentially statistical relationships between parameters, *the Pearson correlation coefficient* (Snedecor & Cochran, 1989) was calculated using the command *proc corr* in SAS 9.4 (2016). The results from the statistical analysis were presented as least square means (lsmeans). Differences were considered statistically significant when $P < 0.05$, and trends were apparent when $0.05 \leq P < 0.10$. Differences between lsmeans were tested based on least square differences using the default pairwise t-test in the pdiff option of the lsmeans statement. Differences were considered statistically significant when $P < 0.05$, and trends were apparent when $0.05 \leq P < 0.10$.

## 3.2 D-174

The experiment *D174* was originally designed to study the tolerance limit for use of Norwegian barley in feed diet for dairy goats (Martinsen, 2020). In this thesis, the experiment has been used to study the association between milk urea levels and dietary crude protein, protein balance in the rumen (PBV), and amino acids absorbed in the small intestine per feed unit milk (AAT/FEm), as well as the suitability of FTIR-analysis as a tool for measuring urea levels in goat milk The coming research description will therefore focus on aspects related to milk urea. See the master thesis of Martinsen (2020) here for additional research description.

### 3.2.1 Experimental design, test animals and treatment

The experiment was performed indoors at the Animal Production Experimental Centre within the Norwegian University of Life Sciences in the period of March to September 2018. Nine multiparous rumen cannulated goat of the Norwegian Dairy Goats breed were involved in the experiment.

The experiment was based on the principals of a 3x3 Latin square design with three replicates. The experiment was divided into three experiment periods: *period 1*, *period 2*, and *period 3*. Period 1 was performed in lactation week 11-16, period 2 was performed in lactation week 21-26, and period 3 was performed in lactation week 28-33. The goats were assigned to three different treatments consisting of concentrate based on alkaline treated rolled barley (Concentrate A), untreated rolled barley (Concentrate B), and untreated grounded barley (Concentrate C). Concentrate A was treated with Maxammon (Harbro Quality Livestock Nutrition, UK). See Table 3.5, 3.6, and 3.7, for replicate 1, replicate 2, and replicate 3, respectively.

*Table 3.5. Replicate 1. Three goats fed three different concentrates (A, B, and C) over three periods.*

| **Goat nr.** / **Experiment period** | 1 | 4/10* | 7 |
|---|---|---|---|
| 1 | A | B | C |
| 2 | C | A | B |
| 3 | B | C | A |

**Goat 4 had to be replaced by goat 10 from period 2 and on, due to rumen acidosis.*

*Table 3.6. Replicate 2. Three goats fed three different concentrates (A, B, and C) over three periods.*

| **Goat nr.** / **Experiment period** | 2 | 5 | 8 |
|---|---|---|---|
| 1 | A | B | C |
| 2 | C | A | B |
| 3 | B | C | A |

*Table 3.7. Replicate 3. Three goats fed three different concentrates (A, B, and C) over three periods.*

| **Goat nr.** / **Experiment period** | 3 | 6 | 9 |
|---|---|---|---|
| 1 | A | B | C |
| 2 | C | A | B |
| 3 | B | C | A |

The Maxammon treatment converts feed urea to ammonia, leading to increased pH and increased crude protein content in the concentrate. In order to balance the three concentrate types in regard to crude protein content, 1.75% of the barley content in concentrate B and concentrate C was replaced by urea. The experiment concentrate was produced in two batches. The nutritional content of each concentrate type is presented in Table 3.8 and Table 3.9, for batch 1 and batch 2, respectively.

*Table 3.8. Chemical composition of batch 1.*

| | **Alkaline treated, rolled barley** | **Untreated rolled barley** | **Untreated grounded barley** |
|---|---|---|---|
| DM[1] (g/kg DM) | 846 | 848 | 854 |
| Starch (g/kg DM) | 398 | 372 | 384 |
| CP[2] (g/kg DM) | 236 | 239 | 248 |
| aNDF[3] (g/kg DM) | 148 | 139 | 156 |
| CFat[4] (g/kg DM) | 45 | 44 | 43 |
| Ash (g/kg DM) | 74 | 76 | 76 |

[1]Dry matter
[2]Crude protein
[3] Amylase-treated neutral detergent fibre
[4]Crude fat

*Table 3.9. Chemical composition of batch 2.*

| | **Alkaline treated rolled barley** | **Untreated rolled barley** | **Untreated grounded barley** |
|---|---|---|---|
| DM[1] (g/kg DM) | 873 | 878 | 867 |
| Starch (g/kg DM) | 422 | 417 | 413 |
| CP[2](g/kg DM) | 207 | 223 | 221 |
| Total-N[3] (g/kg DM) | 34 | 35 | 37 |
| aNDF[4] (g/kg DM) | 149 | 159 | 156 |
| CFat[5] (g/kg DM) | 33 | 38 | 39 |
| Ash (g/kg DM) | 73 | 70 | 73 |

[1]Dry matter
[2]Crude protein
[3]Total nitrogen
[4] Amylase-treated neutral detergent fibre
[5]Crude fat

The goats were fed concentrate six times a day: at 08.00, 12.00, 16.00, 20.00, 00,00, and 04.00. Originally, it was planned to distribute concentrate through an automatic concentrate feeder. However, technical problems occurred, and the concentrate was therefore given to the goats manually through the entire experiment.

Each experimental period was divided into an *adaption period, a challenge period, and* a *recovery period.*

- The *adaption period* lasted for four days. During the adaptation period, each goat was adapted to a new concentrate type by substituting 50% of the preceding concentrate with either of the experiment concentrate types. During the adaption period, the goats were fed 1500 g concentrate per day.
- During the *challenge period*, the level of concentrate for each goat was increased by 150 g dry matter every fourth day. The concentrates level was increased eight times maximum, resulting in eight *challenge levels.*
- If a goat developed subacute rumen acidosis (SARA) indicated by low rumen pH (<5.6) for three consecutive measurements for two consecutive days or lack of appetite for two consecutive days, the goat was moved from the challenge period into a *recovery period.* If a goat were put into a recovery period, the level of concentrate was reduced to 1500 g dry matter. The recovery period lasted for eight days in order to

ensure a rumen pH above 6. When the recovery period was completed, the goat was put into a new adaption period.

- The periods between each challenge period are referred to as *baseline periods*.

### 3.2.2 Roughage

The roughage used in the experiment was first-cut, harvested on the 14th of June 2017. The roughage was pressed into round balls. Preservatives were not used. The chemical composition of the roughage can be seen in **Feil! Fant ikke referansekilden.**Table 3.10.

*Table 3.10. Nutritional content of roughage used during the entire experiment.*

| DM[1] (%) | FEm[2]/kg DM | AAT[3] (g/kg DM) | PBV[4] (g/kg DM) | CP[5] (g/kg DM) | NDF[6] (g/kg DM) | iNDF[7] (g/kg NDF) | Feed intake (%) |
|---|---|---|---|---|---|---|---|
| 22,2 | 0,86 | 82 | 10 | 121 | 535 | 156 | 98 |

[1]Dry matter
[2]Feed unit milk
[3]Amino acids absorbed in the small intestine
[4]Protein balance in the rumen
[5]Crude protein
[6]Neutral detergent fibre
[7]Indigestible neutral detergent fibre

In order to obtain a homogenous mixture with limited possibilities for feed selection, the roughage was cut to a median particle length of 3 mm in a feed mixer (Siloking, Kverneland). After cutting, the roughage was packed in plastic bags, where each bag had a net weight of 20 kg. The bags were frozen directly after packing. The aforementioned procedure was performed before each period, such as the amount of packed roughage was equivalent to the amount of roughage used during the following experiment period.

The goats were given roughage *ad libitum* through the entire experiment. If the goats, nevertheless, had consumed the entire roughage amount distributed, 1 kg of extra roughage was given to the goat of interest.

### 3.2.3 Milking

The goats were milked with a portable milking machine. Milking was performed morning and evening.

### 3.2.4 Registration and sampling

#### i. Feed uptake

Feed uptake for each goat was registered daily in the challenge period and the recovery period. The goat's consummation of roughage was not registered in the change period. Any residue of concentrate was registered every day during the entire experiment.

#### ii. Collection and analysis of feed samples

**S**amples of roughage à 10 kg were collected at day 9, 10, and 11 in each baseline period. Samples of roughage from the same experiment period were merged into one sample à 30 kg. The feed samples from each experiment period were sent to Eurofins for chemical analysis of dry matter (DM), ash, feed unit milk (FEm), crude protein (CP), total nitrogen (total-N), crude fat (CFat), aNDF (amylase-treated neutral detergent fibre), $NH_3$-N (ammonia nitrogen), ethanol, fermentation acids and pH.

In order to detect feed selection against dry matter content and content of aNDF, any residue of roughage was collected on day 11 in each baseline period, the first day in every challenge period, and the first day in every recovery period. The roughage residue from each goat was collected separately, such as one obtained samples from individual goats. The samples were sent to Eurofins for chemical analysis of DM and aNDF.

Samples of concentrate à 1 dl were collected on day 9, 10, and 11 in each baseline period. Samples of concentrate from each concentrate type were merged to one sample. The concentrate samples were sent to Eurofins for chemical analysis of DM, ash, total-N, CP, CFat, starch and pellet quality.

#### iii. Collection of milk samples and milk yield measurements

Milk yield measurements was performed every day in the challenge period and every day in the recovery period. Milk samples from individual goats were collected on day 3 and day 4 at each challenge level, evening and morning, respectively. The milk samples were combined and transferred to a 40 mL container where one tablet of Bronopol were added in order to avoid microbial growth. The milk samples were analysed for urea by both FTIR-analysis and chemical analysis as described in Chapter 3.1.4.

### 3.2.5 Calculations

The calculations of AAT, PBV, and FEm were performed according to the AAT/PBV-system, based on values from silage samples analysed by Eurofins and estimated values for the different concentrate ingredients. See calculation used in the AAT/PBV-system in Chapter 2.6.

### 3.2.6 Statistical analysis

The data collected during the experiment were statistical analysed in SAS 9.4 (2016). The analysis of variance was done by mixed procedure (Littell et al., 1998), where each measurement was repeated several times for each goat and appeared correlated. Consequently, these correlations were taken into account in the statistical model. A covariance structure of repeated measurements was chosen by comparing potential structures using Akaikes' and Schwarz' Bayesian information criterion (Wolfinger, 1996) and first order autoregressive covariance structure proved useful for all data.

