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# Relationships between dietary factors and milk urea nitrogen level in goats grazing herbaceous pasture

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## ABSTRACT

This investigation aimed to individuate the dietary factors affecting the milk urea nitrogen (MUN) concentration in goats grazing herbaceous pasture and, particularly, to verify the relationship linking the diet crude protein (CP) content to MUN.

A total of 205 individual observations regarding dietary and milk variables of 37 Girgentana goats involved in two experiments were used. Goats, averaging 154±14 days in milk and 38.1±5.4 kg of live weight, grazed on swards and received 500 g/d of barley meal. Sward biomass, herbage selected by goats and individual milk yield were measured and sampled weekly. The herbage intake and diet digestibility were estimated by the *n*-alkane method. Milk urea content was determined by an enzymatic method and transformed in MUN (MUN=urea\*0.4665).

The MUN concentration (9.7-35.4 mg/dl) was positively correlated with diet CP content (13.7-26.0% of dry matter (DM);  $r=0.76$ ;  $P<0.001$ ), pasture allowance (39-151 kg DM/goat;  $r=0.42$ ;  $P<0.001$ ), diet net energy for lactation concentration (NE<sub>L</sub>) (1.5-1.9 Mcal/kg DM;  $r=0.37$ ;  $P<0.001$ ) and milk yield (320-2250 g/d;  $r=0.25$ ;  $P<0.001$ ), and negatively related with NDF (18.7-37.4% DM;  $r=-0.69$ ;  $P<0.001$ ) and diet digestibility (72.6-92.5%;  $r=-0.33$ ;  $P<0.001$ ).

The stepwise selection from dietary variables and milk yield showed dietary CP percentage to be the single variable explaining the most variation in MUN ( $R^2=0.56$ ;  $P<0.0001$ ). The other variables entering into the model were diet NDF, 3.5% fat-corrected milk (FCM), DM intake and NDF intake (total  $R^2=0.66$ ). Including the CP/NE<sub>L</sub> and CP/NDF ratios of diet in the stepwise regression model, the CP/NDF ratio alone explained 60.1% of MUN variability, followed by barley proportion in the diet, FCM and diet CP concentration, absorbing an extra 4.6% of MUN variability.

A linear regression, fitting mean feeding treatment per time data of MUN and dietary CP concentration ( $n=28$ ) [ $CP(\% \text{ of DM})=6.91\pm 1.42+0.61\pm 0.06\cdot \text{MUN (mg/dl)}$ ;  $R^2=0.79$ ;  $P<0.0001$ ], suggests that MUN could be used for predicting the CP content of the diet, as a tool for developing feeding strategies aimed at balancing the rations of grazing goats through adequate supplementation. Further data from experiments on grazing goats in different environmental conditions are required in order to define a more robust relationship by which to predict the dietary CP content by MUN.

*Key words:* Milk urea nitrogen, Dietary protein, Goat, Grazing.

## RIASSUNTO

## RELAZIONE TRA LE CARATTERISTICHE DELLA DIETA E LA CONCENTRAZIONE DI AZOTO UREICO NEL LATTE DI CAPRE ALIMENTATE SU PASCOLI ERBACEI

*Gli obiettivi dell'indagine sono stati quelli di individuare i fattori nutrizionali in grado di influenzare la concentrazione di azoto ureico nel latte (AUL) di capre alimentate su pascoli erbacei, e di verificare in particolare la relazione che lega il contenuto in proteina grezza (PG) della dieta al livello di AUL.*

*A tale scopo sono state utilizzate 205 osservazioni individuali relative alla dieta ed alla produzione di latte di 37 capre di razza Girgentana a 154±14 giorni di lattazione e con un peso vivo di 38,1±5,4 kg, che pascolavano su erbai e ricevevano un'integrazione di 500 g/d di farina di orzo. I rilevamenti, effettuati con cadenza settimanale, hanno interessato la biomassa foraggera disponibile, l'erba selezionata dalle capre al pascolo e la produzione individuale di latte. L'ingestione di erba al pascolo e la digeribilità della dieta sono state stimare con la tecnica degli n-alcani. Il livello di urea è stato determinato con metodo enzimatico e trasformato in AUL ( $AUL=urea*0,4665$ ).*

*La concentrazione di AUL (9,7-35,4 mg/dl) è risultata direttamente correlata con la PG della dieta (13,7-26,0% della sostanza secca (SS);  $r=0,76$ ;  $P<0,001$ ), la disponibilità di foraggio al pascolo (39-151 kg di SS/capra;  $r=0,42$ ;  $P<0,001$ ), l'energia netta per la lattazione ( $EN_L$ ) della dieta (1,5-1,9 Mcal/kg di SS;  $r=0,37$ ;  $P<0,001$ ) e la produzione di latte (320-2250 g/d;  $r=0,23$ ;  $P<0,001$ ), mentre correlazioni negative sono emerse con il tenore in NDF (18,7-37,4% SS;  $r=-0,69$ ;  $P<0,001$ ) e con la digeribilità della dieta (72,6-92,5%;  $r=-0,33$ ;  $P<0,001$ ).*

