Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Veterinary Medicine and
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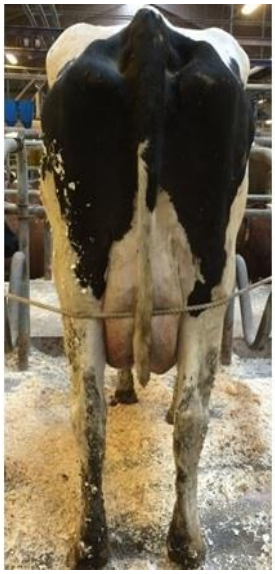


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Abstract

A feeding trial investigating the effect of a concentrate diet consisting only of cereals on milk production, protein utilization and nitrogen efficiency was conducted on Swedish dairy cows. 13 SRB and 12 SH cows were randomly assigned to one of two groups: group 1 was fed a concentrate feed (Cereal), mainly consisting of barley (36%), wheat (34%), oats (25%) as well as molasses (2%) and a mineral and vitamin mix (3%), whereas group 2 had access to both Cereal and a protein rich concentrate feed (Prot) based mainly on soya bean (51%) and rapeseed cake (28%). A high quality grass silage (energy 11.4 MJ ME, CP 14.8%) was fed *ad libitum*, and during summer months the cows were kept on pasture, where the pasture allowance was sufficient for maintaining the production level. The cows were fed according to Swedish regulations for organic production. Milk production data as well as BCS and LW were obtained for the entire lactation, whereas blood samples and urine spot samples were collected for the first three months of lactation. Total CP content in the feed ratio was 14.0% and 16.4% for group 1 and 2, respectively. Group 1 had lower CP and energy intake, whereas the total intake and starch intake did not differ between the groups. The concentrate intake was higher in group 2 at all stages of lactation, whereas the roughage intake was higher in group 1 in mid-lactation. Total ECM production over the whole lactation did not differ between the groups, being 9760 and 9706 kg for group 1 and 2, respectively. Daily milk yield did not differ between the two groups either, being 30.5 kg/day and 31.7 kg/day for group 1 and 2, respectively. Neither did the daily ECM yield, milk fat nor protein concentrations differ between the groups. The lactose content in milk was higher in group 1 (4.78%) compared to group 2 (4.71%). When MY and ECM were grouped by early, mid and late lactation stage, group 2 achieved higher daily MY in early lactation while group 1 had higher ECM yields in late lactation. N-efficiency was higher in group 1 (36.5%) compared to group 2 (32.0%). Milk urea concentration was lower in group 1 (3.85 vs. 4.27 mmol/l), as was the daily UUN excretion (66.08 vs. 150.58 g/d). Plasma histidine concentration differed between the groups, being 34.96 and 47.7 nmol/ml in group 1 and 2, respectively. All in all, the results obtained in this study indicates that high producing dairy cows can be fed with a diet consisting only of silage and cereal concentrate without any significant reductions in production levels. Further, an increased nitrogen efficiency as well as a more persistent lactation can be seen as a beneficial effect of the used diet and the reduced CP content in the dairy cow diet.

Sammanfattning

Ett utfodringsförsök genomfördes på svenska mjölkkor för att utforska effekten av ett kraftfoder som består enbart av spannmål på mjölkavkastning och protein utnyttjandet. 13 SRB och 12 SH kor delades i två grupper: grupp 1 utfodrades med ett kraftfoder (Cereal) som innehöll korn (36%), vete (34%), havre (25%) samt melass (2%) och en mineral och vitamin mix (3%), medan grupp 2 hade tillgång på både Cereal-fodret och ett proteinrikt kraftfoder (Prot) som huvudsakligen bestod av sojabönor (51%) och rapskaka (28%). Korna hade fritillgång på ett ensilage av högkvalitet, och under sommarmånader var de på bete där betestillgång var tillräcklig för höga betesintag. All utfodring var utformad enligt KRAVs regler för ekologisk produktion. Produktionsdata samt hullbedömningar och viktdata insamlades för hela laktationen medan blod- och urinprover samlades in för 3 första månader av laktationen. Totala råprotein innehållet i foderstaten var 14.0% respektive 16.4% för grupp 1 och 2. Råprotein och energi intaget var lägre i grupp 1, medan totala intaget samt stärkelse intaget var likadana mellan de två grupperna. Kraftfoder intaget var högre i grupp 2 i alla laktationsstadier medan grovfoder intaget var högre i grupp 1 i mitten av laktationen. Totala ECM produktion över hela laktationen skiljde sig inte mellan grupperna, 9760 kg respektive 9706 kg, för grupp 1 och 2. Inte heller mjölkavkastningen i kg mjölk, 30.5 kg/dag och 31.7 kg/dag för grupp 1 respektive 2 , fetthalten eller proteinhalten skiljde sig mellan grupperna. Laktoshalten var däremot högre i grupp 1 (4.78%) jämförd med grupp 2 (4.71%). När mjölkavkastning och ECM avkastning grupperades efter tidig, mitten, och sen laktation, hade grupp 2 högre mjölkavkastning i tidig laktation medan grupp 1 hade högre ECM avkastning i sen laktation. N-effektivitet var högre i grupp 1 (36.5%) jämförd med grupp 2 (32.0%). Koncentration mjölkurea var lägre i grupp 1 (3.85 vs. 4.27 mmol/l), liksom den dagliga utsöndring av UUN (66.08 vs. 150.58 g/d). Halten histidin i plasma skilde sig mellan grupperna, och var 34.96 respektive 47.7 nmol/ml för grupp 1 och 2. Resultaten från denna studie tyder på att högproducerande mjölkkor kan utfodras med bara spannmål och vall foder utan minskningar i produktionsnivåer. Det ger dessutom ökad N-effektivitet och mer uthållig mjölkavkastning mot slutet av laktationen.

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1. Introduction

The current situation with the changing climate and decreasing access to land and recourses creates an urgent need to find and develop new ways for ensuring a sustainable and secure food production in the future world (Eisler *et al.*, 2014). It is crucial to utilize the available resources efficiently and wisely so that over-utilization and wasteful use of resources can be avoided (Röös *et al.*, 2016). Especially in animal production, which has been increasingly questioned and criticized in the light of climate change and decreasing access to land and food, use of resources and use of human edible feeds (especially protein sources) should be reconsidered and reevaluated (Korhonen *et al.*, 2000; Wilkinson, 2011; Ertl *et al.*, 2016). Interestingly, already in 1960s there were concerns on whether in the future the production of animal protein would be possible in an overcrowded world (Virtanen, 1966).

To produce milk, and more importantly, to achieve high yields, and thereby exploiting the whole production potential of modern cows, high inputs of feed with high protein contents have become a common practice in dairy farming (Eastridge, 2006; Aguilar *et al.*, 2012). However, many studies have shown that high inclusion of protein in the diets of dairy cows leads to a decreased nitrogen (N) efficiency (the ratio between N content in the diet and N in produced milk) and increased excretion of N via urine and milk (Colmenero and Broderick, 2006; Huhtanen and Hristov, 2009; Broderick *et al.* 2015; Hristov *et al.*, 2016). A significant part of the dietary N is thereby lost, in both environmental and economic terms, leading to decreased production efficiency of dairy systems (Børsting *et al.*, 2003). High-input also means high-costs, and the increase in milk production is often not covering the extra costs of high protein supplementation even though the production might seemingly be higher (Colmenero and Broderick, 2006).

This is of special interest for organic dairy producers, as the protein concentrates (especially soya) that are produced according to the regulations for organic production are not as readily available, and therefore are considerably more expensive to purchase (Heimer, 2009). The high prices, limited production of protein feeds as well as strict regulations make it difficult to achieve a profitable production on organic dairy farms that strive for high production levels, and thereby high inputs and high inclusions of protein concentrates in the feed ratios.

In Swedish dairy production (both conventional and organic) protein supplements are for the most part fed in form of soya bean meal and rapeseed meal. All of the soya is imported, mainly from Brazil, whereas the rapeseed is both produced in Sweden and imported from

other European countries (Heimer, 2009; Gustafsson *et al.*, 2013). The importation of soya is a multifactorial issue; importing a protein feed all the way from Brazil to be consumed by dairy cows in Sweden causes deforestation and loss of rainforest as well as the emissions of the transportation, and also includes the question of feeding the animals with a high quality protein source that could be consumed by humans (preferably in Brazil) (Gustafsson *et al.*, 2013). Further, the use of resources becomes unbalanced when a great amount of the resources in one part of the world are used to produce protein for human consumption in another country. In other words, high amounts of protein are produced in Brazil to produce relatively small amounts of protein in other countries, such as Sweden, and a great part of the plant protein that is being converted to milk protein is lost during the process and excreted as urinary urea N (UUN) and milk urea N (MUN), and will not be utilized either by the animals or by humans (Virtanen, 1966). Therefore, exploring and investigating new options for more efficient as well as economically and environmentally sustainable ways of feeding dairy cattle is of great interest for dairy farmers and advisors as it is for the general public as consumers of the produced product (Gustafsson *et al.*, 2013).

The aim of the study was to investigate whether high producing dairy cows can be fed with a low protein diet based solely on cereals and silage without any significant reductions in production levels over a whole lactation. Further, the N-utilization and efficiency was investigated. The hypothesis was that cows fed without protein supplements from the onset of lactation would utilize the available protein in the forage and cereal more efficiently, resulting in a higher N-efficiency and lower excretion of N, and with no reduction in milk yield.

2. Literature review

2.1 Ruminal digestion and metabolism of dietary protein

When considering the nutrient demands of a ruminant it is important to keep in mind that the consumed feed will not only provide nutrients and energy for the animal but a significant part of the dietary protein and carbohydrates are ingested by rumen bacteria (McDonald *et al.*, 2011; Patton *et al.*, 2014). This balance between the two different but nonetheless linked nutrient requirements of ruminal microbes and the host animal complicates the establishment of optimal ratio of N to energy in the diet formulation for a cow (Clark *et al.*, 1992). It is crucial to take into account the rumen metabolism and processes involved in protein

metabolism when discussing the feeding ratios and protein requirements of a ruminant, as a significant part of a ruminant's amino acid (AA) requirements are provided by microbial protein synthesis (Russel *et al.*, 1992). In this section, protein metabolism in ruminants in the light of ruminal protein degradation and synthesis as well as N-metabolism will be reviewed. An overview of protein metabolism in a lactating ruminant is shown in figure 1.

2. 1.1 Microbial protein degradation and synthesis

As mentioned above a notable amount, around 50-80% (which equals to around 525-2888 g per day), of the AAs absorbed in the small intestine of a ruminant are provided by microbial protein synthesis in the rumen, while the remaining 20-50% originates from rumen ungradable proteins (RUP) (figure 1; Clark *et al.*, 1992; Kung and Rode, 1997; Bach *et al.*, 2005; Patton *et al.*, 2014). The amount and quality of the microbial protein produced is highly variable and depends to a great extent on the nutrient availability and the efficiency of the bacterial use of these nutrients (Clark *et al.*, 1992; Bach *et al.*, 2005; Patton *et al.*, 2014). Hence, it is difficult to predict the quality and amount of absorbed AA in ruminants (Kung and Rode, 1997).

There are several factors affecting the protein degradation and synthesis in the rumen, and thereby the availability of AA for milk production, including feed intake, the type of dietary protein (rumen degradable (RDP) or RUP), interactions with other nutrients, such as carbohydrates (and energy), as well as the microbial population in the rumen, which in turn is affected by the feed ratio, and thereby ruminal passage rate and pH (Clark *et al.*, 1992; Bach *et al.*, 2005).

Nutrient intake and balance in the diet

Both carbohydrates and proteins can be used as energy sources by rumen bacteria, carbohydrates being the main source of energy (Russel *et al.*, 1992; Bach *et al.*, 2005). When considering the nutritional needs of rumen microbes, the main aim is to maximize microbial growth and the amount of RDP captured into rumen microbial cells, which in turn improves the microbial AA supply to the small intestine (Bach *et al.*, 2005).

Increased amounts of readily fermentable carbohydrates (most commonly starch) in the diet improves the N uptake by the ruminal microbes as these carbohydrates are more effective

source of energy for microbial protein synthesis compared to other types of carbohydrates, for example cellulose (Huntington, 1997; Bach *et al.,* 2005). Indeed, Broderick (2003) observed a linear increase in allantoin (a substance produced when microbes are digested) excretion in milk with increased amounts of non-fiber carbohydrate in the diet (from 64% to 72%), indicating increased microbial protein synthesis.

