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Faculty of Veterinary Medicine and Animal Science

Indirect estimation of feed intake in dairy cows on pasture based on urine volume or water consumption

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Indirect estimation of feed intake in dairy cows on pasture based on urine volume or water consumption

Skattning av foderintag hos mjölkkor på bete baserat på urinvolym eller vattenintag

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Abstract

There is a need to be able to estimate the feed intake of dairy cows on pasture. Since feed intake is correlated to factors such as the drinking water intake and urine volume, the possibility to estimate the pasture intake based on those parameters were investigated. It was also investigated whether the pasture intake could be predicted from the estimated K intake based on the estimated urine volume when the K concentration in the feed was known. High yielding dairy cows of the breeds Swedish Holstein (SH) and Swedish Red (SR) were given access to fresh pasture or an exercise area during daytime and kept indoors at night. The cows in the experiment were divided into two treatment groups; one experimental group with an unknown pasture intake offered new production pasture daily and one control group that only had access to a small exercise area. Both groups were offered concentrate supplementation at the same level according to the lactation curve, but the group with exercise pasture has access to full indoor feeding *ad libitum* both day and night while the group with production pasture had a restricted silage ration (6 kg DM) given only at night. The feed intake of silage and concentrates indoors was registered individually in both treatment groups. Intake of feed was therefore known in the exercise group while the intake in the pasture group had an unknown element: pasture intake during daytime. The daily water intake was registered and measured by water meters installed in the water bowls in the feeding area. The daily urine volume was predicted in each cow by collecting urine spot samples that were analysed for the content of urea and creatinine. The urine volume was thus estimated based on the cow's weight and creatinine concentration in the urine. Grass samples from pasture as well as silage and concentrate samples were analysed to determine the content of dry matter (DM), crude protein (CP), ash, energy, potassium (K) and other minerals. Based on dry matter intake (DMI), mineral intake, water intake and urine volume in the control group, simple linear regression and mixed linear regression were made to predict the total feed intake in the experimental group and hence pasture intake by subtracting the known amount of silage and concentrate intake. Drinking water intake (L/day) and urine volume (L/day) in the control group was plotted against the DMI (kg/day) by simple linear regression and gave the equations ($y = 0.167 x + 8.66$; $R^2 = 0.448$) based on the water intake and ($y = 0.459 x + 11.83$; $R^2 = 0.307$) based on the urine volume. When plotting the K intake (g/day) against the estimated urine volume (L/day) by simple linear regression the following equation could be derived; ($y = 9.321x + 255$; $R^2 = 0.334$) and by using mixed linear regression the equation resulted in ($y = 5.036x + 366.9$; $R^2 = 0.468$). Based on those equations the pasture DMI was estimated in the experimental group and gave reasonable intake volumes with the simple linear regression based on drinking water intake and urine volume and also by the simple and the mixed linear model based on the estimated intake of K. Which method that is preferred depends on the conditions since collection of urine spot samples may be more tediously compared to automatic registrations of drinking water intake from water bowls.

Sammanfattning

Det finns ett behov av att kunna skatta kornas foderintag på bete. Eftersom foderintaget är korrelaterat till faktorer som dricksvattenintag och urinvolym undersöktes möjligheten att skatta betesintaget baserat på dessa parametrar. Det undersöktes också huruvida foderintaget kunde beräknas utifrån det skattade kaliumintaget baserat på en uppskattning av urinvolymen när koncentrationen av kalium i fodret var känt. Högavkastande mjölkkor av raserna Svensk låglandsboskap (SLB) och Svensk röd och vit boskap (SRB) ingick i ett försök där alla djur erbjöds möjlighet till utevistelse dagtid antingen på produktionsbete eller i rasthage och hölls inomhus under natten. Korna i experimentet delades in i två grupper; en försöksgrupp som erbjöds nytt produktionsbete dagligen och därmed hade ett okänt foderintag på bete och en

kontrollgrupp som enbart hade tillgång till en mindre rasthage. Kraftfoder utfodrades i samma nivå i förhållande till laktationskurvan i båda grupperna men kontrollgruppen som endast fick tillgång till en rasthage dagtid erhöll foder *ad libitum* inne dygnet runt medan korna i försöksgruppen utfodrades med en restriktiv ensilagegiva (6 kg DM) enbart under natten. Mängden foder (ensilage och kraftfoder) som korna konsumerade i stallet registrerades på individnivå i båda grupperna. Foderintaget hos djuren i kontrollgruppen var därför känd medan intaget i gruppen med produktionsbete innehöll en okänd faktor, mängden konsumerat bete dagtid. Det dagliga vattenintaget registrerades och mättes genom vattenmätare som installerades i vattenskålarna i utfodringsområdet. Den dagliga konsumtionen av vatten registrerades således. Den dagliga urinvolymen beräknades för varje ko genom att samla stickprover av urin som analyserades för innehållet av urea och kreatinin. Urinvolymen skattades baserat på kornas vikt och kreatininkoncentrationen i urinen. Betesprover samt prover av ensilage och koncentrat analyserades för innehållet av torrsubstans (TS), råprotein (RP), aska, energi, kalium (K) samt andra mineraler. Baserat på TS-intag, mineral intag, vattenintag och urinvolym kunde enkel linjär regression samt mixed linjär regression användas för att förutsäga det totala foderintaget i försöksgruppen och därmed betesintaget genom att subtrahera intaget av ensilage och kraftfoder. Dricksvattenintaget (L/dag) och urinvolymen (L/dag) i kontrollgruppen plottades mot TS-intaget (kg/dag) genom enkel linjär regression och gav ekvationerna ($y = 0.167\ x + 8.66$; $R^2 = 0.448$) baserat på vattenintag och ($y = 0.459\ x + 11.83$; $R^2 = 0.307$) baserat på urinvolym. Genom att plotta kaliumintaget (g/dag) mot den skattade urinvolymen (L/dag) med enkel linjär regression gavs följande ekvation ($y = 9.321x + 255$; $R^2 = 0.334$) jämfört med mixed linjär regression som resulterade i ($y = 5.036x + 366.9$; $R^2 = 0.468$). Baserat på dessa ekvationer kunde TS-intaget på bete skattas i experimentgruppen och gav rimliga intagsvolymer både med enkel linjär regression baserat på dricksvattenintag och urin volym samt med enkel och mixed linjär regression baserat på det skattade intaget av K. Vilken metod som är att föredra beror på förhållandena då insamling av urinprover kan vara omständligt jämfört med att automatiskt registrera dricksvattenintagen från vattenkoppar.

Table of content

1. Introduction

The knowledge of dairy cows feed intake on pasture is important for optimization of production systems as well as for future milk production that aim for sustainability. Pasture utilization is also an important economical aspect in dairy production since grass is a cheap feed source compared to concentrates and stored forages. High pasture utilization will reduce the cost of the production and thus making it more effective (Holden *et al.*, 1994). The knowledge of dairy cows feed intake on pasture gives the producers a tool for making practical decisions concerning feeding of the animals which could improve the nutrient management (Frame & Laidlaw, 2011). Feeding inside the barn with stored forage must be regulated according the herbage intake on pasture (Hellwing *et al.*, 2015). Knowledge of the pasture intake is also crucial to be able to balance the feed ration and allocate the right proportion of concentrates. Concentrates are needed to maintain the milk yield throughout the grazing season, but too high a concentrate allowance will result in lower pasture utilization (Frame & Laidlaw, 2011). Utilizing the cow's ability to transform fibrous non-human consumables as pasture grass into high value protein is also an important environmental and sustainability aspect (Nousiainen *et al*., 2004).

The exact individual feed intake of roughage in dairy cows is complicated to measure in commercial production systems since dairy cows often are fed *ad libitum* in larger groups. Feed intake on pasture is even more complicated to measure, both under practical and experimental conditions. The available methods for estimating dairy cow's total feed intake on pasture are rather complicated. The easy methods are often unreliable and inaccurate and to get a more exact estimate more advanced methods must be used that are not applicable in commercial herds since they require advanced equipment (Hellwing *et al.*, 2015). Most estimations of feed intake involve feeding the cows with tracer substances, collection of manure and vast laboratory analyses. Since the dairy cows feed intake on pasture is difficult to measure there is a need for accurate prediction equations to be able to make satisfactory estimations that can be used both under research and farm conditions (Nennich *et al.*, 2006).

Several studies conclude that the DMI in dairy cows can be used as a factor to predict drinking water intake, mineral intake, urine volume and even the excretion of urine components such as urea (Maltz & Silanikove, 1996; Bannink *et al.*, 1999; Nennich *et al*., 2006). Since these factors are shown to be correlated to each other, the predictions could theoretically be used in the opposite direction to estimate the DMI. By measuring the drinking water intake, the feed intake could thus be estimated provided that the DM concentration of the silage and concentrates is known. The water consumption is a realistic and inexpensive measurement that could be registered and used in commercial dairy farms. Urine volume has also been shown to be related to the DMI and could theoretically be used to estimate the feed intake on pasture. However, total collection of urine is not possible on pasture and hence estimations of urine volume require spot sampling procedures (Chizzotti *et al*., 2008). Even urine components such as urea could probably be used to estimate the DMI if the crude protein (CP) content in the feed is known, since all excess nitrogen is excreted as urea in the urine. Instead of estimating DMI from urine volume, the urine volume could be used to predict the total intake of minerals like K, Na and nitrogen. From the estimated total mineral intake, combined with the known mineral content in silage, concentrates and pasture, the DMI on pasture could then be estimated. This would possibly allow for a more accurate estimation of DMI since minerals like K, Na and nitrogen are the main driving force behind urine volume (Nennich *et al.*, 2006). In practice, K may be the most influential element under Scandinavian conditions. Other minerals like Na may have a bigger impact per see on the urine volume, but since dietary K concentration usually is much larger and also varies more

than dietary Na concentration under Scandinavian conditions (Åkerlind, 2013) it may also have bigger influence on urine volume (Eriksson, 2011). However, the use of urine components for DMI estimations would rather be applicable in research than on commercial farms.

2. Aims and objectives

The main objective with this study was to investigate if dairy cows DMI on pasture could be predicted by using simple linear regression equations as well as mixed linear regression equations based on drinking water intake or urine volume. It was also investigated whether the pasture intake could be estimated based on the known K concentrations in the feed together with the estimated dietary K intake based on urine volume.

3. Literature Review

3.1 Feed intake in ruminants on pasture

According to Swedish law (SJVFS 2010:15, §25, §26) dairy cows should have access to pasture both for a certain period of the year and a certain length of time per day. In most European countries where pasture access is voluntary, dairy herds with pasture allowance have decreased over the two last decades. One reason is that feed intake on pasture is hard to estimate which makes optimal feeding based on supplementation of concentrates and silage difficult to manage (Hellwing *et al*., 2015). To be able to use grazing as a major or sole source of feed to dairy cows reliable estimations of the DMI on pasture are needed (Holden *et al.*, 1994).

Feed intake is seldom registered in commercial herds since roughage usually is given *ad libitum* and fed according to appetite (McDonald *et al.*, 2011). There are several factors that influence feed intake, ranging from the prerequisites of the animal to the characteristics of the feed and also climate conditions (Holden *et al.*, 1994; McDonald *et al.*, 2011). Feed intake varies depending of the type of forage, resulting in different feed intake of for example silage and pasture herbage. On pasture, the distribution and availability of the herbage are important factors affecting the feed intake as well as the cow's preferences for herbage species (Holden *et al.*, 1994). The feed intake on pasture can be described as the grazing time combined with the intake rate (Hellwing *et al.*, 2015). The feed intake on pasture can further be described by the cows bite size times the bite rate. The bite size is the quantity and weight of DM that the cow can harvest in one bite and the bite rate is the number of bites per minute (McDonald *et al.*, 2011). According to McDonald *et al.* (2011) a grazing dairy cow (600 kg) has a bite size of approximately 0.6 g DM. The time spent grazing is also important in pasture management. Given a bite size of 0.6 g DM combined with a bite rate of 60 times/minute the cows will need to graze for at least 7.4 hours/day to be able to achieve an intake of 16 kg DM/day and thus get their major feed intake on pasture (McDonald *et al.*, 2011). Feed intake on pasture is directly related to sward height and density since this affects the bite size in dairy cows (Frame & Laidlaw, 2011). The optimum sward is relatively short, 12-15 cm high (McDonald *et al.*, 2011). According to Frame & Laidlaw (2011), the sward height should not be below 10 cm. Herbage allowance on pasture has a linear impact on intake but at higher allowances the impact decreases (Frame & Laidlaw, 2011). Environment and climate conditions have an effect on DMI, and the feed intake has been shown to be reduced with an increase in ambient temperature and relative humidity (West *et al*., 2003). However, West *et al*. (2003) observed that the signs of reduction in DMI were delayed since it was most affected by the air temperature two days earlier. The delay could probably be explained by the time required for

a cow to consume, digest and metabolize feed (West *et al*., 2003). Contrary to that, other studies have shown that warm temperatures have an immediate negative effect on DMI in Holstein cows (Holter *et al*., 1997).