*Sottoponendo le variabili della dieta e della produzione di latte ad una regressione multipla con metodo stepwise, il tenore in PG della dieta è stato in grado di spiegare la maggiore variabilità dell'AUL ( $R^2=0,56$ ;  $P<0,0001$ ). Le altre variabili entrate nel modello sono state, nell'ordine, il tenore in NDF della dieta, il latte corretto al 3,5% di grasso (LC), l'ingestione di SS e l'ingestione in NDF ( $R^2$  totale=0,66). Inserendo i rapporti  $PG/EN_L$  e  $PG/NDF$  della dieta nel modello di regressione stepwise, il rapporto  $PG/NDF$  da solo ha spiegato il 60,1% della variabilità dell'AUL, seguito dalla percentuale di orzo nella dieta, il LC e il tenore in PG della dieta, che hanno assorbito un ulteriore 4,6% di variabilità.*

*La regressione lineare tra i valori medi di AUL e tenore in PG della dieta relativi al trattamento alimentare e al periodo sperimentale ( $n=28$ ) [ $PG$  (% SS) =  $6,91 \pm 1,42 + 0,61 \pm 0,06 * AUL$  (mg/dl);  $R^2=0,79$ ;  $P<0,0001$ ], suggerisce come il livello di AUL, utilizzato per la stima del contenuto in PG della dieta, possa costituire un utile strumento per sviluppare strategie alimentari idonee a bilanciare la razione di capre al pascolo attraverso il ricorso all'integrazione. La disponibilità di ulteriori dati sperimentali di capre alimentate al pascolo in ambienti diversificati renderebbe possibile la definizione di relazioni di più robusta capacità previsionale della PG della dieta a partire dall'AUL.*

*Parole chiave: Azoto ureico del latte, Proteina della dieta, Capre, Pascolamento.*

**Introduction**

Urea is formed in the liver and mainly results from a surplus of protein degraded in the rumen, an excess of true protein digested in the small intestine and gluconeogenesis from amino acids (Schepers and Meijer, 1998).

Even though the heritability of milk urea nitrogen (MUN) in dairy cows was shown to vary between 0.15 and 0.22 (Mitchell *et al.*, 2005), and the MUN is shown to be in-

fluenced by environmental factors, such as parity, season, stage of lactation, milk yield and herd (Schepers and Meijer, 1998; Giaccone *et al.*, 2007), the major determinants of urea formation are the amount of daily crude protein (CP) intake and the dietary ratio of CP to energy intake. Particularly in the rumen, an increased CP in diet that is not balanced with available energy causes a surplus of N used for microbial growth, which is transformed into ammonia. The extra ammonia, which is toxic for animals,

is absorbed into the digestive tract and enhances the formation of urea in the liver. From there the urea passes into the plasma (Harmeyer and Martens, 1980). Entry of the urea into the rumen from the plasma constitutes a recycling mechanism of the urea that improves N utilization efficiency, since urea is used as a precursor of microbial protein synthesis (Brun-Bellut, 1996).

Urea concentrations in urine, blood and milk are closely correlated. In goat milk, the MUN concentration was demonstrated to be lower than the plasma urea nitrogen (PUN), to which it is highly correlated (Cabiddu *et al.*, 1999; Bava *et al.*, 2001), in a similar way to cow (Hof *et al.*, 1997) and sheep milk (Jelinek *et al.*, 1996), as a consequence of the free diffusion of the urea molecule throughout the mammary epithelium.

High MUN concentrations have been shown to be negatively related to health and fertility in dairy cattle (Ferguson and Chalupa, 1989; Guo *et al.*, 2004). As regards reproductive performance, significant values of genetic correlations were found in dairy cows between MUN and days open, ranging from 0.21 to 0.41 (Mitchell *et al.*, 2005). In goats, levels of plasma urea higher than 10 mg/dl were found to be associated with increased risk of abortion and low conception and kidding rates (Mellado *et al.*, 2004).

Both PUN and MUN are considered to be good indicators of protein intake, ammonia loss from the rumen and the efficiency of processes related to protein metabolism in dairy cows (Roseler *et al.*, 1993; Hof *et al.*, 1997; Shepers and Meijer, 1998) and are currently used in the diagnosis of protein feeding. Because milk is easier to collect than blood, and milk urea can be accurately determined by enzymatic or physical methods, it is suggested that milk urea determination can be used in bulk tank milk to evaluate the on-farm efficiency of dietary N utilisation and the adequacy and balancing

of diets (Cannas *et al.*, 1998; Jonker *et al.*, 1998; Shepers and Meijer, 1998).