On the other hand, too high inclusions of concentrate feeds may lead to unpaired rumen function, as the energy is released much faster than the ruminal bacteria can capture it for growth (Clark *et al.,* 1992; Russel *et al.,* 1992; Broderick, 2003). However, diets with high forage content may decrease the quantity and efficiency of microbial protein passage to the intestine because this type of diet may have too little amounts of available energy for the rumen microbes, which slows down the growth of microbes. Also, microbes attach to the larger forage particles, and thereby the outflow of microbes from the rumen will decrease. This in turn increases the recycling of energy and N in the rumen leading to a larger part of the energy to be used for maintenance rather than growth by the rumen microbes (Clark *et al.,* 1992; Russel *et al.,* 1995). Hence, a more efficient microbial growth is achieved by including a mixture of forage and concentrate in the diet: the concentrate provides more rapidly available energy for the microbes while the forage contributes to a more efficient and balanced fermentation providing a more uniform release of energy over time (Clark *et al.,*1992).

Not only the energy but also dietary protein is of great importance when considering the efficiency of microbial protein synthesis. In ruminants, the consumed crude protein (CP) contributes to both N for microbial growth in the rumen as well as to AA for maintenance and milk production for the cow (Clark *et al.,* 1992). Both the proteolytic activity of rumen microbes and the type of dietary protein affect the rate and extent of protein degradation in rumen. When energy is not limiting, AA will be used for microbial protein synthesis, but if sufficient energy is not available, AA will be deaminated and the carbon skeleton will be used for volatile fatty acid (VFA) formation (Bach *et al.,* 2005). The degradation rate of protein increases with increasing CP content in the feed (Colmenero and Broderick, 2006). Argyle and Baldwin (1989) noted that growth of ruminal bacteria is not only stimulated by increased carbohydrate fermentation, but that it is also greatly enhanced by dietary peptides and AA. Further, this stimulation of growth is more dependent on the variety of available AA for bacteria in a given mixture rather than on a specific growth limiting AA (Argyle and Baldwin, 1989).

Also non-protein nitrogen (NPN), which include free AA, urea, ammonia, amines, nitrates and nitrites as well as small peptides, contributes to microbial protein synthesis (Russel *et al.,* 1992; Patton *et al.,* 2014). Dietary NPN is rapidly degraded and converted to ammonia when entering the rumen (figure 1). The role of NPN will be discussed in detail later on as a part of ammonia utilization.

All in all, it is important to consider the synergy of energy and N utilization by the ruminal microbes and by the tissues of the cow. In Nordic feed evaluation this balance between dietary energy and protein has been addressed as the protein balance in the rumen (PBV) with a recommended levels of 0±300 g/kg DM, where the optimal value is 0 (Spörndly, 2003; Børsting *et al.,* 2003). A positive value indicates an overfeeding of dietary CP in relation to energy, whereas a negative value indicates the opposite. Further, it should be kept in mind that not only the quantity but also the quality of both dietary proteins and carbohydrates are crucial for an optimal microbial growth and rumen function, and thereby even the N-utilization. While high inclusions of RUP in the diet might increase the amount of AA absorbed in small intestine (AAT), and thereby providing a more accurate estimations of the quality and quantity of protein utilized by the cow, the available AA for microbes (RDP) might not be sufficient for an efficient microbial growth in diets with a high RUP content, which in turn will affect the rumen fermentation negatively (Nadeu *et al.,* 2007) leading to suboptimal rumen fermentation, decreased energy availability and passage rate.

This balance between energy and AAT/PBV have been taken into account and further developed in the new feed evaluation system (NorFor) that has been introduced in the Nordic countries (Volden and Nielsen, 2011). In this system, the composition of the feed ratio, the feed intake of the cow as well as the interaction between the animal and the feed in terms of digestibility and microbial activity are taken into account, thereby allowing a more accurate balancing of the ratio for the lactating cows (Volden and Nielsen, 2011).

2.1.2 Ruminal ammonia utilization and urea formation

Ammonia in the rumen

Another important aspect of protein metabolism in ruminants, closely linked to microbial protein synthesis and dietary nitrogen utilization, is the formation of ammonia nitrogen in the rumen (see figure 1). The ammonia is the main intermediate in the microbial degradation and synthesis of protein. The amount of rumen ammonia is dependent on the amount of protein in

the diet, as well as on the degree and rate of ruminal protein degradation (Russel *et al.,* 1992; McDonald *et al.,* 2011).

Rumen microbes can convert NPN to protein-N, and use it for microbial protein synthesis (Dijkstra *et al.,* 2011). Early work of Virtanen (1966) demonstrated how the rumen microbes of lactating dairy cows fed a diet where the protein was replaced entirely by urea and ammonium salts were able to synthetize all the protein AAs, and that those AAs were subsequently used for milk protein synthesis. However, the microbial growth rates on ammonia are lower compared to the growth rates when AA and peptides are available, and the use of ammonium-N is not as efficient as the use of N from AA and peptides as was found by Argyle and Baldwin (1989). A more recent work by Broderick and Reynal (2009) further confirmed this, as a stepwise replacement of RDP from soya beans with RDP from urea showed a linear decrease in omasal flow of microbial non-ammonia-N (NAN) (from 440 g/d to 342 g/d when the urea concentration in the diet was increased from 0% to 3.7%) and microbial growth efficiency (by ~16%). Hence, illustrating a depression in the microbial protein synthesis in the rumen (Broderick and Reynal, 2009).

Nonetheless, ammonia-N is needed for an optimal microbial growth as some of the cellulolytic bacteria use only ammonia as N-source (Russel *et al.*, 1992), although the concentration at which the optimal growth is achieved is debatable (Calsamiglia *et al.,* 2010). Relatively high ammonia concentrations above 5 mmol/l will increase the rumen organic matter digestibility (OMD) by stimulating the cellulolytic bacteria, but unavoidable N-losses as ammonia absorption through rumen wall will occur at high concentrations of ammonia-N (Calsamiglia *et al.,* 2010; Spek *et al.,* 2013). Indeed, Huhtanen *et al.* (2015) noted that increased rumen ammonia concentration increased the OMD, but that the optimal rumen ammonia concentration for OMD seems to be higher than that of optimal microbial growth. On the other hand, increased OMD in turn leads to an increased availability of carbohydrates for the rumen microbes. This improves the N uptake by the ruminal microbes, subsequently leading to decreased concentrations of ammonia-N (Huntington, 1997; Bach *et al.,* 2005). Therefore, when considering the optimal ammonia-N concentration for microbial growth the above discussed balance between AA and energy supply for rumen microbiota needs to be taken into account (Argyle and Baldwin, 1989; Calsamiglia *et al.,* 2010).

Urea formation, excretion and recycling

As mentioned above, accumulation of ammonia in the rumen leads to its absorption to the blood circulation, and transportation to the liver, where it is converted to urea (figure 1). Indeed, most of the urea formation in the liver occurs as a process of detoxification of ammonia from the circulation (Spek *et al.*, 2013), but even excess of absorbed AA and peptides in the small intestine are deaminated and converted to urea, and thereby urea synthesis is proportional to the balance of dietary N, hence the ammonia formation in the rumen, and the use of N in animal tissues (figure 1; Baker *et al.*, 1995; Aguilar *et al.*, 2012).

The urea formed in the liver is then transported in blood plasma as plasma urea nitrogen (PUN) diffusing and being transported to other fluid pools such as milk in the udder or is recirculated to the rumen (figure 1; Spek *et al.*, 2013). The recirculated urea enters the ruminal ammonia pool getting a second chance to enhance the microbial growth and protein synthesis. Decreased dietary protein intake increases the nitrogen recirculation to the rumen (Russel *et al.*, 1992). A recent study showed that lowering CP content in the diet (from 17.1% to 12.9%) increases the blood urea recirculation from blood to rumen compensating for the lower availability of dietary protein and thereby maintaining a relatively unchanged microbial protein synthesis even at low dietary CP-levels (Kristensen *et al.*, 2010).

Urea excretion occurs mainly via urine as urinary urea nitrogen (UUN), and most of the PUN is eliminated by the urinary N excretion, but a notable amount of urea is also excreted to milk as milk urea nitrogen (MUN) (see figure 1). It is a well-known fact that increases in N intake leads to a subsequent increase in both UUN and MUN concentrations, and hence contributes to decreased N-efficiency (Jonker *et al.*, 1998; Nousiainen *et al*,. 2004; Aguilar *et al.*, 2012; Spek *et al.*, 2013). These aspects of N-efficiency, among others, in dairy cattle will be discussed next.

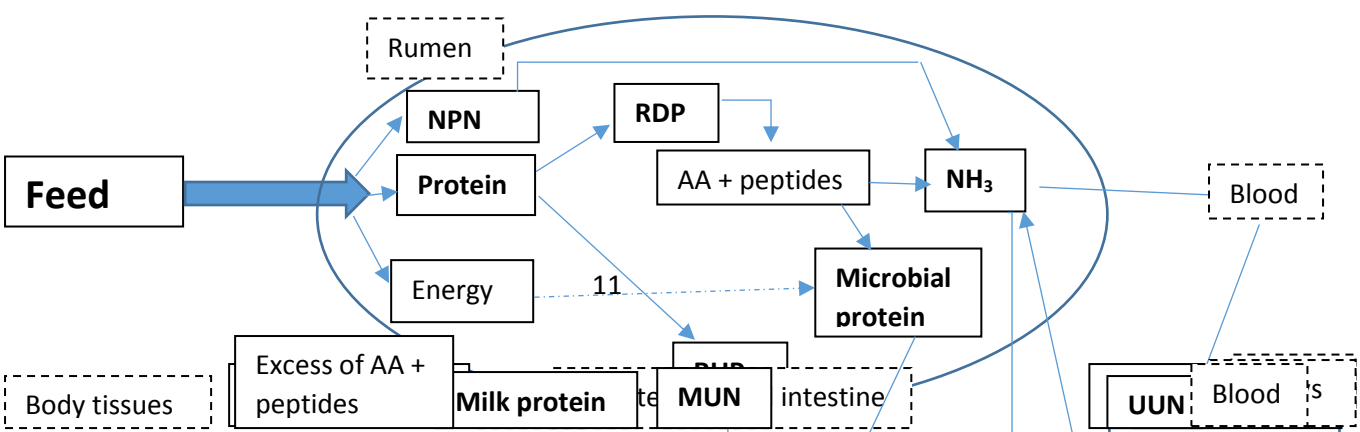

Figure 1. Digestion and metabolism of dietary protein in lactating ruminants. Boxes with dashed lines indicates different organs. Solid arrows represent fluxes of the different compounds. Dashed arrow represents the part of dietary energy (carbohydrates) that contributes to microbial growth and protein synthesis. Abbreviations: RDP = rumen degradable protein, RUP = rumen undegradable protein, NPN= non-protein-nitrogen, AA = amino acids, NH_3=ammonia PUN = plasma urea nitrogen, UUN = urinary urea nitrogen, MUN = Milk urea nitrogen, MG = mammary gland. (Modified from McDonald *et al.,* 2011 and Spek *et al.,* 2013).

2.2 Nitrogen efficiency in dairy cows

N-efficiency refers to the proportion of dietary N that is converted to N in the product (g N in product/g N intake) (Calsamiglia *et al.,* 2010; Dijkrsta *et al.,* 2011). The utilization of dietary protein in ruminants is generally poor compared to monogastric animals (Kohn *et al.,* 2005); in average 25% (varying from 15-40%) of dietary N is converted to milk protein (Huhtanen and Hristov, 2009; Calsamiglia *et al.,* 2010 Dijkstra *et al.,* 2011). The above discussed protein metabolism and especially the ruminal degradation of protein are the main contributors to the poor N-efficiency in ruminants (Calsamiglia *et al.,* 2010).

It is worth noting that there are several actions and strategies that can be exploited when striving for decreased N-emissions and increased N-efficiency on dairy farms. It is quite obvious that farming in general is a complex and comprehensive system with many interactions within the systems but also with the surrounding environment and society (Børsting *et al.,* 2003). Therefore, when considering the possible ways of improving the N-efficiency on dairy farms, a holistic approach to finding solutions is crucial (Børsting *et al.,* 2003). However, other than animal related factors are not in the scope of this paper. In this section the main focus will be on feeding strategies for optimizing nitrogen use by ruminants.

2.2.1 Feeding management and optimization of nitrogen use by ruminants

The most important consideration in the light of maximizing N efficiency in dairy cows is to reduce N losses without any losses of the product (saleable milk) (Reynal and Broderick, 2005). Namely, if striving for a maximal N-efficiency, losses in production performance will occur (Reynal and Broderick, 2005; Calsmamiglia *et al.,* 2010). The most efficient way of reducing N-leakage and losses from dairy production is to avoid overfeeding of dietary protein, especially RDP (Huhtanen *et al.,* 2008; Huhtanen and Hristov, 2009; Huhtanen *et al.* 2015), as the part of dietary N that is in excess of microbial requirements as well as AA utilization in tissues will be wasted as urea N via milk and urine (figure 1; Børsting *et al.,* 2003) and thereby reduces the efficiency of N utilization for product formation (Baker *et al.,* 1995). Also, increased milk yield without any increase in dietary CP increases milk N efficiency, but not to the same extend as reducing CP intake (Huhtanen and Hristov, 2009).