Several studies have investigated the DMI on pasture using different methods (see section 3.2). In a Danish study, Hellwing *et al*. (2015) compared nine different methods to estimate pasture DMI during the spring and the autumn, where the average estimates of the different methods was 2.2-7.6 kg DM/day for dairy cows grazing 7 hours/day in addition to TMR feeding. According to a North American study by Holden *et al.* (1994), the total daily DMI in dairy cows increased during the pasture season from 21.3 kg DM in the early spring to 22.4 kg DM in the late spring. The daily DMI on pasture consisted of 11.6-15.6 kg DM. In this study, the cows were allowed to pasture fulltime and were only fed concentrates during milking and some additional grass silage (2 kg DM/cow/day) (Holden *et al.*, 1994). The total feed intake then decreased along with the ongoing lactation. The pasture intake observed by Holden *et al.* (1994) almost corresponds to the intake of 16 kg DM/cow/day that is observed by McDonald *et al.* (2011).

Under Swedish conditions, the nutritional value differs between pasture and stored feeds such as silage. Forage tends to have a CP content around 140-150 g/kg DM (Åkerlind, 2013). Additionally, fresh grass has a high content of rumen degradable protein even if the levels are higher in silage (Frame & Laidlaw, 2011). The ash content in fresh grass range between 65-90 g/kg DM (Åkerlind, 2013), however, pasture rich in clover and other legumes can have an ash content of 85-100 g/kg DM (Åkerlind, 2013). Under Scandinavian conditions, K is the most variable mineral in forage and usually range between 20-25 g/kg DM (Åkerlind, 2013). Na contents in forage are low and range between 0.5-1.5 g/kg DM (Åkerlind, 2013). The amount of Na in grass and forage reflects how close the soil is to the sea and differs depending on location. The digestibility of the feed depends on the vegetative state of the grass when the forage is harvested and the digestibility is higher in fresh grass compared to silage (Åkerlind, 2013).

3.2 Methods to estimate feed intake on pasture

3.2.1 Prediction of feed intake

There is a need for more simple and elemental estimation methods of feed intake on pasture that could be used both in commercial dairy cow herds as well as in research situations. The models and methods that currently exist are complicated or insecure and often labour demanding and not adapted to the range of quality of the grass. Most methods involve vast collection of data, registrations and even laboratory analyses. Because of this, there is no method to estimate feed intake on pasture that has been approved to be used as a general standard method (Penning, 2004; Hellwing *et al*., 2015). Feed intake on pasture can be estimated with different methods, depending on what purpose the estimations are for. On farm level, the best methods to estimate feed intake on pasture are based on animal performance records (Hellwing *et al.*, 2015). In experimental studies, animal-based methods may give more accurate measurements of feed intake but involve laboratory analysis that are based on collection of manure and the use of external or internal markers (Penning, 2004). Feed intake on pasture can also be estimated in other ways, by sward measuring methods or by observational methods based on grazing behaviour.

3.2.2 Animal performance

Methods to estimate feed intake on pasture based on animal performance include information of the cow's energy requirements and the energy value of the feed (Penning, 2004; Hellwing *et al.*, 2015). These methods are often used in commercial farms combined with the known intake from barn feeding and daily registrations of milk yield (Hellwing *et al.*, 2015). Methods based on animal performance data are less labour demanding and do not require advanced equipment. However, these methods require adequate energy standards and accurate measurements of the cow's production. Calculations of energy and nutrient requirements for the grazing cow predicted from feeding standards could be used as a measurement of feed intake. Careful control of body weight and milk production is needed to confirm the calculations and to control systematic and random error bias. The methods based on animal performance can give useful, relative measures of herbage intake and pasture productivity. These methods are best used as comparison of farm-scale production and performance of larger groups and as a control method (cross-check) to other feed intake prediction methods and is not recommended to be used in critical studies (Penning, 2004).

Energy requirement in cows is either calculated from the metabolizable energy (ME) or the net energy (NE) required for the cows maintenance ($W^{0.75}$), production and physiological state and together with the energy required for activities like grazing it will affect the herbage intake. Animals will use 10-20 % additional energy when grazing (Penning, 2004) and this is often not accounted for in the calculations, leading to inaccurate estimates of herbage intake. Another assumption that may result in an underestimation of herbage intake is that the cows will have no further live weight gain since requirements for growth in adult animals is constant (Penning, 2004; Hellwing *et al.*, 2015). The production of the animal, such as milk yield but also gestation, will affect the energy requirement. Milk yield is registered daily in automatic milking systems and other values such as milk fat and protein percentage is also recorded regularly. The energy value of the herbage can be calculated by predictions based on tables or by more precise chemical analysis of the feed (Penning, 2004). However, in a report by Andrée *et al*. (2011) it is concluded that the nutritional value of Swedish semi-natural pasture significantly varies depending on the type of vegetation. The variation in nutritional value of the pasture could probably also apply to some extent to Swedish production pastures for dairy cows because they are commonly mixtures of several grass and legume species. This fact will complicate the process of determining the energy and nutritional value of the pasture compared to when cows are grazing on mono-cultural pastures that is the case in the United Kingdom. There are many prediction equations to link the digestibility and chemical composition to the energy value of the feed and the estimation of the herbage intake will differ depending on which equation that is used (Penning, 2004).

3.2.3 Comparisons with housed animals

It is relatively easy to calculate feed intake in housed animals fed in troughs since the forage can be weighed and leftovers taken into consideration. One way to calculate herbage intake in cows is to feed them known amounts of freshly cut herbage inside to be able to calculate their intake. This method gives a low between-cow variance and provides the closest control of random error bias in intake measurements. It is a method that is suitable for studies on ruminant nutrition but is not fully representative for estimations of herbage intake since the feed intake does not occur on pasture and the activity and hence energy requirements differ between housed and grazing animals. Those kind of feed intake measurements are often combined with measurements of the ingestive behaviour of the cows (Penning, 2004). Under Swedish as well as in Scandinavian conditions, there is a large variation of vegetation species on pasture. The more available grass and herb species on the pasture, the more will the cows

conduct selective behaviour when grazing (Andrée *et al*., 2011). Cows tend to select more energy and protein containing herbage species before species containing high levels of fibre (McDonald *et al.*, 2011).

The feed intake can be limited by physiological parameters, both within the animal and based on the feed values (Frame & Laidlaw, 2011; McDonald *et al.*, 2011). When using equations based on intake capacity to estimate the feed intake in cows, it is of great importance to take into consideration different physiological states of the animals, such as age, weight, pregnancy and the stage of lactation. For example, it is very easy to overestimate the herbage intake in late lactation. DMI calculations based on intake capacity often give higher results of intake compared to animal performance methods calculating DMI from energy requirements (Hellwing *et al.*, 2015).

3.2.4 Animal-based methods

Feed intake on pasture can also be estimated by animal-based techniques. Three basic techniques are included in the category; faecal output combined with diet digestibility techniques (marker methods), weighing methods and energy expenditure (estimates of feed intake based on energy requirements are discussed above).

Feed intake (I) can be estimated based on faecal output (FO) and digestibility (D) of the feed and is the most widely used method to determine feed intake in ruminants (Penning, 2004; Dove & Mayes, 2005).

$$I = FO / (1-D)$$

Systematic and random errors tend to occur both when estimating faecal output and digestibility of the diet but tend to be more concerning in the latter and can seriously reduce the accuracy of the estimated intake (Dove & Mayes, 2005). The faecal output can either be measured by quantitative collection or by estimation methods involving markers (Penning, 2004). Total collection of faeces is not suitable on pasture since it can disturb normal grazing behaviour in the ruminants and thus affect feed intake (Dove & Mayes, 2005). Marker techniques can be based on natural plant or feed constituents called internal markers, or introduced markers and tracer substances, called external markers. The markers or indicators consist of non-toxic substances that are not absorbed or retained in the digestive tract and could be quantitatively recovered in the faeces. To be able to use the markers the substances should also be present in small amounts in the original diet and be easily analyzed by laboratory methods. The use of markers allow estimation of herbage intake across a wide range of pasture conditions and grazing management systems and allow prediction of feed intake even in individual animals (Penning, 2004). To date, internal markers are preferred over external markers since no external marker yet has met the criteria required to be considered as an ideal marker. Internal markers have an advantage since they can be used even if the diet consists of different components with different digestibility, as often is the case on pasture and also if the animals are receiving additional supplements. Though, the intake and the concentration of the internal marker in the supplemental feed must be known (Dove & Mayes, 2005). However, when Hellwing *et al*. (2015) simultaneously used two internal markers (ingestible neutral detergent fiber and acid detergent lignin) to predict DMI on pasture the INDF: ADL ratio gave negative estimates of average DMI as well as negative intakes for individual cows. This could be explained by that the difference between herbage and barn feed in the ratio INDF: ADL was too small. The bigger difference in this ratio between pasture herbage and stored forage, the more reliable results of feed intake on pasture

(Hellwing *et al.*, 2015). Random error and bias occur with these techniques and should be combined with other control procedures (Penning, 2004).

Weighing is an animal-based technique to estimate feed intake in cows over short periods of time (hours). This method requires that the animal is weighed before and after grazing, taking the weights of faeces, urine, insensible weight loss and the amount of consumed water into consideration in the equation (Penning, 2004).

3.2.5 Sward methods

Sward methods are based on the difference between measurements made on pasture before and after grazing. The feed intake thus corresponds to the herbage offered on pasture minus the herbage refused (Penning, 2004). The total mass of herbage per unit area of ground, or the herbage mass, is estimated at the beginning and the end of the grazing period and it is the difference between these two measurements that can give an estimate of the quantity of herbage consumed per unit area (Penning, 2004). However, the herbage will grow during the pasture period, which gives a need for a correction factor. The intake of animal/day could be estimated by dividing the total estimated herbage consumption by the number of animals grazing days per unit area. This estimation of individual intake may not be very accurate since there is a variance in herbage intake between animals, and to be able to truly estimate the individual herbage intake the animals must be kept in individual pens, although, this is labour demanding and limits natural grazing behaviour in cows (Penning, 2004). The method provides a good control of random variation and the measurements of herbage mass could be done with acceptable accuracy, though it can be subject to systematic errors. The method is best suited in short grazing periods with larger groups and with a high grazing pressure which minimizes the regrowth of grass and thus the bias in estimating herbage intake (Penning, 2004).

3.2.6 Observational methods of grazing behaviour

Estimations of feed intake in grazing animals are often based on behavioural data such as grazing time and bite size. Analysis of ingestive and grazing behaviour provides better understanding of the variation of intake (Penning, 2004). Hellwing *et al*. (2015) found that observation of time spent grazing in dairy cows not is a reliable method to estimate DMI of herbage. The observed correlation between grazing time and estimated DMI is often very low and the efficiency of grazing differs between cows. Selection of different grass species on herbage would affect the results from the methods including intake capacity, digestibility and internal markers (Hellwing *et al.* 2015).

3.3 Water intake and its regulation

3.3.1 Physiology and water balance

Water is considered to be the most important nutrient for lactating dairy cows and they require large amounts of water each day (NRC, 2001). Water intake is essential for organisms and is needed for cell function, osmotic pressure, thermoregulation and the elimination of waste products from the body by urine, faeces and respiration (Sjaastad *et al.*, 2010). Cows on average meet 83 % (NCR, 2001) of their water demand only by drinking. A restriction in water intake will lead to reduced DMI and suppressed milk production (Murphy, 1992; Kume *et al*., 2010; Khelil-Arfa *et al.*, 2012).

The water balance is a concept of homeostasis that is maintained by an approximately equal amount of water intake and water loss (Sjaastad *et al.*, 2010). The amount of water in the

body can vary between days (Sjaastad *et al.*, 2010) and the live weight of a dairy cow consists of 56-81 % water (Khelil-Arfa *et al.*, 2012). Water can be gained through drinking water, water in food and metabolic oxidation of body tissue (Sjaastad *et al.*, 2010). Though, metabolic water constitutes such an insignificant amount in ruminants that the total water intake often is counted as the drinking water intake and the feed water intake only (Kume *et al*., 2010). Kume *et al*. (2010) found that 79 % of the cows total water intake came from intake of drinking water when fed a silage-based diet (51 % DM concentration). In a study by Cardot *et al*. (2008) it was shown that dairy cows consumed water during 7.3 ± 2.8 drinking occasions per day with an average intake of 12.9 litres at each drinking occasion, resulting in a water intake of approximately 94 L/day. According to Sjaastad *et al.*, (2010) a lactating cow needs to drink 84 L of water/day compared to only 23 L during the dry period. Similar results of daily water consumption in dairy cows can be found in other studies (Table 1). The majority of the drinking occasions happen during day time and the peaks in water intake are associated with feeding and milking times (Dahlborn *et al.*, 1998; Cardot *et al*., 2008). The osmolarity of urine is related to the total water intake, and the higher water intake, the lower osmolarity and the more diluted urine. The osmolarity of the urine indicates how much water is excreted in the urine and thus describes the water balance within the animal (Sjaastad *et al.*, 2010). Loss of water from the body occurs through milking, urine and faecal excretion, sweat and vapour loss from the lungs (NCR, 2001). In ruminants, loss of water from the digestive tract is significant (Khelil-Arfa *et al.*, 2012). Though, it is the loss of water through urination that the animal can regulate to maintain water balance (Sjaastad *et al.*, 2010).