As is well known, in the Mediterranean areas the breeding system of dairy sheep and goats is mainly based on grazing pasture. In these conditions, an accurate prediction of pasture quality and an easy indicator for monitoring the feeding ration should permit the development of appropriate feeding strategies, providing the adequate supplementary feed aimed at balancing grazed forage. In this way, MUN might represent a fundamental nutritional tool for grazing small ruminants as well. However, this knowledge of an exact relationship between MUN and dietary CP mainly regards dairy cows (Shepers and Meijer, 1998; Jonker *et al.*, 1999; Nousiainen *et al.*, 2004), whereas there is little knowledge regarding dairy ewes (Cannas *et al.*, 1998) and none for dairy goats. Moreover, the absence of any definition of MUN reference values prevents the utilisation of MUN as indicator of protein metabolism in sheep and goats.

This study aimed to individuate the dietary factors affecting MUN concentration in lactating goats grazing herbaceous pasture, and verify the relationship linking dietary CP content to MUN.

## Material and methods

### Datasets

In this study 205 individual observations regarding dietary and milk variables of 37 Girgentana goats, averaging 154±14 days in milk (DIM) and 38.1±5.4 kg of live weight, were used. Data were derived from two grazing experiments contemporarily carried out in spring over a 45-day period, in the experimental farm "Pietranera" (Fondazione Lima-Mancuso, Università di Palermo), located in a typical hilly semi-arid area of Sicily (S. Stefano Quisquina, Agrigento, 37°37'N; 13°29'E; 178 m a.s.l.).

### Experimental diets

The first experiment involved 21 goats of  $130 \pm 6$  DIM subdivided into 3 balanced groups for milk yield and body weight. From 9 April to 24 May, each group was allowed to continuously graze (9:00-16:00 h) a mixed sward of ryegrass (*Lolium multiflorum* Lam. subsp. *Westerwoldicum*, var. *Elunaria*) and berseem clover (*Trifolium alexandrinum* L., var. *Lilibeo*) at a different stocking rate (36, 48 and 72 goats/ha).

In the second experiment, 16 goats of  $135 \pm 4$  DIM were subdivided into 2 balanced groups for milk yield and body weight. From 9 April to 17 May, each group was left to continuously graze a different plot of ryegrass sward (*Lolium multiflorum* Lam. subsp. *Westerwoldicum*, var. *Elunaria*) at a stocking rate of 64 goats/ha, one group during the morning (9:00-13:00 h), the other one during the afternoon (12:00-16:00 h).

Before and after grazing, the goats in both experiments were housed in a semi-open shelter, tethered in individual wooden stalls equipped with trough and bucket where they were hand-milked twice daily (at 7:00 and 16:30 h). They received water *ad libitum* and 500 g/d per head of barley coarsely ground (DM 87.8%, CP 15.0% of DM, NDF 14.8% of DM,  $NE_L$  1.9 Mcal/kg DM) divided into two meals provided during the morning and the afternoon milking.

### Data collection

Over the experimental period, measurements and sampling were done weekly (6 times in the first experiment and 5 times in the second one). The available herbage mass (t DM/ha) was estimated by clipping areas of 0.72 m<sup>2</sup> at ground level. Pasture allowance (kg DM/goat) was determined by dividing the herbage mass on offer by the number of grazing goats per hectare.

Selected herbage samples were collected by hand-plucking plants and parts of plants

after monitoring by direct observations and recording the goats prehensions of different grass species during grazing. From both morning and afternoon daily milking, weight of individual milk production was recorded and 50 ml milk samples were collected. Individual milk samples from both milkings were immediately stored at 4 °C without any preservative and analysed in the following day.

### Intake and digestibility estimation

The herbage DM intake of grazing goats and the *in vivo* diet DM digestibility were assessed weekly by the *n*-alkane method (Mayes *et al.*, 1986). In the course of both trials, goats were continuously dosed orally twice daily, after morning and afternoon milking (at 7:30 and 17:00 h), with a pure cellulose stopper containing 30 mg of the C<sub>32</sub>-alkane. Faecal grab samples were collected twice daily after milking from each goat during a 4-day period in each week. The concentrations of the natural odd-chain alkanes and the dosed even-chain C<sub>32</sub>-alkane in faeces, herbage and barley were determined by gas-chromatograph following the method of Mayes *et al.* (1986) and used to estimate voluntary DM intake (Mayes and Dove, 2000) and diet DM digestibility (Dove and Mayes, 1991).

