# Feed intake and urinary excretion of nitrogen and purine derivatives in pregnant suckler cows fed alternative roughage-based diets

Jardstedt, M.; Hessle, A.; Nørgaard, P.; Richardt, W.; Nadeau, E.

*Published in:*
Livestock Science

*DOI:*
10.1016/j.livsci.2017.05.026

*Publication date:*
2017

*Document version*
Publisher's PDF, also known as Version of record

Livestock Science 202 (2017) 82–88

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

# Feed intake and urinary excretion of nitrogen and purine derivatives in pregnant suckler cows fed alternative roughage-based diets

M. Jardstedt[a,*], A. Hessle[a], P. Nørgaard[c], W. Richardt[d], E. Nadeau[a,b]

[a] Department of Animal Environment and Health, Swedish University of Agricultural Sciences, Box 234, 532 23 Skara, Sweden
[b] The Rural Economy and Agricultural Society Sjuhärad, Rådde Gård, 514 05 Länghem, Sweden
[c] Department of Veterinary Clinics and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Grønnegårdsvej 3, 1870 Frederiksberg C, Denmark
[d] LKS mbH, August-Bebel Str. 6, 09577 Lichtenwalde, Germany

ARTICLE INFO

*Keywords:*
Beef cow
Creatinine
Festulolium
Nitrogen utilization
Reed canarygrass
Urea

ABSTRACT

This study compared intake of alternative roughage-based diets and of common late-cut grass silage and related intake to urinary nitrogen (N), urea-N and purine derivative (PD) excretion, where PD is an indicator of rumen microbial crude protein (MCP) synthesis. Total urine was collected from 36 Hereford cows, blocked into three groups based on expected calving date. Cows within calving groups were randomly assigned to one of four roughage diets: common mixed grass silage (MGS), festulolium silage plus urea (FLS), reed canarygrass silage (RCS) and barley straw plus urea and rapeseed meal (BRM). Diet crude protein (CP) content was classified into five fractions (A, $B_1$, $B_2$, $B_3$ and C), based on degradability characteristics. Feed intake and urinary excretion data were analysed by ANOVA in a randomised block design. To further explain the ANOVA results, multiple regression analyses were conducted to study relationships between intakes of total N (g/d); sum of the CP fractions A, $B_1$ and $B_2$ ($AB_1B_2$; g/d), most of which is considered rumen-degradable; digestible organic matter (DOMI; kg/d); protein balance in the rumen (g/kg dry matter); and urinary excretion of N, urea-N (g/d) and PD (mmol/d). Urinary N and urea-N excretion was positively related to N intake and was better explained by N intake than intake of $AB_1B_2$. Feeding BRM resulted in the lowest N intake and urinary N output ($P < 0.001$). Cows fed MGS, FLS and RCS had similar N intake, but urinary N and urea-N excretion was significantly higher ($P < 0.001$) in cows fed RCS, which probably was attributable to the significantly lower DOMI of this diet ($P < 0.001$). Furthermore, addition of DOMI to N intake in the multiple regression analysis increased the proportion of explained variation in urinary N and urea-N excretion. The MGS and FLS diets stimulated rumen MCP production to a greater extent than the BRM diet, as indicated by the higher urinary output of PD in cows fed the grass silage-based diets ($P < 0.001$). Diet had no significant effect on urinary PD excretion when expressed per kg DOMI. Overall mean urinary creatinine excretion was 0.197 ± 0.047 mmol/kg body weight, with no significant effect of diet. This study showed that intake of both N and DOM need to be assessed when choosing a suitable alternative roughage diet for suckler cows, in order to prevent undesirable losses of urinary N.

## 1. Introduction

Ammonia is a major air and water pollutant that causes acidification and eutrophication of the environment. Ammonia emitted from cattle manure is responsible for a significant proportion of anthropogenic nitrogen (N) emissions, contributing 30% of total emissions in EU28 (European Environment Agency, 2016). Intake of N has been identified as the main driver of cattle N excretion, especially in urine (Dong et al., 2014; Huhtanen et al., 2008). An essential constituent of urinary N is urea (Bernier et al., 2014; Dijkstra et al., 2013a), which is rapidly hydrolysed to ammonia upon deposition on barn floor or soil. Fresh faeces, on the other hand, contain low amounts of rapidly decomposable N, suggesting that urinary N, at least in the short term, is more susceptible to losses (Bussink and Oenema, 1998).

Around one-third of the 36 million cows in the EU cattle stock are suckler cows (Swedish board of agriculture, 2016). Nevertheless, while much attention has been devoted to investigating dietary effects on urinary N excretion in dairy cattle (Huhtanen et al., 2008; Spek et al., 2013; Weiss et al., 2009), the number of studies on suckler cows is limited (Bernier et al., 2014). Evidently, more knowledge about diet effects and N output in suckler cows is required in order to improve sustainability in this essential sector of livestock production.

* Corresponding author.
*E-mail address:* Mikaela.jardstedt@slu.se (M. Jardstedt).

http://dx.doi.org/10.1016/j.livsci.2017.05.026
Received 2 November 2016; Received in revised form 24 April 2017; Accepted 27 May 2017

Spring-calving suckler cows have low nutritional requirements during winter and are commonly fed grass silage as the main winter feed in Northern Europe (Drennan and McGee, 2009). In the Nordic countries, grass mixtures of timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* L.) and perennial ryegrass (*Lolium perenne* L.) are the most commonly ensiled forages. However, as winter feed production is the largest single cost in cow-calf operations (Kumm, 2009), there is growing interest in alternative grass species that are higher yielding and more persistent, such as festulolium and reed canarygrass (*Phalaris arundinacea* L.). In intensive grain cultivation areas, straw may also be an economically alternative feed for pregnant suckler cows if supplemented with protein.

Delaying the harvest of common grass mixtures for silage is a strategy to increase yield and to avoid overfeeding of pregnant suckler cows fed *ad libitum*. In grass silage, a great proportion of the crude protein (CP) is rumen-degradable protein (RDP) (Merchen and Bourquin, 1994). Provided that energy is not limiting, dietary RDP can be used for microbial protein synthesis. However, this may be a challenge when late-cut grass silage is fed, because of the low availability of fermentable substrates. The excess RDP not used in microbial crude protein (MCP) synthesis is then degraded to ammonia, most of which is excreted as urea in the urine (Nocek and Russell, 1988), while a smaller portion is recycled by the saliva to the rumen (Reynolds and Kristensen, 2008). Thus, the efficiency of RDP use in the rumen plays a central role in determining the environmental impact of production. A minimum amount of RDP must be provided to avoid N constraints on MCP synthesis (Clark et al., 1992), which is essential for cows fed only roughages where microbial protein is the major source of metabolizable protein (NRC, 2000).