3.3.2 Factors affecting drinking water intake

The drinking water intake is correlated to several factors such as the feed composition, milk yield and environmental factors such as heat and additional losses to the surroundings (Bannink *et al*., 1999: Murphy, 1992). Body weight and overstocking of animals are also factors that influence the drinking water intake since the animals will have less access to the water bowls (Meyer *et al.*, 2004; Cardot *et al*., 2008). The free water intake most likely varies with the stage of lactation since the milk yield and DMI changes with the course of lactation (Cardot *et al*., 2008). The drinking water intake in dairy cows varies between individuals (Murphy, 1992). In a study conducted by Murphy *et al*. (1983) the standard deviation (SD) in drinking water intake between cows was 19.1 L/day and exactly the same number was shown in another study by Meyer *et al*. (2004). Similar variations in drinking water intake between cows have been found in other studies and Cardot *et al*. (2008) found a variation of 17.1 L/day. Dahlborn *et al.* (1998) also found individual variations of drinking water intake even if the cows were maintained in the same environment.

Drinking water intake is highly related to DMI and the DM concentration in the diet (Bannink *et al*., 1999; Kume *et al*., 2010; Khelil-Arfa *et al.*, 2012). On the other hand, Khelil-Arfa *et al.* (2012) found a weak correlation between the total water intake and the DM concentration of the feed. DMI is the factor explaining most of the variation in water intake, even if milk yield is closely related (Murphy *et al*., 1983). Dahlborn *et al.* (1998) found that an increase in feed DM concentration increased the drinking water intake. It was observed that a hay based diet (high DM concentration) increased the drinking water intake compared to if the cows were fed silage (lower DM concentration). This was also confirmed by Kume *et al.* (2010) that found that the drinking water intake increased when the DM concentration of the feed increased and that the cows compensated the lowered feed water intake by drinking more.

The water intake is highly determined by the mineral intake of K and Na from the feed ration when other factors remain unchanged (Bannink *et al*., 1999; Nennich *et al.*, 2006).

Additionally, diets rich in protein seem to stimulate drinking water intake in dairy cows and this may be related to the increased need to excrete nitrogen with the urine. In both dry and lactating cows, there is a relationship between the dietary CP and K content and the total water intake, especially between dietary K and total water intake (Kume *et al*., 2010). Kume *et al*. (2010) concluded in their study that the cows will increase their drinking water intake in order to excrete excess nitrogen and K in the urine. There is a positive linear relationship between dietary Na content and Na intake and water intake. Murphy *et al*. (1983) showed that the drinking water intake increased with 0.05-0.20 L for every gram of Na added to the diet. Spek *et al*. (2012) concluded that for every gram increase of Na in the diet, the drinking water intake would increase by 0.14 L (± 0.017 L)

Drinking water intake is also influenced by the amount of water excreted in milk (Bannink *et al.*, 1999). In a study by Murphy *et al*. (1983) the relationship between milk production and water intake showed that a cow will drink 0.9 L of extra water/kg milk produced. Milk consists of 85-88 % water and water loss through milking can be up to 10 % of the body water in high yielding dairy cows (Dahlborn *et al.*, 1998). The water intake is influenced by the fat-percentage in the milk. Dahlborn *et al.* (1998) showed that cows selected for a low milk-fat percentage drank 12 % more water compared to cows producing equal amounts of ECM with high milk-fat percentage, independent of diet composition. This can be explained by an increased production of lactose in the milk with low fat percentage which acts as an osmotic drive of water which in turn increases the milk yield. An increased milk yield will result in an increase of water intake.

Environmental factors such as ambient temperature and relative humidity affect water intake and water balance in dairy cows (Bannink *et al*., 1999; Kume *et al*., 2010). Khelil-Arfa *et al.* (2012) found that prediction equations tended to underestimate the feed water intake and total water intake when ambient temperatures increased above 25 °C. This may be due to the increased water loss by evaporation that occurs when the ambient temperature rises (Khelil-Arfa *et al.*, 2012). Meyer *et al*. (2004) found that for each degree Celsius that the ambient temperature exceed 21 °C the drinking water intake in dairy cows increased with 1.52 L/day. Murphy *et al*. (1983) also concluded that temperature and humidity affected the drinking water intake in cows. For every degree change in recorded minimum temperature in the experiment, the water consumption would increase by 1.20 L.

Murphy (1992) studied factors that affected drinking behaviour in dairy cows and found that eating patterns and water temperature had a significant influence. Drinking behaviour was also affected if water was consumed in a trough or in a water bowl, and also by the flow rate in the water bowl. The number of drinking occasions tends to be higher in cows consuming water from bowls compared to from troughs. Since water bowls often are shared, dominant cows can restrict submissive cows in their water intake, suggesting that animal dominance also affects drinking behaviour (Murphy, 1992). However, water consumption in grazing dairy cows does not seem to be affected by the location of the water source (Spörndly & Wredle, 2005), and neither by the type of milking system (milking parlour/automatic milking) (Meyer *et al*., 2004). Spörndly & Wredle (2005) found no significant difference in water intake if the cows were offered water only inside the barn or both in the barn and on pasture, although cows offered water on pasture spend more time grazing compared to cows that had to go inside the barn to drink. The availability of water on pasture is important from an animal welfare point of view (Spörndly & Wredle, 2005).

3.4 Nitrogen metabolism and urine excretion

3.4.1 Nitrogen metabolism, urea and creatinine

Nitrogen intake from feed (expressed as CP) is mainly composed of proteins and non-protein nitrogen (NPN). Some NPN compounds that can be ingested with the feed are urea, ammonia, amides, amines, peptides and amino acids. Dietary protein consists of 160 g N/kg (16 %) and by multiplying the nitrogen content by the coefficient 6.25 (1000/160) the CP value of the feed can be calculated (McDonald *et al.*, 2011). NPN constitutes 5-15 % (Sjaastad *et al.*, 2010) of the total nitrogen content in fresh grass but in fermented feed, like silages, a large proportion of the proteins are converted into NPN by microorganisms, increasing the NPN content to approximately 70 % (Sjaastad *et al.*, 2010). The CP concentration in the feed affects nitrogen intake, protein synthesis, nitrogen digestibility and efficiency, plasma urea nitrogen, milk urea nitrogen and faecal and urinary excretion of nitrogen (Kauffman & St Pierre, 2001). Most of the ingested nitrogen will be used for microbial protein synthesis. In ruminants, proteins are degraded and synthesized in the rumen and the components that become available for post-ruminal digestion may vary a lot from that originally ingested with the feed (McDonald *et al.*, 2011). The microbes in the rumen have the first access to dietary proteins and more than two-thirds of the CP intake is ruminally degradable (Frame & Laidlaw, 2011). Most of the degradable protein together with NPN will be converted into microbial proteins, provided that there is a fermentable energy source available. The microbial protein is then absorbed in the duodenum. The protein that does not undergo degradation by microbes but goes directly to the duodenum is known as rumen escape protein, bypass protein or ruminal un-degradable protein. To be able to use a large amount of NPN for protein synthesis, the microbes need access to energy, often from easily fermentable carbohydrates. NPN substrates are essential for maintenance of rumen bacteria growth, fermentation and the ruminants' protein and energy supply since feed in ruminants usually have a low content of true protein (Sjaastad *et al.*, 2010).

Ammonia in the rumen comprises a dynamic nitrogen pool and is derived from different sources. Ammonia can either be a result of degradation of dietary protein or hydrolysis of dietary NPN or recycled urea (Owens & Bergen, 1983). The ammonia produced can be used by microbes and 50-70 % of the nitrogen content in microbial organisms in the rumen is derived from ammonia (McDonald *et al*., 2011). The ammonia that is not used by the microbes diffuses easily through the rumen epithelium into the plasma (Owens & Bergen, 1983). However, the levels of ammonia in the blood must be kept low since high concentrations of ammonia will be toxic to the central nervous system. Therefore nitrogen must be removed from peripheral tissues in a non-toxic state so it can be disposed of as urea in the liver, which is the major disposal form of ammonia in this organ (Harvey & Ferrier, 2011). Urea ($CO(NH_2)_2$) is a small, soluble nitrogen compound and is also the major end product of nitrogen metabolism in ruminants (Nousiainen *et al*., 2004). Blood urea nitrogen is affected by the level of CP intake (Kauffman & St Pierre, 2001). Urea has the ability to diffuse into various body fluids such as plasma, milk and urine (Nennich *et al.*, 2006). Urea freely diffuses between the blood:milk barrier and hence the plasma urea concentrations affect the concentrations of milk urea. Urea can either be excreted in the urine, be transported to the milk or recycled and used as a substrate in the rumen (Kauffman & St Pierre, 2001). Between 23-92 % of the plasma urea is recycled (Owens & Bergen, 1983) depending on the nitrogen content of the diet. The amount of nitrogen recycled to the rumen is positively correlated to the plasma urea concentrations and negatively correlated to ruminal ammonia concentrations (Owens & Bergen, 1983). When ruminants receive feed with low nitrogen content, less urea will be excreted in the urine and instead sent to the liver where it can be transferred to the

rumen and used for microbial protein synthesis. On the other hand, a high level of protein in the feed will allow more urea to be produced, resulting in an increased ability to concentrate urine compared to when the amount of protein is low in the feed (Sjaastad *et al.*, 2010). Excess of dietary nitrogen will be excreted as urea in the urine (Harvey & Ferrier, 2011). After excretion urinary urea is hydrolyzed by the microbial enzyme urease to ammonia and carbon dioxide, contributing to environmental emissions from animal production (Bannink *et al*., 1999).

Creatinine ($C_4H_7N_3O$) (with a molar mass of 113.12 g mol $^{-1}$), is derived from creatine and phosphocreatine in the muscles which are high-energy nitrogen containing compounds that provides a small but easily accessible energy reserve during the first minutes of intense muscular contraction. Creatinine is excreted in the urine and the excretion is positively correlated to the animal's muscle mass (Harvey & Ferrier, 2011). The excretion has been shown to be relatively constant for individual animals when expressed as mmol/kg $W^{0.75}$ (Chizzotti *et al*., 2008) and could be used as a urine volume marker since the concentration of creatinine should be independent from the urine volume (Chen *et al.*, 2004). In a small study (10 Friesian dairy cows) by Bristow *et al*. (1992) it was shown that the creatinine concentration of urine ranged between 540-1750 mg/L with an average of 980 mg/L. Eriksson *et al*. (2009) that fed different rations of silage, fodder beets and potatoes to cows with an average body weight of 638 kg reported an average creatinine concentration of 630 mg/L of urine with a range of 69-1470 mg/L for individual spot-samples. Since creatinine is correlated to the body weight of the animal, creatinine excretion in urine most likely varies with the stage of maturity. Chizzotti *et al*. (2008) showed that the creatinine excretion per kg BW decreases linearly as the body weight increases in growing heifers. The reason to why it varies in growing animals could be because of change in proportion of tissue over time. However, adult animals have less variation in body composition and body weight compared to growing animals, making creatinine excretion as a function of body weight less variable and using a fixed creatinine excretion index is usable when predicting urine volume (Chizzotti *et al*., 2008). The results in the study by Chizzotti *et al*. (2008) showed that the daily average creatinine excretion in high and medium yielding dairy cows with a urine volume of 21.6 L/day ranged between 127-135 mmol/day.

3.4.2 Excretion of urine and urine volume

The function of urination is to expel waste material and by-products from cellular metabolism in the body such as water, nitrogen, minerals and pigments (Sjaastad *et al.*, 2010). The urinary excretion of excess nitrogen and minerals is a way to maintain homeostasis in the body (Nennich *et al.*, 2006). In this process the body gets rid of nitrogenous waste products to maintain the blood electrolyte balance (Maltz & Silanikove, 1996; Khelil-Arfa *et al*. 2012). Some nitrogenous constituents of urine are allantoin, hippuric acid, creatinine, creatine, uric acid, free amino acids and ammonia. However, urea is the dominant form of nitrogen in the urine but the proportion of the substance varies with both species and diet (Bristow *et al*., 1992). In an experiment by Eriksson *et al*. (2009) were dairy cows received feed with different proportions of potatoes and fodder beets the average urea concentration in urine was 4187 mg/L and ranged between 545-10824 mg/L of urine. Bristow *et al*. (1992) examined the nitrogenous content in urine and it was concluded that the proportion of the urea and other nitrogenous constituents in the urine was highly reflected by the diet (Bristow *et al*., 1992). The difference in nitrogen excretion between diets can be explained by the differences in ammonia and CP content of the feed (Frame & Laidlaw, 2011). The ability to concentrate urine is a way to handle limited water intake, however, this ability is poor in cattle compared to other species (Sjaastad *et al.*, 2010). Cattle rather seem to be specialized towards nitrogen

conservation instead of water conservation (Maltz & Silanikove, 1996). Cows have a so called urinary fixed osmotic ceiling meaning that unlike humans and dogs, they cannot enhance urine osmolarity by the excretion of high amount of urea. During high intakes of protein rich diets a larger amount of nitrogen needs to be excreted, leading to an increase in urinary volume instead of an increased concentration of urine (Maltz & Silanikove, 1996).