### Chemical analysis

The samples of herbage were dried in a forced-air oven at 60 °C for 48 h and ground with a 1-mm screen for subsequent chemical analysis. For herbage and barley, the DM content was determined by drying samples at 105 °C until reaching constant weight, and ash content in a muffle furnace at 550 °C for 3 h. CP was determined as N\*6.25 using the Kjeldahl method and ether extract with the Soxhlet method (AOAC, 1990). Neutral-detergent fibre (NDF) was determined according to Van Soest *et al.* (1991).

The net energy for lactation ( $NE_L$ ) of diets was calculated on the basis of the estimated diet DM digestibility and the equations proposed by Van Soest and Fox (1992).

Milk fat content was determined by Fourier Transform Infrared (FTIR) spectroscopy method (Milkoscan FT 6000, Foss Electric, Hillerød, Denmark). Milk yield was corrected at 3.5% fat (FCM) according to the formula of Pulina *et al.* (1991) [ $FCM = \text{Milk (g)} * (0.634 + 0.1046 * \text{Fat}(\%))$ ]. Total nitrogen (TN) and non-casein nitrogen (NCN) were determined by FIL-IDF standard procedures (1964, 1993) using Kjeldahl method. From these nitrogen fractions, total protein ( $TN * 6.38$ ) and casein ( $(TN - NCN * 0.994) * 6.38$ ) were calculated. Milk urea was determined by enzymatic method using difference in pH (CL-10 Plus, Eurochem, Italy); MUN values were obtained by multiplying milk urea values for the conversion factor 0.4665.

### *Statistical analyses*

Dietary measurements and milk constituents were analysed using MEAN, CORR, REG and MIXED procedures of SAS 9.1.2 (2004). The Pearson correlations were calculated among all variables utilised. To investigate the relationships between MUN and dietary variables and milk yield, a multiple stepwise regression model was run setting the level of significance at 0.15. With the aim of estimating the CP content of a diet based on MUN values, simple and multiple regression models were fitted linking the dietary CP values to MUN concentration and other variables that could be easily determined on-farm, using individual data ( $n=205$ ). Further simple and multiple regressions were fitted using mean values of goats receiving the same feeding treatment at the same time ( $n=28$ ). The MUN mean values were used for simulating the on-farm urea measurement in bulk tank milk, and

also for reducing the large individual variation in MUN concentration of animals fed the same ration, as suggested by Cannas *et al.* (1998). In order to take into account the effect of goat, another regression approach between diet CP and MUN individual values was attempted using a mixed effects regression model, adapted from the methodology described by St-Pierre (2001); in this model, the fixed effects were the overall intercept and slope, and the random effects were the intercepts and slopes of each goat. The coefficient of determination ( $R^2$ ) was derived from simple regression between CP values adjusted by the mixed model and measured values of MUN; CP adjusted values were generated by adding each residual to its corresponding predicted value. The adequacy of fit of the predictive regression models was assessed by comparing actual and predicted CP values. Criteria for comparison were: Pearson and rank correlations between actual and predicted values; the difference between their standard deviation; the standard deviation of differences between actual and predicted values (mean square error predicted=MSEP); the prediction bias; the Wilkink test, corresponding to 100 times the ratio between the standard deviation of differences between actual and predicted values and the mean value (Macciotta *et al.*, 2000).

## **Results and discussion**

### *Dietary and milk variables*

A description of dietary and milk variables observed on grazing goats is summarized in Table 1.

All dietary parameters showed a wide variability. During the grazing period, changes in pasture allowance (PA) might be directly linked to the combined effect of herbage growth and grazing pressure of goats which are particularly affected by

Table 1. Description of data used as respects diet composition, nutrient intake and milk production of grazing goats (n=205).