Diets formulated for absorbed protein rather than according to CP requirements have been shown to be more efficient at converting dietary N into milk true protein (TP), where N-efficiency was reported to be 25.5 and 23.1% for diets formulated for CP (where dietary CP-contents were 15.1 and 17.5%, respectively) and 27.4 and 28.3% for diets formulated for absorbed protein (CP 14.5 and 15.1%, respectively) (Baker *et al.,* 1995), suggesting that the N in the latter diets are more efficiently utilized in both rumen and tissues. Hence, diets should be formulated keeping the protein quality, not just quantity, in mind in order to achieve high TP concentration and low urea concentrations in milk (Baker *et al.,* 1995), and hence, improve the N-efficiency of lactating cows.

However, a recent study by Apelo *et al.* (2014) showed that diets formulated by using a fixed post absorptive efficiency of metabolizable protein (MP) for milk protein synthesis overestimates the efficiency of AA used for milk protein synthesis leading to overfeeding of dietary N. Therefore, it was suggested that rather than assuming a fixed efficiency of MP used for milk protein synthesis, the individual essential AA (EAA) requirements should be included in the diet recommendations. Thereby, a specific EAA that are known to stimulate milk protein synthesis can be supplemented, the dietary CP levels reduced (in this case from 17 to 15%) and the N-efficiency increased (from 32.8 to 33.0-35.1% depending on the supplemented AAs) (Apelo *et al.,* 2014).

2.2.2 Assessing the N-efficiency in lactating dairy cows

As mentioned above, the N-efficiency refers to the amount of dietary N that is converted to N in the product (milk in the case of lactating cows). However, other measures should also be used to estimate the N-utilization and N-losses in lactating dairy cows, by the side of the obtained value for N-efficiency. Indeed, according to Baker *et al.* (1995), milk CP is too general of a value to assess the efficiency of diet formulation and protein supply as it contains all N fractions in milk. Therefore when evaluating the efficiency in dietary protein supply, TP and urea content in milk should be known. High TP and low urea concentrations in milk reflect an efficient use of dietary N in a lactating dairy cow (Baker *et al.*, 1995).

Further, urea N concentrations, not only in milk but also in both plasma and urine are indicators of the balance between the dietary protein supply and protein requirements; increased dietary CP is the main factor affecting PUN and MUN levels (Baker *et al.*, 1995). Inefficient utilization of dietary N is reflected in high PUN and MUN concentrations as urea is the major end product of N metabolism in ruminants (Nousiainen *et al.*, 2004). Increased MUN in turn has been shown to be reflected even in higher excretion of N via urine, and also in decreased milk N-efficiency (Nousiainen *et al.*, 2004; Huhtanen *et al.*, 2015), as discussed above. Milk integrates variation in plasma urea concentration, and is therefore suggested to be a better indicator of mean PUN concentration than PUN itself (Baker *et al.*, 1995). Similarly to MUN and PUN, even UUN concentration is increased with increasing CP content in the diet, and excess of N is excreted to the environment (Colmenero and Broderick, 2006).

In practical terms, when studying the nutrient utilization of the lactating cow and by the ruminal microbes, assessing the amounts of MUN, UUN and microbial protein synthesis is of great importance. Daily MUN excretion is obviously relatively easy to obtain and analyze as the produced milk is readily available for analysis. It has been shown that MUN is closely related to UUN and PUN concentrations (Nousiainen *et al.*, 2004; Huhtanen *et al.*, 2015) and therefore is a very feasible way of assessing N-excretion by a lactating cow. Indeed, MUN is widely used to estimate the dietary N-balance on farm level (Nousiainen *et al.*, 2004).

UUN and microbial protein synthesis on the other hand are less assessable. Microbial protein synthesis can be indirectly estimated by measuring the purine derivatives, such as allantoin, in urine as they are the end product of the degradation of ruminal purines (protein) that are excreted through urine (Chen *et al.*, 1990). Similarly, daily UUN excretion is obtained by

analyzing the urine. However, total collection of urine is problematic and impractical to perform, and therefore an alternative method has been developed. Urine spot sampling for assessing the excretion of urinary nitrogen compounds have been shown to give similar results to that of total urine collection (Chizzotti *et al.* 2008), and is widely used in research. It has been established that creatinine is excreted at a constant level in relation to body weight in lactating cows (Chizzotti *et al.* 2008), and therefore by obtaining the creatinine concertation in the spot sample, the total daily urine volume can be calculated with the help of the creatinine concentration, whereby both daily excretion of UUN and allantoin can be estimated.

2.3 Production levels and N-efficiency at different dietary CP rations

Lastly, the effect of dietary CP levels on both production levels as well as N-efficiency will be discussed in detail. Several studies have investigated the effect of different protein levels in the diet on production levels as well as on the N-efficiency. Data from these studies will be reviewed in the following sections. Also the protein and AA requirements of dairy cows will be discussed briefly.

2.3.1 Protein requirements of a lactating cow

A common practice is to feed dairy cows with diets containing more than 16% of CP to ensure a maximal milk production (Aguilar *et al.,* 2012), as it is a general belief that increased CP leads to increased milk yield (MY) (Huhtanen and Hristov, 2009). According to Swedish recommendations cows producing ≥35 kg ECM should receive diets with CP content of 16.5 to 18.0% of DM and cows producing ≤25-30 kg ECM should have dietary CP contents of 15.0-17.0%, when calculating according to NorFor system (Lidström and Persson, 2014). According to NRC (2001) large cows (680 kg) producing 30 kg milk with a milk protein content of 3.0% require dietary CP levels of 19.3%. If the protein content in the milk is to be increased to 3.5% the CP intake should be increased to 21% of the DM (NRC, 2001). It is important to note that recommended CP levels will vary not only according to production levels, diet and production system, but also to which system have been used to calculate the requirements and appropriate protein levels in the ratio in question (Gustafsson *et al.,* 2013).

Further, it should be kept in mind that these high levels of CP in the diet will lead to a relatively low N efficiency in lactating dairy cattle (Huhtanen and Hristov, 2009; Aguilar *et al.*, 2012), as discussed before. Therefore, it has been the object of many studies to investigate possibilities of decreasing dietary CP content without decreases in production levels. Also more focus has been shifted from absolute levels of CP in the feed to the quality of the protein, as well as to the proportion of RUP and RDP (Kalscheur *et al.*, 1999; Korhonen *et al.* 2002; Reynal and Broderick, 2005), and to the balance between, and the quality of the supplied energy and N (Broderick, 2003; Cantalapiedra-Hijar *et al.*, 2014).

What it comes to the requirements of individual AAs in ruminants, a relatively complex and less well established recommendations have been made on the limiting AAs for a lactating dairy cow. According to Kung and Rode (1997) and Schwab *et al.* (1992) methionine and lysine are considered as the first limiting AAs for milk production. However, Vanhatalo *et al.* (1999) and Korhonen *et al.* (2000) showed that in silage and cereal based diets histidine is the first limiting AA for production.

There are no theoretical requirements for AA in ruminant diets because of the extensive microbial protein synthesis, but to achieve an optimal production microbial protein production alone will not be enough (Kung and Rode, 1997). Further, Patton *et al.* (2014) and Sinclair *et al.* (2014) noted that despite the wide range of research done on the limiting AAs, especially methionine and lysine, the role of these AAs as limiting for production cannot be established, as dietary factors like diet composition and CP content (both quantity and quality) in the diet as well as animal factors, such as stage of lactation, energy balance and milk yield has a great effect on the utilization and metabolism of AAs, and thereby the optimal levels of specific AAs in the diet are difficult to generalize. The most important consideration in the light of AA supplementation for lactating dairy cows is that the dietary AAs escaping rumen fermentation should be complementary to the microbial AA absorbed in the small intestine (Clark et al., 1992; Broderick, 2003).

2.3.2 Effect of dietary protein content on production levels

Milk production data from six different feeding trials where cows have been fed different levels of CP in the diets are presented in table 1. Most of the available research done on the relation between different dietary CP levels, production levels and N-efficiency have been conducted in early or mid-lactation and with a Latin-Square design where all the cows

included in the study are assigned for all the different diets, and the feeding regimens are changed after around one month (including two week adaptation period) on the diet (table 1). It is worth noting that the level of feeding in terms of both energy and protein in early lactation have been shown to affect the milk yield later in lactation (Jørgensen *et al.*, 2016), and changing the diet several times during a lactation, as is the case in most of the reviewed studies here, might partly affect the obtained results. Further, the diets were in most cases fed as total mixed ratios (TMR), that is roughage and concentrates are not fed separately, which might also give different results from that of separate feeding.

Table 1. Dry matter intake (DMI), milk yield (MY) and energy corrected milk (ECM) yield of cows fed with different dietary CP levels. Values within a cell with different superscripts are significantly different from each other ($p<0.05$).

Authors	Study period	Dietary CP%	DMI	MY	ECM
Korhonen *et al.* (2002)	28x4 from 51±6 DIM	13.4/17.0	19.8/19.7	23.1^{a}/26.1^{b}	26.6/27.3
Broderick (2003)	4x4w from 126±64 DIM	15.1/16.7/ 18.4	21.1^{a}/22.1^{b}/ 22.6^{c}	33.0^{a}/34.1^{b}/ 34.1^{b}	33.1^{a}/34.6^{b} /34.3^{b}
Reynal and Broderick (2005)	28dx4 from 72±62 DIM	17.2/18.8	25.4/25.1	41.5/42.3	39.4/38.4
Colmenero and Broderick (2006)	28dx5 from 120±76 DIM	13.5/15.0/ 16.5	22.3/22.2/2 3.0	36.3/37.2/3 8.3	34.2/35.6/ 36.7
Spek *et al.* (2013b)	38d from 146±29 DIM	11.6/15.4	19.2/19.6	22.4^{a}/25.0^{b}	23.3/25.0
Hymøller e*t al.* (2014)	2x2, from week 8 to 30	14/16	20.2^{a}/21.0^{b}	28.2^{a}/30.7^{b}	29.3^{a}/31.8^{b}
Cantalapiedra-Hijar *et al.* (2014)	21dx4 from 211±13 DIM	12/16.5	13.5/13.8	13.5^{a}/15.5^{b}	17.4^{a}/20.1^{b}
Broderick *et al.*(2015)	21dx5 from 81±29 DIM	15/17	24.9/25	39.5/40.1	38.6/39.3
Mutsvangwa *et al.* (2016)	29dx4 from 109±36 DIM	15/17	31.3/30.9	43.2/44.1	43.2/43.5

As can be seen in table 1, in general dry matter intake (DMI) did not differ at different CP levels, but similar intakes were achieved irrespective diet. One exception was the data obtained by Hymøller *et al.* (2014): Holstein cows at higher CP diets, in TMR feeding regimen, had a significantly higher intake compared to those at lower CP diets, the DMI being in average 0.8 kg higher in group with dietary CP of 16% compared to group with dietary CP of 14%. They further observed significant differences in MY and ECM, those being 2.5 and 1.1 kg higher, respectively, for cows receiving diets with higher CP. This is in

agreement with Broderick (2003), who reported higher linear increase in DMI with increasing dietary CP content (from 15.1 to 18.4%), also in a TMR feeding. Further, MY was lowest in cows on low CP diet, but interestingly increasing CP from 16.7 to 18.4% did not show any significant response in MY.

Even Cantalapiedra-Hijar *et al.* (2014) found significant differences in MY and ECM in Jersey cows receiving diets with low (12%) and high (16.5%) CP contents in late lactation. MY was 2.0 kg and ECM 2.7 kg higher in cows on high CP diets. Further, Spek *et al.* (2013b) observed a higher MY in Holstein cows receiving a diet containing 15.4% CP compared to cows on low CP diet (11.6%), being 25.0 and 22.4 kg respectively.

Interestingly, Korhonen *et al.* (2002), did not observe any differences in DMI of cows having a separate feeding of roughage and concentrates. However, the silage intake of cows receiving the low CP diet was higher (11.2 kg) compared to cows on high CP diets (10.5 kg), while cows on high CP diet had higher concentrate intake (9.2 vs. 8,6 kg), hence resulting in similar total DMI. The MY was nonetheless 3 kg higher in cows with high CP (17 vs. 13.4%) levels in the diet, as a result of a higher RUP flow and a more balanced AA supply to the intestine in these cows (Korhonen *et al.* 2002).