DMI, dietary CP intake and mineral intake of K and Na have been directly linked to the urine volume in dairy cows (Maltz & Silanikove, 1996; Bannink *et al.*, 1999; Nennich *et al*., 2006). Dairy cows that consume a large amount of nitrogen in the diet will get an increased water intake and thus an increased urine volume and nitrogen excretion (Bannink *et al.*, 1999; Eriksson *et al.*, 2004). Nennich *et al*. (2006) concluded that the urine volume is greater when dairy cows are fed diets rich in protein compared to diets with a low protein content. The study showed that an increase of dietary CP content in the feed by 3.3 % units (from 15.1 % to 18.4 % of DM) increased the urinary volume by 6.5 L/day (Nennich *et al*., 2006). However, the CP content in feed does not explain the variation in urinary volume alone. Khelil-Arfa *et al.* (2012) suggests that variations in urine volume can be explained by the variation of CP in the forage associated with DMI (Khelil-Arfa *et al.*, 2012). Further, Spek *et al*. (2013) found that an increased CP intake not always had an effect on urine volume. In this study with incremental dietary Na concentration, the urine volume was closer associated to the Na content of the diet rather than the CP content, even if the urinary urea nitrogen excretion was positively correlated to the CP intake (Spek *et al*., 2013).

Urine excretion is the main mechanism to maintain homeostasis for K and Na in the body. Thus, intake of those minerals will directly affect the urine volume (Nennich *et al.*, 2006; Leiber *et al*., 2009). Under practical conditions, high yielding dairy cows are often fed rations containing excess minerals that must be excreted in the urine. The kidneys are limited in increasing the concentrations of minerals that need to be excreted, causing the urine volume to increase instead (Bannink *et al*., 1999). Measurements of dietary intake and concentration and excretion of K and Na in the urine have successfully been used to predict urine volume in dairy cows (Nennich *et al*., 2006). According to Bannink *et* al. (1999) the mineral concentrations in urine are better to use when predicting urine volume compared to the mineral intake. When predicting urine volume from mineral intake, the estimations could be improved if digestion and milk production is taken into account (Bannink *et al.*, 1999). What mineral that affects urine volume the most depends on which mineral that is most variable within the feed ration or experiment. Bannink *et al*. (1999) found that Na has a bigger effect on urine volume compared to K, giving a urine volume twice as high per unit weight. This is probably correlated to the molar mass of the two different minerals with K (39.1 g/mol) having almost twice the mass compared to Na (23.0 g/mol). This was confirmed by Nennich *et al*. (2006) that found that Na intake had a greater effect (ml urine/g Na) on urine excretion than nitrogen and K intake. Spek *et al*. (2012) showed a linear relationship between Na intake and urine volume and concluded that for every additional gram of Na intake, the urine volume would increase with 0.136 kg (Spek *et al*., 2012). The reasons to why the urine volume increases with a higher Na intake is because of the dilation of blood vessels that cause an increase in the kidneys glomerular filtration rate as well as a reduction of the release of ADH that will lead to a decrease in renal reabsorption of water (Spek *et al*., 2012). Even if Na is a mineral that has a strong influence on the urine excretion, some studies claim that predicting urine volume by assessing Na intake is an unreliable method when mineral blocks are available, making the variation increase within individuals (Khelil-Arfa *et al.*, 2012). In a study by Leiber *et al*. (2009) the K concentration and urine volume was compared in dairy cows that received feeds that differed in fibre and K content. The high K diet consisted of

lowland hay (28.7 g K/kg DM) and the low K diet consisted of alpine hay (10.1 g K/kg DM). The concentration of urinary K varied with the K intake with 15.2 g K/L urine for the lowland hay and 12.8 g K/L urine for the alpine hay (Leiber *et al*., 2009). If urinary K concentration reaches an asymptote, as has been suggested by studies where K concentration leveled out at approximately 13 g/L (Kume, 2008; Eriksson & Rustas, 2014), K intake would above this asymptote regulate urine volume linearly. A compilation of Swedish cattle feeding trials (Eriksson, 2011) suggested that urinary volume is predominantly regulated by the dietary intake of K in Swedish Red cattle fed typically local feed in form of grass-legume forages. In an experiment by Eriksson & Rustas, (2014) dairy cows were fed three different diets containing low, medium or high amounts of K. The results showed the urinary volume increased linearly with increasing K intake. A relationship between K intake and urine excretion that has been confirmed by Eriksson (2011) and Bannink *et al*. (1999) is 0.056 L urine/ g K intake. Nennich *et al*. (2006) also found that the concentration of K in urine was a good indicator for urine volume but that in the case of Na (and nitrogen) the total excretion in the urine was better to use compared to the concentrations.

Urine volume is also affected by the water intake in dairy cows. A high water intake results in a reduction of the protein-osmotic pressure and the concentration of ADH. This will lead to an increase in arterial blood pressure which in turn increases the urine volume (Sjaastad *et al.*, 2010). The large day-to-day variation in urine volume within cows can be explained by the large variation in water intake between days. Changes in water consumption are immediately reflected in the urine production (Bannink *et al.*, 1999). When high quality drinking water is not restricted, urine volume is considered to be in excess compared to the amount needed to excrete body wastes (Bannink *et al*., 1999). Urine production has not been shown to be indirectly predicted from water intake (Bannink *et al.*, 1999). However, Kume *et al.* (2010) found that there was also a strong positive correlation between N and K intake or urine volume and total water intake (rather than drinking water intake) in dry and lactating cows.

3.4.3 Estimation of urine volume

The exact urine volume in dairy cows takes an extensive amount of time to measure. Total collection of urine allows a direct measurement of the urine volume but often involves the use of urine sampling funnels or catheters that may cause discomfort in the animals. Additionally, total collection of urine is hard to perform on pasture since it requires that the cows are kept in tied-up barns (Eriksson *et al*., 2004; Chizzotti *et al*., 2008). The use of total collection of urine is restricted under commercial farm conditions due to it being too complicated (Chen *et al.*, 2004). Another method is to estimate the daily urine volume by measuring the creatinine concentrations in spot samples of urine (Chizzotti *et al*., 2008). The excretion of creatinine is correlated to the urine volume at a certain body weight and therefore the body weight of the cows is needed when predicting urine volume (Maltz & Silanikove, 1996). Chizzotti *et al*. (2008) found that the average creatinine excretion in lactating dairy cows was 0.212-0.213 mmol/kg BW (or 1.06-1.07 mmol/kg $BW^{0.75}$). The average creatinine excretion 0.213 mmol/kg BW (Chizzotti *et al*., 2008) corresponds to 24.1 mg creatinine/kg BW. In another study by Valadares *et al*. (1999) the average creatinine excretion in the urine was 29 mg creatinine/kg BW. The urine volume can thus be estimated from the average BW of the cow and the mean concentration of creatinine mg/L. The use of the coefficient 24.1 mg/kg BW in the equation (Chizzotti *et al*., 2008) is justified by similar results obtained in total collection studies performed at the same facility and analysed by the same techniques(Eriksson, 2010; Eriksson and Rustas, 2014).

Spot sampling procedure of urine is often used in studies of ruminal microbial protein synthesis that aim to predict the purine derivatives (that is used as a marker) excretion in cows based on the estimated urine volume from the daily creatinine excretion. To be able to use spot sampling of urine to predict urine volume the excretion of creatinine must be constant. Several studies have investigated this and Chen *et al*. (2004) concluded that there was a diurnal variation of creatinine. However, other studies like Chizzotti *et al*. (2008), indicates that the rate of creatinine excretion in lactating dairy cows is constant over the day. Valadares *et al*. (1999) concluded that the excretion of urinary creatinine did not vary between the time intervals from 05:00-17:00 but there was 5 % increase in creatinine excretion between the hours 17:00-05:00. However, the apparent increase in creatinine excretion during night could in that study be due to a loss of urine (total collection) when cows were moved for the morning milking. Eriksson *et al*. (2009) recommend that spot sampling of urine should be done multiple times within the same day to be sure to minimize variations associated to animal, sampling day and time. Valadares *et al*. (1999) also concluded that there was no significant difference when estimating the purine derivatives when comparing urine volume from total collection and estimations from spot sampling. The excretion of creatinine in the urine has been showed not to be influenced by the diet, minerals or lactational stage of cattle (Valadares *et al*., 1999; Chizzotti *et al*., 2008; Eriksson & Rustas, 2014). Valadares *et al*. (1999) found that the creatinine excretion in urine was unaffected by diet when comparing different moisture content in alfalfa silage and different proportion of silage and concentrates. However, the urine creatinine concentrations are higher during the prepartum period (10.5 mmol/L) compared to during the lactation period (5.0 mmol/L) but this is probably caused by differences in urine volume (Maltz & Silanikove, 1996).

Automated weighing systems are useful tools when calculating the body weights of cows. However, registrations of body weight can contain systematic errors and show daily variations for individual cows. The body weight has a diurnal variation within cow and will vary with the level of gut- , udder- and bladder fill (Mäntysaari & Mäntysaari, 2015). Mäntysaari & Mäntysaari (2015) concluded in their study that the morning body weight on average was 7.3 kg less compared to the afternoon body weight in Nordic red cattle. It was also concluded that the within-cow variation of body weight was 6.4 % of the total variance and could be reduced with different modelling methods. Modelling methods can thus be used to make predicted values and increase the reliability of the body weight measurements.

3.5 Predicting DMI from water intake, urine volume and K intake

Several studies have investigated the possibility to predict drinking water intake and urine volume from DMI (Bannink *et al*., 1999). Since those factors have been found to be correlated there should be a reversed method to be able to predict the DMI from water intake and urine volume. Cardot *et al*. (2008) found that the ratio between drinking water intake and DMI in cows was 4.1 L water/kg DMI and this is similar to the results from other studies; 4.7 L/kg DMI (Murphy *et al*., 1983), 4.0 L/kg DMI (Meyer *et al*., 2004) and 3.7 L/kg DMI (Kume *et al*., 2010). Khelil-Arfa *et al*. (2012) predicted the water intake in Holstein dairy cows by several equations. In most equations the DMI was an important parameter and when not including DMI in the equation to predict water intake the dataset would need to come from cows with a narrow range of milk yield and dietary DM variation.

Table 1. Summary of result from different studies showing the average DMI, DM concentration of the feed, the daily milk yield, drinking water intake and urine volume

Study	DMI (kg/day)	DM content (%)	Milk yield (l/day)	Drinking water intake (l/day)	Urine volume (l/day)
Paquay *et al.* (1970)			15.0		34.0
Murphy *et al.* (1983)	19.0	62.0	33.1	89.2	
Dahlborn *et al.* (1998)	18.4	46.0		61.1	16.0
Bannink *et al*. (1999)	19.4	67.8	25.2		30.9
Meyer *et al.* (2004)	20.5	54.5	31.1	81.5	
Nennich *et al.* (2006)	22.2		32.7		24.1
Cardot *et al*. (2008)	20.6	47.9	26.5	83.6	
Kume *et al.* (2010)	20.7	51.2	29.9	77.6	21.9
Gustafson (2001)	16.8	46.0	22.3	62.0	17.3
Khelil-Arfa *et al.* (2012)	17.3	57.4	28.8	62.7	21.5

Nennich *et al*. (2006) found that DMI was one among several factors that affected the urine volume in dairy cows. No study has reported that urine volume can be predicted from DMI alone (or vice versa) but that the equation needs additional factors to be valid. Other factors such as the CP intake, mineral intake and the concentration of milk urea nitrogen has been found to be more closely related to urine excretion than the DMI (Nennich *et al*., 2006). Leiber *et al*. (2009) made a regression on urinary excretion on DMI (kg/100 kg BW) ($y = 1.321x + 0.789$; $R^2 = 0.58$) and found a correlation when the cow were fed lowland hay containing a high amount of K. The K intake was correlated to the DMI when cows had a high intake of K corresponding to 576 g K/day but not when they had a lower K intake of 198 g K/day (Leiber *et al*., 2009). This suggests that the correlation between urine volume and DMI is dependent on other factors such as mineral intake.

Table 2. Coefficients and relationship between K intake and urine volume

Study	Slope, urine (L)/K intake (g/d)	g K intake/ L urine
Bannink *et al*. (1999)	0.056	
Gustafson (2001)		12.0
Kume *et al*. (2008)		
Leiber *et al*. (2009)	0.041	15.2
Eriksson *et al*. (2011)	0.056	17.7

Eriksson (2011) produced an equation based on a simple linear regression allowing the urine volume to be estimated from the known K intake ($y = 1.9 + 0.056x$) (coefficients of g K/L urine volume from different studies are shown in Table 2). By using the equation in the other direction, the total dietary K intake could be estimated from the urine volume. When

combining the total intake of K with the known concentrations of K/ kg DM in the feed, estimations of the feed intake based on the K intake can be done.

4. Material and Methods

4.1 Animals, housing and treatments

This experiment was part of a larger study that examined a pasture model during 7 weeks with part-time pasture allowance for dairy cows in an automated milking system. In total, 43 lactating cows of the breeds Swedish Holstein (SH) and Swedish Red (SR) from the Swedish livestock research centre at Lövsta (Swedish University of Agriculture) were used in the experiment, of which one cow left the experiment in the middle of the sampling periods because of health reasons. Cows included were both primiparous (25 %) and multiparous (75 %) with DIM (days in milk) ranging between 102-192 days (9^{th} of June). The cows were selected based on previously registered DIM and health and the groups were formed to get an even distribution between breeds, milk yield and lactation number. The experimental design of the study consisted of one experimental treatment that was compared with one control treatment.