The objective of this study was to compare intake of alternative roughage-based diets and of common mixed grass silage and to relate intake to urinary excretions of N and urea-N and to MCP synthesis, based on urinary excretion of purine derivatives (PD). Furthermore, relationships between output of urinary N, urea-N and PD and intakes of N and digestible organic matter were investigated.

## 2. Materials and methods

The experiment was conducted during winter 2013–2014 at Götala Beef and Lamb Research Centre, south-western Sweden, and was approved by the Gothenburg Research Animal Ethics Committee (case number 175–2012, 181–2013).

### 2.1. Animals and experimental design

The experiment involved 36 Hereford cows that were blocked into three calving groups, with 12 cows per group, according to expected date of calving: early (E; expected calving 18 February-2 March), medium (M; expected calving 13 March-4 April) and late (L; expected calving 12 April-12 May). Within each calving group, cows were randomly assigned to one of four diets: mixed grass silage (MGS), festulolium silage plus urea (FLS), reed canarygrass silage (RCS) and barley straw plus urea and rapeseed meal (BRM) (Fig. 1). There were nine cows in total per diet. Six urine collection harnesses were available and each calving group was therefore further divided into two subgroups: E = E1, E2; M = M1, M2; L = L1, L2, with six cows and two diets per subgroup. The four diets were randomised between subgroups within calving group. Mean cow body weight (BW), age and body condition score (BCS; 1 = emaciated, 9 = obese; adapted from Herd and Sprott, 1986) at the start of total urine collection was 719 ± 85 kg, 4.4 ± 1.5 years and 5.9 ± 0.9, respectively.

### 2.2. Experimental diets

The MGS was harvested from a mixed sward dominated by timothy and meadow fescue, the festulolium silage from a monoculture sward of the festulolium hybrid Hykor of Italian ryegrass (*Lolium multiflorum* L.) and tall fescue (*Festulolium arundinacea* L.), and the RCS from a monoculture sward of reed canarygrass (cv Palaton). The MGS and RCS were spring-fertilised with 90 kg N/ha and the festulolium with 80 kg N/ha. The barley straw was fertilised with 113 kg N/ha before harvest. All grass silages were first cuts harvested at the bloom stage of maturity, on 4–8 July. The grasses were pre-wilted and preserved in round bales with addition of a chemical additive (2 L/t; Kofasil LP; nitrite, hexamine, benzoate; Addcon Europe GmbH). The barley straw in the BRM diet (*Hordeum vulgare*; cv. Rosalina) was baled after threshing.

The protein balance in the rumen (PBV) value of the roughages was calculated using the NorFor digestive model at an intake level of 8 kg dry matter (DM). The PBV is calculated as: RDP + recirculation of urea in saliva – microbial protein. In the NorFor feed model, recirculation of urea is estimated to be 4.6% of the CP concentration in the diet (Volden and Larsen, 2011). The festulolium silage and barley straw had negative PBV values and were therefore supplemented with 6.9 and 11.3 g urea per kg DM, respectively, to avoid N constraints on rumen MCP synthesis (Clark et al., 1992). The PBV of the festulolium silage plus urea (FLS) and the barley straw plus urea (BarS) was −3 and −16 g/kg DM, respectively, after supplementation (Table 1). Each cow fed BarS was also given 0.5 kg DM per day of rapeseed meal (DM 869 g/kg, CP 378 g/kg DM, ash 69 g/kg DM, OM-corrected neutral detergent fibre (NDFom) 275 g/kg DM, PBV 22 g/kg DM and 78.5% *in vivo* organic matter digestibility (OMD)) to further increase PBV. The final PBV of the BRM diet (BarS plus rapeseed meal) was 14 g/kg DM. All roughage diets were considered to be balanced for PBV (range −3–14 g/kg DM). Urea was suspended in water and mixed thoroughly into the roughages in a mixer wagon before feeding and feed sampling.

Cows were acclimatised to their experimental diet for three weeks before the start of urine collection and were fed individually *ad libitum*, allowing 10% refusals. Cows had free access to water and a salt block and received approximately 100 g vitaminised minerals per cow daily. Feed was delivered once a day and refusals were removed daily. Feed intake was recorded daily. Roughages were sampled at feeding and pooled to one sample per roughage and calving group. Refusals were sampled at removal from each individual cow and were pooled to one sample per cow. All samples were stored frozen at −20 °C until analysis.

### 2.3. Urine collection

Cows were moved from a loose-house system, with individual feed intake recording, to individual tie-stalls with sawdust or peat as bedding 1.5 days before the start of the urine collection. Total urine collection was performed during 48 consecutive hours for each cow using a rubber/latex mould, which was fitted tightly over the vulva and maintained in place by a harness. Total collection of urine was conducted for each respective calving group when cows were in the beginning of their third trimester and was performed on each subgroup on the following dates: 19–21 (E1) and 23–25 November (E2), 10–12 (M1) and 14–16 (M2) December, 7–9 (L1) and 11–13 January (L2). Cows were weighed and BCS were assessed independently by two observers in the morning of two consecutive days just before urine collection commenced.

| Early calving | | | | Medium calving | | | | Late calving | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| E1 | | E2 | | M1 | | M2 | | L1 | | L2 | |
| MGS | BRM | RCS | FLS | FLS | BRM | RCS | MGS | FLS | BRM | RCS | MGS |

**Fig. 1.** Experimental design of the study. Cows were blocked according to expected calving date into three calving groups (early, medium, late) with two subgroups (1, 2) and four diets in each calving group. The diets were: MGS = mixed grass silage, BRM = barley straw plus urea and 0.5 kg dry matter rapeseed meal per cow and day, RCS = reed canarygrass silage, FLS = festulolium silage plus urea.

**Table 1**
Chemical composition of the experimental roughages mixed grass silage (MGS), festulolium silage plus urea (FLS), reed canarygrass silage (RCS) and barley straw plus urea (BarS), means and standard deviations within brackets, $n = 3$.