Analysis of variance for repeated measurements was performed according to the model: $Y_{ijkl} = \mu + A_i + B_j + A \times B_{(ij)} + C_k + \varepsilon_{ijkl}$, where $\mu$ represented the mean value, $A_i$ represented the fixed effect of concentrate types, $i$=1,2,3 (Alkaline, Rolled, Grounded), $B_j$ represented the fixed effect of concentrate level, $j$ =1,2,…,8 (1,5, 1,65, 1,80, 1,95, 2,10, 2,25, 2,40, 2,55 kg DM/day), A x $B_{(ij)}$ represented the effect of interaction between concentrate type $i$ and concentrate level $j$, and $C_k$ represented the fixed effect of experimental period, $k$=1,2,3, and $\varepsilon_{ijkl}$ represented the residuals.

In order to find potentially statistical relationship between parameters, *the Pearson correlation coefficient* (Snedecor & Cochran, 1989) was calculated using the command *proc corr* in SAS 9.4 (2016). The results from the statistical analysis were presented as least square means (lsmeans). Differences were considered statistically significant when $P < 0.05$, and trends were apparent when $0.05 \leq P < 0.10$. Differences between lsmeans were tested based on least square differences using the default pairwise t-test in the pdiff option of the lsmeans statement. Differences were considered statistically significant when $P < 0.05$, and trends were apparent when $0.05 \leq P < 0.10$.

# 4 Results

## 4.1 Feed parameters

The results of feed analysis for the G110 experiment and the D174 experiment is shown in Table 4.1 and Table 4.2, respectively. Compared to the results of G110, the results of D174 showed a slightly larger numerical variation in mean values of PBV (g/day), crude protein (g/kg DM), g AAT kg/DM, g AAT/kg ECM, and AAT/kg milk when looking at the entire experiments.

*Table 4.1. Mean values of feed variables in the G110.*

| Variable | N[7] | Mean | SD[8] | Minimum | Maximum |
|---|---|---|---|---|---|
| **FEm[1]/day** | 239 | 2.1 | 0.26 | 1.3 | 3.0 |
| **AAT[2] (g/day)** | 239 | 197 | 23.7 | 131 | 281 |
| **PBV[3] (g/day)** | 239 | -4 | 12.1 | -34 | 17 |
| **CP[4] (g/day)** | 239 | 322 | 39.0 | 212 | 462 |
| **FEm/ kg DM[5]** | 239 | 1.0 | 0.03 | 0.9 | 1.1 |
| **AAT (g/kg DM)** | 239 | 89 | 2.6 | 83 | 100 |
| **PBV (g/kg DM)** | 239 | -2 | 5.4 | -13 | 9 |
| **CP (g/kg DM)** | 239 | 145 | 5.5 | 133 | 162 |
| **FEm-balance** | 239 | -0.10 | 0.305 | -1.49 | 0.74 |
| **FEm/ kg milk** | 239 | 1.32 | 0.255 | 0.50 | 2.17 |
| **FEm/kg ECM[6]** | 239 | 0.45 | 0.096 | 0.12 | 0.77 |
| **AAT-balance** | 239 | -16 | 31.1 | -149 | 60 |
| **g AAT/FEm** | 239 | 93 | 2.5 | 89 | 101 |
| **g AAT/ kg milk** | 239 | 128 | 23.2 | 58 | 208 |
| **g AAT/ kg ECM** | 239 | 43 | 8.9 | 14 | 71 |

[1]Feed unit milk
[2]Amino acids absorbed in the small intestine
[3]Protein balance in the rumen
[4]Crude protein
[5] Dry matter
[6]Energy corrected milk
[7]Number of observations
[8]Standard deviation

*Table 4.2 Mean values of feed variables in the D174.*

| | N[7] | Mean | SD[8] | Minimum | Maximum |
|---|---|---|---|---|---|
| **FEm[1]/day** | 144 | 2.8 | 0.32 | 2.1 | 3.6 |
| **AAT[2] (g/day)** | 144 | 291 | 34.3 | 217 | 364 |
| **PBV[3] (g/day)** | 144 | 94 | 16.1 | 64 | 127 |
| **CP[4] (g/day)** | 144 | 545 | 66.4 | 412 | 688 |
| **FEm/ kg DM[5]** | 144 | 1.0 | 0.02 | 1.0 | 1.1 |
| **AAT (g/kg DM)** | 144 | 106 | 4.0 | 97 | 116 |
| **PBV (g/kg DM)** | 144 | 34 | 3.9 | 26 | 44 |
| **CP (g/kg DM)** | 144 | 200 | 8.0 | 183 | 220 |
| **FEm-balance** | 124 | 0.47 | 0.272 | -0.13 | 1.26 |
| **FEm/ kg milk** | 144 | 2.03 | 0.337 | 1.26 | 2.78 |
| **FEm/kg ECM[6]** | 124 | 0.63 | 0.111 | 0.45 | 1.02 |
| **AAT-balance** | 124 | 59 | 28.6 | -10 | 141 |
| **g AAT/FEm** | 144 | 102 | 1.6 | 98 | 106 |
| **g AAT/ kg milk** | 144 | 219 | 35.4 | 142 | 295 |
| **g AAT/ kg ECM** | 124 | 67 | 11.6 | 48 | 106 |

[1]Feed unit milk
[2]Amino acids absorbed in the small intestine
[3]Protein balance in the rumen
[4]Crude protein
[5] Dry matter
[6]Energy corrected milk
[7]Number of observations

## 4.2 Results of G110

Least square means (lsmeans) of dietary protein and energy content in the experiment, G110, is presented in Table 4.3. The dietary content of crude protein (g/kg DM) and AAT (g/kg DM) were highest at 115 days in milk (DIM), while the dietary content of PBV was highest at 225 DIM. The amount of AAT/FEm was lowest at 225 DIM, while the content of FEm was kept relatively stable through the entire trial. No significant effect of concentrate types on milk urea levels analysed chemically, was found in G110.

*Table 4.3. Least square means ± standard error of dietary energy and protein variables on different days in milk (DIM).*

| | **30 DIM** | **55 DIM** | **85 DIM** | **115 DIM** | **185 DIM** | **225 DIM** |
|---|---|---|---|---|---|---|
| **CP[1] (g/kg DM[2])** | 139±0.7 | 146±0.7 | 144±0.7 | 149±0.7 | GP[6] | 148±0.7 |
| **PBV[3] (g/kg DM)** | -10 ±0.4 | 1±0.4 | -3±0.4 | -1±0.4 | GP | 5±0.4 |
| **AAT[4] (g/kg DM)** | 89±0.3 | 88±0.3 | 89±0.3 | 91±0.3 | GP | 86±0.3 |
| **FEm[5]/kg DM** | 1.0±0.00 | 0.9±0.00 | 0.9± 0.00 | 1.0±0.00 | GP | 0.9±0.00 |
| **g AAT/FEm** | 94± 0.2 | 93± 0.2 | 95± 0.2 | 94± 0.2 | GP | 91± 0.2 |

[1]Crude protein
[2]Dry matter
[3]Protein balance in rumen
[4] Amino acids absorbed in the small intestine
[5] Feed unit milk
[6]Grazing period

### 4.2.1 Effect of lactation stage on milk urea levels

Lactation stage had a significant effect (P<0.001) on milk urea levels. Milk urea levels were significantly higher (P<0.005) on 115DIM, compared to milk urea levels at 30, 85, 185, and 225 DIM. The milk urea level was significantly lower (P<0.001) at 185 DIM, compared to other test days (Figure 4.1).

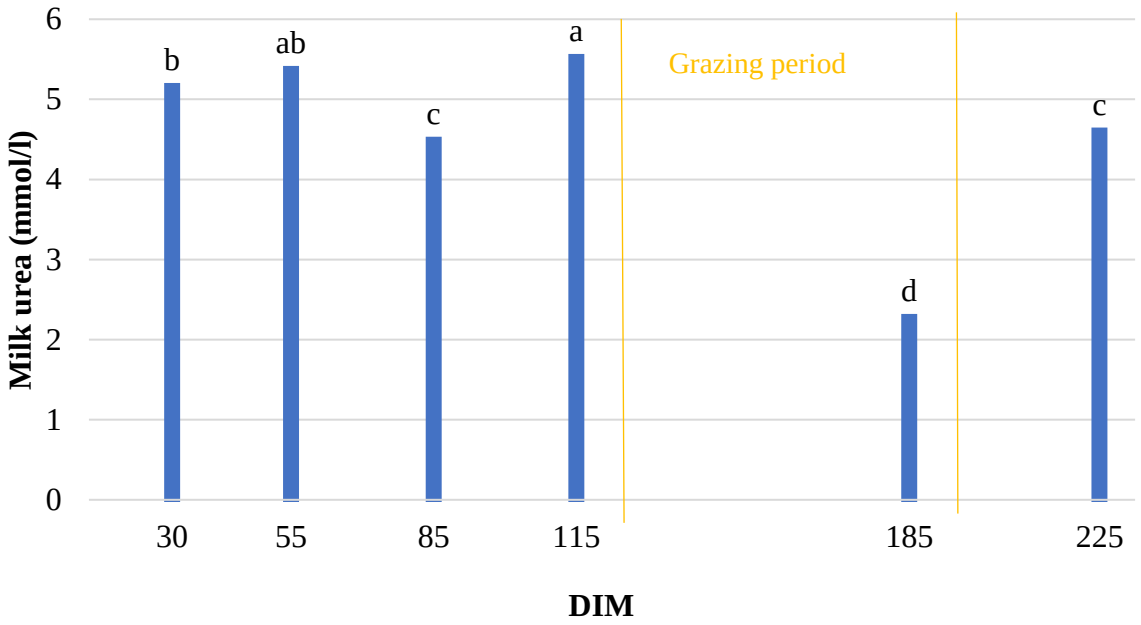


*Figure 4.1. Milk urea concentrations (least square means (lsmeans)) on different days in milk (DIM). Standard error for all concentrations of milk urea = 0.14. a-d: lsmeans with different letters are significantly different (P<0.05).*

### 4.2.2 Associations between milk yield and milk urea levels

The observed average daily milk yield was significantly highest (P<0.05) at 30 and 55 DIM, and significantly lowest (P<0.001) at 225 DIM. A small increase of milk yield from 115 to 185 DIM was observed (Figure 4.2). A significant negative correlation between milk yield and milk urea levels was observed at 185 DIM (r=-0.40, P<0.01) and 225 DIM (r= -0.44, P<0.01).