It is, however, notable that the MY did not decrease significantly when CP-content was decreased from 17 to 15% (Colmenero and Broderick, 2006; Broderick *et al.*, 2015; Mutsvangwa *et al.*, 2016), from 18.8 to 17.2% (Reynal and Broderick, 2005) or from 18.4 to 16.7% (Broderick, 2003). Similar results were observed in a case study conducted by Histrov *et al.* (2016), where the effect of dietary CP content on N-efficiency and production levels was investigated on commercial dairy farms. During the first year the average dietary CP content was 16.5%, and during a subsequent year it was decreased to 15.4%. No significant effects on MY or milk composition was observed between the two years (Hristov *et al.*, 2016). All in all, it seems that decreasing CP content in the diet below 12% will have negative effects on production levels (Huhtanen and Hristov, 2009; Cantalapiedra-Hijar *et al.*, 2014), but that there is a margin for reducing dietary CP contents from the current recommendations with CP levels over 15%. Indeed, Broderick (2003), stated that increases in MY and ECM were only modest when the CP content was increased from 15.1 to 16.7%, as can be seen in table 1.

It is important to consider the quality (and not only quantity) of the dietary protein, the balance between RUP and RDP in the diets as well as the energy and protein balance in the

diet, as discussed in previous sections. Indeed, a starch rich diet with low CP did have a positive effect on MY, being around 1 kg higher compared to a fiber rich diet with low CP (Cantalapiedra-Hijar *et al.,* 2014). Both microbial N-flow and the efficiency of microbial protein synthesis were higher in cows receiving the starch rich diet, showing that starch rich diets can at least partly compensate for a lower dietary CP (Cantalapiedra-Hijar *et al.,* 2014).

Further, it is important to keep in mind that the nutrient requirements in a lactating cow change over the course of lactation. Indeed, Kalscheur *et al.,* (1999) showed that in early lactation high dietary CP (17.4%) had a positive effect on MY over lower dietary CP content (~15%), being 1.5-4.9 kg higher in cows on high CP diets than cows receiving diets with lower CP (with increasing levels of RUP). However, in later stages of lactation cows fed a diet with 17% CP had a similar MY to cows fed with diets containing ~15% CP (Kalscheur *et al.* 1999). These results are in agreement with another whole lactation study where a higher CP content in the diet (17.3 vs. 14.4%) resulted in a higher MY in early lactation (35.4 vs. 31.8 kg) whereas no differences were observed in late lactation (29.8 vs. 28.8 kg) (Law *et al.,* 2009). The authors stated that high levels of CP ensure high DMI and production levels in early lactation but in late lactation the dietary CP level can be decreased to 14.4% without any effects on animal performance. However, cows on a diet with a very low CP content of 11.4% had lower yields throughout the whole lactation compared to cows on diets with CP content of 14.4 and 17.3% (Law *et al.,* 2009), which further indicates that the critical dietary CP content for achieving high yields seems to be around 12% of DM, as discussed above.

Kalscheur *et al.* (1999) noted that this lack of response to increased CP and RUP levels in later stages of lactation indicates that the recommended levels of dietary CP content over 17% of DM in dairy cow diets might be overestimated (Kalscheur *et al.,* 1999). They further discussed that high DMI may ensure a sufficient protein intake even at lower CP concentrations, and therefore additional protein supplementation might not be necessary. Furthermore, high DMI may increase passage rate of the digesta, and thereby decrease the protein degradation in rumen leading to higher amounts of AA to be absorbed in the small intestine (Kalscheur *et al.,* 1999).

2.3.3. *N-efficiency in lactating cows fed different levels of dietary protein*

The effects of different dietary CP levels on MUN, UUN and N-efficiency are presented in table 2. Overwhelmingly consistent results have been obtained across different trials; significantly lower MUN and UUN levels, and thereby lower N-excretion have been observed for cows receiving diets with lower CP contents. Further, an increased N-efficiency is achieved by lowering the CP level in the diet, ranging from 36.5 to 30.4% with CP contents of 12-17.2% whereas at the higher CP levels (16.5-18.8%) the N-efficiency ranged from 33.0 to 26.4%. As discussed in previous sections, lower MUN and UUN concentrations indicate a more efficient N-utilization leading to a higher N-efficiency, that is a higher proportion of dietary N is converted to N in the product.

Table 2. Milk urea nitrogen (MUN) concentration (mmol/l), urinary urea nitrogen (UUN) amount (g/d) and nitrogen efficiency (dietary N/N in milk) of cows fed different dietary CP levels. Values within a cell with different superscripts are significantly different from each other ($p<0.05$).

Authors	Dietary CP, %	MUN (mmol/l)	UUN (g/d)	N-efficiency, %
Korhonen *et al.* (2002)	13.4/17.0	$4.53^{a}/9.5^{b}$	$103^{a}/169^{b}$	30.65/27.6*
Broderick (2003)	15.1/16.7/18.4	$3.28^{a}/4.43^{b}/5.68^{c}$	$119^{a}/172^{b}/216^{c}$	$30.3^{a}/27.0^{b}/23.9^{c}$
Reynal and Broderick (2005)	17.2/18.8	$4.57^{a}/5.68^{b}$	$163^{a}/240^{b}$	$30.4^{a}/29.6^{b}$
Colmenero and Broderick (2006)	13.5/15.0/16.5	$2.75^{a}/3.03^{b}/4.0^{c}$	$63^{a}/91^{b}/128^{c}$	$36.5^{a}/34.0^{b}/30.8^{c}$
Spek *et al.* (2013b)	11.6/15.4	$1.90^{a}/3.32^{b}$	$28^{a}/74^{b}$	34.9/29.3
Hymøller e*t al.* (2014)	14/16	$2.54^{a}/3.07^{b}$	-	$35^{a}/33^{b}$
Cantalapiedra-Hijar *et al.* (2014)	12/16.5	-	$58.6^{a}/131^{b}$	$31.3^{a}/26.4^{b}$
Broderick *et al.* (2015)	15/17	$3.32^{2}/4.46^{b}$	$96^{a}/161^{b}$	$32.1^{a}/28.8^{b}$

*Calculated from the daily milk protein N yield and daily N intake reported in the paper, significance level cannot therefore be given.

Cantalapiedra-Hijar *et al.* (2014) showed that decreasing dietary CP content from 16.5% to 12.0% in Jersey cows in late lactation decreased the urinary N excretion by more than 50% and increased the N-efficiency from 26.4 to 31.3%, but this came in the expense of decrease in both MY by 2 kg/day and milk protein yield by 18%. Similarly, Hymøller et al. (2014), Spek *et al.* (2013b) and Korhonen *et al.* (2002) showed an increased N-efficiency and decreased N excretion with lowered CP levels at the expense of decreased milk yields.

However, more moderate decreases in dietary CP levels allows increased N-efficiency and decreased N excretion without any significant losses on production levels (Reynal and Broderick, 2005; Colmenero and Broderick, 2006; Brodercik *et al.*, 2015). This might be achieved by decreasing dietary CP with a simultaneous increase in RUP or starch content (Kalscheur *et al.*, 1999; Reynal and Broderick, 2005; Cantalapiedra-Hijar *et al.*, 2014). Interestingly, maximum microbial protein yield did not give the optimal N-efficiency, but the optimum was achieved by balancing the dietary protein degradation and microbial protein synthesis (Reynal and Broderick, 2005). Indeed, Nadeu *et al.* (2007) showed that energy availability from carbohydrates as well as synchronization of protein and carbohydrate metabolism are important for efficient nitrogen utilization by dairy cows, as was also discussed by Clark *et al.* (1992).

The optimal CP level was further discussed by Reynal and Broderick (2005), and they suggested the optimal dietary CP level to be 17.7% (11.7% RDP) when striving for an optimal the N-efficiency, that is balancing the need for a high profitability of the production system and the need for minimizing the negative environmental effects of excessive N-excretion. However, they further noted that the recommend dietary protein levels will depend on the criteria used for defining the optimum N use, and also that there is an increasing amount of evidence showing that MY and N-efficiency can be maximized with diets containing substantially lower than 17.7% of CP (Reynal and Broderick, 2005), as can be seen in table 1.

3. Material and Methods

All procedures involving animals were approved by the Uppsala local ethics committee (Ref. C119/14). The data analyzed in this report is part of a 2-year study conducted at the Lövsta Livestock Research Centre in Uppsala, Sweden. In total 50 cows are included in the study, 25 cows each year. Data and results from the first year of the study (from October 2014 to October 2015) will be presented and discussed in this paper.

3.1 Animals and housing

Data from 25 dairy cows, 13 Swedish red (SRB) (of which 3 primiparous cows) and 12 Holstein Friesian (SH) (of which 3 primiparous cows), were collected for entire lactation (10 months). Two of the SH cows were excluded from the study after 4 months of lactation due to illness not associated with the treatments, and therefore only urea and blood data (see below) from the first three months of lactation from these 2 cows were analyzed and included in the dataset. Furthermore, one cow was sent to slaughter after 9 months of lactation (due to an administrative error), and the data for the last month was estimated in statistical analysis. The number of animals included in the study by treatment group, breed, parity and initial live weight (LW) is presented in table 3.

Table 3. The number of animals used in the study by treatment group, and by breed, parity and initial live weight (LW).

		Breed:		**Parity:**		**Initial LW:**	
	N	SH	SRB	1	>1	SH	SRB
Group 1	12	6	6	3	9	666	703
Group 2	11	4	7	3	8	682	631
Total	23	10	13	6	17		

The cows were housed in a loose-house barn with automatic feeding system, and were milked twice a day in automatic milking rotary (AMR™ DeLaval International, Tumba, Sweden). Feed intake and milk yield were automatically registered at each feeding and milking occasion individually for each cow.

3.2 Diets and feeding

All the cows were fed a high quality silage *ad libitum*, the dominating forage being Timothy grass. Feeding regime followed the Swedish KRAV standard for organic milk production (KRAV, 2015) stating that for the first 3 months of lactation $\geq$50% and from the 3rd month until the end of lactation $\geq$60% of the diet should consist of roughage. Concentrate allotment was adjusted every month according to milk yield using NorFor-system by IndiviRAM (IndiviRAM, 2015), and thereby ensuring the roughage to concentrate ratio as stipulated by KRAV regulations.

During summer months (from 5th of May until the end of August) the cows were at grazing, but had access to silage inside the barn during the first weeks on pasture (between 5th and 21st of May), and at least half of the roughage intake was from pasture. From 22nd of May until 4th

of July the cows were fed entirely on pasture and concentrates, and did not have access to silage. They were then gradually housed inside having access to silage from 5th of July until 1st of September, where after the cows were housed inside for the winter, and fed on silage and concentrates.

For the concentrate diet the cows were randomly assigned for one of the two treatment groups: group 1 received concentrate feed without protein supplements (Cereal), while group 2 received both Cereal and a protein-rich concentrate feed (Prot). The composition of the two diets are described in table 4. All concentrate components were certified according to KRAV regulations for organic production (KRAV 2015).

Table 4. Ingredients of the concentrate diets used in the study.

Ingredients (% of DM)	Cereal	Prot
Barley	36	-
Wheat	34	3
Oats	25	15
Molasses	2	1
Soya expeller	-	47
Rapeseed cake	-	16
Crushed rapeseed	-	12
Crushed soybean	-	4
Mineral and vitamin mix	3	2

3.3 Feed analysis

During the experiment silage and pasture samples were analyzed for dry matter (DM), energy (MJ ME) (Rumen *in vitro* organic matter digestibility-method), crude protein (CP) (Kjeldahl method) and neutral detergent fibre (NDF) (amylase neutral detergent fiber method (aNDFom)) for chemical and nutritional composition. Am-N and pH were analyzed to assess the silage quality. Silage and pasture samples were collected every weekday and analyzed as pooled samples for two week periods for silage and one week periods for pasture. For both silage and pasture, AAT and PBV values were calculated from tabulated values (Spörndly, 2003). The concentrates were sampled at each delivery from the feed factory, and each batch analyzed for DM, NDF, CP, EE and starch. Further, AAT and PBV values were obtained from the manufacturer.

The results from the feed analysis (both pasture, silage and concentrates) are presented in table 5. The values were averaged for each lactation month, whereby nutrient intake of each cow and lactation month were obtained. Further, an average value of chemical and nutritional composition of the diets for the entire study period was also calculated.

Table 5. Chemical and nutritional composition of the diets used in the study.