| Item[a] | MGS | FLS[b] | RCS | BarS[b] |
|---|---|---|---|---|
| Dry matter (DM), % | 46.7 (18.3) | 36.7 (6.79) | 44.3 (20.3) | 78.4 (7.63) |
| Ash, g/kg DM | 56.6 (3.10) | 67.4 (0.92) | 41.7 (2.85) | 51.9 (3.73) |
| aNDFom, g/kg DM | 564 (11.0) | 517 (8.25) | 654 (17.2) | 794 (23.3) |
| ADFom, g/kg DM | 330 (7.53) | 293 (6.50) | 394 (23.8) | 451 (12.4) |
| ADL, g/kg DM | 41.8 (5.06) | 33.0 (2.72) | 57.0 (7.25) | 62.3 (4.46) |
| OMD, % | 60.7 (2.89) | 67.0 (1.79) | 51.6 (0.17) | 51.4[c] |
| ME, MJ/kg DM | 8.7 (0.50) | 9.64 (0.30) | 7.3 (0.04) | 7.1 (0.09) |
| MP, g/kg DM | 71 (0.58) | 67 (1.00) | 71 (0.58) | 47 (0.58) |
| WSC, g/kg DM | 190 (1.07) | 206 (2.25) | 61.1 (1.54) | – |
| PBV, g/kg DM | 5 (13) | −3 (1) | 7 (12) | −16 (6) |
| CP, g/kg DM | 102 (15.1) | 97.3 (3.88) | 129 (4.30) | 66.9 (5.84) |
| TP, g/kg DM | 55.9 (7.31) | 28.7 (2.58) | 48.3 (5.13) | 31.3 (1.68) |
| A, % of CP | 45.1 (1.33) | 70.5 (1.50) | 62.7 (3.63) | 52.9 (4.10) |
| $B_1$, % of CP | 1.25 (0.15) | 1.50 (0.78) | 2.76 (0.44) | 1.42 (0.57) |
| $B_2$, % of CP | 34.0 (1.14) | 19.3 (1.35) | 21.6 (1.59) | 22.2 (3.85) |
| $B_3$, % of CP | 13.3 (0.62) | 4.60 (0.80) | 7.67 (2.69) | 9.39 (1.18) |
| C, % of CP | 6.36 (0.29) | 4.11 (0.15) | 5.29 (0.19) | 14.1 (1.08) |

[a] aNDFom = neutral detergent fibre, ADFom = acid detergent fibre, ADL = acid detergent lignin, OMD = *in vivo* digestibility of organic matter calculated using *in vitro* organic matter digestibility, ME = metabolizable energy, MP = metabolizable protein, WSC = water-soluble carbohydrates, PBV = protein balance in the rumen, CP = crude protein, TP = true protein (CP minus fraction A), A = non-protein nitrogen, $B_1$ = buffer-soluble protein, $B_2$ = neutral detergent-soluble protein, $B_3$ = acid detergent-soluble protein, C = acid detergent-insoluble protein.
[b] Results include CP from added urea, which supplied 20 and 33 g CP/kg DM to FLS and BarS, respectively.
[c] $n = 1$.

Cows were allowed to become accustomed to wearing the urine collection harness for half a day before the collection period started and were monitored continuously by personnel. Urine was collected in 20-L vessels containing 10% $H_2SO_4$, to ensure that the pH was decreased below 3.0 in order to prevent bacterial destruction of PD and creatinine. Urine vessels were replaced every 12 h, the volume of acidified urine was measured and a subsample of 200 mL per cow and 12-h period was immediately frozen at −20 °C. For three cows, the urinary excretion data are based on three 12-h intervals, because of dysfunction of their harnesses during one interval.

### 2.4. Chemical analyses

The DM of the feed and refusal samples was determined in a drying cabinet at 60 °C for 24 h. Pooled samples of feeds and refusals were analysed for concentrations of aNDFom, OM-corrected acid detergent fibre (ADFom) and acid detergent lignin (ADL) by the FiberTech method according to Van Soest et al. (1991), including α-amylase in the NDF analysis but not sodium sulphite. Crude ash concentration was determined at 525 °C for 16 h. Total N concentration was analysed using the Kjeldahl procedure and the concentration of CP was calculated as total N x 6.25. The concentration of metabolizable protein (MP) was calculated using the NorFor digestive model (Volden and Nilsen, 2011). The MGS, FLS and RCS feed samples were analysed for *in vitro* organic matter digestibility (IVOMD) by the VOS method (incubation of 0.5 g dried sample in 49 mL buffer and 1 mL rumen fluid at 38 °C for 96 h) according to Lindgren, (1979, 1983, 1988) and metabolizable energy (ME) was calculated according to Lindgren (1983). *In vivo* OMD for MGS, FLS and RCS was calculated using the VOS value (Lindgren, 1983). The BarS and rapeseed meal were analysed for IVOMD by the EFOS method (Weisbjerg and Hvelplund, 1993). The EFOS values were used to calculate *in vivo* OMD for BarS (Hvelplund et al., 1999) and for rapeseed meal (Weisbjerg and Hvelplund, 1993). For rapeseed meal, the NDFom concentration was analysed according to Weisbjerg and Hvelplund (1993), crude ash was determined at 550 °C for 6 h and the CP concentration was determined by Dumas combustion.

The CP content was classified into five fractions (A, $B_1$, $B_2$, $B_3$ and C) (Table 1), based on degradability characteristics according to the Cornell Net Carbohydrate and Protein System (Sniffen et al., 1992), using analyses according to Licitra et al. (1996). The A fraction is non-protein nitrogen (NPN), which is the N recovered in the filtrate after precipitation with tungstic acid. The B fraction is degradable true protein, which is further divided into fraction $B_1$, which is soluble in borate-phosphate buffer at rumen pH and rapidly degraded in the rumen; fraction $B_2$, which is insoluble in borate-phosphate buffer, but soluble in neutral detergent (ND) solution and has variable degradation; and fraction $B_3$, which is insoluble in ND solution but soluble in acid detergent (AD) solution. Fraction $B_3$ is digestible but slowly degradable, most of which occurs post-ruminally (Licitra et al., 1996). Fraction C is considered to be indigestible and is insoluble in AD solution. True protein is the CP concentration minus the A fraction.

For the grass silages, concentrations of acetate, propionate, butyrate and ethanol were determined by gas chromatography (Weiss, 2001), lactic acid concentration by high-pressure liquid chromatography (HPLC) (Weiss and Kaiser, 1995) and ammonia concentration photometrically by Scalar (CFSA, 2004) based on the Berthelot reaction. The pH was determined potentiometrically using a calibrated pH electrode and concentration of water-soluble carbohydrates (WSC) was determined according to Lengerken and Zimmermann (1991).

The N concentration in urine was analysed with a Kjeldahl procedure. Urine concentrations of creatinine, allantoin and uric acid were analysed by HPLC as described by Shingfield and Offer (1999), but with the modification that a second mobile phase containing methanol, acetonitrile and distilled water (45/45/10) and a Kinetex XB-C18 column (150 × 4.6 mm, 5 µm) was used. Urinary Urea concentration was analysed with HPLC (LKS, 2006).

### 2.5. Statistical analysis

One cow fed the BRM diet in subgroup L1 was excluded from statistical analysis of data because her feed intake decreased by 51% during the urine collection period compared with her mean feed intake during seven days prior to urine collection. Three cows fed the FLS diet in subgroup E2 were excluded from the statistical analysis of urinary creatinine and PD excretion, as their excretion values were identified as outliers using the generalised extreme studentised deviate (ESD) test (Rosner, 1983).