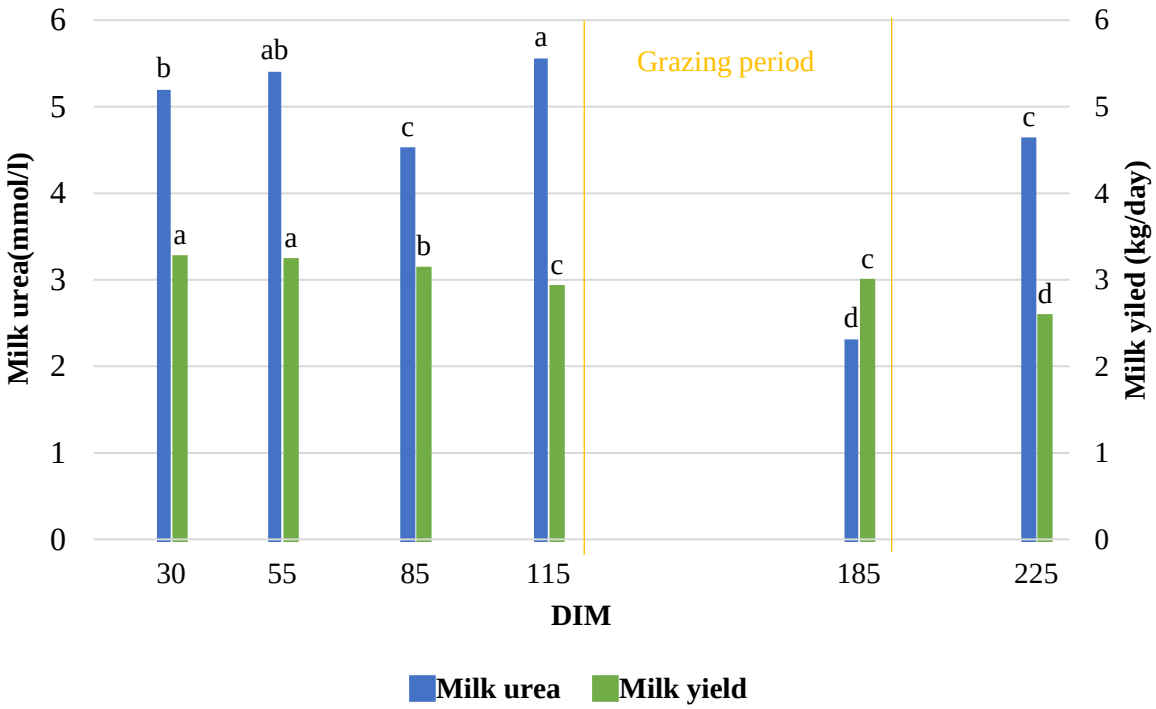


*Figure 4.2. Least square means (lsmeans) of milk urea levels and milk yield on different days in milk (DIM). a-d: lsmeans with different letters within same series are significantly different (P<0.05). Standard error (SE) for all lsmeans of milk urea=0.14. SE for all lsmeans of milk yield=0.06.*

### 4.2.3 Effect of parity on milk urea levels

No significant effect of parity on milk urea levels was observed. The numerically highest urea levels were observed in milk from goats in their sixth lactation (5.06 mmol/l), while the numerically lowest urea level was observed in milk from goats in their fifth lactation (4.18 mmol/l) (Figure 4.3).

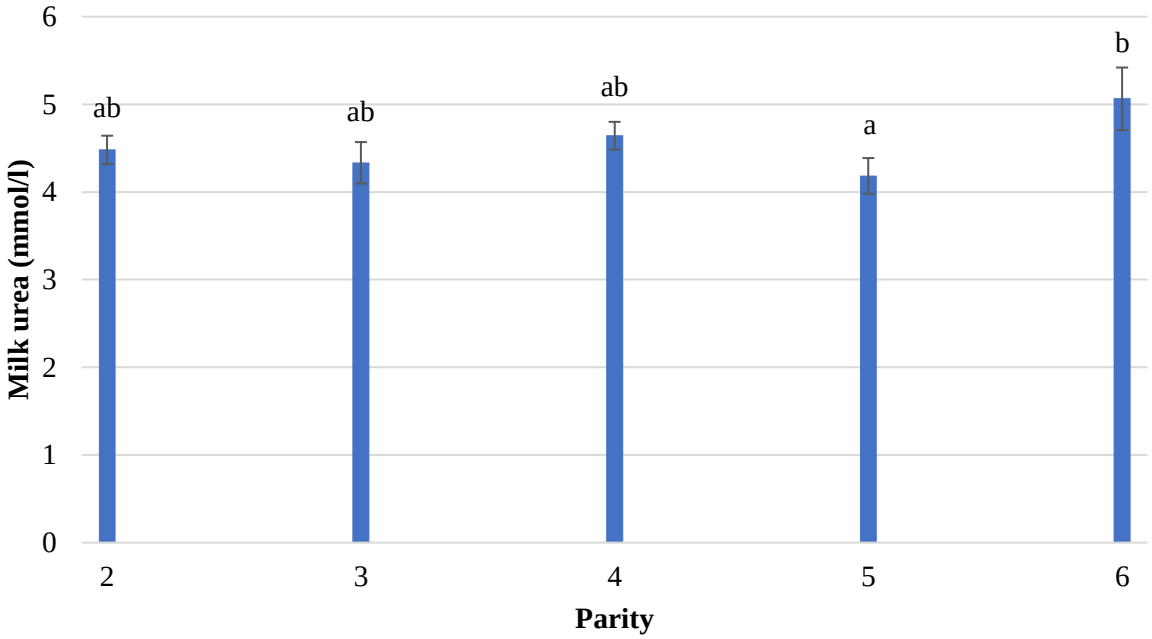


*Figure 4.3. Least square means ± standard error (lsmeans ± SE) for milk urea levels at different lactation numbers. a-d: lsmeans with different letters are significantly different (P<0.05).*

### 4.2.4 Associations between protein percentage in milk and milk urea levels

Milk protein percentage was significantly higher (P<0.001) at 185 DIM, compared to milk protein percentage on other test days (Figure 4.4).

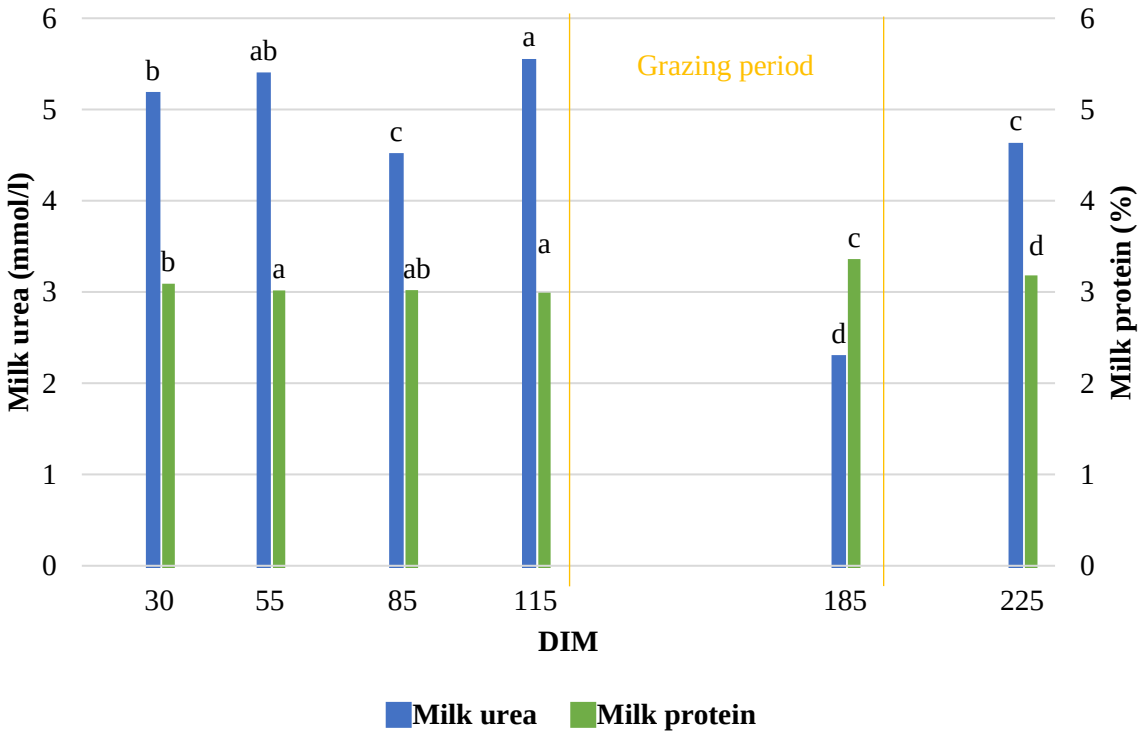


*Figure 4.4. Least square means (lsmeans) for milk urea levels and milk protein percentage on different days in milk (DIM). a-d: lsmeans with different letters within the same series are significantly different (P<0.05). Standard error (SE) for all lsmeans of milk urea=0.14. SE for all lsmeans of milk yield=0.05.*

A significant negative correlation between milk protein percentage was observed at 55 DIM (r=-0.32, P<0.05) and 85 DIM (r=-0.42, P<0.001), while no significant correlation between milk urea and milk protein percentage was observed on other test days. When looking at the entire G110 experiment, no significant correlation between milk urea levels and milk protein percentage was observed. The degree of explanation ($R^2$=0.107) between milk urea and milk protein percentage was low when looking at the entire G110 experiment (Figure 4.5).

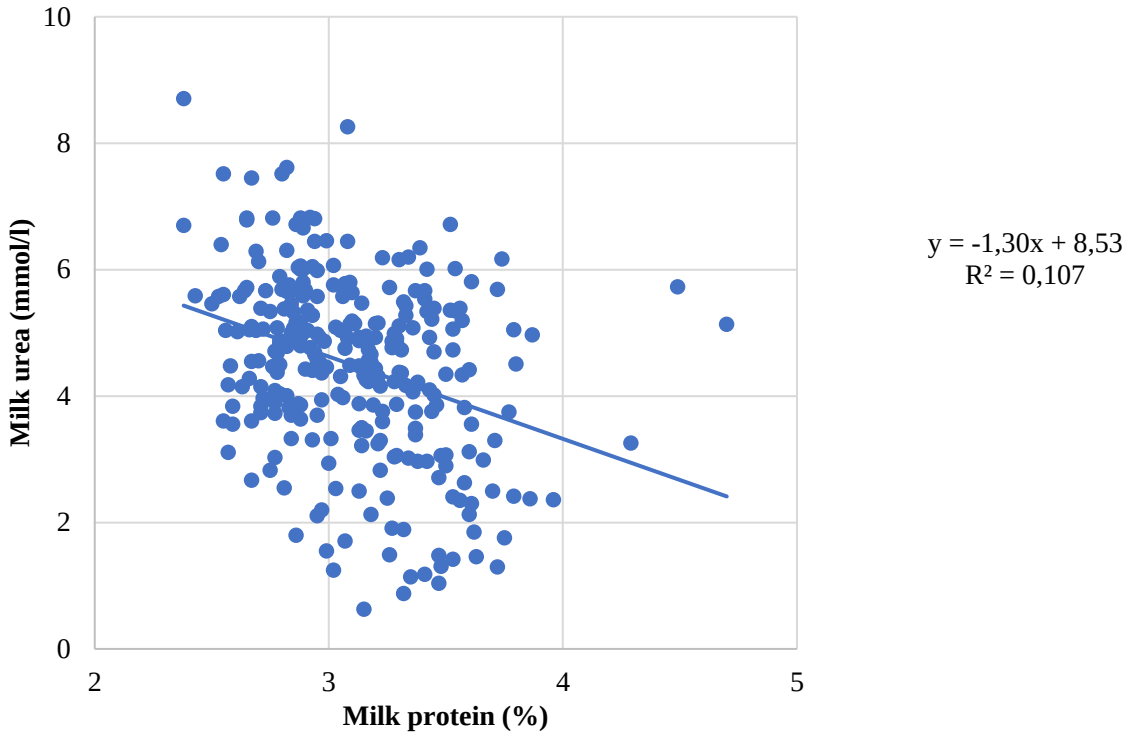


*Figure 4.5. Relationship between milk urea levels (y) and milk protein % (x). $R^2$= coefficient of determination.*

### 4.2.5 Association between blood urea levels and milk urea levels

The observed average blood urea levels were significantly higher (P<0.001) at 115 DIM compared to blood urea levels on other test days (Figure 4.6).