	Cereal*	**Prot.***	**Silage****	**Pasture****
DM, %	89.6	92.0	32.0	23.7
Energy, MJ ME/ kg DM	12.7	16.1	11.4	11.8
CP, g/kg DM	125.2	333.3	148.0	148.0
AAT, g/kg DM	84.9	158	72.7	78.5
PBV, g/kg DM	- 24.5	96.1	22.7	12.9
Fat, g/kg DM	25.6	119.3	NA	NA
Ash, g/kg DM	56.1	75.6	85.9	83.0
Starch, g/kg DM	530.4	105.7	NA	NA
NDF, g/kg DM	159.1	167.9	-	422
Am-N, %	-	-	7.4	-
pH	-	-	4.2	-
OMD (%)	-	-	78.9	80.3

* Energy content, AAT (AA absorbed in the small intestine) and PBV (protein balance in the rumen) values for concentrates were obtained from the manufacturer. **AAT and PBV values for silage and pasture were calculated from tabulated values (Spörndly, 2003).

3.4 Feed intake and production data

Both silage and concentrate intake of the cows were registered automatically at each feeding occasion. The silage was fed from individual boxes that are attached to a weighing scale, which registers the amount of feed consumed by each cow (Biocontrol, Rakkestad, Norway). Similarly, concentrate feeds were fed from feeding automates that identifies each cow individually and register the feed intake as well as regulate the total amount and type of the concentrate feed consumed by the cow (DeLaval, Tumba, Sweden). The data was accessed

from a database summarizing the intake for each cow and day of lactation, and an average daily feed intake of each feed was calculated for each cow and lactation month.

The pasture allowance (kg DM/cow and day) was calculated from pasture mass (kg DM/ha) and stocking rate (cows/ha). To calculate the pasture mass, pasture height was measured on weekdays (Monday to Friday) on each paddock using an electronic pasture meter (EC-09 Electronic Counter Kit, Jenquip New Zealand). An estimation of average daily pasture intake for each cow and month on pasture was done by calculating the daily energy requirement of each cow based on the average live weight (LW) as well as daily LW-change and milk yield for each month on pasture by using following equations according to Spörndly (2003):

If LW-change positive: $((0.507*LW^{0.75}) + (5.0*$ kg ECM) + (35.8*kg LW gain))-13.6

If LW-change negative: $((0.507*LW^{0.75}) + (5.0*$ kg ECM) + (34.5*kg LW gain))-13.6

There after the energy intake from the concentrates and silage was calculated, whereby the remaining amount of required energy was assumed to be obtained from pasture. The pasture intake (kg/DM/day) was then calculated based on the energy content of pasture.

All the cows were milked in an automatic milking rotary (AMR) system, where milk yield (MY) is automatically registered at every milking. An average daily MY for each lactation month was calculated from the daily MY registrations. Milk composition (fat%, protein%, milk urea and somatic cell count (SCC)) for each cow and lactation month was obtained by using two different sources. Milk composition was analyzed once a month by the national cow control database for fat, protein, SCC and urea using FTIR/IR and fluorescence technique (CombiFoss™ FT+). Further, monthly analysis of milk fat, protein and SCC were performed by the university lab for research purposes using a MilkoScan FT 120 (FOSS Electric A / S Instruments) for milk composition analysis and Fossomatic 5000 (FOSS Electric A / S Instruments) for SCC analysis. Thereby, milk composition data for each cow was analyzed with a two-week interval and the lactation month average was calculated using two different sources. Energy corrected milk (ECM) yield was calculated from the average daily MY and milk composition for each month as described by Sjaunja *et al.* (1990).

Live weight (LW) of each cow was registered once a week on a scale in conjuction to the milking machine. An average weight for each cow and each lactation month was calculated based on the weekly registrations. Furthermore, BCS on a scale of 1-5 (according to Geno,

Norway) with 0.25 accuracy was estimated at lactation week 2-3, 5-6 and thereafter once a month.

3.5 Urine and blood sampling and analysis

In total three blood samples and three urine spot samples were collected during the lactation (lactation week 2-3, 5-6 and 11-12) from each cow. The blood samples were analyzed for plasma histidine concentration to determine a possible histidine deficiency by using Waters UHPLC Amino Acid Analysis Solution (System Guide 71500129702).

Urine was analyzed for urea, allantoin and creatinine using AutoAnalyzer III (SEAL Analytical GmbH, Norderstedt, Germany). Allantoin was analyzed for estimation of microbial protein production, and urea for the determination of daily nitrogen excretion. As the urine spot sampling was used (that is only three sampling occasions per cow were performed), creatinine concentration was analyzed for the estimation of the total daily amount of urine excreted, and thereby the daily amount of allantoin and urea produced could be obtained.

3.6 Statistical Analysis

The statistical analysis of the data was performed in SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). Least square means (LSmeans) for dependent variables of milk production data (kg milk, kg ECM, milk composition), feed and nutrient intake, LW and BCS were calculated for the entire lactation, while a separate analysis for plasma histidine concentration as well as the amount of allantoin and urea were performed for early lactation. Both of these datasets were analysed by using proc GLM procedure in SAS. Breed and parity were included as independent variables in the models. Also the interactions between group and breed as well as group and parity were tested, and if significant included in the model.

4. Results

4.1 Pasture allowance and feed intake

As can be seen in the table 6 the pasture allowance exceeded the planned 25 kg DM/cow/day for the entire pasture period, being over 60 kg DM/cow/day during June and July when the cows did not have access to silage.

Table 6. Average pasture allowance during the grazing season (May-August 2015).

Month	Pasture allowance (kg DM/cow/day)
May	35.6
June	64.0
July	66.2
August	47.6

Intake of concentrates (intake of Cereal for group 1 and intake of Cereal + Prot for group 2), silage and total roughage (silage + pasture) and total DMI for both groups are presented in table 7. The total intake of concentrate feeds was higher ($p<0.001$) in group 2 compared to group 1. Also, primiparous cows had lower Prot and total concentrate intakes compared to multiparous cows, but no interaction between group and parity was found. The difference in silage intake did not reach significant levels, but the total roughage intake (including silage intake for the whole lactation and pasture intake for the last 4 months of lactation) on the other hand, was higher in group 1. Pasture intake (for lactation months 7-10), was calculated backwards based on the energy requirements and concentrate intake, and thereby was higher in group 1 (10 kg) compared to group 2 (7.0 kg). Breed had significant effect on silage intake and total DMI being higher in SH cows. Also, primiparous cows had lower total DMI compared to multiparous cows.

The nutrient intake was also calculated for energy, CP and starch (table 7). The overall CP content for the feed ratios was calculated based on the intake proportion of the individual concentrate feeds. The Prot feed made up to 12% of total concentrate intake of group 2, and thereby the total CP content in their feed ratio was 16.4%, whereas the CP content in the feed ratio of group 1 was 14.0%. In group 1 the roughage made up to 66% of the diet, whereas the same number for group 2 was 58%. Both energy and CP intake were lower in group 1, whereas starch intake did not differ between the groups. However, when viewing the results

by lactation stage group 1 had higher (p=0.009) starch intake in early lactation compared to group 2 (5118 vs. 4538 g/d). Similarly, to feed intakes, SH cows and multiparous cows achieved higher energy and CP intakes compared to SRB and primiparous cows, respectively.

Table 7. LSmeans for total daily concentrate (Cereal/Cereal+Prot) intake, silage intake and total feed intake as well as energy, CP, NDF and starch intakes for each group over the whole lactation. Total CP content as well as the proportion of roughage in the diet are also presented.

	Group 1	**Group 2**	**Breed**		**Parity**		**P-value**		
	N=12	N=11	SRB	SH	1	>1	Group	Breed	Parity
Cereal intake, kg DM	7.3	6.5	7.1	7.1	6.8	7.0	<.0001	NS	NS
Prot. Intake, kg DM	-	2.5	2.6	2.7	2.3	2.7	NA	NS	0.022
Total conc. intake, kg DM	7.2	9.0	8.3	8.5	7.8	8.5	<.0001	NS	0.005
Silage, kg DM	13.4	12.9	12.0	14.3	12.4	13.4	NS	<.0001	NS
Total roughage, kg DM*	14.4	12.7	12.3	14.7	13.2	14.2	0.0039	<.0001	NS
Total DMI, kg	22.3	22.6	20.7	22.8	21.3	23.6	NS	0.004	0.023
Energy intake, MJ	256	277	254	280	253	281	0.05	0.0017	0.02
CP intake, g	3055	3697	3207	3545	3197	3555	<.0001	0.014	0.02
Starch intake, g	3984	3866	3844	3840	3670	4014	NS	NS	NS
AAT, g	1676	2017	1750	1944	1754	1939	<.0001	0.0068	0.019
PBV, g	-3.4	378	174	201	174	201	<.0001	0.009	0.017
Roughage%**	0.66	0.58	0.60	0.63	0.61	0.62	<0.001	0.03	NS
Total CP%***	14.0	16.4	-	-	-	-	-	-	-

* Total roughage intake includes silage intake for lactation months 1-6 and the sum of pasture and silage intakes for lactation months 7-10. **Proportion of roughage in the total feed ratio. ***the total CP% content in the diet was calculated based on the intake proportion of different feed types (group 1: roughage and Cereal, group 2: roughage, Cereal and Prot).

Lactation stage had a significant effect on the total roughage intake. As can be seen in figure 1, total roughage intakes in early and late lactation did not differ between the groups, but was higher (p<0.0001) in group 1 in mid-lactation. Further, group 1 had a significantly lower total concentrate intake in all lactation stages but the higher roughage intake resulted in a similar total DMI between the groups.

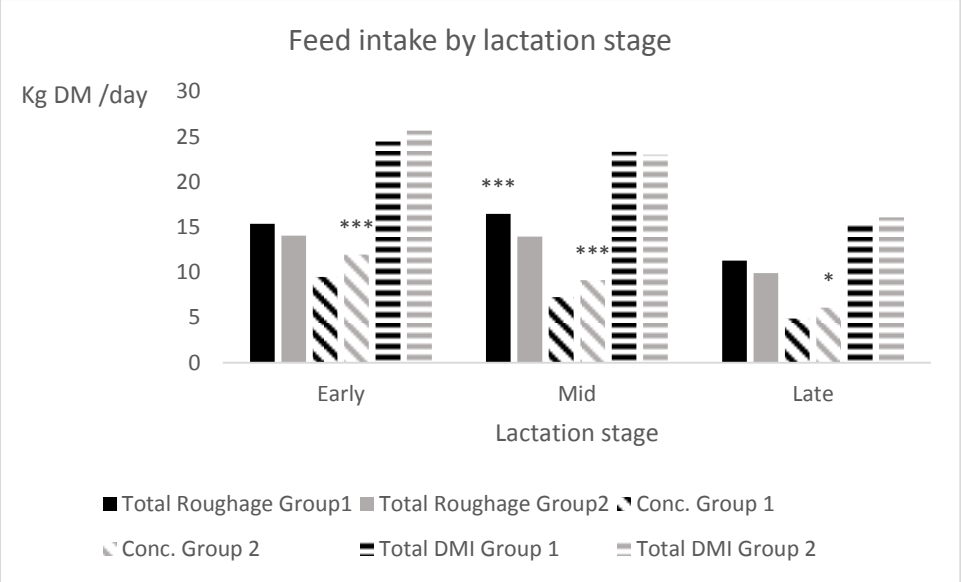

Figure 1. Total daily feed intake as well as daily feed intake of silage and concentrate by lactation stage (early (months 1-3), mid (months 4-7) and late (months 8-10) lactation). **p<0.05, ***p<0.001.

When comparing the Cereal intake over the whole lactation, group 1 had a significantly (p<0.001) higher Cereal intake for the first 3 months of lactation (figure 2). The total concentrate intake in group 2 (Cereal+Prot) was significantly higher throughout the lactation (with an exception for months 8 and 9).

