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# Relationship between feed protein content and faeces nitrogen content in early lactation dairy cows

Diana Ruska*, Daina Jonkus

*Latvia University of Life Sciences and Technologies, Faculty of Agriculture, Institute of Animal Sciences, Jelgava, Latvia*



The increase of milk production at the farm level requires an accurate balancing of the diet and the nitrogen supply also to minimise the possible environmental pollution deriving from dairy farming. The aim of this study was to evaluate dietary protein utilization at different crude protein (CP) levels and to predict nitrogen content in faeces on the basis of nutritional parameters and milk urea nitrogen content (MUN, mg $dL^{-1}$). The study was conducted on three groups (A, B, C) of lactating dairy cows (8 cows per group, including Latvian Brown and Holstein Black and White breeds) from 10 to 30 days in milk. Total mixed rations containing different levels of CP (approximately 18.0%, 17.5% and 17.0% for A, B and C, respectively) were fed. The amount of feed consumed by each cow was measured and feed samples collected during the trial. Milk yield (kg $d^{-1}$) and faeces amount were recorded, and samples were collected at day 21 of the study for further analysis. Feed samples were analysed for CP, net energy for lactation (NEL, MJ $kg^{-1}$) and other parameters. Milk samples were analysed for fat (%), total protein (%), casein (%) and urea content (mg $dL^{-1}$). The statistical investigation was conducted using ANOVA, and correlation and regression analyses. The results showed that milk yield, fat, total protein, casein, urea, and MUN were not significantly different among groups being not affected by the dietary CP levels. The correlation between faecal nitrogen content and CP content in feed was moderately positive and statistically significant (r=0.44, P=0.03), while the correlation between faecal nitrogen content and MUN was moderately negative and showed tendency towards significance (r=-0.39, P=0.06). The regression analysis showed that feed CP explained approximately 20% of faeces nitrogen content.

**Keywords:** dairy cow, milk urea, faeces nitrogen, feed crude protein

## 1 Introduction

The interest in environmental pollution associated to livestock farming has been growing around the world in the last decades. Dairy farmers are therefore looking for ways to properly manage cattle nutrition in a sustainable way. Among the most important nutrients for humans and animals are proteins. Therefore, the study and control of nitrogen balance at the farm level in relation also to milk production traits, profit and environmental pollution is becoming an essential step in dairy farming. To increase milk production farmers tend to increase protein content in the diet. Data of the National Research Council in USA demonstrates that nitrogen amount in feed of dairy cattle exceeds the requirements on average by 6.6%, leading to an increase of nitrogen content in faeces and urine. Information available at farm level can be used for the production process control and management. Examples are milk production data and nutritional parameters that can be obtained without need to collect and testing special samples of urine and faeces (Broderick and Huhtanen, 2020). High protein concentrations in feed ration generally contribute to increase milk production levels, but part of the nitrogen is not partitioned to milk and is excreted in faeces and urine (Dijkstra et al., 2011). Straalen (1995) reported that nitrogen distribution from feed are excreted in faeces (37%), urine (35%), and milk (27%). Several countries in Europe have already regulatory enactments and control possible environmental pollution that may arise from agricultural activities. Urea content in milk is one of the parameters that can be used for nitrogen pollution monitoring from farms (Bijgaart, 2003). Other

* **Corresponding Author:** Diana Ruska. Latvia University of Life Sciences and Technologies, Faculty of Agriculture, Institute of Animal Sciences, Liela str.2, LV-3001, Jelgava, Latvia, E-mail: diana.ruska@llu.lv

parameters are nutritional parameters, like crude protein (CP) content in feed, and CP intake and net energy for lactation (NEL) intake (Rotz et al., 1999). In addition, information about predicted nitrogen content in faeces is a useful tool for planning manure management and soil fertilization in the farm (Powell and Rotz, 2015).

The objective of this study was to evaluate feed protein utilization with different CP levels and to predict nitrogen content in faeces starting from on farm readily available information like nutritional parameters and milk urea nitrogen (MUN) content.