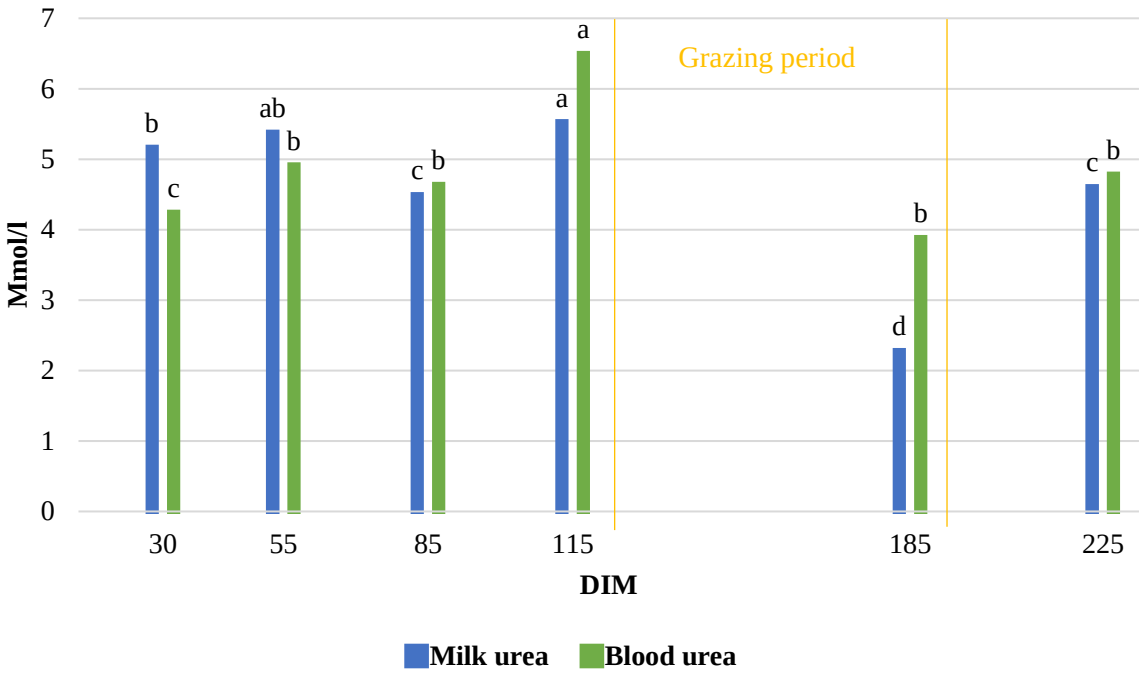


*Figure 4.6. Least square means (lsmeans) for milk urea levels and blood urea levels on different days in milk (DIM). a-d: lsmeans with different letters within the same series are significantly different (P<0,05). Standard error (SE) for all lsmeans of milk urea=0.14. SE for all lsmeans of blood urea=0.17.*

A significant positive correlation (P<0.001) between milk urea levels and blood urea levels was observed at all test days (Table 4.4). The degree of explanation between milk urea levels and blood urea levels was rather low for the entire experiment ($R^2$=0.45) (Figure 4.7).

*Table 4.4. Pearson correlation coefficient (r) between blood urea levels (BU) and milk urea levels (MU) on different days in milk (DIM).*

| | **30 DIM** | **55 DIM** | **85 DIM** | **115 DIM** | **185 DIM** | **225DIM** |
|---|---|---|---|---|---|---|
| **r(BU, MU)** | 0.87*** | 0.59*** | 0.74*** | 0.82*** | 0.54*** | 0.85*** |

*** p<0.001

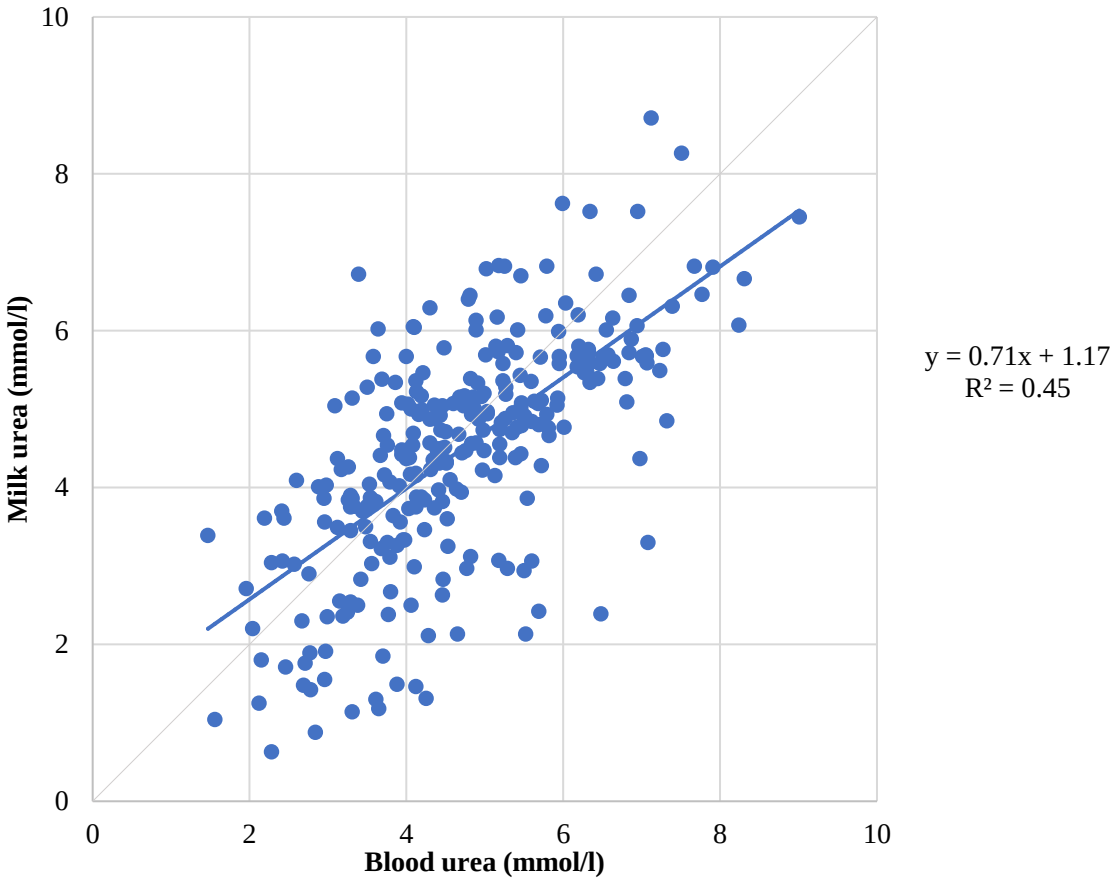


*Figure 4.7. Relationship between milk urea (y) and blood urea (x). $R^2$= coefficient of determination.*

## 4.3 Results of D174

Milk urea levels were significantly higher (P<0.001) in period 1, compared to period 2 and period 3. No significant difference in milk urea levels was observed between period 2 and period 3. Numerically smaller levels of dietary crude protein (g/kg DM), PBV (g/kg DM), AAT (g/kg DM), FEm (g/kg DM), and g AAT/FEm were observed in period 1, compared to period 2 and period 3 (Table 4.5). A significant effect (P<0,05) of concentrate levels (kg DM) on milk urea levels analysed chemically was observed in D174.

*Table 4.5. Least square means ± standard error (lsmeans± SE) of milk urea analysed chemically and dietary energy and protein variables for each period. Period 1= 11th -16th week of lactation. Period 2= 21st- 26th week of lactation. Period 3=28th -33rd week of lactation*

| | **Period 1** | **Period 2** | **Period 3** |
|---|---|---|---|
| **MU (ch)[1]** | 12.30±0,241 | 10.81±0.296 | 9.98±0.285 |
| **CP[2] (g/ kg DM)** | 198±1.0 | 204±1.2 | 204±1.2 |
| **PBV[3] (g/kg DM)** | 34±0.4 | 36±0.5 | 36±0,5 |
| **AAT[4] (g/kg DM)** | 106±0,5 | 109±0,6 | 109±0,6 |
| **FEm[5]/kg DM** | 1.0±0.00 | 1.1±0.00 | 1.0±0.00 |
| **g AAT/FEm** | 102±0.2 | 103±0.3 | 103±0.2 |

[1]Milk urea analysed chemically
[2]Crude protein
[3]Protein balance in rumen
[4]Amino acids absorbed in the small intestine
[5]Feed unit milk

## 4.4 Correlations

When looking at the entire D174 experiment, no significant correlation was observed between milk urea and crude protein (g/kg DM), AAT (g/kg DM), FEm (g/kg DM), and AAT/FEm. A significant positive correlation (P<0.01) was observed between milk urea levels and PBV (Table 4.6).