|                              |            | Mean | SD   | Min  | Max   |
|------------------------------|------------|------|------|------|-------|
| DIM                          | d          | 154  | 14.4 | 120  | 185   |
| Live weight                  | kg         | 38.1 | 5.4  | 27.0 | 52.7  |
| Pasture allowance            | kg DM/goat | 75.3 | 31.9 | 38.9 | 151.1 |
| Diet composition:            |            |      |      |      |       |
| DM                           | %          | 23.6 | 3.9  | 14.9 | 37.6  |
| OM                           | % DM       | 90.5 | 1.1  | 87.4 | 93.8  |
| CP                           | "          | 20.5 | 3.3  | 13.7 | 26.0  |
| Ether extract                | "          | 3.88 | 0.71 | 2.46 | 5.51  |
| NDF                          | "          | 27.9 | 4.5  | 18.7 | 37.4  |
| <sup>1</sup> NFC             | "          | 38.3 | 4.3  | 27.1 | 50.4  |
| <sup>2</sup> NE <sub>L</sub> | Mcal/kg DM | 1.70 | 0.08 | 1.50 | 1.89  |
| DM digestibility             | %          | 84.2 | 3.1  | 72.6 | 92.5  |
| Intake:                      |            |      |      |      |       |
| Herbage                      | g DM/d     | 723  | 209  | 217  | 1422  |
| Diet                         | "          | 1162 | 209  | 656  | 1861  |
| Barley proportion            | % DM       | 39.1 | 7.7  | 23.6 | 67.0  |
| CP                           | g/d        | 240  | 63.5 | 102  | 426   |
| NDF                          | "          | 327  | 90.4 | 135  | 643   |
| <sup>1</sup> NFC             | "          | 438  | 52.8 | 321  | 625   |
| <sup>2</sup> NE <sub>L</sub> | Mcal/d     | 1.97 | 0.38 | 1.10 | 3.33  |
| Milk yield and composition:  |            |      |      |      |       |
| Milk                         | g/d        | 1160 | 354  | 320  | 2250  |
| <sup>3</sup> FCM             | "          | 1186 | 347  | 376  | 2115  |
| Fat                          | %          | 3.78 | 0.57 | 2.51 | 6.03  |
| Protein (N*6.38)             | "          | 3.86 | 0.43 | 2.56 | 5.87  |
| Casein                       | "          | 3.00 | 0.39 | 1.92 | 5.03  |
| <sup>4</sup> MUN             | mg/dl      | 22.5 | 5.3  | 9.7  | 35.4  |

DIM: days in milk.

<sup>1</sup>NFC=non-fibrous carbohydrates [100-(EE+CP+NDF+ash)].

<sup>2</sup>NE<sub>L</sub>=net energy for lactation.

<sup>3</sup>FCM=3.5% fat-corrected milk, according to: Milk (g)\*(0.634+0.1046\*Fat %) (Pulina *et al.*, 1991).

<sup>4</sup>MUN=milk urea nitrogen.

stocking rate (Bonanno *et al.*, 2007), which varied in our experimental conditions. The high variation in the DM intake of goats must refer only to different levels of herbage consumption at pasture, since all goats were supplemented with the same amount of barley (500 g/d per goat). The DM intake esti-

mated for the grazing Girgentana goats was 76 g/kg of metabolic body weight (BW<sup>0.75</sup>) on average (range 48-110 g DM/kg BW<sup>0.75</sup>). This value agrees with those reported by other authors for lactating Mediterranean goat breeds (Fedele *et al.*, 2002), but it was slightly lower compared with breeds hav-

ing higher body weight or milk production (Bava *et al.*, 2001) than Girgentana goats. The variability of the chemical composition of diet,  $NE_L$  value and digestibility must be related in particular to the responses of the grazing goats, in terms of selective behaviour, to the changes in PA and botanical and chemical composition of herbage mass at pasture (Baumont *et al.*, 2000; Bonanno *et al.*, 2007). These changes have to be linked to the physiological development of plants and the grazing intensity of animals. The mean CP and NDF contents of selected herbage were 24.1% (range 13.2-31.4%) and 36.3% (range 21.7-47.7%) of DM, respectively. The barley supplementation contributed to balancing the high nitrogen content of herbage in the diet with non-structural degradable carbohydrates, which are important in optimising the fermentation activities and growth of ruminal microflora. In most cases, the CP was higher and the NDF was lower in the diet than those observed by Fedele *et al.* (2002) in goats fed "free-choice," for which CP settled on values between 12 and 13% DM and NDF was constantly kept at a level around 40%. DM digestibility was comparable with values found in goats similarly grazing herbaceous vegetation (Soryal *et al.*, 2004), but was noticeably higher than levels reported for goats fed indoors according to the "free-choice" feeding system (Fedele *et al.*, 2002) or with total mixed rations (Bava *et al.*, 2001).

With regard to milk variables, the daily milk yield of goats was 1160 g on average, with 3.8% of fat, 3.9% of protein and a mean value of ratio between fat and protein equal to  $0.99 \pm 0.19$  (range 0.57-1.73). These mean productive parameters and their variations were in accordance with other results reported in literature for the same breed reared in analogous environmental conditions (Todaro *et al.*, 2005). A very wide range was observed for MUN. The mean

(22.5 mg/dl) and extreme values of MUN (9.7-35.4 mg/dl) were analogous to those reported by Todaro *et al.* (2005) for goats of the same breed grazing on herbaceous pasture. The MUN value range was narrower in goats browsing on shrubland (12.1-22.5 mg/dl, Cabiddu *et al.*, 1999), but similar in non-grazing goats (13.9-31.5 mg/dl, Bava *et al.*, 2001).