## 2 Material and methods

The study was conducted at the research and study farm Vecauce of the Latvia University of Life Sciences and Technologies (LLU MPS Vecauce) between May and July 2019. For the study, three groups of dairy cows, each including 8 animals of Latvian Brown (n=3) and Holstein Black and White (n=5) breeds were created. Cows were in early lactation, from 10 to 30 days in milk, and each group included second lactation (n=5) and third lactation (n=3) cows. The experimental design was a 3x3 Latin square where three diets were administered over three periods, each lasting 21 days. The diets were represented by total mixed ration (TMR) prepared directly in the farm, differing for CP content (diet A: 18.0% CP; diet B: 17.5% CP; diet C: 17.0% CP). The TMR was comprised of maize silage, barley and wheat grain, soya seed, rapeseed cakes and mineral additives. The different protein levels were obtained through the variation of soya seeds levels in diets. During the experiment, cows were housed in tie stalls and individually fed; individual water sources with automatically water counter were provided. Cows were fed *ad libitum*. Refused feed were collected, weighted and their amount recorded every day for each cow. Total mixed ration samples were taken for testing every two or three days (n=24). The samples were immediately frozen and later analysed in accredited laboratory by international or validate methods. The average chemical compositions of TMR is presented in Table 1.

**Table 1** Chemical composition (mean ± standard error) of diets tested in the study

| Trait | A group (n=8) | B group (n=8) | C group (n=8) |
|---|---|---|---|
| Dry matter (DM, %) | 45.62±1.03$^{a}$ | 40.57±0.85$^{b}$ | 38.29±1.30$^{b}$ |
| Crude protein (CP, % DM) | 18.02±0.46$^{a}$ | 17.89±0.49$^{a}$ | 16.99±0.66$^{a}$ |
| Unavailable protein (% DM) | 0.74±0.06$^{a}$ | 0.89±0.10$^{a}$ | 0.88±0.09$^{a}$ |
| Soluble protein (% DM) | 1.77±0.15$^{a}$ | 1.78±0.16$^{a}$ | 1.92±0.10$^{a}$ |
| Insoluble available protein (% CP) | 59.88±2.10$^{a}$ | 57.84±0.93$^{a}$ | 49.55±2.72$^{b}$ |
| Ash (% DM) | 6.88±0.16$^{a}$ | 7.14±0.10$^{a}$ | 8.40±0.24$^{b}$ |
| Crude fibre (% DM) | 13.46±0.65$^{a}$ | 15.27±0.53$^{a}$ | 19.17±1.42$^{b}$ |
| NDF (% DM) | 28.94±0.58$^{a}$ | 32.15±0.42$^{b}$ | 36.62±1.38$^{c}$ |
| ADF (% DM) | 16.78±0.60$^{a}$ | 19.46±0.62$^{a}$ | 23.40±1.75$^{b}$ |
| pdNDF (% DM) | 72.78±1.58$^{a}$ | 78.17±2.11$^{a}$ | 76.36±2.42$^{a}$ |
| NEL (MJ kg$^{-1}$ DM) | 7.27±0.05$^{a}$ | 7.06±0.05$^{a}$ | 6.74±0.14$^{b}$ |
| Ether extract (% DM) | 3.30±0.18$^{a}$ | 3.20±0.09$^{a}$ | 3.17±0.20$^{a}$ |

$^{a,b,c}$ – means with different letters within a row differed significantly (P<0.05)

The CP content of the TMR ranged from 16.99% to 18.02%, depending on the dietary group tested and met the requirements of dairy cows according to milk yield. Insoluble available protein in dietary group C was significantly lower than in A and B dietary groups. Neutral detergent fibre (NDF) in TMR samples were significantly different in diet C and ranged from 28.94% of diet A to 36.63% of diet C; however, potentially digestible NDF (pdNDF) was not significantly different among diets. The NEL was significantly lower (6.74 MJ kg$^{-1}$) in diet C and similar in diet A and B (7.27 MJ kg$^{-1}$ and 7.06 MJ kg$^{-1}$, respectively).

To individually measure cow milk production, milk yield (kg d$^{-1}$) was recorded and milk samples collected on day 7, 11, 15 and 21, separately for each milking time. Milk composition was analysed for the content of fat (%), total protein (%) and urea (mg dL$^{-1}$) with infrared spectroscopy method, and somatic cell count in accredited laboratory for milk quality control.

 

After 21 days, total faeces amount was collected over 72 hours from each cow separately (n=24 samples), weighed, mixed thoroughly, and subsampled. Faeces composition was determined in accredited laboratory for dry matter (%, LVS EN 13040:2008, 8.1; 9-11; LVS, 2008) and nitrogen in raw faeces (N, %, LVS EN 13654-1/NAC:2004; LVS, 2004).