*Table 4.6. Pearson correlation coefficient for urea and dietary protein and energy variables in D174.*

| | **MU** | **CP (g/kg DM)** | **AAT (g/kg DM)** | **PBV (g/kg DM)** | **FEm/kg DM)** | **gAAT/FEm** |
|---|---|---|---|---|---|---|
| **MU[1]** | 1.00 | | | | | |
| **CP[2] (g/kg DM[3])** | 0.15 | 1.00 | | | | |
| **AAT[4] (g/kg DM)** | 0.02 | 0.94*** | 1.00 | | | |
| **PBV[5] (g/kg DM)** | 0.27** | 0.92*** | 0.74*** | 1.00 | | |
| **FEm[6]/kg DM** | -0.01 | 0.92*** | 0.99*** | 0.69*** | 1.00 | |
| **gAAT/FEm** | 0.05 | 0.97*** | 0.99*** | 0.80*** | 0.99*** | 1.00 |

[1]Milk urea analysed chemically
[2]Crude protein
[3]Dry matter
[4]Amino acids absorbed in the small intestine
[5]Protein balance in the rumen
[6]Feed unit milk
* p<0.05
**p<0.01
*** p<0.001

## 4.5 Dietary crude protein and milk urea levels

A significant positive correlation between CP (g/kg DM) was observed in period 1 (r=0.46, P<0.001) and period 3 (r=0.44, P<0.01), while no correlation between milk urea and CP (g/kg DM) was observed in period 2. The degree of explanation between milk urea levels and CP (g/ kg DM) was low for all periods (Figure 4.8).

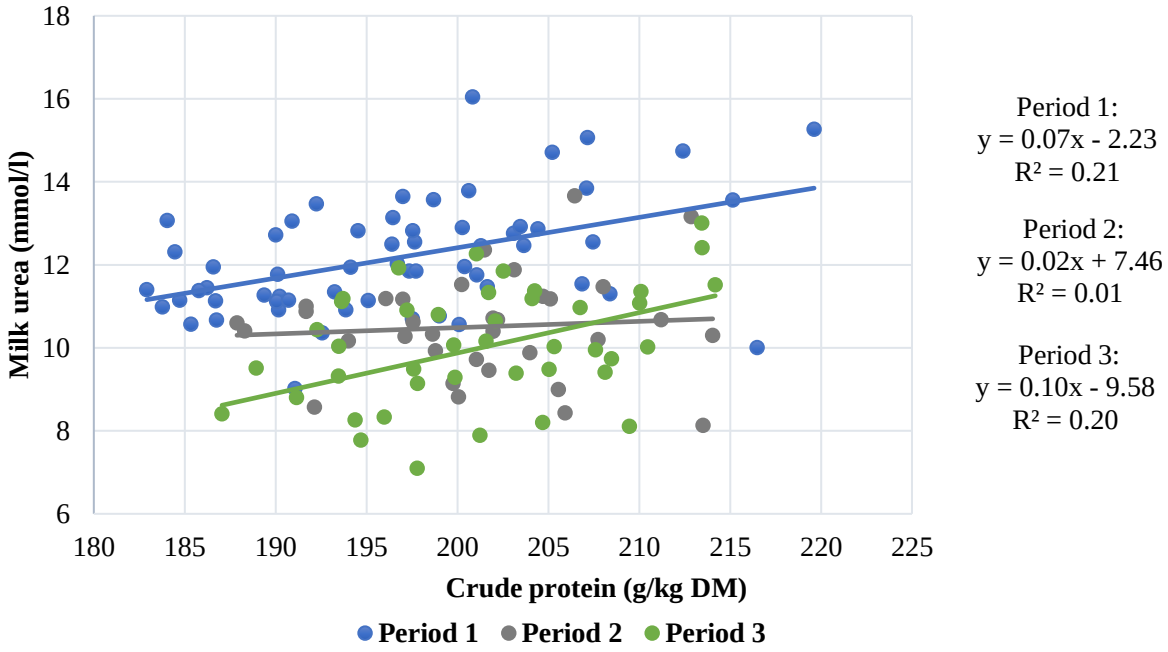


*Figure 4.8. Relationship between milk urea levels (y) and dietary crude protein (x). Period 1= 11$^{th}$ - 16$^{th}$ week of lactation. Period 2= 21$^{st}$- 26$^{th}$ week of lactation. Period 3=28$^{th}$ -33$^{rd}$ week of lactation. $R^2$= coefficient of determination.*

## 4.6 PBV and milk urea levels

A significant positive correlation between milk urea levels and PBV (g/kg DM) was observed in period 1 (r=0.54. P<0.001) and period 3 (r=0.64. P<0.001), while no correlation between milk urea levels and PBV (g/kg DM) was observed in period 2. The degree of explanation between milk urea levels and PBV (g/kg DM) was low for all periods (Figure 4.9).

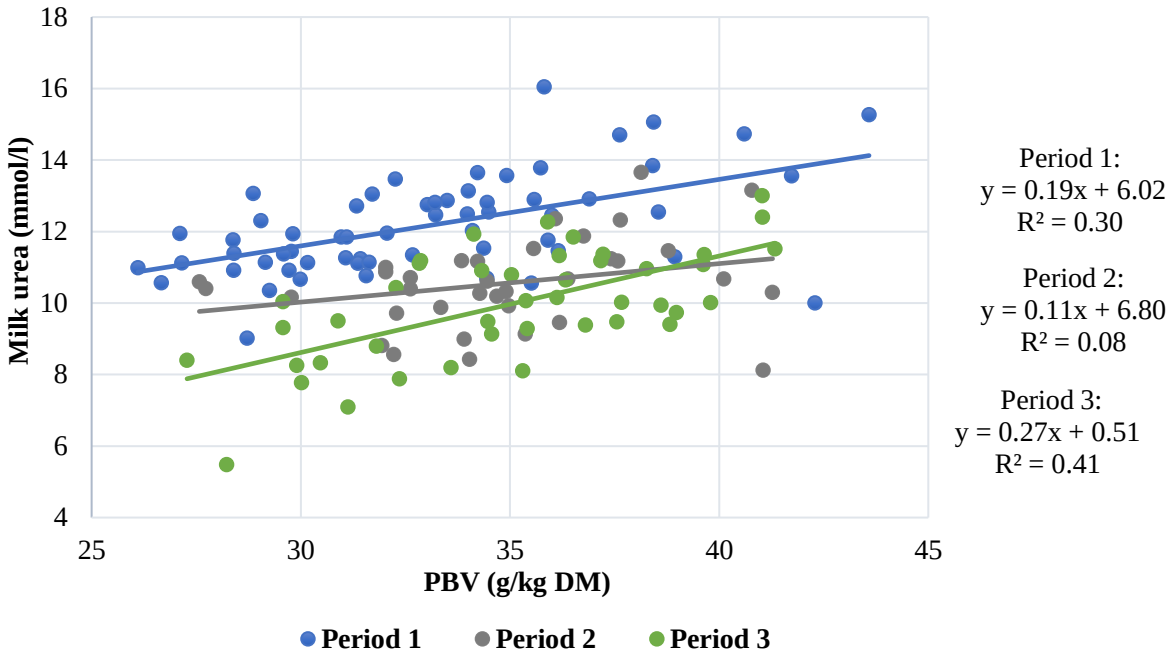


*Figure 4.9. Relationship between milk urea levels (y) and PBV (x). Period 1= 11$^{th}$ -16$^{th}$ week of lactation. Period 2= 21$^{st}$- 26$^{th}$ week of lactation. Period 3=28$^{th}$ -33$^{rd}$ week of lactation. $R^2$=coefficient of determination.*

## 4.7 AAT per FEm and milk urea levels

A significant positive correlation between milk urea levels and g AAT per FEm was observed in period 1 (r=0.48. P<0.001) and period 3 (r=0.30. P<0.01), while no correlation between milk urea levels and g AAT/FEm was observed in period 2. The degree of explanation between milk urea levels and g AAT per FEm was rather low for all periods (Figure 4.10).

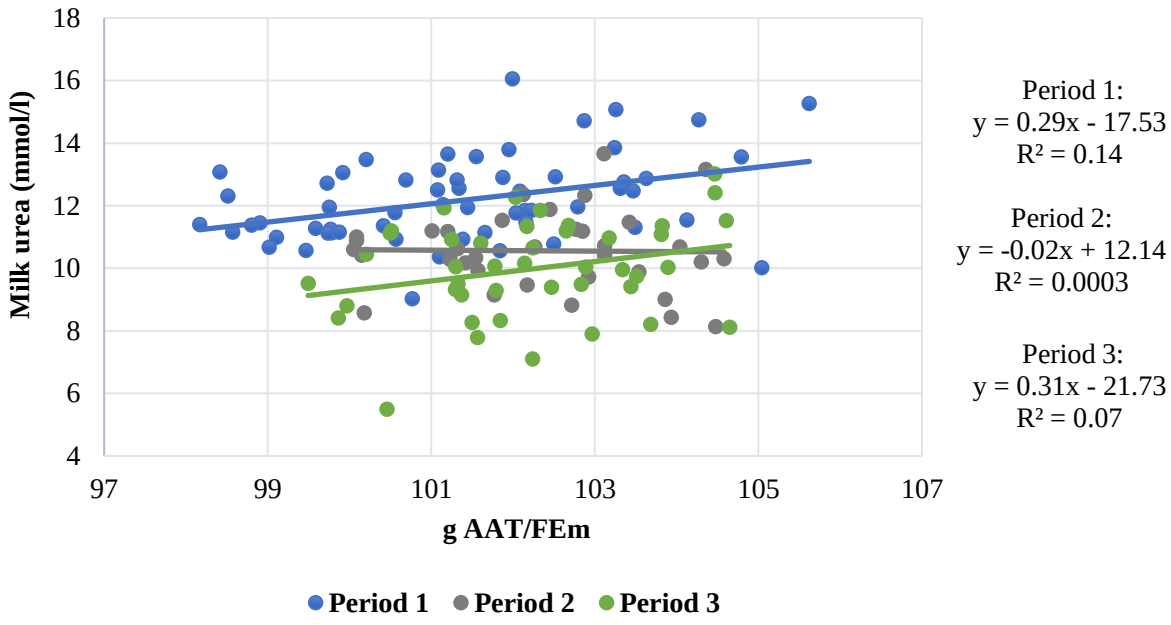


*Figure 4.10. Relationship between milk urea levels (y) and g AAT/FEm in the diet (x). Period 1= 11$^{th}$ - 16$^{th}$ week of lactation. Period 2= 21$^{st}$- 26$^{th}$ week of lactation. Period 3=28$^{th}$ -33$^{rd}$ week of lactation. $R^2$= coefficient of determination.*

## 4.8 Associations between MUL analysed chemically and by FTIR

### 4.8.1 G110

In the G110 experiment, milk urea levels analysed chemically were significantly lower (P<0.001) on all test days, compared to milk urea levels analysed by FTIR (Figure 4.11).