Data were analysed by ANOVA in a randomised block design using the MIXED procedure in SAS (SAS Inst. Inc., NC, USA, 2012), with diet (MGS, FLS, RCS, BRM) as fixed effect, calving group (E, M, L) as random effect and sub-group (E1, E2, M1, M2, L1, L2) nested within calving group as random effect. Differences between treatments in the *F* test were significant at $P < 0.05$ and a tendency for significance was assumed at $0.05 < P < 0.10$. Pair-wise comparisons between least squares (LS) means of treatments were conducted according to Tukey's test. Data reported are LSmeans and standard error of the mean (SEM) (Tables 2, 3).

To further explain the results from ANOVA, multiple regression analyses were conducted using Minitab® statistical software (version 16 © 2016 Minitab Inc.) to study the relationships between total intake of N (g/d), digestible organic matter (DOMI; kg/d) and diet PBV concentration (g/kg DM), and urinary excretion (g/d) of N and urea-N. In addition, total N intake was replaced by intake (g/d) of the sum of the protein fractions A, $B_1$ and $B_2$ ($AB_1B_2$), most of which is considered rumen-degradable. For urinary PD excretion (mmol/d), the effects of all above-mentioned intake variables except PBV were tested. Pearson correlations were calculated between all explanatory variables to check for collinearity. Random effect of calving group and fixed effect of diet were included in all models and retained even at $P > 0.05$. The models for each response variable were constructed by first testing all single explanatory variable models and then including the other intake variables one by one until all possible combinations had been tested. The

**Table 2**
Effects of diet on body weight and daily intake in suckler cows fed mixed grass silage (MGS), festulolium silage plus urea (FLS), reed canarygrass silage (RCS) or barley straw plus urea and 0.5 kg rapeseed meal per cow and day (BRM), $N = 35$.

| Item[1] | MGS | FLS | RCS | BRM | SEM | *P*-values |
|---|---|---|---|---|---|---|
| Body weight, kg | 710 | 782 | 704 | 706 | 26.3 | n.s. |
| Dry matter (DM), kg | 12.4$^{a}$ | 11.8$^{a}$ | 9.38$^{b}$ | 6.63$^{c}$ | 0.70 | *** |
| DM, % of body weight | 1.79$^{a}$ | 1.55$^{ab}$ | 1.39$^{b}$ | 1.00$^{c}$ | 0.12 | *** |
| ME, MJ | 109$^{a}$ | 117$^{a}$ | 68.6$^{b}$ | 53.6$^{b}$ | 6.11 | *** |
| MP, g | 883$^{a}$ | 815$^{ab}$ | 684$^{b}$ | 303$^{c}$ | 43.9 | *** |
| DOM, kg | 7.14$^{a}$ | 7.63$^{a}$ | 4.67$^{b}$ | 3.39$^{b}$ | 0.40 | *** |
| aNDFom, kg | 7.08$^{a}$ | 6.27$^{ab}$ | 6.31$^{ab}$ | 5.32$^{b}$ | 0.44 | ** |
| WSC, kg | 2.35$^{a}$ | 2.54$^{a}$ | 0.56$^{b}$ | – | 0.20 | *** |
| CP, kg | 1.28$^{a}$ | 1.18$^{a}$ | 1.29$^{a}$ | 0.56$^{b}$ | 0.08 | *** |
| A, g | 578$^{b}$ | 802$^{a}$ | 773$^{a}$ | 208$^{c}$ | 49.0 | *** |
| $B_1$, g | 15.9$^{b}$ | 17.2$^{b}$ | 32.8$^{a}$ | 37.0$^{a}$ | 2.38 | *** |
| $B_2$, g | 426$^{a}$ | 227$^{bc}$ | 282$^{b}$ | 188$^{c}$ | 22.1 | *** |
| $B_3$, g | 165$^{a}$ | 58.3$^{c}$ | 105$^{b}$ | 39.1$^{c}$ | 11.9 | *** |
| $AB_1B_2$, g/d | 1022$^{a}$ | 1044$^{a}$ | 1086$^{a}$ | 436$^{b}$ | 70.5 | *** |

[1]ME = metabolizable energy, MP = metabolizable protein, DOM = *in vivo* digestible organic matter, calculated from *in vitro* organic matter digestibility, aNDFom = neutral detergent fibre, WSC = water-soluble carbohydrates, CP = crude protein, A = non-protein nitrogen, $B_1$ = buffer-soluble protein, $B_2$ = neutral detergent-soluble protein, $B_3$ = acid detergent-soluble protein, $AB_1B_2$ = sum of protein fractions A, $B_1$ and $B_2$.
***$P < 0.001$, **$P < 0.01$, n.s. = non-significant.
[a-c] Means within row with different superscripts differ significantly ($P < 0.05$).

**Table 3**
Effects of diet on nitrogen (N) intake, urine volume, urinary excretion of N, urea-N and purine derivatives (allantoin plus uric acid; PD) in suckler cows fed mixed grass silage (MGS), festulolium silage plus urea (FLS), reed canarygrass silage (RCS) or barley straw plus urea and 0.5 kg DM rapeseed meal per cow and day (BRM), $N = 35$ for N intake and N excretion parameters and $N = 32$ for PD excretion parameters.

| Item[1] | MGS | FLS | RCS | BRM | SEM | *P*-values |
|---|---|---|---|---|---|---|
| N intake, g/d | 205$^{a}$ | 188$^{a}$ | 207$^{a}$ | 88.9$^{b}$ | 13.1 | *** |
| *Urinary excretion* | | | | | | |
| Urine, L/d | 7.79$^{b}$ | 11.9$^{a}$ | 7.53$^{bc}$ | 5.46$^{c}$ | 0.74 | *** |
| N, g/d | 66.3$^{b}$ | 62.8$^{b}$ | 91.5$^{a}$ | 40.7$^{c}$ | 6.26 | *** |
| Urea-N, g/d | 31.3$^{b}$ | 27.2$^{b}$ | 61.1$^{a}$ | 25.1$^{b}$ | 3.67 | *** |
| Urea-N, % of total N-excretion | 46.7$^{b}$ | 42.9$^{b}$ | 68.0$^{a}$ | 60.4$^{a}$ | 2.58 | *** |
| N-excretion, % of N-intake | 31.3$^{b}$ | 35.4$^{b}$ | 44.9$^{a}$ | 45.1$^{a}$ | 3.50 | *** |
| Allantoin, mmol/d[2] | 148$^{a}$ | 174$^{a}$ | 119$^{ab}$ | 85.0$^{b}$ | 14.3 | *** |
| Uric acid, mmol/d | 10.5 | 10.6 | 9.10 | 5.00 | 2.33 | n.s. |
| PD, mmol/d[3] | 158$^{a}$ | 186$^{a}$ | 127$^{ab}$ | 91.1$^{b}$ | 15.1 | *** |
| Allantoin, mmol/kg DOMI | 20.8 | 24.5 | 24.7 | 26.2 | 2.78 | n.s. |
| PD, mmol/kg DOMI | 22.1 | 26.1 | 26.9 | 27.6 | 3.13 | n.s. |

[1]DOMI = digestible organic matter intake.
***$P < 0.001$, n.s. = non-significant.
[2]$P = 0.069$ for the difference between the FLS and RCS diets.
[3]$P = 0.051$ for the difference between the FLS and RCS diets.
[a-c] Means within rows with different superscripts differ significantly ($P < 0.05$).

regressions were evaluated by visual inspection of residual plots and, based on the $R^2$-adjusted value ($R^2$-adj), it was decided whether an additional variable improved the explanation of the observed variation.