#### *Correlation between variables*

The Pearson correlation coefficients between dietary and milk factors are reported in Table 2.

The PA, being elevated also to the minimum level recorded (39 kg DM/goat), was not correlated to diet DM intake, but was linked to dietary CP (0.50), NDF (-0.34),  $NE_L$  (0.21) and digestibility (-0.28). These relationships showed as adequate forage availability at pasture allowed the goats to express their selective behaviour, according to which they chose a forage of higher quality than that being offered, seeking the more proteic and less fibrous parts of plants (Baumont *et al.*, 2000; Avondo *et al.*, 2007; Bonanno *et al.*, 2007, 2008). Other than to PA, the CP of diet resulted positively correlated to  $NE_L$  (0.43) and DM intake (0.20), and negatively correlated to NDF (-0.67) and digestibility (-0.38). The negative correlations of both PA and CP with digestibility were the direct consequence of the positive correlation between NDF and digestibility (0.46). Moreover, DM intake was not related to digestibility, whereas it was directly correlated to NDF (0.35). In practice, the increased NDF of diet raised the DM intake and digestibility. In accordance with these results, Fedele *et al.* (2002) recognized that in goats NDF does not give an adequate fill effect, which might restrict intake. Hadji-georgiou *et al.* (2001) noted that the high degree of selectivity by goats resulted in a greater intake of a diet of smaller particles,



Table 2. Matrix of Pearson correlations among dietary and milk variables of grazing goats (n=205).

|                              | DIM      | LW       | PA       | Diet composition |          |          |                  |                              |          |  |
|------------------------------|----------|----------|----------|------------------|----------|----------|------------------|------------------------------|----------|--|
|                              |          |          |          | DM               | CP       | NDF      | <sup>1</sup> NFC | <sup>2</sup> NE <sub>L</sub> | DIG      |  |
| Live weight (LW)             | -0.02    |          |          |                  |          |          |                  |                              |          |  |
| Pasture allowance (PA)       | 0.27***  | 0.13+    |          |                  |          |          |                  |                              |          |  |
| kg                           |          |          |          |                  |          |          |                  |                              |          |  |
| kg DM/goat                   |          |          |          |                  |          |          |                  |                              |          |  |
| Diet composition:            |          |          |          |                  |          |          |                  |                              |          |  |
| DM                           | 0.50***  | -0.21**  | 0.02     |                  |          |          |                  |                              |          |  |
| CP                           | 0.30***  | 0.21**   | 0.50***  | -0.18*           |          |          |                  |                              |          |  |
| NDF                          | -0.30*** | -0.04    | -0.34*** | -0.26***         | -0.67*** |          |                  |                              |          |  |
| <sup>1</sup> NFC             | 0.14*    | -0.21**  | -0.03    | 0.63***          | -0.16*   | -0.61*** |                  |                              |          |  |
| <sup>2</sup> NE <sub>L</sub> | -0.09    | 0.02     | 0.21**   | -0.13+           | 0.43***  | -0.41*** | 0.12+            |                              |          |  |
| DM digestibility (DIG)       | -0.02    | -0.17*   | -0.28*** | 0.07             | -0.38*** | 0.46***  | -0.17*           | 0.26***                      |          |  |
| Intake:                      |          |          |          |                  |          |          |                  |                              |          |  |
| Herbage                      | 0.25***  | 0.35***  | 0.11     | -0.72***         | 0.20**   | 0.35***  | -0.78***         | 0.05                         | -0.01    |  |
| Diet                         | 0.25***  | 0.35***  | 0.11     | -0.72***         | 0.20**   | 0.35***  | -0.78***         | 0.05                         | -0.01    |  |
| Barley proportion            | 0.20**   | -0.36*** | -0.13+   | 0.75***          | -0.19**  | -0.37*** | 0.80***          | 0.01                         | 0.05     |  |
| CP                           | -0.01    | 0.35***  | 0.39***  | -0.60***         | 0.74***  | -0.18*   | -0.61***         | 0.33***                      | -0.23*** |  |
| NDF                          | -0.31*** | 0.20**   | -0.14    | -0.58***         | -0.27*** | 0.80***  | -0.83***         | -0.22***                     | 0.26***  |  |
| <sup>1</sup> NFC             | -0.23*** | 0.37***  | -0.15*   | -0.58***         | 0.16*    | -0.01    | -0.29***         | 0.16*                        | -0.21*** |  |
| <sup>2</sup> NE <sub>L</sub> | -0.25*** | 0.34***  | 0.17*    | -0.72***         | 0.30***  | 0.22**   | -0.71***         | 0.31***                      | 0.05     |  |
| Mcal/d                       |          |          |          |                  |          |          |                  |                              |          |  |
| Milk yield and composition:  |          |          |          |                  |          |          |                  |                              |          |  |
| Milk                         | -0.19**  | 0.27***  | 0.41***  | -0.47***         | 0.39***  | -0.11    | -0.33***         | 0.18*                        | -0.32*** |  |
| <sup>3</sup> FCM             | -0.20**  | 0.27***  | 0.38***  | -0.46***         | 0.35***  | -0.08    | -0.34***         | 0.15*                        | -0.32*** |  |
| Fat                          | 0.01     | -0.12+   | -0.25*** | 0.25***          | -0.27*** | 0.15*    | 0.12+            | -0.10                        | 0.17*    |  |
| Protein (N*6.38)             | 0.06     | -0.13+   | 0.15*    | 0.07             | 0.13+    | -0.06    | -0.03            | 0.08                         | 0.03     |  |
| Casein                       | 0.001    | -0.26*** | 0.19**   | 0.07             | 0.09     | -0.09    | 0.05             | 0.06                         | -0.02    |  |
| <sup>4</sup> MUN             | 0.27***  | 0.11     | 0.42***  | 0.03             | 0.76***  | -0.69*** | 0.15*            | 0.37***                      | -0.33*** |  |
| (continued)                  |          |          |          |                  |          |          |                  |                              |          |  |