For statistical data processing, milk urea content (mg dL$^{-1}$) was recalculated to MUN (mg dL$^{-1}$) content according to Spiekers and Obermaier (2007):

$$MUN = milk\ urea\ content \times 0.46 \quad (1)$$

The contents of CP and NEL, which were measured in laboratory as % and MJ kg$^{-1}$, respectively, were recalculated to intake per day in compliance with ICAR guidelines (ICAR, 2017).

$$Intake\ amount, kg = (dry\ matter\ intake, kg \times content)/100 \quad (2)$$

In order to compare and evaluate results among groups, milk yield and contents were transformed to energy corrected milk (ECM, kg d$^{-1}$; ICAR, 2017) as follows:

$$ECM = (fat\ yield, kg \times 38.3 + protein\ yield, kg \times 24.2 + milk\ yield, kg \times 0.7832)/3.14 \quad (3)$$

A one-way ANOVA was conducted to analyse the data by including the dietary group (3 levels: A, B, C) as fixed effect, with Bonferroni *t* test correction for testing if groups differed significantly ($P<0.05$). Also, Pearson's correlations of nitrogen content in faeces with feed and milk composition indicators were estimated. Statistical processing was carried out with MS for SPSS (SPSS Inc. Chicago, Illinois, USA) and Microsoft Excel (Microsoft Corporation, Redmond, Washington, USA).

## 3 Results and discussion

Dry matter intake (DMI) did not differ among groups and ranged from 16.1 kg d$^{-1}$ in dietary group B to 17.1 kg d$^{-1}$ in dietary group A (Table 2). In a previous study, researchers did not find decreased DMI in early lactation cows when CP ranged from 13.5% to 19.4% (Colmenero and Broderick, 2006). During lactation, and especially in the early lactation stage, it is very important to balance energy and protein to avoid rumen fermentation anomalies, which result in reduced milk yield, and fat and protein content. After calving, and especially at the end of the first week of lactation, cows may experience negative energy balance (Ng-Kwai-Hang et al., 1985). In our study DMI did not meet the recommended amount for multiparous cows in early lactation (NRC, 2001).

**Table 2** Dry matter intake and cow milk traits (mean ± standard error) by dietary group

| Trait | Group | | |
|---|---|---|---|
| | A (n=8) | B (n=8) | C (n=8) |
| Dry matter intake (kg d$^{-1}$) | 17.1±0.87 | 16.1±1.08 | 16.8±0.78 |
| Milk yield (kg d$^{-1}$) | 40.5±3.68 | 39.8±3.71 | 39.7±4.36 |
| Fat content (%) | 3.46±0.19 | 3.68±0.16 | 3.56±0.20 |
| Crude protein content (%) | 3.14±0.13 | 3.06±0.09 | 2.83±0.11 |
| Casein content (%) | 2.53±0.10 | 2.47±0.07 | 2.30±0.09 |
| Urea content (mg dL$^{-1}$) | 27.0±1.40 | 24.9±1.88 | 30.9±1.81 |
| MUN (mg dL$^{-1}$) | 12.4±0.64 | 11.4±0.86 | 14.2±0.83 |
| ECM (kg d$^{-1}$) | 36.3±2.51 | 36.8±2.87 | 34.9±3.04 |

Milk yield, fat, total protein, casein, urea, and MUN content did not differ among dietary groups (Table 2). Milk yield ranged from 39.7 kg d$^{-1}$ in dietary group C to 40.5 kg d$^{-1}$ in dietary group A. Fat content was the lowest in dietary group A (3.46%) and total protein content in dietary group C (2.83%). Milk urea and MUN contents were similar in all groups and did not exceed the recommended levels since milk urea levels between 20 and 30 mg dL$^{-1}$ are generally considered as normal for cow's milk in

 

Europe (Bijgaart, 2003), consistently with the MUN level of 12.0 mg $dL^{-1}$ recommended in USA (Bucholtz et al., 2007). Milk urea content was higher in dietary group C probably due to insufficient NEL content in ration, which is related to differences in chemical content of dietary group C. The ECM in dietary group C was reduced by 5.4% compared with group B. Milk traits did not differ among dietary groups consistently with previous studies where the decrease of CP in feed ration did not lead to significant effects on milk production traits (Kalscheur et al., 1999, Amanlou et al., 2017, Kidane et al., 2018).