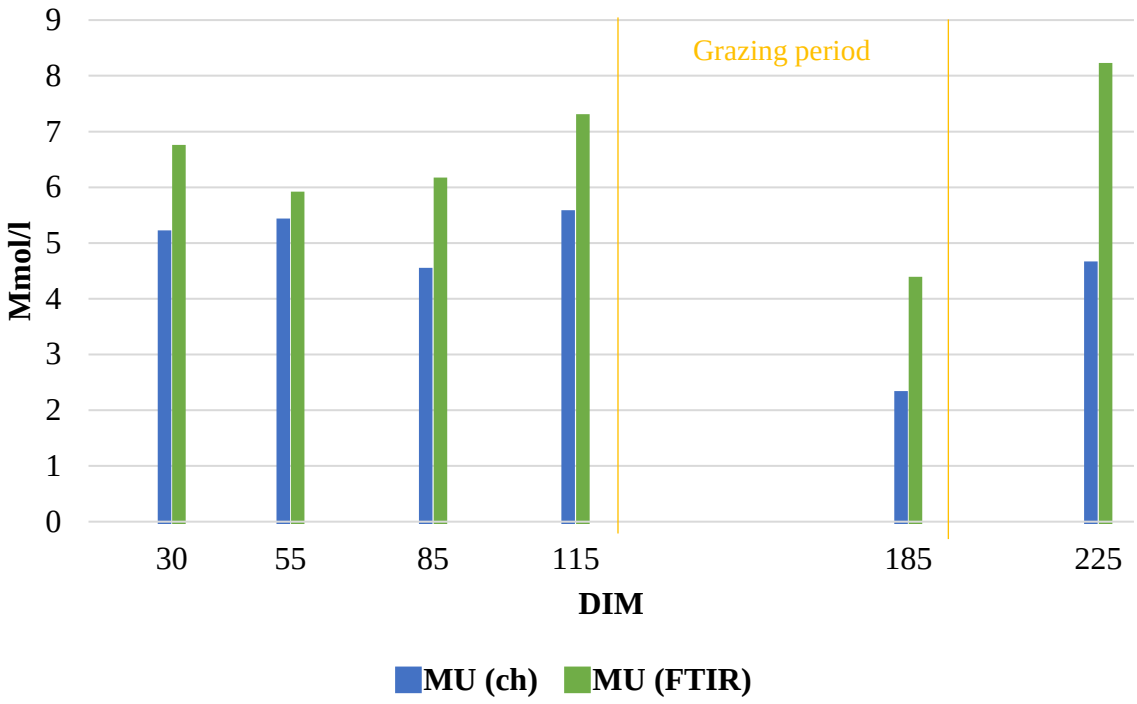


*Figure 4.11. Least square means (lsmeans) for urea levels analysed chemically (MU(ch)) and milk urea levels analysed by FTIR (MU (FTIR)) at different days in milk (DIM). Standard error (SE) for all lsmeans of milk urea=0.14. SE for all lsmeans of milk yield=0.22.*

A significant positive correlation (P<0.001) between milk urea levels analysed chemically and milk urea levels analysed by FTIR was observed when looking at the entire G110 experiment. A significant positive correlation was observed on all test days. The correlation coefficient was highest at 30 DIM (r=0.75) and lowest at 85 DIM (r=0.38) (Table 4.7).

*Table 4.7. Pearson correlation coefficient (r) between milk urea levels analysed chemically (MU(ch)) and milk urea levels analysed by FTIR (MU(FTIR)) on different days in milk (DIM).*

| DIM | G110 | 30 DIM | 55 DIM | 85 DIM | 115 DIM | 185 DIM | 225 DIM |
|---|---|---|---|---|---|---|---|
| **r (MU(ch), MU(FTIR))** | 0.63*** | 0.75*** | 0.55*** | 0.38** | 0.55*** | 0.45** | 0.72*** |

**p<0.01
*** p<0.001

The degree of explanation between milk urea levels analysed chemically and milk urea levels analysed by FTIR was rather low when looking at the entire G110 experiment ($R^2$=0.39) (Figure 4.12).

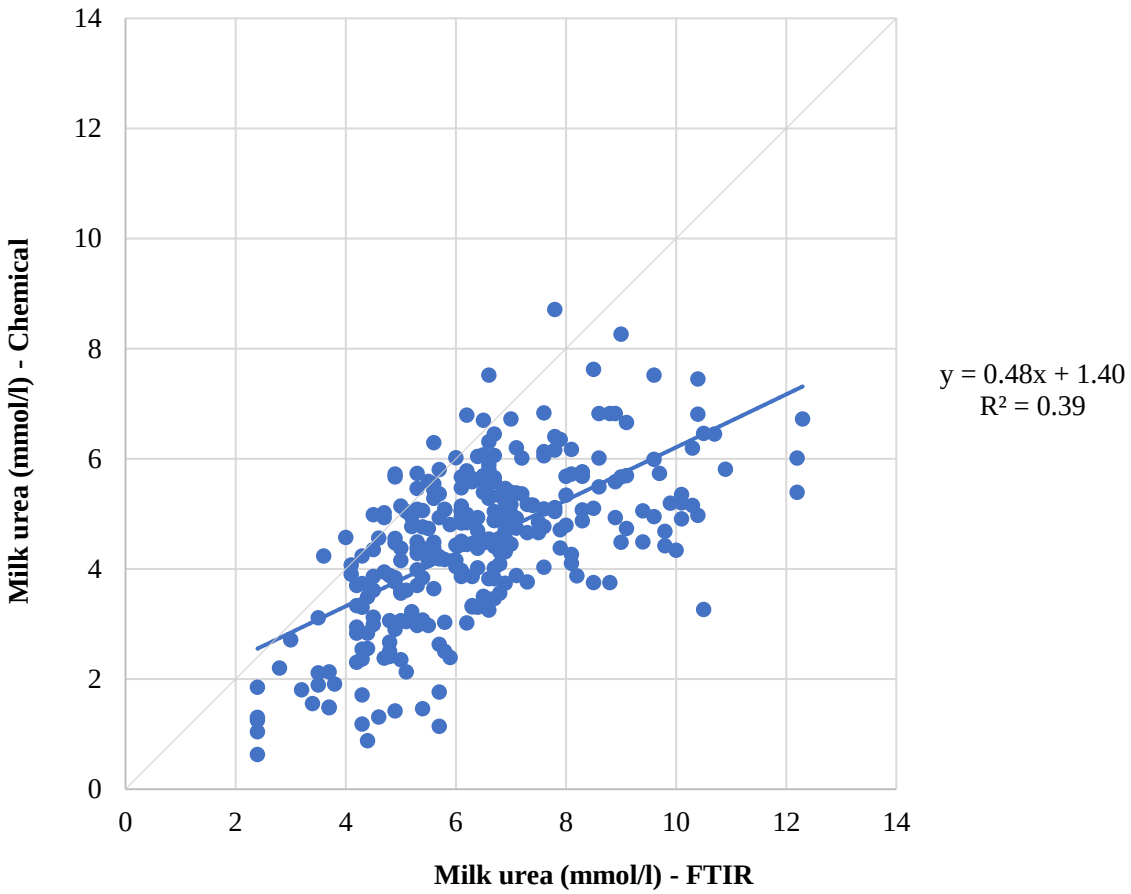


*Figure 4.12. Relationship between milk urea levels analysed by chemical method (y) and milk urea levels analysed by FTIR (x). $R^2$= coefficient of determination.*

### 4.8.2 D174

In the D174 experiment, milk urea levels analysed chemically were significantly higher (P<0.001) on all test days, compared to milk urea levels analysed by FTIR (Figure 4.13).

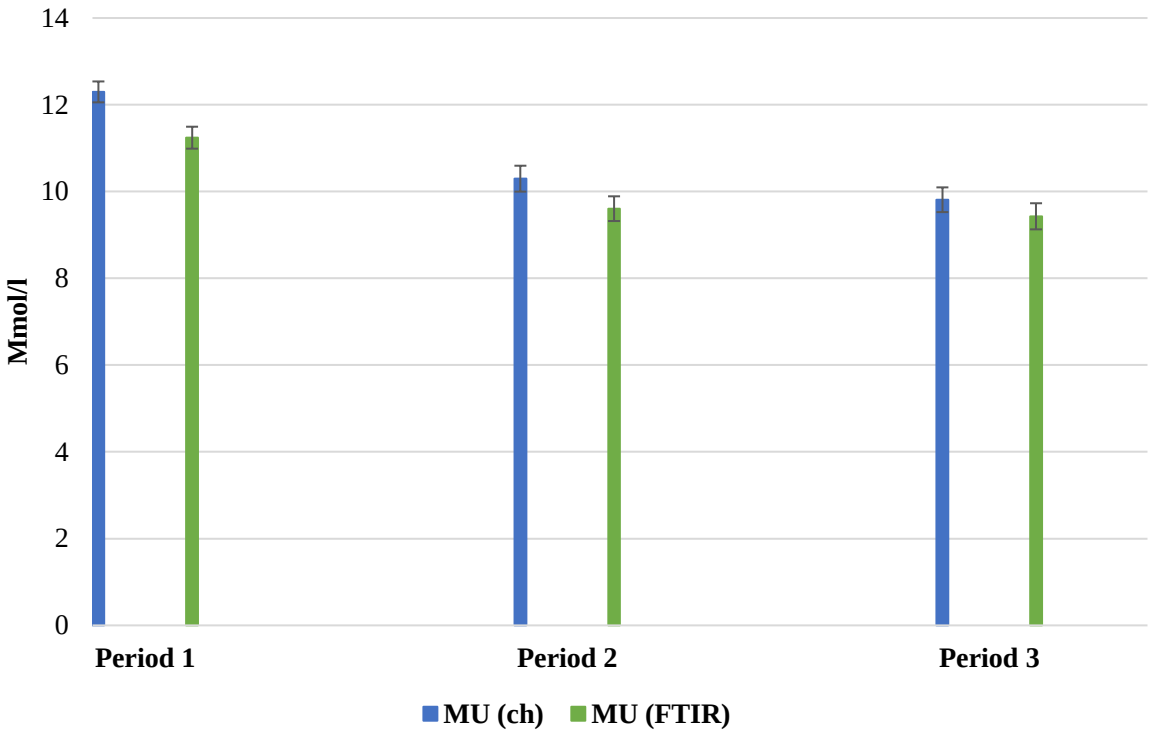


*Figure 4.13. Least square means ± standard error (lsmeans ± SE) for milk urea levels analysed chemically (MU (ch)) and by FTIR (MU (FTIR). Period 1= 11$^{th}$ -16$^{th}$ week of lactation. Period 2= 21$^{st}$- 26$^{th}$ week of lactation. Period 3=28$^{th}$ -33$^{rd}$ week of lactation.*

A significant positive correlation (P<0.001) between milk urea levels analysed chemically and milk urea levels analysed by FTIR was observed when looking at the entire D174 experiment. The correlation coefficient was higher in period 1 (r=0.62. P<0.001) and period 3 (r= 0.67, P<0.001), compared to period 2 (r=0.42, P<0.05). The degree of explanation between milk urea levels analysed chemically and milk urea levels analysed by FTIR was low when looking at the entire D174 experiment ($R^2$=0.52) (Figure 4.14).