Table 2. Continuation

|                              | Intake  |          |          |          |          | Milk yield and composition |                              |          |                  |          |                    |         |
|------------------------------|---------|----------|----------|----------|----------|----------------------------|------------------------------|----------|------------------|----------|--------------------|---------|
|                              | Herbage | Diet     | Barley   | CP       | NDF      | <sup>1</sup> NFC           | <sup>2</sup> NE <sub>L</sub> | Milk     | <sup>3</sup> FCM | Fat      | Protein            | Casein  |
| Intake:                      |         |          |          |          |          |                            |                              |          |                  |          |                    |         |
| Diet                         | g DM/d  | 1.00***  |          |          |          |                            |                              |          |                  |          |                    |         |
| Barley prop.                 | % DM    | -0.96*** | -0.96*** |          |          |                            |                              |          |                  |          |                    |         |
| CP                           | g/d     | 0.80***  | 0.80***  | -0.76*** |          |                            |                              |          |                  |          |                    |         |
| NDF                          | "       | 0.83***  | 0.83***  | -0.81*** | 0.39***  |                            |                              |          |                  |          |                    |         |
| <sup>1</sup> NFC             | "       | 0.81***  | 0.81***  | -0.78*** | 0.64***  | 0.49***                    |                              |          |                  |          |                    |         |
| <sup>2</sup> NE <sub>L</sub> | Mcal/d  | 0.96***  | 0.96***  | -0.91*** | 0.85***  | 0.73***                    | 0.81***                      |          |                  |          |                    |         |
| Milk yield and composition:  |         |          |          |          |          |                            |                              |          |                  |          |                    |         |
| Milk                         | g/d     | 0.60***  | 0.60***  | -0.59*** | 0.64***  | 0.30***                    | 0.62***                      | 0.62***  |                  |          |                    |         |
| <sup>3</sup> FCM             | "       | 0.60***  | 0.60***  | -0.59*** | 0.62***  | 0.32***                    | 0.62***                      | 0.61***  | 0.98***          |          |                    |         |
| Fat                          | %       | -0.21**  | -0.21**  | 0.26***  | -0.31*** | -0.04                      | -0.24***                     | -0.24*** | -0.38***         | -0.23*** |                    |         |
| Protein (N*6.38)             | "       | -0.05    | -0.05    | 0.07     | 0.04     | -0.05                      | -0.12 <sup>+</sup>           | -0.03    | -0.09            | -0.09    | 0.009              |         |
| Casein                       | "       | -0.08    | -0.08    | 0.09     | 0.0004   | -0.10                      | -0.09                        | -0.06    | 0.01             | 0.005    | -0.06              | 0.85*** |
| <sup>4</sup> MUN             | mg/dl   | -0.08    | -0.08    | 0.11     | 0.40***  | -0.44***                   | -0.01                        | 0.02     | 0.25***          | 0.23***  | -0.13 <sup>+</sup> | 0.25*** |

Barley prop.: barley proportion.

<sup>+</sup> P<0.10; \*P<0.05; \*\* P<0.01; \*\*\*P<0.001.

<sup>1</sup>NFC=non-fibrous carbohydrates (100-(EE+CP+NDF+ash)).

<sup>2</sup>NE<sub>L</sub>=net energy for lactation.