Crude protein and NEL intake, and nitrogen content and amount in faeces for each study group were estimated in order to evaluate nitrogen utilization by the animals (Table 3). Crude protein intake ranged from 2.86 kg $d^{-1}$ in dietary C group to 3.08 kg $d^{-1}$ in dietary group A, but this difference was not statistically significant. The NEL intake was lower in dietary C group and higher in A diet-fed group (113.3 MJ $kg^{-1}$ and 124.6 MJ $kg^{-1}$, respectively), but this difference was not significant among groups.

**Table 3** Nutrients intake and output in faeces (mean ± standard error) by dietary group

| Trait | A group (n=8) | B group (n=8) | C group (n=8) |
|---|---|---|---|
| Crude protein intake (kg $d^{-1}$) | $3.08±0.16^{a}$ | $2.88±0.19^{a}$ | $2.86±0.13^{a}$ |
| NEL intake (MJ $kg^{-1}$) | $124.6±6.30^{a}$ | $116.6±8.03^{a}$ | $113.3±5.23^{a}$ |
| Nitrogen content in faeces (%) | $0.43±0.01^{a}$ | $0.41±0.02^{a}$ | $0.35±0.01^{b}$ |
| Nitrogen in faeces (kg $d^{-1}$) | $0.15±0.01^{a}$ | $0.16±0.02^{a}$ | $0.15±0.02^{a}$ |

$^{a,b}$ – means with different letters within a row differed significantly (P<0.05)

Nitrogen content in raw faeces was similar in A and B groups (0.43% and 0.41%, respectively) and significantly lower in C group (0.35%, P<0.05). Results from previous studies supported that decreasing CP content in feed decreases faeces nitrogen content (Arunvipas et al., 2008). Indeed, Norway researchers found a tendency for increased faeces nitrogen excretion with increasing CP level in ration (P=0.063; Kidane, 2018). Nitrogen excretion in faeces in the present study ranged from 0.16 kg $d^{-1}$ in B group to 0.15 kg $d^{-1}$ in C group, but the difference was not significant. In a study dealing with high CP (16.6% and 16.7%) and low CP (15.2% and 15.1%), and low and high starch intake from ration, Recktenwald (2014) found that N excretion in faeces was higher for high CP diet.

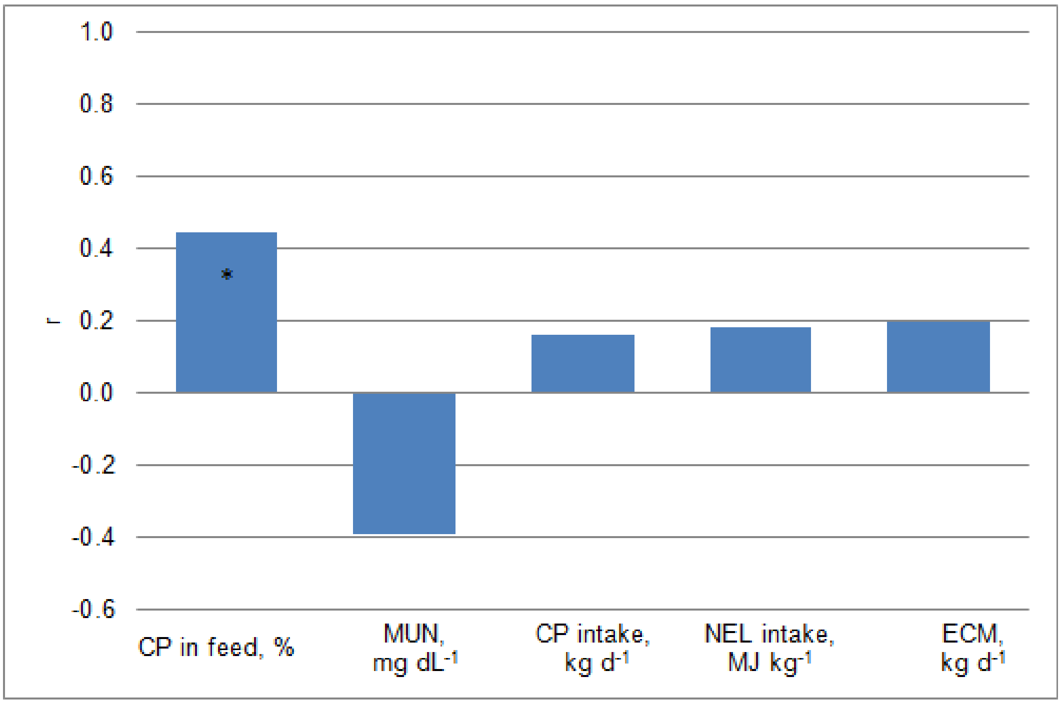


**Figure 1** Correlations of nitrogen content in faeces with feed crude protein (CP), milk urea nitrogen (MUN), CP intake, net energy for lactation (NEL) intake, and energy corrected milk (ECM) (*P<0.05)