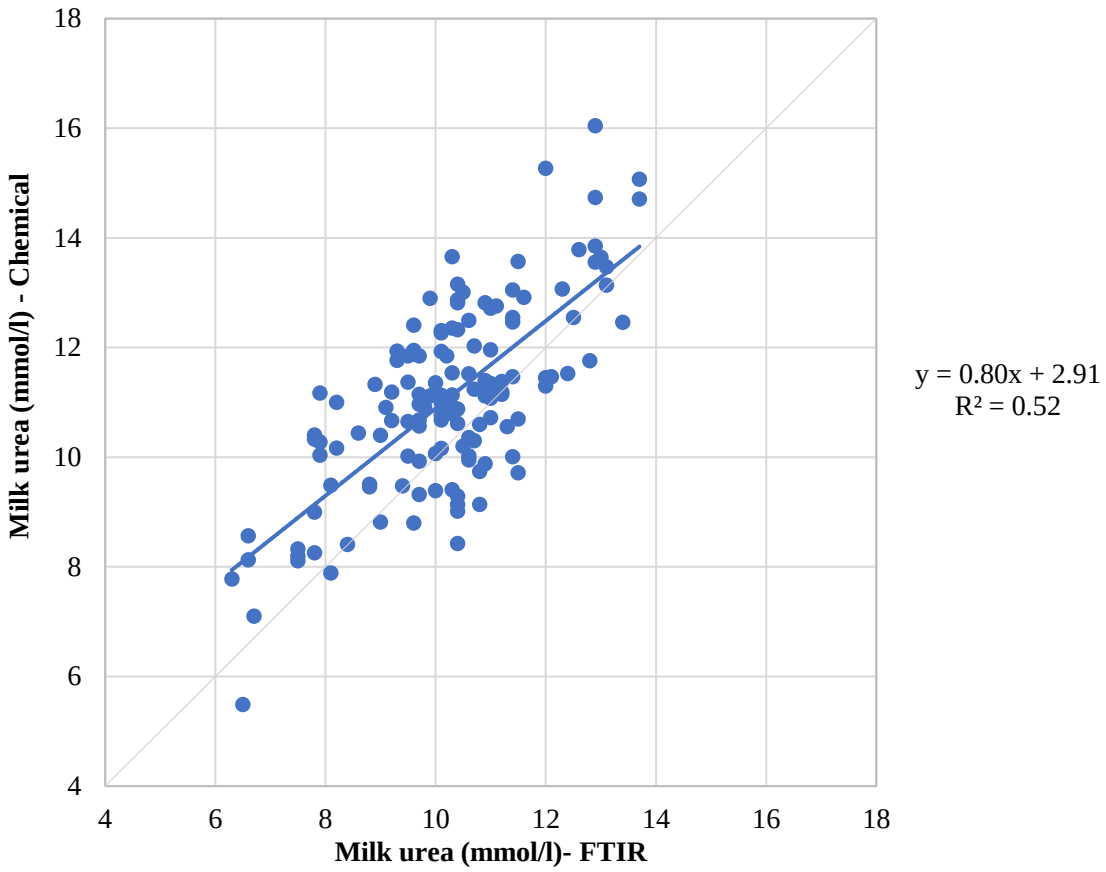


*Figure 4.14. Relationship between milk urea levels analysed by chemical method (y) and milk urea levels analysed by FTIR (x). $R^2$= coefficient of determination.*

# 5 Discussion

## 5.1 Limitation of the study

Due to a limited number of reports on urea concentration in goat milk, the results of G110 and D174 will, in addition to research done on goats, be compared to research done on dairy sheep and dairy cows. When comparing different ruminant species, one should especially be aware of differences in grazing pattern, feed selection and efficiency of nitrogen metabolization between ruminant species (Morand-Fehr & Sauvant, 1991).

No significant effect of parity on milk urea levels were found. This result is probably coloured by the fact that no first lactation goats were included in the trial of G110. Further research on the effect of parity on milk urea levels should be performed in order to achieve a better understanding of variations in milk urea levels of Norwegian dairy goats.

In order to study the effect of dietary factors, a larger variation in dietary energy and protein variables should have been present in the dataset of D174. This is probably the largest weakness of this study's methodology. The amount of dietary protein and energy in the trial of D174 were also relatively high, compared to normal feeding standards of Norwegian dairy goats. This makes the trial of D174 unsuited for studying optimal levels of milk urea within normal feeding standards. However, the results give an impression on how milk urea levels turn out at high concentrations of dietary energy and protein. In general, further research on the area should be performed if to achieve a better understanding of dietary factors affecting milk urea levels.

## 5.2 The effect of lactation stage on milk urea levels

A significant effect of DIM on milk urea levels were found in the G110 trial. A similar conclusion was reached by Giaccone et al. (2007), wherein milk urea levels were significantly affected by DIM-classes. On the other hand, Pazzola et al. (2011) did not find milk urea levels to be significantly affected by DIM alone, but rather significantly affected by the interaction *DIM x milk yield*. The variation in results of aforementioned studies indicates that milk urea levels are affected by other factors as well as lactation stages.

Previous studies report an increase of milk urea concentration from the beginning of lactation, until maximum concentration has been reached in early to mid-lactation. Antunović et al. (2017) found that milk urea levels increased from beginning of lactation until maximum

concentration were reached at 140 DIM. Giaccone et al. (2007) report similar results, showing that milk urea levels increased steadily from first day of lactation until a maximum concentration was reached at 60 DIM. In contrast, the trend of milk urea in the G110 trial fluctuated more than the aforementioned studies. A sudden drop of milk urea levels occurred at 85 DIM, before peaking at 115 DIM. A drop in milk urea levels was also found at 185 DIM, before reaching a higher concentration once more at 225 DIM. The drop of milk urea levels at 85 DIM is difficult to explain, but more frequent measurements of milk urea levels could possibly have helped identify a reasonable explanation. Regarding the drop at 185 DIM, it is likely to assume that the nutritive value of pasture affected milk urea levels. The effect of pasture will be further discussed in Chapter 5.7.

## 5.3 Associations between milk urea and milk yield

Studies done on Sarda goats showed a significant positive correlation between milk yield and milk urea levels (Pazzola et al., 2011). Likewise, studies done on Girgentana goats have also shown milk yield and milk urea to be significant positively correlated (Bonanno et al., 2008; Giaccone et al., 2007). Comparably, similar results been reported for dairy cows (Godden et al., 2001; Hojman et al., 2004; Jílek et al., 2006; Johnson & Young, 2003). In contrast, Rapetti et al. (2014) did not find any relationship between milk urea levels and milk yield for Saanen goats, nor did Volden (1997) in a study including several experiments on dairy cows, corresponding to the results obtained in G110. The discrepancy of results from different authors may be explained by variables such as feed and physiological differences. For instance, results obtained by Brun-Bellut (1997) indicated that high yielding goats could to a larger extent recycle rumen urea, compared to low yielding goats. Furthermore, Wattiaux et al. (2005) found that the association between milk yield (fat corrected) and milk urea nitrogen were different in primiparous cows, compared to multiparous cows. Oltner et al. (1985), on the other hand, found a positive correlation between milk yield and milk urea levels for dairy cows fed 18,2 g digestible crude protein per mega joule metabolizable energy (DCP/MJ ME), while no correlation between milk yield and milk urea were found for cows fed 24 g DCP/MJ ME. In the case of G110, a significant negative correlation was found at 185 DIM and 225 DIM. However, no correlation was found on other DIM. The negative correlation between milk urea levels and milk yield at 185 DIM is likely due to the fact that that milk yield was kept relatively constant, despite a conceivable low crude protein content of pasture (see Chapter 5.7). The negative correlation between milk yield and milk urea levels at 225 DIM is most likely due to a reduced milk production at the end lactation.

## 5.4 The effect of parity on milk urea levels

No significant effect of parity on milk urea levels were found in G110. In contrast, Giaccone et al. (2007) report a significant influence of parity on milk urea levels for Girgentana goats. When comparing the result of G110 against Giaccone et al. (2007), it is important to note that primiparous goats were not included in the experiment of G110. Giaccone et al. (2007) found that milk urea levels were significantly lower in primiparous goats, compared to multiparous goats, while no significant differences were found between goats in their second, third or greater lactation. The fact that no significant differences in milk urea levels were found between goats in their second, third or greater lactation, tie well with the results of G110. In general, the aforementioned results may presumably be explained by the fact that younger animals have a more efficient nitrogen metabolism due to their ongoing growth, compared to older animals (Oltner et al., 1985).

The nitrogen metabolism may also be affected by the interaction between breed an age. A retrospective study performed by Johnson and Young (2003) found that milk urea nitrogen levels were significant lower for Holstein cows in their second lactation, compared to cows in their first, third or greater lactation. In contrast, they found that MUN levels were significant lower for Jersey cows in their third or greater lactation, compared to primiparous cows. The authors further argue that the differences in milk urea levels were small and question its biological significance (Johnson & Young, 2003). However, the results of Johnson and Young (2003) vaguely indicate that the pattern of nitrogen metabolism differs between breeds of cows. One may assume that the dissimilarities between breeds of cows can be transferred to different breed of goats. When comparing the result of G110 and Giaccone et al. (2007) the factor of breed (Norwegian dairy goats VS. Girgentana goats) should therefore be taken into account. However, little available research has been done on this and further discussion is difficult to obtain.

## 5.5 Associations between blood urea and milk urea (G110)

The results of G110 showed a significant positive correlation between blood urea levels and milk urea levels on all stages of lactation. This is in accordance to the results obtained by Pazzola et al. (2011), that showed a significant positive correlation between that blood urea and milk urea levels irrespectively of milk yield. The aforementioned results tie well with reports involving other ruminant animals. Jelinek et al. (1996) found a positive correlation between blood plasma urea and milk urea levels for dairy sheep. In addition, several authors

reports similar results for dairy cows (Broderick & Clayton, 1997). In general, it seems to be an agreement among authors that blood urea and milk urea are closely correlated to each other.

A close correlation between blood urea and milk urea were expected to occur in G110, since both parameters is a product of nitrogen metabolism, hence, likely to be affected by similar variables. For instance, authors reports that both milk urea levels and blood urea levels are significantly affected by the concentration of dietary crude protein. Akhtar et al. (2020) found that the concentration of blood urea nitrogen (BUN) was significantly different between Beetal goats fed high levels of rumen degradable protein (RDP), compared to goats fed lower concentrations of RDP. Similar effect of crude protein content on milk urea levels of goats have been reported by several authors (Bava et al., 2001; Bonanno et al., 2008; Rapetti et al., 2014; Sahoo & Walli, 2008). The effect of feed parameters on blood urea levels has not been study in G110, however, the results of G110 show that both milk urea levels and blood urea levels varied between different DIM: both blood urea and milk urea were significantly higher on 115 DIM, compared to 30 DIM, 85 DIM, 185 DIM and 225 DIM. In accordance, Antunović et al. (2017) reports that both milk urea and blood urea of dairy goats differs significantly between different DIM.