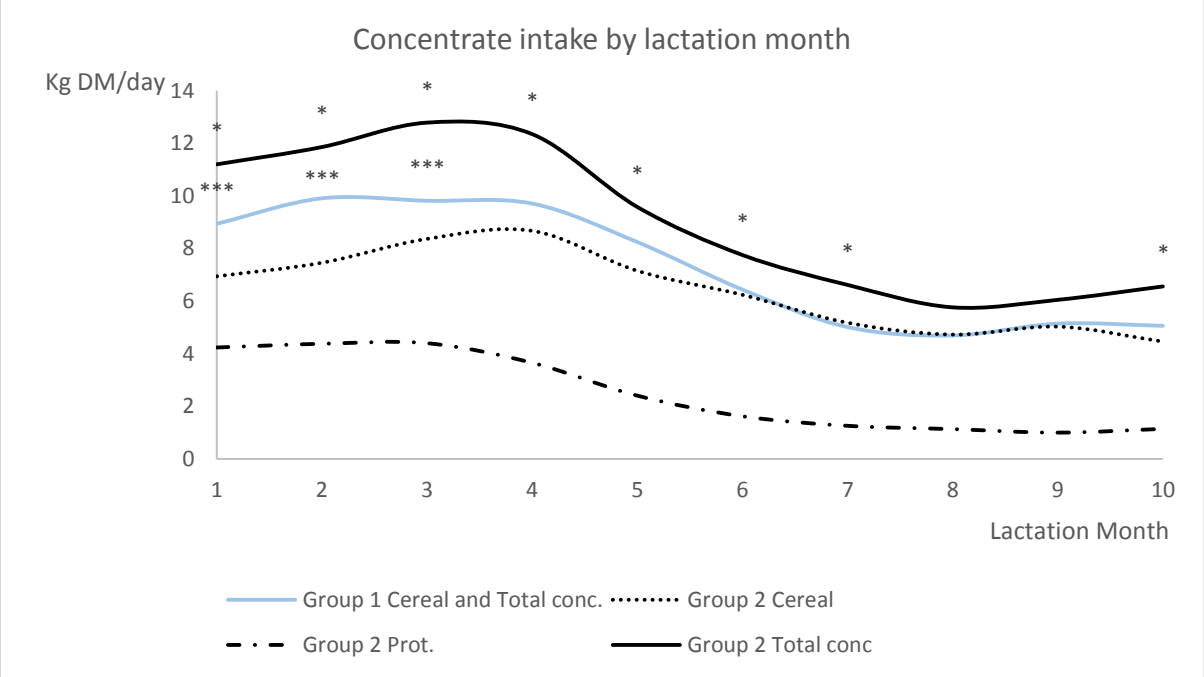

Figure 2. LSmeans for daily Cereal intake for group 1 and 2, Prot intake for group 2 as well as the total concentrate intake by lactation month. * p<0.05 (Total conc. group 1 vs. group 2). ***, p<0.001 (Cereal intake group 1 vs. group 2)..

4.2 Production and milk composition data

The total milk production over the whole lactation (305d) did not differ between the groups, being in average 9760 and 9706 kg ECM for group 1 and 2, respectively. The LSmeans of production and milk composition as well as LW and BCS change over the entire study period are presented in table 8. Neither MY nor ECM differed significantly between group 1 and 2. For milk composition data, lactose concentration tended to be higher in group 1 compared to group 2 ($p=0.056$). Neither protein nor fat concentrations differed between the groups. Cell count was higher ($p=0.04$) in group 2 as was the milk urea concentration ($p=0.021$).

As expected from a higher CP intake and similar ECM yields, the N-efficiency was significantly ($p=0.013$) lower in group 2 compared to group 1. Lastly, the BCS change between 2nd and 9th lactation month differed ($p=0.017$) between the groups. LW change did not differ between the groups.

Further, SH cows achieved higher MY and ECM yields, had higher fat% but lower protein% and N-efficiency compared to SRB cows. They also decreased in body condition and had a relatively low increase in LW, while SRB cows increased in both BCS and LW. Similar effect of parity was observed: multiparous cows had higher MY and ECM yields, and increased in body condition while primiparous cows had a decrease in BCS.

Table 8. Production and milk composition data presented as LSmeans over 10 months of lactation. P-values for the main effect as well as p-values for the interaction between the main effect and breed are presented.

	Group 1	**Group 2**	**Breed**		**Parity**		**P-value**			
	N=12	N=11	SRB	SH	1	>1	Group	Breed	Parity	Breed*group
MY, kg/day	30.5	31.7	29.6	32.6	28.9	33.3	NS	0.0004	<.0001	0.02
ECM, kg/day	31.0	31.2.	29.5	32.7	29.2	33.0	NS	0.0003	<.0001	NS
Fat%	4.10	3.99	3.94	4.11	4.10	4.00	NS	0.0157	NS	0.02
Fat, g/d	1.24	1.24	1.15	1.32	1.17	1.31	NS	<0.0001	0.0009	NS
Protein%	3.47	3.42	3.50	3.40	3.47	3.43	NS	0.0126	NS	0.002
Protein, g/d	1.04	1.08	1.03	1.10	1.00	1.13	NS	0.023	0.0007	0.04
Lactose%	4.78	4.71	4.73	4.76	4.80	4.69	0.056	NS	0.0031	NS
Lactose, g/d	1.46	1.51	1.46	1.60	1.39	1.58	NS	0.0073	0.0032	0.04
Cell count	88.4	171	142	105	107	129	0.04	NS	NS	NS
MU, mmol/l	3.85	4.27	4.02	4.09	4.20	3.91	0.021	NS	NS	NS
N-eff.*	36.5	32.0	36.0	33.0	33.0	35.0	0.014	0.07	NS	NS
BCS	3.49	3.51	3.89	3.11	3.44	3.56	NS	<0.0001	NS	NS
LW	655	635	643	650	592	698	0.017	NS	<0.0001	NS
BCS change**	-0.10	0.26	0.3	-0.14	-0.16	0.32	0.017	0.0051	0.0055	0.013
LW change, kg**	4.1	24.7	18.3	8.3	11.4	15.3	NS	NS	NS	NS

*N-efficiency was calculated as the proportion of N in milk produced and N intake from the feed **BCS and LW change were calculated as LSmeans of the difference between BCS (or LW) at month 9 and 2 for each group.

The interaction between group and breed for milk yield was significant (p=0.02) as can be seen in table 8. The average daily milk yields by breed within each group are presented in figure 3. It can be seen that SH cows in group 2 had higher (p<0.001) yields than SRB cows in the same group, whereas the milk yields between SH and SRB cows in group 1 did not differ, and were similar to the milk yield of SRB cows in group 2.

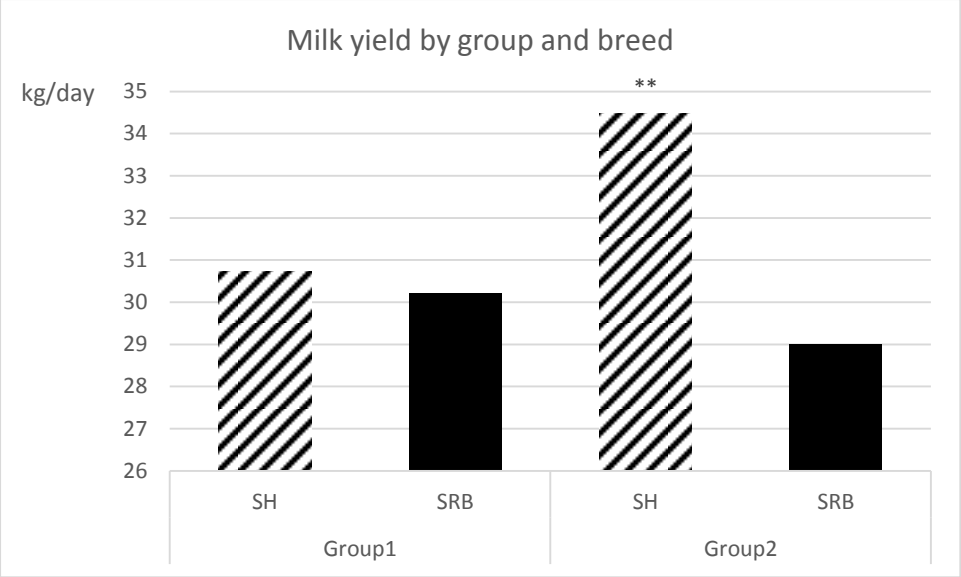

Figure 3. Daily milk yield by group and breed presented as LSmeans. ** the value differs significantly from all other values over the groups, $p<0.005$.

Even milk fat% and protein% were affected by breed and group (see table 8). The differences in fat and protein concentrations by group and breed are presented in figure 4. The fat concentration was highest in SH cows in group 1 compared to SRB cows in group 1 as well as both SH and SRB cows in group 2. In contrast, protein concentration was lowest in SH cows in group 2, whereas the values between SH and SRB cows in group 1 did not differ significantly and were similar to the value of SRB cows in group 2.

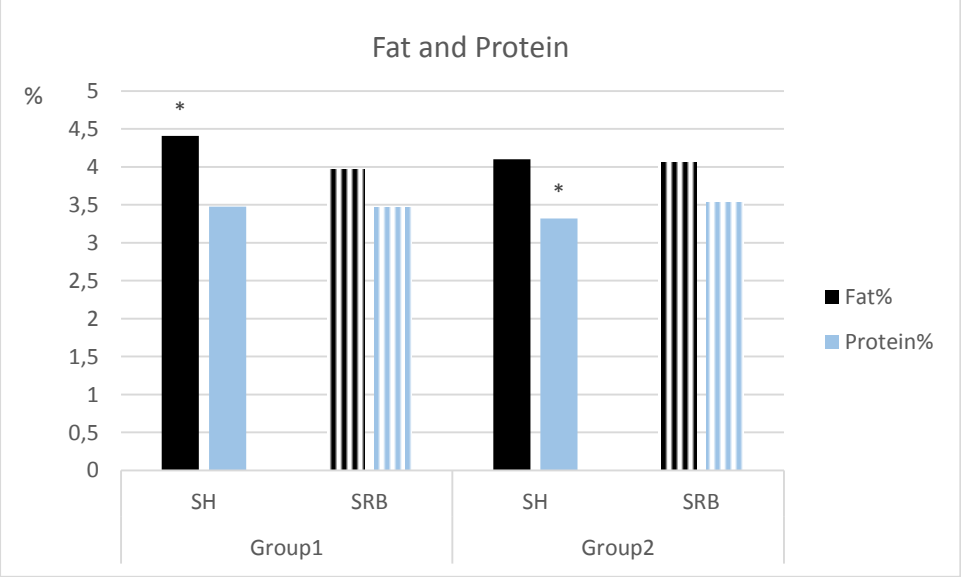

Figure 4. Proportion of fat and protein in milk by group and breed. *the value differs significantly from all other values over the groups, $p<0.05$.

When the data was grouped by stage of lactation, a significant interaction between lactation stage and group was observed: group 2 had a higher MY in early lactation (p=0.015), but in mid and late lactation the groups had similar yields (see figure 5). Furthermore, ECM yield did not differ between the groups in early or mid-lactation, but in late lactation group 1 had higher ECM yields compared to group 2.

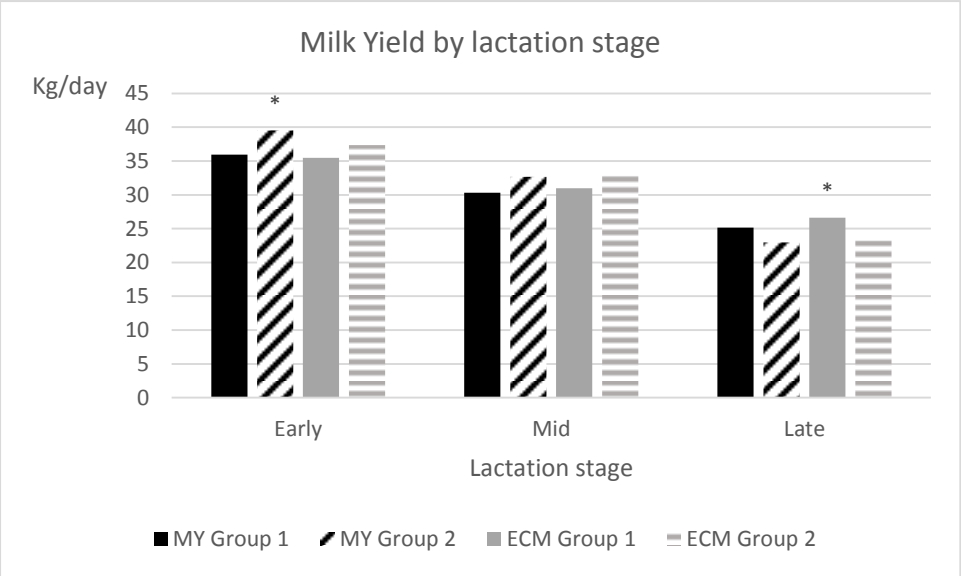

Figure 5. LSmeans for daily MY and ECM by lactation stage (early (months 1-3), mid (months 4-7) and late (months 8-10) lactation). *p<0.05

The milk solid yields by lactation stage did also differ between the groups so that the protein yield was higher (p=0.034) in group 1 (0.904 g/day) in late lactation compared to group 2 (0.795 g/d), but did not differ between the groups in early or mid lactation. Similarly, fat yield was higher (p=0.033) in group 1 compared to group 2, being 1.20 and 1.03 g/day, respectively. Lactose tended to be higher (p=0.063) in group 1 (1.08 g/d) than in group 2 (0.947 g/d).