The correlation between faecal nitrogen content and CP content in feed was moderately positive and significant (r=0.44, P=0.03; Figure 1), meaning that, on average, the reduction of CP content in feed is associated to a reduction of nitrogen content in faeces. The correlation between CP and NEL intake, and ECM was not significant, whereas the correlation between faecal nitrogen content and MUN was moderately negative and exhibited a tendency towards significance (r=-0.39, P=0.06). Canadian researchers reported consistent results on the correlation between faecal nitrogen content and CP and NEL intake but, in contrast to our results, the correlation between faecal nitrogen content and feed CP (r=0.146) and MUN (r=0.028) was slightly positive (Arunvipas et al., 2008).

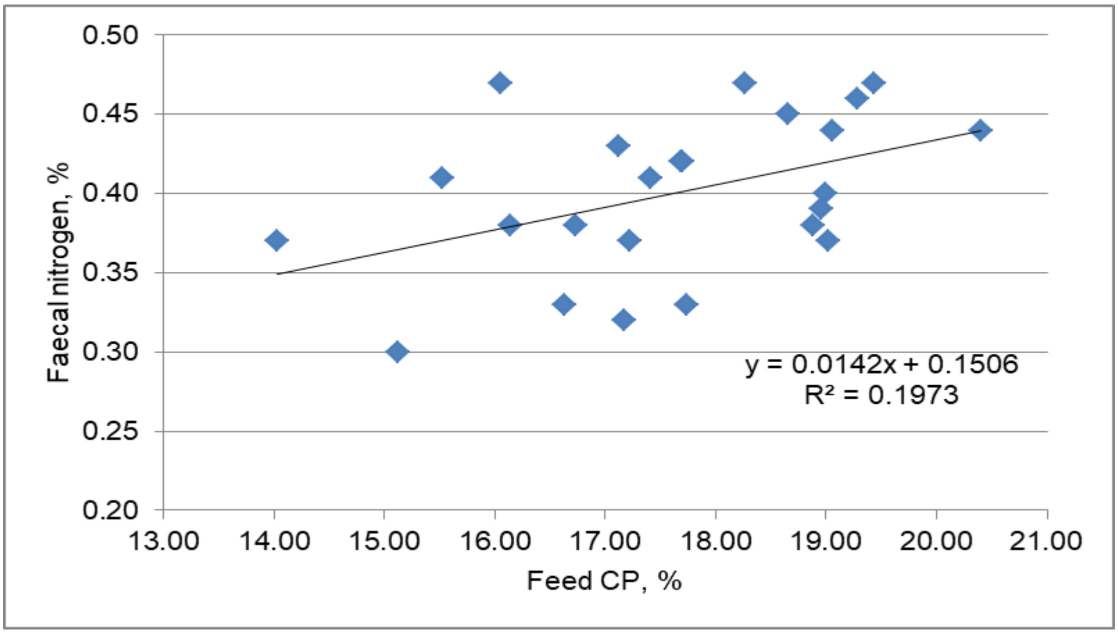


**Figure 2** Regression analysis of nitrogen content in faeces on feed crude protein (CP) ($P<0.05$)

Feed CP content explained approximately 20% of the variation of faecal nitrogen content (Figure 2), meaning that the feed CP can affect faecal nitrogen content and thus that there is room to manage CP content in feed to reduce faecal nitrogen content. Also, there is room to reduce environmental pollution and costs of milk production. Nevertheless, faecal nitrogen content is influenced by several factors (about 80% of faecal nitrogen content variation remained unexplained). One of them was probably water intake which ranged from 96.8 L $d^{-1}$ in dietary group C to 105.9 L $d^{-1}$ in dietary group B in summer season. These values were higher than those from mathematical models that predict water intake by DMI, where on average DMI of 18.3 kg $d^{-1}$ per day requires 75.2 kg $d^{-1}$ of water for lactating cows in neutral temperature condition (+18°C; Appuhamy et al., 2016).

## 4 Conclusions

Dietary CP level ranging from 16.99% to 18.02% under the tested conditions did not affect milk production traits. Correlation between feed CP and faeces nitrogen content was moderately positive and significant. The CP content in feed explained about 20% of the variation of faecal nitrogen content.

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