## 3. Results

### 3.1. Chemical composition of feeds

The nutrient composition of the experimental roughages is shown in Table 1. For rapeseed meal, the percentages of the different CP fractions were: 8.1% A, 17% $B_1$, 63% $B_2$, 6.0% $B_3$ and 6.3% C in CP. The range of fermentation product concentrations (g/kg DM) for the silages was 17–32 for lactic acid, 7.5–11 for acetic acid, 0.0–0.5 for butyric acid and 5.1–11 for ethanol. Ammonia-N, including N from the silage additive and urea, was 8.2–12% of total N and pH was 4.5–4.8.

### 3.2. Nutrient intake

Body weight and BCS of cows did not differ between diets (Table 2). Cows fed MGS and FLS had higher intake of DM and digestible organic matter (DOM) than cows fed RCS and BRM (Table 2). Intake of CP and sum of the protein fractions A, $B_1$ and $B_2$ was similar for cows fed MGS, FLS and RCS, which differed from BRM. The intake of protein fraction A was higher for cows fed FLS and RCS than for cows fed MGS and BRM, and higher for cows fed MGS than for cows fed BRM. The RCS and BRM diets resulted in higher intake of fraction $B_1$ than MGS and FLS. Feeding MGS resulted in the highest intake of fraction $B_2$. The highest intake of fraction $B_3$ was recorded for MGS, followed by RCS, with the lowest intakes observed for FLS and BRM.

### 3.3. Urinary N, urea-N, PD and creatinine excretion

Urine volume, urinary excretion of N and urea-N, proportion of urea in total urinary N excreted, proportion of N intake excreted as urinary N, urinary allantoin and PD excretions were all affected by diet (Table 3). Feeding RCS resulted in the greatest urinary output of N, while the MGS and FLS diets resulted in higher N excretion than the BRM diet. The RCS gave about twice as high urea-N output as the other diets. In cows fed RCS and BRM, urea-N accounted for a larger proportion of total urinary N excretion than in cows fed MGS and FLS. In addition, cows fed RCS and BRM excreted a larger proportion of their total N intake as N in urine.

Allantoin and PD excretions were significantly higher in cows fed MGS and FLS than in cows fed BRM (Table 3). The FLS diet tended to result in higher allantoin ($P = 0.069$) and PD ($P = 0.051$) excretion than RCS. When the endogenous contribution of allantoin ($0.385 * BW^{0.75}$) (Verbic et al., 1990) was taken into account, the differences in PD excretion between diets persisted (data not shown). Diet had no effect on excretion of PD per kg DOMI.

Creatinine excretion in urine was not affected by diet and overall mean excretion ($N = 32$) was 141 ± 36.5 mmol/day and 0.197 ± 0.047 mmol/kg BW (min = 0.120, max = 0.325 mmol/kg BW).

### 3.4. Relationship between intake of N and DOM and urinary excretion of N, urea-N and PD

Urinary N and urea-N excretion were positively related to N intake, with $R^2$-adj of 0.66 and 0.79, respectively. The variation in urinary N and urea-N output was better explained by total N intake than intake of $AB_1B_2$. Combining total N intake with DOMI improved the explanation of observed variation in urinary N excretion (N excretion = 28.9 + 0.39 x N intake – 4.83 x DOMI; $R^2$-adj = 0.67) and urea-N excretion (urea-N excretion = 10.3 + 0.25 x N intake – 3.40 x DOMI; $R^2$-adj = 0.80), where an increase in DOMI decreased N and urea-N output. Addition of diet PBV concentration to N intake did not increase the proportion of explained variation in N and urea-N excretion. Urinary PD output was more closely associated with DOMI (PD excretion = −24 + 26.8 x DOMI; $R^2$-adj = 0.67) than N intake ($R^2$-adj = 0.63), where DOMI had a positive effect on PD excretion. Addition of N intake to DOMI did not increase the $R^2$-adj value for observed variation in PD output.

## 4. Discussion

### 4.1. Urinary N and urea-N excretion

As expected, increased N intake led to increased urinary N and urea-N output in this experiment, which is in agreement with other studies on suckler cows fed grass silage (Zou et al., 2016) and low quality forage with protein supplement (Bernier et al., 2014). Nitrogen intake explained a larger proportion of urea-N excretion than N excretion,

which implies that the former was more sensitive to N intake (Bernier et al., 2014).

Cows fed the three grass silage-based diets had similar daily intakes of N and $AB_1B_2$. However, cows fed RCS excreted greater amounts of N and urea-N in urine than cows fed MGS and FLS, indicating that factors other than N intake were important. The rumen is an essential source of N losses in cattle (Tamminga, 1992). Efficient rumen microbial N use requires a balance between available N and supply of fermentable substrates, mainly carbohydrates. If energy becomes limiting, for example when poorly digestible roughages are fed, excess rumen-degradable N is excreted in urine as urea (Nocek and Russell, 1988). It is therefore reasonable to believe that the 35% and 39% lower DOMI of RCS compared with MGS and FLS, respectively, was the reason for the greater N and urea-N excretion by cows fed RCS. Adding DOMI to the regression model with N intake increased the proportion of explained variation in N and urea-N excretion, which further suggests that DOMI needs to be considered in order to achieve a reduction in urinary N output. This result is in agreement with previous findings in lactating and dry dairy cows (Kebreab et al., 2010; Stergiadis et al., 2015), where intake of metabolizable energy was negatively related to urinary N excretion.