The cows were kept in an automatic, voluntary milking system (VMS™, DeLaval International AB, Tumba, Sweden), in a feed first system. The housing consisted of cubicles with rubber mats and sawdust bedding in the lying area. In the feeding area the cows where fed grass-silage from individual troughs with automatic registration of feed intake in a database (BioControl AS, Rakkestad, Norway). There were in total seven water bowls that were located in the feeding area that were equipped with custom-made water flow meters that registered individual water consumption (BioControl AS, Rakkestad, Norway). Concentrates were distributed from three different stations placed in the lying area as well as in the milking unit (DeLaval International AB, Tumba, Sweden).

The cows were divided into two groups, either the experimental group (22 cows) or the control group (21 cows). The experimental group received a restricted grass-silage ration (6 kg of DM/day/cow) and commercial concentrates, Solid 620 and Unik 82 (Lantmännen, Stockholm, Sweden) indoors and had access to pasture during 8.5 hours/day between 06:00-10:30 h and between 16:00-20:00 h. The experimental group was given access to fresh pasture daily with a pasture allowance of 15 kg DM/cow/day. The cows in the experimental group were assumed to eat at least 12 kg roughage daily with 6 kg DM silage allowance indoors during non-grazing hours at night and the remaining intake was assumed to come from pasture. The pasture allowance of 15 kg DM/cow/day could therefore without difficulties support an intake of 7.5-10.5 kg DM pasture/day in the range of 50-70 % pasture utilization. The control group received grass-silage *ad libitum* inside the barn, and were only allowed an exercise pasture with little or no grass available. The expected pasture intake in the control group was 0 kg DM/cow/day. To ensure that the cows on the exercise pasture (control group) would not consume any pasture, the sward on the exercise pasture was cut at ground level before intake estimations, leaving no green grass and virtually no stubble for the animals to consume. The control group had access to the same exercise paddock (0.2 ha) throughout the whole experiment. Dairy cows at the research facility were routinely supplemented with minerals and NaCl by mixing it into the silage before distributing the feed in the troughs, but during this experiment the supplementation was turned off. Both the control group and experimental groups were given concentrates according to production before experimental onset at the same levels. The concentrate Solid 620 was fed at a maximum allowance of 16 kg/cow/day (as fed) but was adjusted to a standardized lactation

curve (NorFor, Aarhus, Denmark) with an assumed drop in milk yield by 0.33 L/week for multiparous cows and 0.125 L/week for primiparous cow. Thus the concentrate allowance changed weekly during the experimental procedure for each individual cow. Cows milking >40 kg milk/day received extra concentrate, Unik 82 at a maximum allowance of 2.5 kg/cow/day. The dairy cows could consume the concentrates in the feeding stations inside the barn and also twice a day during milking in the robot. The nutritional values of the silage, pasture and concentrates are presented in Table 3.

Table 3. Chemical composition and metabolizable energy in three period samples (P1-P3) of pasture and silage. The results from the analysis of the concentrates were pooled into a total mean for the whole experimental period

	Pasture			**Silage**			**Concentrate**	
	P1	**P2**	**P3**	**P1**	**P2**	**P3**	**Solid 620**	**Unik 82**
DM %	22.0	21.7	23.2	33.4	31.3	32.3	89.5	89.0
g/kg DM								
Ash	99.4	100.2	105.7	93.2	89.7	87.4	69.0	85.0
CP	187	152	171	160	154	157	178.0	296.0
NDF	353	425	373	433	437	419	282.0	243.0
ME MJ/kg DM	11.3	10.9	11.0	11.4	11.6	11.8	13.2	14.0
K	28.3	24.2	32.6	30.9	30.3	29.0	8.7	13.3
Na	0.2	0.1	0.1	1.9	1.9	1.2	3.7	3.5
Ca	8.6	6.8	10.1	5.4	5.0	4.5	8.9	12.1
Mg	1.6	1.3	1.7	1.7	1.8	1.6	4.0	4.6
P	3.3	3.0	3.1	3.2	3.2	3.1	5.7	7.4
S	2.1	2.1	2.2	2.2	2.2	2.1	3.2	5.0

4.2 Data sampling procedure

Spot sampling of urine was performed to be able to estimate the total urine volume. The main sampling procedure was preceded by a covariate period in April and May when the sampling method was tested and evaluated. To get a covariance value from the indoor feeding season with completely known feed intake, one urine sample from each cow in the experiment was collected on the 4th of May.

The main sampling was done during three periods, the first period ranging between 9 – 13 of June, the second period ranging between 22 - 26 of June and the third period ranging between 29 of June – 3 of July. The samples were taken during day time (07:00-13:00) when the cows were inside the barn and since both groups (control and experimental) had access to pasture until 10:30 samples from the animals were taken when they on free will entered the barn. Urine was collected in buckets from voluntary urinations. Samples were then taken using a 3.7 ml tube that was transferred to a Saarstedt tube containing 30 ml of 0.10 M HCl (resulting in a dilution factor of 33.7/3.7 = 9.11) . The tubes were marked with cow ID, date and time.

After collection, each urine sample was further divided into three smaller (ca 10 ml) replicate samples in storage tubes before stored in a freezer at -30 °C until analysis of urea and creatinine. A total of 781 urine samples were collected during the main sampling period with a mean of 6 samples/cow/period (range of 2-10 samples/cow/period), of which 769 samples were entered into the final dataset after discarding samples with outlier results or that belonged to cows not assigned to the experiment.

Pasture samples were taken four times daily in connection to when the cows were let out and in from pasture (06:00, 10:30, 16:00 and 20:00). The fresh pasture samples were weighed and dried in an oven at 60 °C for 12-24 hours. The preliminary DM concentration was then calculated by dividing the weight of the dried pasture sample with the fresh pasture sample weight. Silage samples were collected daily from the feeding troughs and stored in a freezer -30 °C until analyzing. Concentrate samples were also collected and stored until analyzing. The temperature and relative humidity was registered daily outside on pasture with a digital thermometer (Clas Ohlson AB, Insjön, Sweden). Due to some misunderstandings, barn temperature and humidity was only registered once a day during the third experimental period. A weather station close to the barn also registered the ambient temperature and relative humidity outside on an hourly basis.

The consumption of grass-silage from the troughs, concentrates and water intake for each individual cow in both treatment groups was registered. The flow meters in the water bowls registered the water consumption with some bias that was corrected for in the calculations with a calibration factor for each water bowl based on controls. The body weights of the cows were registered with an automatic weighing system 4.2 times daily (mean based on data from the first sampling period) (AWS100; ALPRO™, DeLaval International AB, Tumba, Sweden) when passing a selection gate on the way to milking in the robot or to the lying area. The milk yield was registered during milking in the robot (Delpro™, DeLaval International AB, Tumba, Sweden). Cows were test milked for three consecutive days during each of the three sampling periods and samples were analyzed for fat, crude protein and lactose by FTIR spectroscopy.

4.3 Laboratory analysis

The concentration of urea and creatinine was analyzed on an AutoAnalyser III (SEAL Analytical GmbH, Nordstedt, Germany). The urine samples were defrosted and firmly shaken before dilution. Each urine sample was diluted 2 times before analysis (0.75 ml sample + 0.75 ml dilution) and placed on a rack following the serial numbers. In the analyser the samples were mixed with colour reagents that reacted with either urea (diacetylmonoxime, Technicon methodNo. SE40001FD4) or creatinine (picric acid, Technicon method No. SE4-0011FH4). The absorbance was then measured in a colorimeter and the absorbance correlated to the concentration of urea and creatinine. The results were registered and multiplied with the dilution factor of 18.22 (9.11 * 2) to get the concentration (mg/l) of urea and creatinine in the urine.

The daily silage samples were defrosted and weighed before they were dried at 60 °C in a drying cabinet for at least 24 h. The dry samples were then left to stabilize in room temperature for a minimum of 4 h. The silage samples were then weighed again to calculate a preliminary DM concentration. A hammer mill (KAMAS AB, Malmö, Sweden) with a 1.0 mm screen was used to mill the samples and ca 100 ml of each sample was put in a jar for further laboratory analysis. A portion of each daily sample was used for pooling together weekly samples of 15 g. Those weekly silage samples were then analyzed as described by

Åkerlind *et al*. (2011) for DM at 60 °C (with correction for losses of volatiles), ash, CP, NDF and *in vitro* organic matter digestibility by the method of Lindgren (1979) for calculation of metabolizable energy. Minerals (Ca, K, Mg, Na, P and S) were analysed at Agri-Lab, Uppsala by inductively coupled plasma atomic emissionspectroscopy (Spectroflame; Spectro GmbH,Kleve, Germany) after digestion with nitric acid. Samples collected in the actual bunker silo during the experiment were used for obtaining liquid for subsequent pH measurement, analysis of fermentation products with chromatography (Ericson & André, 2010) and ammonia analysis with flow injection (FOSS-Tecator, 1992). The pasture samples were prepared in a similar way as silage samples and analyzed for the same content (except for analysis of fermentation products and ammonia). Samples of the concentrates were also analyzed for DM, ash, NDF, CP and minerals as described for silage samples. The energy value for the concentrate was adapted from the product declaration of the manufacturer (Lantmännen, Stockholm, Sweden). The results for the concentrate samples were averaged into an experimental mean before further intake calculations.

4.4 Calculations and statistical analysis

All data including drinking water intake, silage intake, concentrate intake, DMI, urea excretion and creatinine concentration, body weight, urine volume and milk yield was calculated to weekly means for each cow and experimental period together with standard deviations (SD) for the different treatment groups. The variation within cow was also calculated for drinking water intake and silage intake. The experimental periods included the three weeks that comprised the main sampling period as well as the covariate period in the spring (1 covariate sampling period + 3 main sampling periods). The covariate period only included one day of urine sampling (4^{th} of May) but the weekly means of silage and water consumption was calculated for four days (1^{st}-4^{th} of May) and the milk yield for five days (2^{nd} -6^{th} of May). Registrations of ambient temperature and relative humidity from the weather station were calculated to an AM/PM average (corresponding to when the cows were allowed to pasture) for each experimental period. To calculate the combined effects of ambient temperature and relative humidity a temperature-humidity index (THI) was calculated according to Mader *et al*. (2006):

$$0.8 \times \text{ambient temperature} + [(\%\ \text{relative humidity} \div 100) \times (\text{ambient temperature} - 14.4)] + 46.4$$

When calculating the weekly means of body weight for each cow, numbers that differed more than ± 50 kg from the cow's mean weight from each experimental period were considered to be outliers and were excluded from the calculations. After excluding the outlier numbers the average weekly weights were recalculated. The outlier numbers could be the cause of the cow not standing properly with all four claws on the scale or that another cow partly stood on the scale as well resulting in an inaccurate registration of body weight. When calculating the urine volume from the creatinine concentration and body weight the formula derived from Chizzotti *et al*. (2008) was used:

$$\text{L urine/day} = (24.1 \times \text{BW}) / (\text{mg creatinine/L urine})$$

Numbers that were considered outliers were excluded when calculating the period means of water intake and silage intake. For example, feeding sessions exceeding over 30 g of consumed silage per second were excluded. The feed intake of silage was then multiplied with the DM concentration derived from the feed analysis to get the total weekly DMI from silage for each cow. The same was done to calculate the intake of concentrates. Milk yield

was compiled from the detailed log files of the milking unit after careful examination for possible erroneous registrations.

Preliminary statistical analysis was done with the application Analysis ToolPak in Microsoft Office Excel 2007 (ver. 12.0.4518.10.14, Microsoft Corporation, Redmond, WA, USA) and results were then verified by using the appropriate procedures in SAS (Ver. 9.3, SAS Institute Inc., Cary, USA). SAS was also used for mixed model analyses and stepwise regressions. Effects of the fixed variables covariate value, treatment, parity (primiparous or multiparous), breed, week and the interaction treatment*week were examined in a model in Proc MIXED that also included cow as a repeated variable. Covariance structure was autoregressive (AR1). Non-significant ($P > 0.05$) factors were successively deleted from the model, starting with the factor with largest P, except for the factor treatment that always was retained. Results are presented as arithmetic means for treatments and treatment*week with significance for treatment differences obtained by the final mixed model for each variable.

Correlations between different factors were calculated for the control group with Proc CORR. Stepwise regressions were done with Proc REG on factors affecting water intake and urine excretion. Variables were allowed to enter the model if $P < 0.15$ and to stay in the model if $P < 0.15$.

Simple and mixed linear regressions for the control group were done with Proc REG and with a Proc MIXED model with random intercept and slope for individual cows. Results were expressed with "adjusted y:s" (St-Pierre, 2001) to obtain R^2 values. The total daily DMI in the control group was regressed against daily drinking water intake and also against daily urine excretion. Daily K intake was also regressed against daily urine excretion. The results are presented in figure 1 and 2.

The regressions for total DMI intake in the control group were directly applied on water intake and urine excretion, respectively, for the experimental group so that a total DMI could be estimated for each cow in each period. Pasture DMI was then calculated by deducting intake of silage and concentrates. The simple and the mixed linear regressions with K intake on urine excretion were applied in a similar manner on the experimental group to estimate a total K intake. After deducting K intake with silage and concentrates, the remaining K estimate was divided by pasture K concentration to obtain an estimate of pasture DMI. DMI based on total K intake estimated from urine volume was also calculated by rearranging the simple linear regression equation obtained from previous N balance trials (Eriksson, 2011):

Urinary excretion (L/D) = 1.9 + 0.056 K intake (g/d)

After rearranging, the equation read:

K intake = (Urinary excretion -1.9)/0.056

This equation was applied on urinary excretion estimates from the experimental group and pasture intake was then calculated after deducting K intake from silage and concentrates as previously described.