## 5.6 Associations between milk urea levels and milk protein percentage

Johnson and Young (2003) found a negative correlation between milk urea levels and milk protein percentage in a study performed on dairy cows. A significant negative correlation between milk urea levels and milk protein percentages was also observed in G110, however, only on 55 and 85 DIM. This might be due to a higher distribution ratio of amino acids to the mammary gland in the beginning of lactation (Sjaastad et al., 2016). A higher distribution of amino acids to the mammary gland would further on lead to a more efficient nitrogen metabolism, where amino acids to a higher degree are utilized for synthesis of milk proteins, rather than being deaminated and transformed to urea (Madsen et al., 2003).

Seen in context with milk yield (Chapter 5.3.), both protein percentage and milk yield are expected to increase at beginning of lactation for the same reasons as explained above – increased nutrient distribution to the mammary gland in early stages of lactation (Madsen et al., 2003). The observed result of G110, does tie well with this expectation in regard to milk yield. However, the highest milk protein percentage was observed in late lactation. Partly in accordance, Fekadu et al. (2005) found that the protein percentage was higher in early and late

lactation, compared to mid-lactation in milk from an Alpine goat herd. As milk protein percentage are affected by several parameters, such as nutrition, a systemic understanding of the variation in milk protein percentage is difficult to obtain from the dataset of G110. However, the high level of milk protein percentage at mountain pasture is interesting and will be further discussed in Chapter 5.7.

## 5.7 Milk urea levels at mountain pasture

The results of the G110 showed a significant drop of milk urea levels at 185 DIM, which was in the middle of August. At 185 DIM, the goats were grazing mountain pasture. The drop of milk urea levels at 185 DIM could be due to either nutritive value of pasture, the activity level of the goats, or most likely a combination of both. However, the nutritive value of pasture was not registerer, so it is only possible to speculate on this issue. In regard to grazing activity, goats are browsers, characterized by their ability to utilize many different types of forage and select for the highest nutritive value (Morand-Fehr & Sauvant, 1991). It is therefore likely to assume that goats are willing to leave behind a longer distance in order to find pasture of best nutritive value. It is also likely to assume that the nutritive value, especially the crude protein content, of pasture was lower in the middle of August, compared to earlier stages of grazing season. Consequently, the goats most likely left behind a longer distance in the middle of August, compared earlier stages of grazing period. An increased activity level would again lead to an increased requirement of nitrogen for work. A combination of reduced crude protein supplementation and a more efficient utilization of nitrogen might explain the drop of milk urea levels at 185 DIM. The aforementioned assumptions are in accordance with Eide (1999): in a study performed on goats grazing in Einundalen, Eide (1999) reported an increased activity level of goats in late summer, compared to early summer. A reduced uptake of FEm and AAT in late summer, compared to early summer was also observed. The study of Eide (1999) was performed in the same grazing area as utilized in G110. The nutritive values of pasture might differ between G110 and due to annually differences in climate, however, an increased activity level and reduced nutritive value of pasture seems to be a likely explanation of the drop in milk urea at 185 DIM.

Despite a drop of milk urea levels, no significant reduction in milk yield and milk protein percentage was observed at 185 DIM. More precisely, a small peak in milk protein percentage was found at 185 DIM, while the milk yield were kept relatively stable. If the nutritive value of pasture did not cover the goat's requirement for milk production, the goat's energy

reservoir as well as body proteins must have been catabolized in order to prevent a drop in milk production. The goat's condition score was not registered in G110. However, the results of Eide (1999) showed that the goat's condition score was lower in late summer, compared to early summer. If a conceivable reduction in body condition score during grazing season also occurred in G110, one may assume that a relatively stable milk yield and an increased milk protein percentage was obtained at 185 DIM due to utilization of body reservoirs.

## 5.8 Effect of dietary energy and protein levels

### 5.8.1 Differences between periods

In general, a higher correlation and degree of explanation between feed parameters and milk urea was found in period 1 and period 3, compared to period 2. It is hard to explain the aforementioned differences, due to the relatively similar nutritive values in all periods. The dietary content of protein and energy were kept relatively similar for all periods, and the goats were housed and treated similarly in all periods. The differences might however be due to high concentrate levels leading to instability of physiological homeostasis and rumen environment (Sjaastad et al., 2016). A potential variation in milk urea levels of goats in instable homeostasis should be further studied, in order to reach a clear conclusion.

### 5.8.2 Associations between milk urea levels and PBV and AAT/FEm

A significant positive correlation between milk urea and PBV concentration was found in period 1 and period 3. The aforementioned findings of period 1 and period 3 were expected, due to the close associations between PBV, protein utilization in the rumen and milk urea levels (Madsen et al., 1995; Volden, 2012). The results of period 1 and period 3 ties well with results obtained by other authors. In a study including several experiments on dairy cows Volden (1997) found that PBV was significantly correlated to milk urea levels. Volden (1997) also found that PBV was the single variable explaining most of the variation in milk urea levels. The latter, differs slightly from the results observed in period 1 and period 3, where the degree of explanation was lower than reported by Volden (1997).

A significant positive correlation was found between milk urea levels and g AAT/FEm in period 1 and period 3. The aforementioned findings of period 1 and period 3 were expected, due to the close associations between the metabolization of amino acids and milk urea levels (McDonald et al., 2011; Sjaastad et al., 2016). In a study including several experiments on dairy cows, Volden (1997) found that milk urea levels were affected by AAT/FEm. Volden

(1997) further on conclude that observed results indicate that AAT not utilized for synthesis of milk protein are deaminated in the liver and transformed to urea. In general, not many studies have been done on the effect of AAT/FEm on milk urea levels. However, several studies have observed the effect of the ratio between crude protein and dietary energy, expressed by variables comparable to AAT/FEm and PBV. In a study done on cows, Oltner and Wiktorsson (1983) found that the amount of crude protein per mega joule metabolizable energy (CP/MJ ME) in the diet explained 94% of the variations in milk urea levels in dairy cows, supporting the correlation between AAT/FEM and milk urea levels, as well as the correlation between PBV and milk urea levels in period 1 and period 3.

In general, both milk yield and milk protein percentage is known to be positively affected by the balance between dietary energy and AAT (Madsen et al., 2003). However, the utilization of dietary AAT to milk production has a declining dividends for increased levels of AAT/energy-ratio, something that is accounted for in the feeding standard of Norwegian dairy cows (NorFor), but not directly in the AAT/PBV-system (Volden, 2006). In the case of D174, a high level of AAT/FEm was present. For further study, it would have been interesting to investigate the context between milk urea levels and utilization of AAT for different levels of AAT/FEm.

### 5.8.3 Association between dietary crude protein and milk urea levels

In a study performed on Girgentana goats on pasture, Bonanno et al. (2008) reported a significant positive correlation between milk urea levels and the dietary crude protein percentage. Bonanno et al. (2008) also found that among crude protein percentage, dietary NDF content, milk yield (fat corrected milk), dry matter intake, and NDF-intake, the crude protein percentage was the single variable explaining most of the variation in milk urea nitrogen ($R^2$=0,76). In a study performed on cross-bred goats (Beetal x Alpine and Beetal x Sannen) Sahoo and Walli (2008) found that the milk urea concentration was significantly reduced when the amount of undegradable protein in diet increased and the amount of rumen degradable protein was reduced. Laudadio and Tufarelli (2010) also found that the concentration of milk urea nitrogen was different between Jonica goats fed high levels of rumen degradable protein and Jonica goats fed low levels of rumen degradable protein. In accordance with aforementioned studies, the results of D174 indicate that milk urea levels are related to the dietary crude protein content. A positive significant correlation between milk urea levels and crude protein and the share of crude protein in diet were found in period 1 and

period 3. However, the small variance in crude protein content through the entire experiment might explain the fact that no significant correlation was found between milk urea level and the share of crude protein content when looking at the entire experiment.

The aforementioned findings of dietary crude protein's effect on milk urea levels is supported by the fact that a significant difference in milk urea levels were found between the results of D174 and G110, where the largest values of milk urea analysed chemically was found in D174. The discrepancy in milk urea levels is likely to be caused by a higher crude protein content in D174, compared to G110.

## 5.9 Analysis of urea in goat milk: Chemical analysis versus FTIR-analysis

The results of G110 showed that milk urea levels analysed by FTIR were higher than milk urea levels analysed by chemical analysis. The two methods showed especially high discrepancy at 225 DIM. In contrast, the results of D174 showed that milk urea levels analysed by FTIR were lower than those analysed chemically. In addition, the numerical differences between chemical analysis and FTIR analysis were larger in G110, compared to results of D174. The discrepancy within and between G110 and D174, indicates that the FTIR method is not calibrated well in regard to urea levels in goat milk. This tie well with the conclusion of Schei (2003), who found that FTIR methods and chemical methods were not satisfactory associated. Similar to the results of G110, Schei (2003) found that the differences between methods were especially pronounced in the last months of lactation. Schei (2003) further on conclude that better calibration of FTIR in regard to goat milk is necessary if to be used in the goat milk industry.

# 6 Conclusion

This study demonstrates that milk urea levels of Norwegian dairy goats are associated to physiological status, as well as dietary energy and protein measures. A significant effect of lactation stage on milk urea levels were found, probably linked to variation of energy distribution and protein turnover through lactation. This interpretation is strengthened by the findings of milk urea to be negatively correlated to both milk yield and milk protein percentage at some stages of lactation. The results indicate that milk urea levels are affected by CP (g/kg DM), PBV (g/kg DM), and g AAT/FEm. The discrepancy between milk urea levels analysed by chemical methods and by FTIR-analysis, indicates that the FTIR-instrument is not well-calibrated regarding milk urea levels of Norwegian dairy goats.

The findings of this study support that further focus should be directed towards recognizing factors influencing variation in milk urea levels of Norwegian dairy goats. The fact that available methods of milk urea analysis are either time consuming or not well-suited for Norwegian dairy goats, makes it hard to investigate the variations in milk urea levels of Norwegian dairy goats. In order to increase further research feasibility, a better calibration of FTIR-instrument is appropriate. With improved methods of milk urea analysis and further research, milk urea levels have a great potential to be used as an indicator of efficiency in protein metabolization of Norwegian dairy goats. Having a clear metric of efficiency in protein metabolization of Norwegian dairy goats, would be an important contributor concerning both economy in production, as well as issues related to Norway’s self-sufficiency and reduced environmental pollutions.

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