Rumen-degradable protein supplied in excess of microbial requirements is mainly lost through N excretion in urine (Hristov et al., 2004) and it has been demonstrated that reducing diet CP and RDP concentrations will produce manure with lower ammonia emissions potential (Agle et al., 2010). Consequently, we tested the hypothesis that the sum of intake of protein fractions A, $B_1$ and $B_2$, most of which is rumen-degradable, would result in a greater effect on urinary N and especially urea-N excretion than total N intake, because total N intake also includes N from rumen undegradable protein. However, both N and urea-N output were better explained by total N intake. The proportion of $AB_1B_2$ in total CP intake was high (77–88%) and the relative difference between the diets in $AB_1B_2$ intake was the same as total N intake. These similarities between total N intake and $AB_1B_2$ intake suggests that urinary N and urea-N excretions would be explained by both N and $AB_1B_2$ intake to the same extent. However, the variation in N intake was smaller than the variation in $AB_1B_2$ intake, which might be the reason for a greater proportion of N and urea-N excretion being explained by the former.

Cows fed the BRM diet had the lowest output of urinary N. When diets with low CP concentration are fed, the proportion of urea produced in the liver that is returned to the gut *via* blood and saliva increases and the proportion of urea excreted in urine decreases (Reynolds and Kristensen, 2008). According to Reynolds and Kristensen (2008), cattle fed a diet with a CP concentration of 9.5%, which was the concentration of the BRM diet, have 85% of the urea produced by the liver returned to the gut and 17% is excreted in urine. In ruminants, there are inevitable losses of N that are related to rumen metabolism, intestinal digestion and post-absorptive metabolism (Dijkstra et al., 2013b). The urinary N and urea-N excretion of cows fed the BRM diet were in the range reported previously for dry pregnant dairy cows fed no dietary N at all (Ørskov and MacLeod, 1982) or diets with 9–10% CP (Wohlt et al., 1978). Based on this, it can be inferred that the urinary N excretion of cows fed the BRM diet might have been close to the lower limit of what is possible.

Even though urinary N excretion as a percentage of N intake was similar for cows fed RCS and BRM, cows fed the former excreted 125% more N in urine (g/d) than cows fed BRM, as the N intake of RCS was more than twice the N intake of BRM.

The MGS, FLS and RCS diets provided about twice the daily amount of MP required according to the Swedish feed table for ruminants (data not shown; Spörndly, 2003). Furthermore, MGS and FLS supplied 60% more ME than required (data not shown; Spörndly, 2003). Hence restrictive feeding could be one strategy to reduce the urinary losses of N associated with those diets. Similar results could probably be obtained by mixing MGS and FLS with straw, which would limit intake and decrease ME and MP concentrations. However, cows offered RCS were fed close to their ME requirements. Thus, restrictive feeding of this diet, to better match the MP requirements of the cows and reduce N excretion, is not an option.

### 4.2. Urinary PD excretion

Measurement of urinary PD excretion is an indirect, non-invasive method for estimating MCP supply to cattle fed different diets (Chen and Gomes, 1992). The MGS and FLS diets appeared to be better stimulants of rumen MCP production than the BRM diet, as indicated by the greater urinary excretion of PD in cows fed those diets. This could probably be explained by the greater DOMI of the MGS and FLS diets compared with BRM (Clark et al., 1992). The main driver of MCP synthesis is the availability of fermentable substrates and urinary excretion of allantoin has previously been reported to increase as intake of digestible DM and organic matter (Südekum et al., 2006; Vercoe, 1976) increases. Furthermore, it was found in this study that DOMI was positively related to urinary PD output, explaining more than 60% of the variation in PD excretion.

It was unexpected that the PD excretion only tended to differ between cows fed the FLS and RCS diets, considering the large numerical differences (Table 3) and the fact that cows fed FLS had higher DOMI than cows fed RCS. This lack of significance could be related to the short duration of the urine collection period, but there could also be other reasons, such as analytical errors.

The final PBV values were positive for all diets except FLS, where cows were fed below requirements ($-172$ g/d) according to the NorFor model (Volden and Larsen, 2011). The negative PBV value of FLS implies that rumen microbial growth might have been constrained by RDP, estimated as $AB_1B_2$ in this study. Urinary PD excretion did not differ significantly between diets when expressed per kg DOMI, which indicates similar efficiency of MCP synthesis per kg DOMI across diets according to our methodology. In addition, total N or $AB_1B_2$ intake did not increase the proportion of explained variation in urinary PD excretion when added to the model that already included DOM intake. This suggests that N supply was not a factor limiting MCP synthesis in this study.

### 4.3. Excretion of creatinine

Creatinine excretion in urine has been validated as a marker to estimate urine volume from urine spot samples in dairy cattle (Chizzotti et al., 2008; Valadares et al., 1999). Creatinine excretion is assumed to be a constant function of BW, but varies with animal breed and animal physiological stage (Chen and Ørskov, 2004) and should be determined by total collection for the animal category for which it will be used. The creatinine excretion per kg BW in this study was approximately 30% lower than reported previously for pregnant suckler cows of Angus × Red Simmental cross (Whittet et al., 2004). Beef breeds are generally considered to have a larger proportion of muscle mass and lower proportion of bone per kg BW than dairy breeds (Clarke et al., 2009). It was therefore unexpected that the beef suckler cows in this study had lower mean excretion of creatinine per kg BW than previously reported for lactating Holstein cows (Chizzotti et al., 2008; Leonardi et al., 2003; Valadares et al., 1999). These contradictory results could be due to a higher proportion of fat deposits and, hence, a lower proportion of lean body tissue per kg BW in the Hereford cows in the present study, resulting in lower creatinine excretion per kg BW compared with dairy breeds. The BCS of the dairy cows in the studies cited above is not reported and a more thorough comparison is therefore not possible.

The current study observed high between-animal variation in creatinine excretion per kg BW, which partly could be a result of the short duration of urine collection. However, Whittet et al. (2004) reported a similar large range in creatinine excretion, 0.15–0.28 mmol/kg BW, in beef suckler cows when determined by 5-day total urine

collection using urethral catheters. This indicates that the use of urine spot sampling as a tool to estimate urinary N and PD excretions, *e.g.* under practical farm conditions, will require use of numerous animals and that its application will be limited to situations where relative measurements are acceptable.

Creatinine excretion per kg BW was not significantly affected by diet, which is in agreement with findings by others (Gonda et al., 1996; Moorby et al., 2006; Valadares et al., 1999).

## 5. Conclusions

This study revealed an interaction between intake of N and digestible organic matter and subsequent effects on urinary N and urea-N output. Despite similar N intake, dietary N was better utilized in cows fed the more digestible MGS and FLS diets compared to the less digestible RCS diet, which was indicated by considerably lower excretion of urinary N and urea-N and by a greater stimulation of rumen MCP synthesis in cows fed MGS and FLS. Thus, it is clear that organic matter digestibility needs to be considered in addition to the N content of alternative roughage-based diets when their suitability as feeds for suckler cows is being evaluated, in order to prevent undesirable losses of urinary N. Urinary PD excretion was mainly related to intake of digestible organic matter across different forage diets, indicating that rumen degradable protein was not a limiting factor for microbial CP synthesis in this study.