5. Results

Table 4. Daily mean values of DMI, water intake, milk yield, urine volume, body weight, excretion of creatinine and urea and K intake for the entire experiment and for each experimental period (P1-P3). The total DMI of silage and concentrates is equal to the total DMI for the control group; however, in the experimental group the DMI from pasture is not included. The P-value indicates the difference between the two groups during all the three periods. P value not calculated for total DMI and silage DMI because of intended difference in experimental design. The standard deviation (SD) is calculated between each observation during all sampling periods for the two treatments

	Experimental group					**Control group**					***P* value**
	P1	**P2**	**P3**	**Total**	**SD**	**P1**	**P2**	**P3**	**Total**	**SD**	
DMI silage kg	5.05	4.77	5.77	**5.20**	1.0	12.79	14.14	13.75	**13.56**	2.2	
DMI conc. kg	10.95	10.87	10.72	**10.85**	2.9	10.51	10.23	9.82	**10.19**	2.8	n.s.
Total DMI (excl. pasture)	16.00	15.64	16.49	**16.05**	3.5	23.30	24.37	23.57	**23.75**	3.9	
Water intake L	83.6	75.7	89.4	**82.9**	18.6	92.0	86.4	93.1	**90.5**	15.5	**
Milk yield L	37.8	37.6	36.9	**37.4**	9.3	36.5	35.8	34.8	**35.7**	7.8	n.s.
Urine volume L	26.6	25.6	25.1	**25.8**	4.6	25.8	27.1	25.0	**26.0**	4.7	n.s.
Body weight kg	666	674	671	**671**	74	653	667	674	**665**	73	n.s.
Creatinine mg/L	628	649	664	**647**	134	621	598	667	**629**	94	n.s
Urea-N g	141	153	146	**147**	23.0	124	129	125	**126**	24.6	***
K intake g (excl. pasture)	253	252	251	**253**	46	495	496	497	**497**	75	

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

5.1 Pasture, silage and concentrate samples

The results from the feed analysis are presented in Table 3. The pasture samples varied in DM concentration, both within and between days as well as between weeks. The total DM mean in the pasture samples was estimated to be 22.3 % and the K concentration was 28.4 g/kg DM for all the three experimental periods. The DM concentration in the silage varied between days and also between the three experimental weeks with a total mean of 32.3 %. The average content of K in the silage samples was 30.1 g/kg DM for the three experimental periods. The results from the analysis of the concentrates were pooled into a total mean for the whole experimental period.

5.2 Silage and concentrate intake

The daily mean consumption of silage in the control group was in line with what the cows were expected to consume with *ad libitum* feeding (12 kg DM/day) and the intake ranged between 9.37-20.50 kg DM/day. The experimental group had a restricted allowance of silage of 6 kg DM/day and the intake ranged between 2.10-6.98 kg DM/day. The average within cow standard variation of daily silage intake was higher in the control group (1.26-3.55 kg DM in the control group, with a variation mean of 2.18 kg DM) compared to the experimental group (0.62-2.41 kg DM with a variation mean of 1.18 kg DM). The concentrate ration was

the same for both the experimental and the control group and the consumption of concentrates did not significantly differ ($P < 0.05$) between the two treatments.

5.3 Drinking water intake

Experimental period means for daily drinking water intake of individual cows ranged between 46.2-123.4 L. The total mean for both treatment groups were 86.6 ± 17.5 L/day. The drinking water intake differed significantly ($P = 0.01$) between the experimental group and the control group. The daily average water consumption in the experimental group was 82.9 ± 18.6 L and the corresponding number in the control group was 90.5 ± 15.5 L. The weekly within cow variation of daily drinking water consumption varied between 1.3-30.9 L/day and the mean within cow variation in both groups were 11.2 L/day. The coefficient of variation was estimated to 13.4 %.

5.4 Urine volume, urine components and body weight

The weekly urine volume was estimated based on the creatinine concentrations in the urine samples and the weekly body weight of the cows. The estimations showed that the daily urine volume ranged between 11.9-39.5 litres with an overall mean of 25.9 ± 4.6 L/day/cow. The urine volume did not significantly differ ($P > 0.05$) between the experimental group (25.8 L/day) and the control group (26.0 L/day).

The average daily concentration of creatinine and excretion of urea in the urine for the two treatment groups is presented in Table 4. In total, the mean concentration of creatinine in the urine was 638.0 ± 116.1 mg/L/day and ranged between 441.7-1139.9 mg/L. The mean concentration of urea N was 5.3 ± 0.92 g/L in both treatment groups and the mean excretion was 136.8 ± 25.9 g/day. The excretion of urea N ranged between 87.2-195.5 g/day in the experimental group and 83.9-183.4 g/day in the control group and differed significantly ($P < 0.001$) between the groups.

In general, the cows increased their body weight with 10 kg from the first sampling period (9 – 13 of June) to the second sampling period (22 - 26 of June) and with 2 kg between the second and the third sampling period (29 of June – 3 of July). The average weight of all cows during the whole experiment was 667.6 ± 72.9 kg.

5.5 Milk yield

The milk yields were calculated from registrations from a database, however, some data needed to be adjusted and some milking occasions (23) were not registered because the cows were milked > 4 times/day. The cows milked on average 2.5 times a day during the three sampling periods and had a total average milk yield of 36.6 ± 8.5 L/day with a range of 19.4-55.4 L/day. There was no significant difference ($P < 0.05$) in milk yield between the two treatment groups.

5.6 Temperature and Humidity

The ambient temperature and relative humidity varied between the different experimental weeks with slightly warmer conditions outside on pasture during the third period (Table 5). Calculations of THI also showed highest values during this period. Barn temperature was only measured during the last experimental week and gives thus no possibility to compare the temperature inside between the different periods.

Table 5. The ambient temperature (degree Celsius) and the relative humidity (%) registered in the weather station close to the barn and the calculated THI. The results are presented as an AM and PM value for each of the three experimental periods (P1-P3)

	P1 AM	P1 PM	P2 AM	P2 PM	P3 AM	P3 PM
Pasture temp C°	14.2	19.7	12.3	16.8	18.0	23.5
Pasture RH %	56.8	41.9	82.9	61.5	71.5	48.0
Pasture THI[1]	57.6	64.4	54.5	61.3	63.4	69.6
Barn temp C°					19.2	22.8
Barn RH %					56.0	60.0

[1]Temperature-humidity index according to Mader et al. (2006)

5.7 Statistical analysis

The effect that different experimental factors had on each other showed that there were effects of the covariate value on milk yield, drinking water intake and urine excretion (Table 6).

Table 6. Effects of experimental factors. Treatment differences were assessed by retaining factors with $P < 0.05$ in the model.

	Covariate period	Parity	Breed	Treatment	Week	Week*Treatment
Milk, kg/d	<.0001	NS	0.01	NS	NS	NS
Drinking water, L/d	<.0001	0.009	0.02	<.0001	<.0001	0.02
Urine excretion, L/d	0.007	NS	NS	NS	NS	NS
Urinary urea N, g/d	0.0001	0.02	NS	0.0004	0.0005	NS
Creatinine, mg/L	NS	0.04	NS	NS	0.011	NS

The results showed that there were correlations between total DMI and drinking water intake as well as between total DMI and urine volume. The K intake also had significant correlations both to total DMI and urine volume (Table 7).

The results from the stepwise regression showed that the factors significantly affecting drinking water intake and urinary excretion were DMI, K intake, Na intake, CP intake, milk yield, body weight and water intake in the covariate period (Table 8). Some factors entering late in the regression equation such as DMI showed negative coefficients to drinking water intake. The results also showed that the CP intake was important in explaining the variation in drinking water intake and the K intake was important in explaining the variation of urine volume.

Table 7. Correlations between different factors obtained from the control group and their significance

	Total DMI	*Water intake*	*Urine volume*	*Milk yield*	*Total DM %*	*MJ ME*	*CP*	*UUN*	*K*
Water intake	*** 0.67								
Urine volume	*** 0.55	** 0.36							
Milk yield	*** 0.79	*** 0.60	*** 0.42						
Total DM %	** 0.36	** 0.38	n.s 0.04	*** 0.69					
MJ ME	*** 1.00	*** 0.68	*** 0.54	*** 0.81	** 0.39				
CP intake	*** 0.99	*** 0.70	*** 0.55	*** 0.84	*** 0.45	*** 1.00			
UUN, g/d [1]	*** 0.70	** 0.37	*** 0.71	*** 0.55	*** 0.22	*** 0.69	*** 0.70		
K intake	*** 0.90	*** 0.55	*** 0.58	*** 0.52	n.s -0.03	*** 0.87	*** 0.86	*** 0.63	
Na intake	*** 0.88	*** 0.57	*** 0.50	*** 0.84	*** 0.58	*** 0.88	*** 0.90	*** 0.61	*** 0.72

[1]UUN = Urinary urea N

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Table 8. Stepwise regressions for factors affecting drinking water intake and urinary excretion. Variables allowed to enter the model if $P < 0.15$ were: DMI, K intake, Na intake, CP intake, milk yield, body weight and water intake in the covariate period

	Intercept/slope	SE	*P* value	R^2 cumulative
Drinking water intake, L/day				
Intercept	35.56	11.04	0.002	
Water intake covariate period	0.47	0.10	<.0001	0.51
CP intake, g/d	0.06	0.02	0.001	0.64
DMI, kg/d	-6.28	2.69	0.02	0.66
Na intake, g/d	-0.67	0.22	0.00	0.69
Body weight, kg	-0.05	0.02	0.03	0.71
Drinking water intake, L/day (covariate value not allowed)				
Intercept	38.93	8.94	<.0001	
CP intake, g/d	0.08	0.02	<.0001	0.49
Na intake, g/d	-0.65	0.25	0.01	0.53
DMI, kg/d	-9.76	3.11	0.003	0.58
Urine, L/d				
Intercept	1.60	4.34	0.71	
K intake, g/d	0.022	0.01	0.01	0.33
Body weight	0.020	0.01	0.03	0.38

5.8 Prediction of pasture intake in the experimental group

In the control group, the DMI explained 44.8 % of the variation of the drinking water intake and 30.7 % of the variation of the urine volume. The simple linear regression models based on data from the experimental period means in the control group had slopes of 0.167 kg DM/L water and 0.459 kg DM/L of urine, respectively (Fig. 1) ($P < 0.001$ for both). The intercept for the water intake regression was 8.66 and 11.83 for the urine volume regression and both were significant ($P < 0.001$). Using the equations from the simple linear regression analysis of the control group, the total DMI in the experimental group was estimated to 22.6 kg/day or 23.7 kg/day for the water and urine regression, respectively. With a total DMI of 16.1 kg/day of silage and concentrates for the cows in experimental group, the pasture intake could be expected to be 6.5 kg DM/day and 7.6 kg DM/day for the water and urine regression, respectively. The estimated pasture DMI for each regression and period is presented in Table 9.

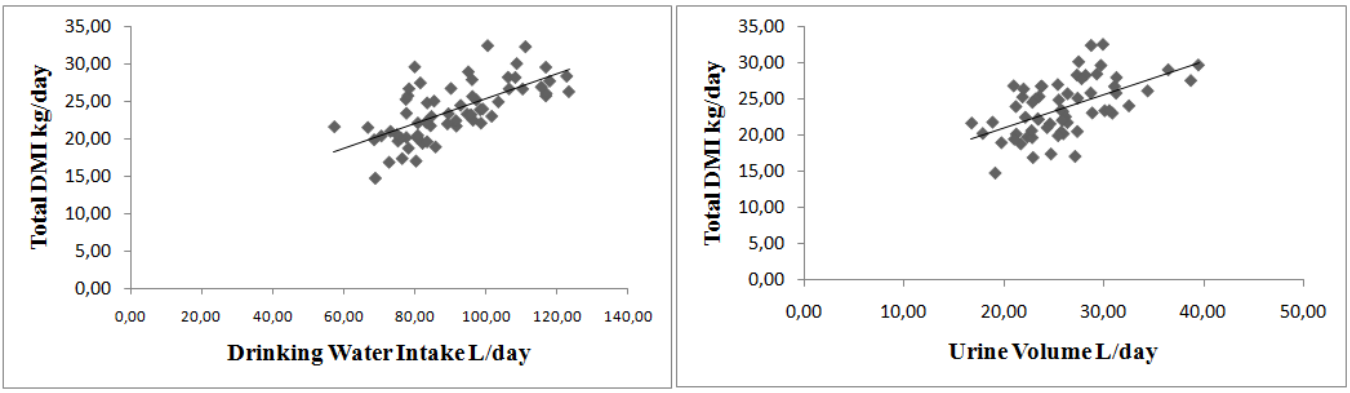

Figure 1. DMI (kg/day) in the control group plotted against drinking water intake (L/day) and urine volume (L/day) by simple linear regression. Left: ($y = 0.167 x + 8.66$; $R^2 = 0.448$). Right: ($y = 0.459 x + 11.83$; $R^2 = 0.307$). Both the slopes and intercept in both equations are significant ($P < 0.001$)

The mixed linear regression was used to adjust for the variation within individual in the control group. Both the regressions based on drinking water intake and urine volume had non-significant slopes of 0.032 kg DM/L of water ($P = 0.20$) and 0.112 kg DM/L of urine ($P = 0.13$), respectively. However, intercepts were both significant for the mixed linear model equations ($P < 0.001$). In the mixed linear model the results was adjusted for variation within individual. The mixed linear regression based on drinking water intake gave the following equation; ($y = 0.032x + 20.97$; $R^2 = 0.255$), while the mixed linear regression based on the urine volume resulted in; ($y = 0.112x + 20.70$; $R^2 = 0.358$).