The lactation curve for ECM is presented in figure 6. It can be seen that group 1 had a relatively even ECM yield throughout the lactation. The reduction in milk yield between the first and last lactation month was 12.2 kg, the same number for group 2 being 14.9 kg. Group 2 had a higher peak yield at lactation month 2, where after the yield decreased steadily ending up at around 20 kg.

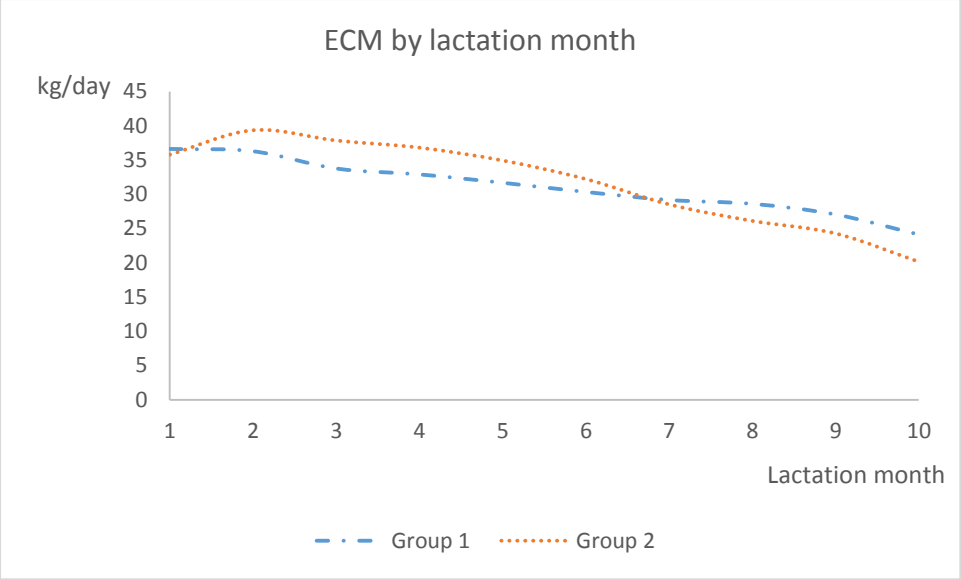

Figure 6. Lactation curve for daily ECM by lactation month.

The average LW and BCS for lactation months 2-9 are presented in figure 6. Several cows were missing BCS and/or LW for the first and/or last month of lactation, and therefore to obtain more balanced data, these two months were excluded. Group 1 started the lactation at higher LW and BCS and maintained a relatively constant LW and BCS throughout the lactation, as can be seen in figure 7. Group 2 had more fluctuation in BCS, and a great increase in LW in late lactation, ending up at similar LW as group 1. The average BCS and LW did not differ significantly between the groups at any lactation month.

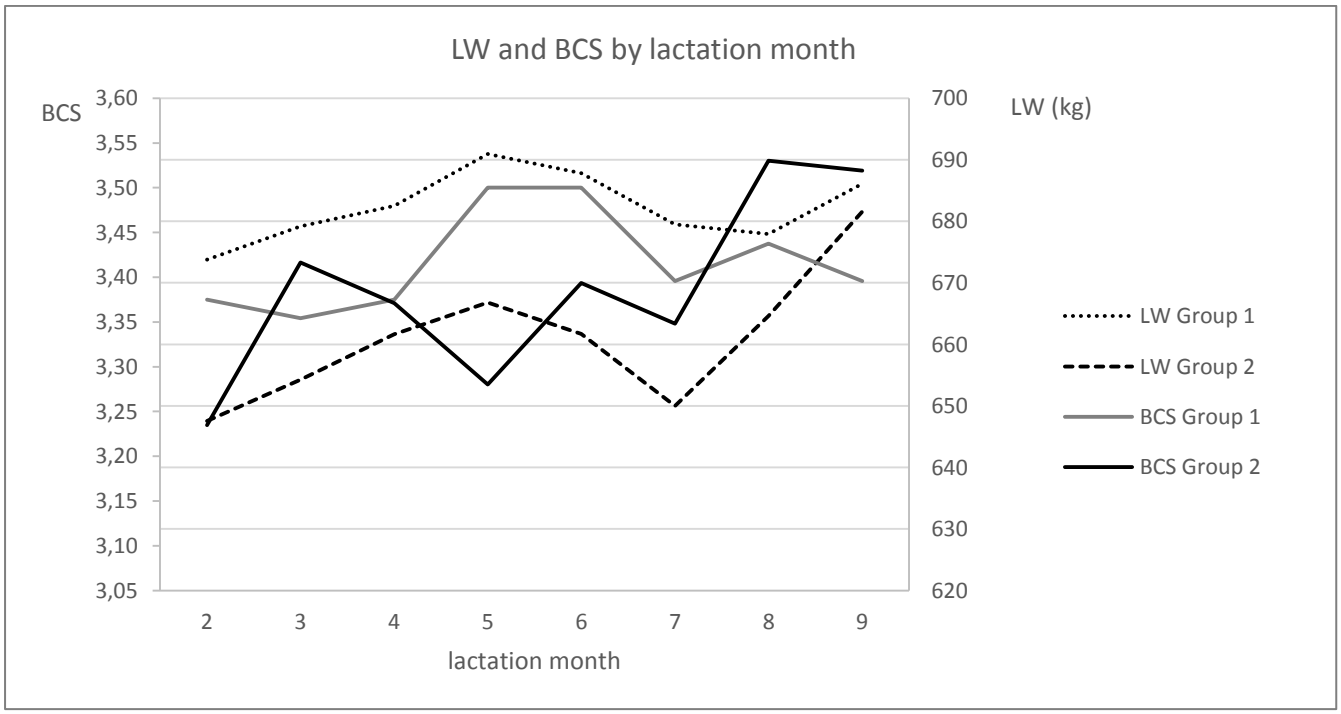

Figure 7. LW and BCS plotted from lactation month 2 to 9 as LSmeans for each group.

4.3 Plasma and urine metabolites

The LSmeans for the total daily urine volume and urine concentration of allantoin, UUN and creatinine as well as the plasma concentration of histidine for the first 3 months of lactation are presented in table 9. Daily urine volume did not differ between the groups. UUN concentration was higher ($p<0.001$) in group 2 compared to group 1, as was the amount of UUN excreted per day ($p<0.001$). Allantoin concentration in urine did not differ between the two groups. Similarly, the amount of allantoin produced per day did not differ between the groups. Plasma concentration of histidine was significantly higher ($p<0.001$) in group 2 compared to group 1.

Table 9. The concentration of urine metabolites and plasma histidine for each group presented as LSmeans for the first 3 months of lactation

Urine	**Group 1**	**Group 2**	**P-value**
Creatinine, mg/l	809	689	0.0167
Urine, l/d	21.8	24.6	NS
UUN, g/l	3.0	6.39	<0.001
UUN, g/d	66.1	150.9	<0.001
Allantoin, g/l	2.88	2.75	NS
Allantoin, g/d	62.6	66.2	NS
Plasma			
Histidine, nmol/ml	35.14	48.25	<0.001

5. Discussion

When reflecting the results from this study, it should be kept in mind that the number of cows was relatively low (N=23), and the individual variation as well as the variation in feed quality from year to year will have an impact on the production response to the diets. It is therefore important to await results for the second year of the experiment, where data from another 25 cows will be obtained, before making the final conclusions.

5.1 Milk production and feed intake

5.1.1 *Milk production levels over the whole lactation in relation to feed intake*

Both groups achieved a high total ECM yield for the whole lactation, 9760 and 9706 kg ECM for group 1 and 2, respectively, which is about 700 kg more than the Swedish average ECM yield for organic cows, and about 300 kg less than the average yield in conventional herds (Husdjursstatistik, 2016). Also, the average daily yields of both MY and ECM were similar between the groups, and similar to the Swedish average. The lack of effect of decreased CP content in the diet is in agreement with several previous short-term studies (Reynal and Broderick, 2005; Colmenero and Broderick, 2006; Broderick *et al.,* 2015; Mutsvangwa *et al.*, 2016) and two whole lactation studies (Kalscheur *et al.,* 1999; Law *et al,*. 2009) investigating the effect of decreased dietary CP on milk production levels.

In this study silage and concentrate feeds were fed separately and the silage was provided *ad libitum,* and thereby a possibility for a compensated roughage intake was provided for the group not receiving any protein concentrates. Indeed, Korhonen *et al.* (2002) observed a higher silage intake of cows on a diet based on silage and barley compared to cows on protein rich diets (11.2 vs. 10.5 kg). This is in agreement with the results in the present study as the cows with low CP diet had in average 1.1 kg higher roughage intake in early lactation and 2.7 kg higher roughage intake in mid-lactation compared to cows on the high CP diet. Interestingly, Hymøller *et al.* (2014) reported a higher DMI by cows achieving diet with a CP content of 16% compared to cows with a diet with CP content of 14% on TMR. Similarly, Brodercik (2003) observed a linear increase in DMI when CP content in the TMR was increased from 12.1 to 18.4% of DM. Hence, a separate feeding of silage and concentrates might counteract the possible negative effects of reduced CP content in the diet by enabling an increased roughage intake, and thereby a compensation for the low dietary CP content. However, it is important to keep in mind that high roughage intakes are achieved only with high feed quality, that is high energy content and CP content as well as low NDF content, which in turn will provide a high rumen digestibility of the forage. Indeed, it has been shown that pasture silages cut in later maturity (hence, are of lower quality) affect the feed intake negatively (Vanhatalo *et al.,* 2009).

Interestingly, group 2 that received the protein concentrate achieved higher total concentrate intakes, and thereby even higher CP and energy intakes, compared to the group fed only with the Cereal concentrate. However, the extra CP and energy consumed by group 2 was not reflected in the milk production levels, but the extra protein was probably lost via urine or milk, and both the consumed extra protein and energy were directed towards deposition of body reserves, as will be discussed further on.

Surprisingly, the starch intake did not differ significantly between the groups ($p=0.397$). It was expected that cows in group 1 would achieve a higher starch intake as their concentrate intake was solely from cereals with a high starch content. The starch intake was indeed higher in group 1 for the first 3 months of lactation, when also the Cereal intake was higher in this group. This might have been enough to suppress the effect of low CP content on ECM in early lactation (see later on). It has been shown that high starch content or decreased NDF-content in the diet increases the microbial protein synthesis (Broderick, 2003; Cantalapiedra-Hijar *et al.*, 2014). Also the passage rate increases with an increased dietary content of starch increasing the amount of RUP reaching the small intestine (Nadeu *et al.*, 2008). As a result, an efficient microbial protein synthesis as well as an increased amount of RUP with increased passage rate could cover the AA needs for maintained milk production in cows receiving a low CP diet (14.0%).

Also, the PBV in group 1 was closer to the optimal value of 0 indicating an optimal energy to N-ratio in the diet (Børsting *et al.*, 2003; Huhtanen *et al.*, 2008), which in turn ensures optimal rumen conditions for an efficient use of N by microbes. This in turn will enhance the rumen fermentation increasing the utilization of forage, further compensating for the lower dietary CP. The PBV value further indicates that the diet with a low CP content used in this study was sufficient in providing both energy and N for an efficient microbial growth.

Indeed, no differences in the amount of daily urinary allantoin excretion was observed between the groups, indicating a similar microbial protein synthesis for both diets. This is in contrast to Broderick (2003), who reported higher total urinary purine derivate (PD) excretion with dietary CP content of 16.7% compared to diet with a lower CP content (15.1%). However, Cantalapiedra-Hijar *et al.* (2014) observed a similar urinary allantoin excretion of 199 and 212 mmol/day for low CP (12.0%) and high CP diets (16.5%). Interestingly, higher starch content increased the allantoin excretion by 32 mmol/day. Similarly, Broderick (2003) reported an increased urinary PD excretion with decreasing dietary NDF-content. In this

study, the overall starch intake, and hence the starch content in the total feed ratio, did not differ between the groups, which might partly explain the lacking difference between the groups. Unfortunately, the total NDF intake could not be calculated as the silage NDF-analysis was delayed, and therefore the effect of fibre on allantoin excretion, and thereby on microbial protein synthesis, could not be obtained.

It should be noted that dietary CP contents under 12% of DM might not be enough to maintain the milk production of high yielding dairy cows, as was shown by Law *et al.* (2009), Spek *et al.* (2013b) and Cantalapiedra-Hijar *et al.* (2014). However, there is a great amount of evidence (e.g. Broderick, 2003; Colmenero and Broderick, 2005; Mutsvangwa *et al.*, 2016), together with the results in this study, showing that the dietary CP content can be decreased to levels of 14-15% of DM without any significant losses in milk production levels.