## Conflict of interest statement

Authors declare no conflict of interest.

## Acknowledgements

This study was funded by the Interreg Öresund-Kattegatt-Skagerrak European Regional Development Fund (Grant number ÖKS-4–2011-47), Västra Götalandsregionen, Agroväst, Skaraborgs kommunalförbund, the Anders Elofsson Fund and the Swedish University of Agricultural Sciences (SLU). The authors wish to thank Emma Duvelid for her great work during the experiment, our colleagues at the Department of Animal Environment and Health for their help in data collection, Jan-Eric Englund, Department of Biosystem and Technology, SLU, for statistical advice and Maria Åkerlind, Växa Sverige, for her help with the NorFor digestive model.

## References

Agle, M., Hristov, A.N., Zaman, S., Schneider, C., Ndegwa, P., Vaddella, V.K., 2010. The effects of ruminally degraded protein on rumen fermentation and ammonia losses from manure in dairy cows. J. Dairy Sci. 93, 1625–1637.

Bernier, J.N., Undi, M., Ominski, K.H., Donohoe, G., Tenuta, M., Platen, D., Plaizier, J.C., Wittenberg, K.M., 2014. Nitrogen and phosphorus utilization and excretion by beef cows fed a low quality forage diet supplemented with dried distillers grains with solubles under thermal neutral and prolonged cold conditions. Anim. Feed Sci. Technol. 193, 9–20.

Bussink, D.W., Oenema, O., 1998. Ammonia volatilization from dairy farming systems in temperate areas: a review. Nutr. Cycl. Agroecosystems 51, 19–33.

Chen, X.B., Gomes, M.J., 1992. Estimation of Microbial Protein Supply to Sheep and Cattle Based on Urinary Excretion of Purine Derivatives – An Overview of the Technical Details. Rowett Res. Institute, Bucksburn, Aberdeen, UK.

Chen, X.B., Ørskov, E.R., 2004. Research on urinary excretion of purine derivatives in ruminants: past, present and future. In: Makkar, H.S., Chen, X.B. (Eds.), Estimation of Microbial Protein Supply in Ruminants Using Urinary Purine Derivatives. Springer, Netherlands, pp. 180–210.

Chizzotti, M.L., Valadares, S.D., Valadares, R.F.D., Chizzotti, F.H.M., Tedeschi, L.O., 2008. Determination of creatinine excretion and evaluation of spot urine sampling in Holstein cattle. Livest. Sci. 113, 218–225.

Clark, J.H., Klusmeyer, T.H., Cameron, M.R., 1992. Microbial protein synthesis and flows of nitrogenous fractions to the duodenum of dairy cows. J. Dairy Sci. 75, 2304–2323.

Clarke, A.M., Drennan, M.J., McGee, M., Kenny, D.A., Evans, R.D., Berry, D.P., 2009. Intake, live animal scores/measurements and carcass composition and value of late-maturing beef and dairy breeds. Livest. Sci. 126, 57–68.

Dijkstra, J., Oenema, O., van Groenigen, J.W., Spek, J.W., van Vuuren, A.M., Bannink, A., 2013a. Diet effects on urine composition of cattle and $N_2O$ emissions. Animal 7, 292–302.

Dijkstra, J., Reynolds, C.K., Kebreab, E., Bannink, A., Ellis, J.L., France, J., van Vuuren, A.M., 2013b. Challenges in ruminant nutrition: towards minimal nitrogen losses in cattle. In: Oltjen, J.W., Kebreab, E., Lapierre, H. (Eds.), Energy and Protein Metabolism and Nutrition in Sustainable Animal Production. Sacramento, CA, USA, pp. 47–58.

Dong, R.L., Zhao, G.Y., Chai, L.L., Beauchemin, K.A., 2014. Prediction of urinary and fecal nitrogen excretion by beef cattle. J. Anim. Sci. 92, 4669–4681.

Drennan, M.J., McGee, M., 2009. Performance of spring-calving beef suckler cows and their progeny to slaughter on intensive and extensive grassland management systems. Livest. Sci. 120, 1–12.

European Environment Agency, 2016. European Union emission inventory report 1990–2014 under the UNECE Convention on Long-range Transboundary Air Pollution (LRTAP). Report no 16. doi:http://dx.doi.org/10.2800/628267.

Gonda, H.L., Emanuelson, M., Murphy, M., 1996. The effect of roughage to concentrate ratio in the diet on nitrogen and purine metabolism in dairy cows. Anim. Feed Sci. Technol. 64, 27–42.

Herd, D.B., Sprott, L.R., 1986. Body Condition, Nutrition and Reproduction of Beef Cows. Texas Agricultural Extension Service, Texas A & M Universitypp. 12.

Hristov, A.N., Etter, R.P., Ropp, J.K., Grandeen, K.L., 2004. Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. J. Anim. Sci. 82, 3219–3229.

Huhtanen, P., Nousiainen, J.I., Rinne, M., Kytola, K., Khalili, H., 2008. Utilization and partition of dietary nitrogen in dairy cows fed grass silage-based diets. J. Dairy Sci. 91, 3589–3599.

Hvelplund, T., Weisbjerg, M.R., Søegaard, K., 1999. Use of *in vitro* digestibility methods to estimate *in vivo* digestibility of straws. In: Tanzania society of animal production conference, pp. 70–78. Arusha, Tanzania 3–6 August.

Kebreab, E., Strathe, A.B., Dijsktra, J., Mills, J.A.N., Reynolds, C.K., Crompton, L.A., Yan T., France, J., 2010. Energy and protein interaction and their effect on nitrogen excretion in dairy cows. In: Crovetto, G.M. (Ed), Energy and protein metabolism and nutrition, pp. 417–425. Parma, Italy 6-10 September.

Kumm, K.-I., 2009. Produktionskostnad för grovfoder till köttdjur (Cost of roughage production to beef cattle). Report no 23. The Department of Animal Environment and Health. The Swedish University of Agricultural Sciences, Skara, Sweden (In Swedish).

Lengerken, J., Zimmermann, K., 1991. Handbuch Futtermittelprüfung, 1. ed. Deutscher Landwirtschaftsverlag, Berlin.

Leonardi, C., Stevenson, M., Armentano, L.E., 2003. Effect of two levels of crude protein and methionine supplementation on performance of dairy cows. J. Dairy Sci. 86, 4033–4042.

Licitra, G., Hernandez, T.M., Van Soest, P.J., 1996. Standardization of procedures for nitrogen fractionation of ruminant feeds. Anim. Feed Sci. Technol. 57, 347–358.