By using simple linear regression to plot the K intake in the control group against the urine volume the following equation could be derived; ($y = 9.321x + 255$; $R^2 = 0.334$). The slope and the intercept were both significant ($P < 0.001$) (Fig. 2). Plotting the K intake against the urine volume by mixed linear regression gave the equation ($y = 5.036x + 366.9$; $R^2 = 0.468$) with a significant slope of 5.036 g K/L of urine ($P < 0.01$) as well as a significant intercept ($P < 0.001$) (Fig. 2). The K intake explained 33 % of the variation of urine volume in the control group according to the simple linear equation and 47 % of the variation according the mixed linear equation. The estimation of pasture intake based on the concentration of K in the feed and the estimated K intake based on urine volume resulted in an overall pasture DMI mean of 8.72 DM/day based on the simple linear regression, 8.78 kg DM/day based on the mixed linear model and 6.26 kg DM/day based on the simple linear regression equation (Eriksson, 2011). Since the mean DMI of silage and concentrates in the experimental group was 16.1 kg this results in a total DMI of 24.9 kg/cow/day and 22.4 kg/cow/day respectively.

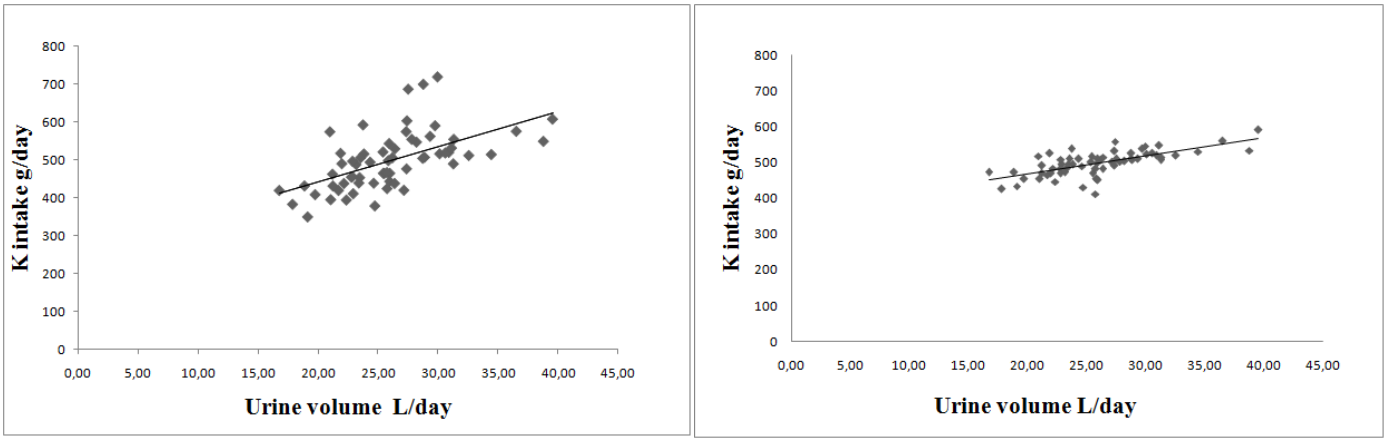

Figure 2. K intake in the control group plotted against urine volume (L/day). Left: Simple linear regression (y = 9.321x + 255; R^2 = 0.334). Right: Mixed linear regression (y = 5.036x + 366.9; R^2 = 0.468). Both the slope for the simple linear regression ($P < 0.001$) and the mixed linear regression ($P < 0.01$) and the intercepts ($P < 0.001$) are significant

Table 9. The estimated pasture intake (kg DM/cow/day) in the experimental group based on the simple linear regressions and mixed linear regressions calculated from the control group. Period values as well as overall means are presented. The min and max values refer to the lowest and the highest estimated value for an individual cow during all the three experimental periods

	Pasture intake kg DM simple linear regression						**Pasture intake kg DM mixed linear regression**					
Regression based on;												
	P1	**P2**	**P3**	**Total Mean**	**Min**	**Max**	**P1**	**P2**	**P3**	**Total Mean**	**Min**	**Max**
Water intake	6.62	5.65	7.09	6.46	0.56	10.42						
Urine volume	8.04	7.96	6.83	7.61	-0.33	15.46						
K intake	8.80	10.45	6.92	8.72	4.00	16.32	8.73	10.53	7.07	8.78	4.56	14.46
K intake[1]	6.62	7.55	4.62	6.26	0.20	17.31						

[1] Simple linear regression based on the total K intake estimated from urine volume using previously mentioned equation (y = 1.9 + 0.056x; R^2 = 0.956) (Eriksson, 2011)

6. Discussion

6.1 Prediction of pasture DMI

The total DMI consisting of both silage and concentrates in the control group was 23.8 ± 3.9 kg DM/cow/day for the whole experimental period. This number can be used as a guideline of how much the total DMI of silage, pasture and concentrate could be in the experimental group. The total DMI of silage and concentrates excluding pasture intake in the experimental group was 16.1 ± 3.5 kg DM/cow/day for the whole experimental period. This will lead to an assumption of a pasture DMI consisting of 23.8-16.1 = 7.7 kg/cow/day in the experimental group, given that the cows in both groups consumed the same amount of kg DM and that the experimental group complemented their restricted silage intake with pasture. This is a credible amount of herbage intake and is in line with what has been found in earlier studies

conducted under Scandinavian conditions (Hellwing *et al*., 2015). The estimations of pasture DMI from the different regression models in Table 9 are close to what was expected and lies within the range of estimated DMI in the study by Hellwing *et al*. (2015). However, the estimated intake is lower compared to the pasture intake suggested by Holden *et al.* (1994) and McDonald *et al*. (2011) which can be expected since the cows in these experiments had almost their solitary feed intake from pasture with no additional feeding. It is also important to consider the geographical difference of where the studies have been conducted which gives different conditions for animal production with respect to climate and the length of the pasture season. Therefore these references may not be applicable to Swedish or Scandinavian conditions.

The slopes in the mixed linear regression for both water intake and urine volume were not significant ($P > 0.05$) and hence the equations should not be used when estimating the pasture DMI in the experimental group. When using the simple linear regression equations, estimations of the pasture DMI in the experimental cows sometimes gave a negative individual value. The same cow could be estimated to have a negative pasture intake with one of the simple regression and a very high pasture intake based on another simple regression for the same week. This tendency can be viewed as one of the consequences of applying regression equations as prediction methods. The negative intake values are consistent with some of the results in the study by Hellwing *et al*. (2015) that also got negative intake values when using regression equations. It is suggested that when the cows are fed forage and concentrates inside the barn as a supplement to grazing, this can result in variations in the estimated DMI on pasture (Hellwing *et al*., 2015). In could also be of interest to investigate whether the estimation equations would give reasonable predictions of DMI in cows with a wider range and larger variation in drinking water intake and urine volume. The case may be that the estimations only fit cows within a certain range of feed intake, water intake and urine volume.

To get a correlation between urine volume and DMI it seems like other factors such as high K intake are important (Nennich *et al*., 2006; Leiber *et al*., 2009). The estimations of pasture DMI based on the concentration of K in the feed and the estimated total K intake from urine volume resulted in a total pasture intake of 8.72 kg DM/day based on the simple linear regression 8.78 kg DM/day based on the mixed linear regression and 6.26 kg DM/day based on the simple linear regression equation derived from Eriksson (2011) as can be seen in Table 9. Both the simple and the mixed linear regression equations derived from this experiment gave similar estimations of pasture intake and the estimations gave a higher pasture intake compared when using the simple linear equation derived from Eriksson (2011). Another important difference is the R^2 values that are much lower in the regression equations based on this experiment compared to the one from Eriksson (2011). According to the results from the statistical analysis the K intake did not explain as much of the variation in urine volume that could have been expected from the earlier study. The correlation between K intake and urine volume was 0.58 suggesting a moderate relationship between these factors but it strengthens the fact that it is possible to estimate one of the factors based on the other. Additionally, when plotting K intake against urine volume in a mixed linear regression equation the slope was significant ($P < 0.01$) and this suggests that the method is useful when estimating pasture DMI. The results can be compared with other studies shown in Table 1.

The statistical analysis showed that the correlation between total DMI and drinking water was 0.67 and the correlation between total DMI and urine volume was 0.55. This could be seen as a moderately strong correlation and supports the method of estimating the dairy cow's DMI

based on their drinking water intake or urine volume. The total DMI had also strong correlations (Table 7) with the total intake of CP, K and Na in the feed suggesting that it would be possible to estimate DMI given the intake of these factors are known. There was a strong positive correlation (0.79) between milk yield and DMI in the control group. This corresponds to earlier studies that also have found significant correlations between DMI and milk yield (Murphy *et al*., 1983). However, the strong correlation between milk yield and DMI is expected since the concentrate allowance was adjusted after the milk yield and an expected intake of silage and pasture.

The stepwise regressions for factors affecting drinking water intake and urinary excretion that can be seen in Table 8 did show that DMI, K intake, Na intake, CP intake, milk yield, body weight and water intake in the covariate period were all significant. This is in line with what have been found in earlier studies (Maltz & Silanikove, 1996; Bannink *et al.*, 1999; Nennich *et al*., 2006). However, many of the factors that were added last in the regression equations had negative coefficients. For example, when it comes to drinking water intake the cows should have consumed 6.28 L/day less for each kg of DM consumed, which is in opposite with what have been found in earlier studies where there is a clear positive correlation between drinking water intake and DMI (Bannink *et al*., 1999; Kume *et al*., 2010; Khelil-Arfa *et al.*, 2012). The reason for the negative coefficients is that the factors added last to the regression explains such as small part of the variation and that previously added factors already explained most of the variation.

6.2 Silage intake and feed samples

The cows in the experimental group had an average silage intake of 5.2 kg DM/day as shown in Table 4. The allowance was 6.0 kg DM/day which means that the cows consumed less silage than expected. This is probably due to that there was a lack of silage during the nights which was concluded after observations of the troughs before the morning feeding. Results from the behavioural study showed that there was a significant difference ($P < 0.001$) between how much time the cows in the experimental group (5.6 h/day) and the control group (2.6 h/day) spent on pasture which shows that there was very little or no grass available, suggesting that when there is no feed available the pasture gets very unattractive. The observation further suggests that the cows in the control group really consumed virtually all their feed indoors from the troughs and concentrate feed stations and that the measurements of their total feed intake is plausible. This is also strengthened by the fact that the cows in the control group on average consumed more silage than expected (13.6 kg DM/day compared to 12 kg DM/day). However, some within cow variation of daily silage intake occurred and was larger in the control group compared to the experimental group. This is expected since the control group had access to silage *ad libitum* while the experimental group had a restricted silage allowance. According to some observations of silage stealing that occurred when more dominant cows from the experimental group (that received a restricted forage ration) could push away cows with lower ranks from their through, the following feed consumption would be registered on the wrong individual. This does not seem to have affected the results since the within cow variation of the daily silage intake was very low.

The results from the feed analysis (Table 3) can be compared with the average results for the years 2010-2013 from Swedish commercial farms connected to the advisory service company Växa Sverige (Åkerlind, 2013). The CP content of the silage and the pasture is similar to earlier reports (Åkerlind, 2013). The relatively high ash content in the pasture samples can be an indication that there was a high inclusion of legumes in the sward or that a lot of dirt followed with the pasture samples. However, the latter is not too credible since the pasture

samples were carefully cut to not include too much soil. The K and Na content in the silage samples are somewhat higher compared to the mean of analyzed Swedish farm samples used as a comparison (Åkerlind, 2013) but are well in line when looking at the pasture samples. It can be very hard to get representative samples of the grass on pasture. Since pasture samples were collected four times a day the possible change in grass quality can be considered to be corrected for. Further, cattle tend to select between the species in the grass sward making it harder to get a representative pasture sample only containing the herbage species that the cows actually consume (Penning, 2004). Cows may not actually consume the same herbage species as present in the pasture samples, making the digestibility of herbage hard to measure (Hellwing *et al*., 2015).

The DM concentration in the silage differed much within weeks. The biggest variation in silage DM concentration was during the first and the second sampling period (variation with 6 % units within both weeks). The third sampling period gave less variation (3 % units within the week) and could thus provide more accurate results. The variation of DM concentration during the second sampling period was probably due to that the cows received silage from a newly opened silo, and the silage in the first part of the silo was probably of varying quality and DM concentration.