Lastly, it has been suggested that diets with high cereal contents are deficient in histidine, which in turn have been shown to be the first limiting AA for milk production in diets based on cereal and silage (Vanhatalo *et al.*, 1999; Korhonen *et al.*, 2000). Indeed, the plasma histidine concentration was observed to be lower in cows receiving the low CP diet (34.96 vs. 47.67 nmol/ml) during the first month of lactation. This was reflected in the lower MY of group 1 in early lactation, but not in either ECM or milk protein yield. The plasma concentrations of histidine in cows on a low CP diet based on silage and cereals used by both Vanhatalo *et al.* (1999) and Korhonen *et al.* (2000) were notably lower (18 nmol/ml and 23 nmol/ml, respectively) than that of observed in this study, which in turn was reflected in both lower MY and protein yield of cows in those studies. Further, Cantalapiedra-Hijar *et al.* (2014) observed higher plasma histidine concentrations (27.3 nmol/ml) than the two above named studies, which was nonetheless lower than the values observed in the present study. They also observed lower MY and milk protein yield in cows with lower plasma histidine concentration. However, neither the milk protein yield nor ECM in early lactation differed between the groups in the present study. It might be so that the plasma histidine level of 34.96 nmol/ml, which is higher than observed by the above named authors, is sufficient for maintained milk protein and ECM production. Hence, the dietary CP content itself does not say much about the quality of the dietary protein or the AA balance in the feed. Therefore, the lack of response in production levels to decreased CP content in the feed may indicate a good AA balance in the silage and Cereal diet used in this study. Also, the AAs produced by the ruminal bacteria in cows receiving the low CP diet might be complementary to the dietary

AAs, thereby ensuring a balanced mixture of AAs required for maintaining high milk production levels.

5.1.2 *Effect of breed and parity on production levels*

It is a well-known fact that SH cows have a higher intake capacity and a greater ability to direct dietary nutrients as well as body reserves towards milk production, which in turn is reflected in a higher milk yield of this breed compared to SRB cows as was observed in this study (32.6 vs. 29.6 kg). Further, there was a significant breed*group interaction: SH cows in group 2 had significantly higher yields over SH cows in group 1 as well as SRB cows in both group 1 and 2. This indicates a greater response of SH cows to protein supplementation in terms of milk yield compared to SRB cows. SH cows had a higher DMI as well as protein and energy intake, which might partly explain the higher yield of SH cows in group 2, as well as in general. Interestingly, it is SRB cows that are more commonly used in organic dairy farms in Sweden (Sundberg, 2010), and therefore feeding the cows on only silage and cereals might be a possible option for increased profitability on dairy farms with SRB cows, as the milk yield is the same with this diet, while the costs for feeding will be reduced.

As expected, the milk yield was significantly lower in primiparous cows than multiparous cows. Primiparous cows usually are still growing during their first lactation, and therefore part of the dietary energy and protein are directed towards growth rather than milk production (McDonald *et al.,* 2011). Also, as was observed, the feed intake capacity of primiparous cows is not as great as that of multiparous cows because of their smaller size, leading to lower total DMI as well as energy and CP intake, which hinders primiparous cows to reach the maximal production potential during their first lactation.

Interestingly, there was no interaction between group and parity for any of the prodcution variables, indicating that primiparous cows receiving a diet with higher CP content did not benefit from the additional protein in the diet. The lack of response in milk production indicates that the Cereal and silage diet is sufficient in energy and protein for feeding of primiparous cows, at least in terms of milk production levels. It might, however, be so that the extra dietary protein is directed towards growth rather than milk production, and thereby it could be beneficial to feed these cows with high protein diets to insure that there is enough nutrients for growth.

Primiparous cows did indeed gain weight during the lactation, but the gain was not different from that of multiparous cows, nor was there any interaction between group and parity in weight gain. Furthermore, parity did have an effect on BCS change over the lactation: primiparous cows lost BCS while multiparous cows gained in body condition, but no interaction between group and parity was found, indicating that the BCS loss of primiparous cows was similar in both groups. However, it should be stressed that the number of primiparous cows in this study was relatively low (n=6), (although balanced between the groups), which might be the reason for the lacking interaction between group and parity.

5.1.3. *Milk production by lactation stage*

When the MY was pooled by lactation stage, group 2 had 3.6 kg higher yields in early lactation compared to group 1, which is in agreement with Kalscheur *et al.* (1999) and Law *et al.* (2009). This might be expected as the nutrient requirements, especially protein, are high in early lactation as the milk production is at its highest levels. Even though the total intake was similar in the early lactation, the protein intake was significantly lower in group 1, which might have been limiting the milk yield at this point, as was suggested by Klauscher *et al.* (1999). Thus, in early lactation DMI is limiting the protein intake in cows on low CP diets. For the later stages of lactation the yields were similar. This can be explained by the observed higher roughage intake in group 1 in the mid lactation as well as similar concentrate and total feed intakes in the late lactation that coincided with decreasing yields with the progressing lactation. It seems like cows with lower CP in the diet can partly compensate the lower protein concentration in the diet by increasing the roughage intake after peak yields to cover the nutrient needs to maintain a high milk production throughout the entire lactation and achieving similar daily yields than cows on a standard diet.

Indeed, the ECM yield did not differ between the groups in early or mid-lactation, and group 1 even achieved over 3 kg higher ECM yields in late lactation. Further, when examining the lactation curves for ECM, it could be seen that group 1 had a lower peak yield, and as follows a more persistent lactation and higher yields in late lactation. A decreased CP content in the diet might therefore be favourable in terms of more even and persistent milk production throughout the lactation.

5.1.4 *Energy balance, LW and BCS change*

Furthermore, high yields in early lactation are often associated with more severe negative energy balance and a higher risk for metabolic disorders (Plaizier *et al.,* 2009). This risk might be decreased by aiming for lower peak yields, and a more persistent lactation so that the total lactation yields are not decreased while the stress and pressure on the cow are reduced. As was seen in both LW and BCS changes, group 1 had a more even and slight changes throughout the lactation, which also might be favourable in terms of metabolic stress that the cow will be exposed to during the lactation: in early lactation a steep decrease in BCS and LW are common where after the cow gains the lost BCS and LW later in lactation. Indeed, cows in group 2 increased heavily in LW and moderately in BCS towards the end of lactation as a result of decreasing yields and high intake levels of concentrate feeds. Hence, the nutrients were directed more towards weight gain and fat deposition as the nutrients demands for milk production decreased with decreasing MY. Similar observation was done by Kalscheur *et al.* (1999) as cows on high CP diet had higher DMI, and thereby higher LW gain in late lactation. These fluctuations in BCS and LW, whilst not unavoidable, should be kept in minimum, and therefore a more even BSC and LW throughout lactation in group 1 might give a better longevity of the cows in long term.

Also, it is interesting to note that there was a significant group*breed interaction for BCS change, and it could be seen that SRB cows in group 2 gained 0.68 BCS whereas both SRB cows in group 2 as well as SH and SRB cows in group 1 decreased in BCS during the lactation. This further indicates that the cereal and silage based diet could be suitable for SRB cows, that are known to easily deposit fat, and that did not respond to the protein supplementation by increasing their milk yield, as discussed above. The heavy deposition in body reserves (seen as increased BCS) further indicates that the consumed extra energy and protein are directed towards body reserves rather than milk production in SRB cows.

However, merely looking at LW and BCS changes does not provide much information about the energy balance and health of high producing cows on cereal and silages diets. A more comprehensive data on energy balance, health and reproduction are needed before drawing too far-reaching conclusions on the possible implications of the low CP diet on the health and energy balance of high producing cows.

5.2 Milk composition

Of the milk components, lactose content was higher in group 1, in line with Broderick *et al.* (2015) with Holstein cows and Catalapiedra-Hijar *et al.* (2014) with Jersey cows. Lactose is a highly hydrophilic compound and is therefore said to be the main determinate of milk volume (Kronfeld, 1982). The higher lactose concentration could be one possible contributor to a remained high milk production in cows on cereal and silage based diets. However, several studies on Holstein cows (e.g Colmenero and Broderick, 2006; Spek *et al.,* 2013b; Mutsvangwa *et al.,* 2016) have not observed any differences in milk lactose content.

Further, the fat and protein contents did not differ between the groups, which in turn is in agreement with Broderick *et al.* (2015), Colmenero and Broderick, (2006), Spek *et al.* (2013b) and Mutsvangwa *et al.* (2016), but in contrast to Reynal and Brodercik (2005) and Cantalapiedra-Hijar *et al.* (2014). However, Cantalapiedra-Hijar *et al.* (2014) did note that high starch diet improved the milk protein yield, and therefore the lacking difference in the present study might be because of the high starch content in low CP diet, which improves the microbial protein synthesis and thereby ensure a sufficient amount of protein and other nutrients to be supplied to mammary gland, as discussed above. All in all, these results indicate that low CP diet based solely on silage and cereals does not have drastic effects neither on the milk composition nor the yield of milk solids.

It is interesting to note that SH cows in group 1 had highest fat content in the milk, which is in contrast to the well-known fact that SH cows in general have lower concentrations of milk components than SRB cows. In line with this fact, SH cows in group 2 had the lowest protein content in the milk. No explanation can be offered to the high fat content of SH cows in group 1.

5.3 N-efficiency and environmental implications

The daily UUN excretion for the first 3 months of lactation was obtained by calculating the total daily urine volume from the urine creatinine concentration. This differed between the groups, being higher in group 1. However, when calculating the daily urine volume no differences were found. The urine volume is calculated based on the daily production of creatinine, which in turn is related to the body weight (Chizzotti *et al.,* 2008). Group 1 had a

higher average weight than group 2, and hence, higher creatinine production, but as this is related to body weight, the daily total urine volume did not differ between the groups.

As expected the overall N-efficiency was higher in the group receiving the diet based solely on silage and cereals. Similarly, the daily UUN and MUN excretion was significantly lower in this group. These observations are in line with a great number of studies (e.g. Korhonen *et al.,* 2002; Broderick, 2003; Hymøller *et al.,* 2014; Broderick *et al.,* 2015) and the general understanding that reducing CP content in the diet will lead to an increased N-efficiency and decreased N excretion. The increased N-utilization observed in this study is a result of increased uptake of both dietary N as well as ammonium-N by the microbes with high starch diets, as noted by Huntington (1997) and Bach *et al.* (2005). Higher uptake of N by the microbes as well as lower concentrations of ammonia-N caused by both increased N uptake but also decreased dietary N content leads to decreased urea formation in the liver, which in turn decreases the N-excretion via both urine and milk (Nousiainen *et al.,* 2004). Further, it has been shown that lower CP content in the diet also increases the N recycling from plasma to rumen (Kristensen *et al.,* 2010), thereby decreasing the N wastage in urine and milk. All in all, cows with lower CP intakes seem to be more efficient in converting dietary N to milk N.

The increased N-efficiency combined with a remained high production levels together point to a greater production efficiency of cows on a diet based on silage and cereal, in both environmental and economic terms. Locally produced feeds are not only cheaper to produce and purchase but also more friendly for the environment (Gustafsson *et al.,* 2013). It is clear that high inclusion of cereal grains might be criticized, as these feeds are usually based on highly human edible products (Wilkinson, 2011). However, the exclusion of soya from the feed will eliminate the environmental problems linked to the production of soya as well as this human edible part of the feed ratio. Further, decreased CP content in the cereal based diet will decrease the N pollution of the milk production, which in turn is beneficial in environmental terms (Calsamiglia *et al.,* 2010). It is also notable that cows with no protein supplementation in the feed increased their roughage intake thereby increasing the amount of human inedible fraction in their feed ratio.

6. Conclusions

The results from this study indicate that a low protein diet (CP=14.0%) based solely on cereal concentrate and high quality silage can be fed to high producing dairy cows without any significant effects on production levels, and with significantly lower MUN and UUN levels, and higher N- efficiency compared to cows receiving a diet with a CP content of 16.4% of DM achieved by protein supplementation with soybean and rapeseed expeller. These results raise interesting opportunities and alternatives for (Swedish) dairy farmers in exploiting a more varied feeding regimens and the use of locally produced feed stuffs. Also, a great possibility for reducing the negative environmental impact of dairy farming is possible with the silage and cereal based diet used in this study, in terms of both decreased N-leakage and increased N-utilization. It should be kept in mind that the results of this study are based on one lactation and 23 cows only. The results from the ongoing second full lactation study with another 25 cows should be weighted in before drawing the final conclusions of this experiment. Also the implications of cereal and silage based diet on reproduction and health of high producing cows will be further addressed.

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