Lindgren, E., 1979. Vallfodrets näringsvärde bestämt *in vivo* och med olika laboratoriemetoder. Report 45. Department of animal nutrition and management, Swedish University of Agricultural Sciences, Uppsala, Sweden (in Swedish).

Lindgren, E., 1983. Nykalibrering av VOS metoden för bestämning av energivärde hos vallfoder. Working paper. Department of animal nutrition and management, Swedish University of Agricultural Sciences, Uppsala, Sweden (in Swedish).

Lindgren, E., 1988. Fodrets energivärde. Course paper Feed Science HNU 3. Department of animal nutrition and management, Swedish University of Agricultural Sciences, Uppsala, Sweden, pp. 49.

LKS – Landwirtschaftliche Kommunikationsund Servicegesellschaft mbH, 2006. Bestimmung des Harnstoff – Gehaltes. Qualitätsmanagementhandbuch, Arbeitsanweisung no.123, 5 pp.

Merchen, N.R., Bourquin, L.D., 1994. Processes of digestion and factors influencing digestion of forage-based diets by ruminants. In: Fahey, G.C., Collins, M., Mertens, D.R., Moser, L.E. (Eds.), Forage, Quality, Evaluation, and Utilization. Lincoln, USA, pp. 564–612.

Moorby, J.M., Dewhurst, R.J., Evans, R.T., Danelon, J.L., 2006. Effects of dairy cow diet forage proportion on duodenal nutrient supply and urinary purine derivative excretion. J. Dairy Sci. 89, 3552–3562.

Nocek, J.E., Russell, J.B., 1988. Protein and energy as an integrated system - relationship of ruminal protein and carbohydrate availability to microbial synthesis and milk production. J. Dairy Sci. 71, 2070–2107.

NRC, 2000. Nutrient Requirements for Beef Cattle: Seventh Revised Edition, Update 2000. Natl. Acad. Sci. Press, Washington, DC.

Ørskov, E.R., MacLeod, N.A., 1982. The determination of the minimal nitrogen excretion in steers and dairy cows and its physiological and practical implications. Br. J. Nutr. 47, 625–636.

Reynolds, C.K., Kristensen, N.B., 2008. Nitrogen recycling through the gut and the nitrogen economy of ruminants: an asynchronous symbiosis. J. Anim. Sci. 86, 293–305.

Rosner, B., 1983. Percentage points for a generalized ESD many-outlier procedure. Technometrics 25, 165–172.

Shingfield, K.J., Offer, N.W., 1999. Simultaneous determination of purine metabolites, creatinine and pseudouridine in ruminant urine by reversed–phase high–performance liquid chromatography. J. Chromatogr. B. 723, 81–94.

Sniffen, C.J., Oconnor, J.D., Vansoest, P.J., Fox, D.G., Russell, J.B., 1992. A net carbohydrate and protein system for evaluating cattle diets. 2. Carbohydrate and protein availability. J. Anim. Sci. 70, 3562–3577.

Spek, J.W., Dijkstra, J., van Duinkerken, G., Hendriks, W.H., Bannink, A., 2013. Prediction of urinary nitrogen and urinary urea nitrogen excretion by lactating dairy cattle in northwestern Europe and North America: a meta-analysis. J. Dairy Sci. 96, 4310–4322.

Spörndly, R., 2003. Fodertabeller för idisslare. Report no. 257. The Swedish University of Agricultural Science, Uppsala, Sweden (in Swedish).

Stergiadis, S., Chen, X.J., Allen, M., Wills, D., Yan, T., 2015. Evaluating nitrogen

utilization efficiency of nonpregnant dry cows offered solely fresh cut grass at maintenance levels. J. Anim. Sci. 93, 709–720.
Südekum, K.H., Brusemeister, F., Schroder, A., Stangassinger, M., 2006. Effects of amount of intake and stage of forage maturity on urinary allantoin excretion and estimated microbial crude protein synthesis in the rumen of steers. J. Anim. Physiol. Anim. Nutr. 90, 136–145.
Swedish board of agriculture, 2016. Jordbruksstatistisk årsbok 2016 (Yearbook of agricultural statistics 2016). Chapter 13, p 188.
Tamminga, S., 1992. Nutrition management of dairy cows as a contribution to pollution control. J. Dairy Sci. 75, 345–357.
Valadares, R.F.D., Broderick, G.A., Valadares Filho, S.C., Clayton, M.K., 1999. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives. J. Dairy Sci. 82, 2686–2696.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.
Vercoe, J.E., 1976. Urinary allantoin excretion and digestible dry matter intake in cattle and buffalo. J. Agric. Sci. Camb. 86, 613–615.
Volden, H., Larsen, M., 2011. Digestion and metabolism in the gastrointestinal tract. In: Volden, H. (Ed.), NorFor – the Nordic Feed Evaluation System. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 59–80.
Volden, H., Nilsen, N.I., 2011. Energy and metabolizable protein supply. In: Volden, H. (Ed.), NorFor – the Nordic Feed Evaluation System. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 81–84.
Weisbjerg, M.R., Hvelplund, T., 1993. Bestemmelse af nettoenergiindhold (FEK) i råvarer og kraftfoderblandinger. Research report 3. Statens husdyrbrugsforsøg, Denmark, pp. 39.
Weiss, K., Kaiser, E., 1995. Milchsäurebestimmung in Silageextrakten mit Hilfe der HPLC. Das. Wirtsch. Futter. 41, 69–80.
Weiss, K., 2001. Gärungsverlauf und Gärqualität von Silagen aus nitratarmem Grünfutter. (Dissertation) Faculty of Agriculture and Horticulture, Humboldt University of Berlin, Berlin.
Weiss, W.P., Willett, L.B., St-Pierre, N.R., Borger, D.C., McKelvey, T.R., Wyatt, D.J., 2009. Varying forage type, metabolizable protein concentration, and carbohydrate source affects manure excretion, manure ammonia, and nitrogen metabolism of dairy cows. J. Dairy Sci. 92, 5607–5619.
Whittet, K.M., Klopfenstein, T.J., Erickson, G.E., Loy, T.W., McDonald, R.A., 2004. Effect of age, pregnancy and diet on urinary creatinine excretion in heifers and cows. Nebraska Beef Cattle Reports. Paper 212. University of Nebraska, Lincoln, US.
Wohlt, J.E., Clark, J.H., Blaisdell, F.S., 1978. Nutritional value of urea versus preformed protein for ruminants. II. Nitrogen utilization by dairy cows fed corn based diets containing supplemental nitrogen from urea and/or soybean meal. J. Dairy Sci. 61, 916–931.
Zou, C.X., Lively, F.O., Wylie, A.R.G., Yan, T., 2016. Estimation of the maintenance energy requirements, methane emissions and nitrogen utilization efficiency of two suckler cow genotypes. Animal 10, 616–622.