6.3 Water intake

The results showed a significant difference ($P = 0.01$) in drinking water consumption from the water bowls between the experimental group (82.9 L/cow/day) that had access to pasture and the control group (90.5 L/cow/day) that only consumed silage and concentrates. The daily drinking water consumption in the treatment groups can be compared to the recorded drinking water intake during the covariate period that corresponded to 83.4 L/cow/day. During the covariate period the cows received silage *ad libitum* similar to the treatment in the control group during the experiment which can lead to the assumption that the drinking water intake hence should have been the same. However, the drinking water intake during the covariate period is more similar to the intake in the experimental group receiving a different feed ration including pasture. The conclusion that could be drawn is that there must have been a variation in DM content since this is an important factor affecting drinking water intake (Bannink *et al*., 1999; Kume *et al*., 2010; Khelil-Arfa *et al.*, 2012). The difference in drinking water intake between the experimental group and the control group could be an effect of the difference in DM concentration in pasture (23.6 %) and silage (33.0 %) which resulted in that the cows in the experimental group had a higher feed water intake. Based on the DM content in the silage and the pasture grass and given the total feed intake of silage, concentrate and pasture, the control group received 28.7 kg water with the feed per day compared to the cows in the experimental group that received 32.7 kg water with the feed (including pasture) per day assumed that the pasture intake was 6.46 kg DM/day (the average estimated pasture intake during the experimental period based on the drinking water intake simple linear regression). This would result in a total water intake of 119.2 L/cow/day in the control group compared to 115.6 L/cow/day in the experimental group. This suggests that a higher feed water intake is not compensated for by an increase in urine volume since there was no significant difference in urine volume between the two groups. However, cows tend to compensate an increase of feed water intake by reducing the intake of drinking water (Dahlborn *et al.*, 1998; Kume *et al*., 2010). Unlike urine volume, the drinking water intake seems to follow the DM concentration in the feed.

The variation of daily drinking water intake between cows (SD 17.5 L) can be considered normal since the drinking water intake in cows is related to milk yield, DMI, DM concentration in food and also to environmental factors (Murphy, 1992: Bannink *et al*., 1999). The number 17.5 L corresponds to result from other studies (Murphy *et al*., 1983; Meyer *et al*., 2004; Cardot *et al*., 2008). However, the weekly variation within cows in drinking water intake is an important factor to look at. The standard deviation within cow within week ranged between 1.3-30.9 L with a mean SD of 11.2 L. Some cows had a larger daily variation in drinking water intake compared to others and the covariate of drinking water intake comprised 13.4 % of the individual weekly mean consumption. However, it has been noted earlier that cows have a large variation in water intake between days (Bannink *et al.*, 1999), hence the variation in drinking water intake found in this experiment seem to be reasonable. The ratio between drinking water intake and DMI also agrees with previous studies (Murphy *et al.*, 1983; Meyer *et al*., 2004; Cardot *et al*., 2008; Kume *et al*., 2010).

The cows showed a tendency to leave water in the bowl after drinking (ca 600 grams, but sometimes up to 1400 grams). This could have affected the registrations and resulted in an over-estimation of drinking water intake since there was residual water left in the bowls. However, it can be assumed that the residual water was somewhat similar for every cow which minimizes the error.

There were positive correlations between drinking water intake and milk yield (0.60), CP intake (0.70) and intake of K (0.55) and Na (0.57). Since the amount of Na in the diet affects the intake of drinking water (Murphy *et al.*, 1983; Spek *et al*., 2012) salt was excluded from the silage mix. However, because of misunderstanding with the farm workers, NaCl was included in the silage during one day of the experiment (9^{th} of June). This is shown in the silage analysis by a higher level of ash and may have affected the drinking water intake that day. It can be discussed however, if the NaCl inclusion in the silage could have affected the results of the study by increasing the drinking water consumption the following days. When comparing the mean drinking water intake in the groups that day with the rest of the week this did not seem to be the case. The mean consumption of water the 9^{th} of June was 99.4 L/cow in the control group and the corresponding number in the experimental group was 85.1 L/cow. The mean drinking water intake for the day when there was Na mixed in the silage did not significantly differ ($P = 0.1$ in the control group and $P = 0.8$ in the experimental group) from the mean drinking water intake the rest of the week.

The effect of different experimental factors that can be viewed in Table 6 which shows that the covariate period, treatment and sampling week had the biggest effects on drinking water intake. This suggests that the DM concentration in the feed that differed between the treatments had an effect on drinking water intake and that the cows in the experimental group that had a lower water intake received more water from the feed (pasture herbage compared to silage). This is also in line with the measured DM concentration in the pasture and silage samples as can be seen in Table 3.

6.4 Weather conditions

The ambient temperature and relative humidity was calculated to weekly experimental period means and presented as a morning (AM) and an afternoon (PM) average as can be seen in Table 5. However, the results of the registrations from the weather station could have underestimated the temperature that the cows were exposed to since the station was placed in a bushy area on the side on the pasture and was sometimes shadowed unlike the pasture where the cows grazed. Therefore the cows were exposed to direct sunlight and the radiant energy

and temperature was probably higher at the pasture. Since environmental factors such as ambient temperature and relative humidity can affect the drinking water intake (Murphy, 1992) it is important to consider this when looking at the results. It can be observed that the cows in the experimental group had a higher drinking water intake during the third experimental period (89 L/cow/day compared to 84 and 76 L/cow/day during the previous experimental periods) and this was certainly an effect of the higher ambient temperature during this time (an average of 23.5 C° in the afternoon, Table 5). The weather conditions could in turn affect the pasture intake of the cows since high ambient temperature and humidity has been shown to affect DMI negatively (Holter *et al*., 1997; West *et al.*, 2003). As can be seen in Table 6, sampling week had a significant effect on drinking water intake, suggesting that the different weather conditions during the experimental periods had an effect on drinking water intake. If comparing the estimated DM pasture intake between the weeks, predictions based on the urine volume regression and the total K intake regression shows that the cows had a lower feed intake during the third experimental period (Table 9). On the other hand, the regression model based on water intake shows an increased feed intake during this period. This is due to the increase in drinking water intake that occurs during warmer temperatures and hence the estimation of DMI based on drinking water intake could be overestimated during those circumstances.

6.5 Urine sampling, volume, components and body weight

In this experiment urine volume was estimated based on collection of urine by a spot sampling procedure. According to earlier studies, the estimations of urine volume based on spot sampling procedure may not be as reliable as the total collection method and may have influenced the results. Chen *et al.* (2004) discuss the weaknesses with using spot sampling of urine compared to total collection and states that the sensitivity of the method is low because of high variability and could thus only be used to detect larger differences among urine components. Additionally, the method requires a lot of urine samples (Chen *et al.*, 2004). There is also a discussion whether or not there is a diurnal variation of creatinine excretion, which requires many samples to lower the variability (Chen *et al.*, 2004; Chizzotti *et al*., 2008). However, the urine volume estimated in this experiment is similar to results from studies using total collection of urine and suggests that spot sampling of urine and then estimation of the urine volume by the creatinine concentration and body weight of the cow is a usable method. Additionally, collecting urine by spot sampling technique inflicts less discomfort of the animals and does not require them to be tied-up (Chizzotti *et al*., 2008). The procedure is also applicable both on farm-level and on pasture (Chen *et al*., 2004). The estimated mean of daily urine volume of 25.9 L is a credible result and close to the values found on the literature (Table 1). The urine volume did not significantly differ ($P < 0.05$) between the experimental group (25.8 L/cow/day) and the control group (26.0 L/cow/day) and there was neither a significant difference ($P < 0.01$) in urine volume during the covariate period (29.8 L/cow/day) and the main sampling period.

In total, the daily mean concentration of creatinine in the urine (638.0 mg/L) lay between the average concentrations of creatinine in the studies by Bristow *et al*. (1992) (980 mg/L) and Eriksson *et al*. (2009) (565 mg/L). In comparison, in this study 769 urine samples were used compared to 10 in the study by Bristow *et al*. (1992) and 356 in the study by Eriksson *et al.* (2009).

There was a great significant difference ($P < 0.001$) in the urinary urea excretion between the control group and the experimental group. The mean urea excretion in the control group was 146.9 g/day compared to the experimental group that had an excretion of 126.2 g/day. The

cows in the experimental group that had access to pasture unlike the cows in the control group that only were fed silage. These results suggest that the cows in the experimental group had a higher nitrogen and CP intake than the cows in the control group. The results from the analysis of the pasture samples showed that the CP content of the grass differed between the experimental periods, ranging between 152-187 g/kg DM. This is probably due to the difference in composition of plant species between the different pastures where the amount of legumes differed. The CP content in the silage varied less than that in the pasture and ranged between 154-160 g/DM during the whole experimental period. Even if the CP content in the pasture grass were slightly higher compared to the silage it is probably not enough to solely give the significant difference in urea excretion between the two groups. The difference in urea excretion could be explained by diurnal variation and by looking at when the urine samples were taken during the day an explanation could be found. If the cows in the experimental group would have had a significant higher intake of CP in the diet, this would probably have been shown by a higher urine volume compared to the control group since dietary nitrogen gives an increased excretion of urine (Bannink *et al.*, 1999; Nennich *et al*., 2006). As mentioned earlier, the urine volume did not significantly differ between the two treatment groups. However, the high excretion of urea shows that the cows in the experimental group had an unnecessary high intake of nitrogen since all excess dietary nitrogen will be excreted as urea in the urine (Harvey & Ferrier, 2011). This is considered a problem from an environmental point of view where excess nitrogen causes eutrophication (Bannink *et al.*, 1999; Nennich *et al.*, 2006). The correlation between CP intake and the total excretion of urea in the urine was 0.70 and corresponds to earlier studies that have concluded that the nitrogen excretion in the urine is correlated to nitrogen intake and is directly reflected by CP content of the diet (Eriksson *et al.*, 2004).

The results showed a positive correlation between urea excretion and urine volume (0.71). The urine volume also showed positive correlations with the intake of CP (0.55), K (0.58) and Na (0.50) and these relationships correspond to earlier studies (Maltz & Silanikove, 1996; Bannink *et al.*, 1999; Nennich *et al*., 2006). The fact that urine volume had a slightly stronger correlation with K intake compared to Na intake is supported by Eriksson (2011) that suggests that K is the mineral that predominantly regulates urine volume in dairy cows in Scandinavian conditions fed grass-legume forages. The effect of K intake on urine volume is based on the assumption that the urinary K concentration has reached an asymptotic value and hence is constant and regulating the urine volume linearly. Further analysis of the K content in the urine could have contributed to more accurate results. However, the correlation between urine volume and Na intake could have been stronger if it was not for the low variation of Na content in the feed. The variation of Na intake between the cows was low and the addition of NaCl in the grass-silage mix was also reduced on purpose. In the results from the feed analysis, K is the most abundant mineral.

The estimations of urine volume were dependent on that the body weights of the cows were somewhat accurate. Some cows varied a lot in weight between and within weeks and the accuracy of the measurement could be questioned. Earlier studies have concluded that cows show a diurnal variation in body weight depending of the fill of the gut and the udder (Mäntysaari & Mäntysaari, 2015). The accuracy of the body weight measurements could be increased if weighing is performed after milking, however, this was not the case in this experiment since the cows had to pass the scale both before and after milking. This could be one of the reasons to the large variation of body weight within cow. There is a need to standardise a method to calculate the body weights of the cows when the results from the scale gives large variations. The reliability of body weight measurements from automated

weighing systems has been evaluated by Mäntysaari & Mäntysaari (2015) that concluded that the reliability of the measurements could be increased with modeling methods since unprocessed data of body weight often show some variation. In this experiment, the registrations of body weights for each week were calculated into a mean value. The mean value was then corrected by exclusion of weights varying more than ± 50 kg from the mean weight and then a new mean value was recalculated. This may not be the most accurate method to calculate body weights, however, the results gave satisfying estimations of the urine volume and the weights could be considered as plausible for this experiment.

7. Conclusions

As concluded in earlier studies, there are positive correlations between the DMI and drinking water intake and urine volume in dairy cows. This is confirmed in this experiment and strengthens the fact that the DMI on pasture can be estimated based on these factors when the intake of silage and concentrates are known. The results suggest that the DMI on pasture in dairy cows can be estimated when applying the equations based on the simple and the mixed linear regressions. The DMI estimations gave reasonable intake volumes with the simple linear regression based on drinking water intake and urine volume and also by the simple and the mixed linear model based on the estimated intake of K. It is hard to conclude which method that gave the most accurate estimation of pasture DMI because the study did not include a treatment with known pasture intake. Basing the pasture DMI estimations on the drinking water intake may be more applicable in commercial farms since registration is possible in individual water bowls. However, the drinking water intake may be affected during warmer weather conditions as well as by the variation of DM concentration in pasture herbage. Because of this, estimations of pasture intake based on urine volume can be more accurate since it is not affected by temperature, climate or the DM concentration in the diet. The DMI can also be predicted based on the estimated total intake of K from urine volume if the K concentration of the feed is known. The method gave reasonable estimated of pasture DMI both based on the mixed and the simple linear regression. There was also a positive, significant correlation between K intake and urine volume which further strengthens the usage of this method. Still, the methods based on urine volume require collection of urine as well as laboratory analysis of urine components and should therefore be less applicable in commercial farms and more suited for experimental situations. Further it can be concluded that the spot sampling procedure of urine seem to be reliable since the estimated urine volumes in this experiment agreed with other studies were total collection of urine was performed. The estimations of pasture DMI was reasonable when it comes to pasture intake in dairy cows in Scandinavian conditions. In future research it could be of interest to investigate whether the pasture DMI could be estimated from the known CP intake since results from the experiment both showed strong positive correlation between those factors as well did the CP intake explain a large proportion of the variation of DMI according to the stepwise regression.

8. References

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