<sup>3</sup>FCM=3.5% fat-corrected milk, according to: Milk (g)\*(0.634+0.1046 \*Fat %)(Pullina et al., 1991).

<sup>4</sup>MUN=milk urea nitrogen.

which did not generate a faster passage rate generally accompanied by a reduction in digestibility. Goats have a better digestive capacity than other ruminants, explained by the longer retention time of digesta and higher fermentation rate in the rumen, which allow the maximising of feed intake and digestibility (Silanikove, 2000). In the cases studied, in explaining the positive relationship between NDF content and both intake and digestibility, it is necessary to take into account that NDF was derived mainly from fresh herbage, which is more degradable, and was kept at a moderate level also at the maximum value recorded. In these conditions, the increase in dietary NDF assured better regulation of the digestive process, stimulating ensalivation and rumination and preventing a fast passage rate (Van Soest *et al.*, 1991), thus improving intake and digestibility.

Daily milk yield resulted positively correlated with DM intake (0.60), PA (0.41), dietary CP (0.39) and  $NE_L$  (0.18), confirming the observations of Bonanno *et al.* (2007) regarding the feeding behaviour of Girgentana goats grazing at a low stocking rate, which selected forage of a high quality, had a higher voluntary DM intake and, consequently, produced more milk than goats at a higher stocking rate. On the contrary, milk yield was negatively correlated with the DM percentage (-0.47) and digestibility (-0.32) of diet; this result can be explained by considering that higher daily milk yield was obtained during the first grazing period, when goats were consuming a higher amount of forage in an early phase of physiological development, with low content of DM and cell wall constituents which are responsible for a faster passage rate in the digestive tract and, thus, a reduced digestibility. On the whole, milk yield was strictly linked to DM intake which, in turn, was progressively reduced when forage advanced

on its development.

Among milk chemical constituents, fat resulted the most related to dietary factors. In fact, milk fat showed positive correlations with dietary DM (0.25) and negative correlations with PA (-0.25), DM intake (-0.21) and CP of diet (-0.27). Nevertheless, the highest negative correlation of fat was found with milk yield (-0.38), through the so called "dilution effect" which reduces milk fat when milk yield increases. Thus the relationships that linked milk fat and dietary variables appear to depend mainly on the influence of dietary factors on milk yield. As regards this, Nudda *et al.* (2002) recognized that the effect on the fat percentage of the fibre, responsible for the ruminal production of acetic acid, precursor of milk short chain fatty acids, is due more to the reduced milk yield than to the direct effect of NDF. This aspect explains the low correlation coefficient between dietary NDF and milk fat (0.15).

It was observed that total protein and casein percentages of milk were not correlated to either feeding or milk variables. The irrelevance of DM intake and diet composition on milk proteic components was also observed in sheep (Cannas *et al.*, 1998; Nudda *et al.*, 2002) and goats (Bava *et al.*, 2001) fed a total mixed ration. On the contrary, Soryal *et al.* (2004) found a positive effect of the concentrate supplementation on protein and casein contents in the milk of goats grazing mixed pasture. An explanation might be provided by hypothesizing the different effects of various dietary factors on milk casein in the course of the grazing period, on the basis of which the relation is invalidated. In the first grazing period, the integration with a source of highly degradable energy, such as barley, which in our experiments all grazing goats received in an equal amount, should have permitted balancing in the rumen of the high level of

soluble nitrogen from young herbage, which favours the synthesis of microbial protein, the precursor of milk casein. This fact, associated with the utilisation in the rumen of recycled urea, which was demonstrated to be higher in lactating goats with high intake and milk yield (Brun-Bellut, 1996), should have improved the milk casein content. On the contrary, at the end of the grazing period, when goats reduced their milk yield as a consequence of lowering their DM and CP intake, the milk casein percentage increased as a result of the well known "dilution effect," according to which milk proteic components rise when milk yield decreases (Todaro *et al.*, 2005).

Contrarily to total protein and casein, the MUN content resulted strongly correlated to dietary variables. The highest correlation of MUN was observed with the CP percentage of diet (0.76). This relationship is well established for dairy cows (Hof *et al.*, 1997; Shepers and Meijer, 1998; Nousiainen *et al.*, 2004) and sheep (Cannas *et al.*, 1998), but is less known for goats. The correlations that link MUN to dietary NDF (-0.69) and  $NE_L$  (0.37) depend on the correlations that link CP to NDF and  $NE_L$  of diet. For the

same reason, MUN was positively correlated to PA (0.42), since a higher availability of forage mass at pasture allowed the goats to select forage with a high nitrogen level and lower NDF content, thus with a higher energy concentration (Bonanno *et al.*, 2008).

The correlations indicated that CP, NDF and  $NE_L$  concentrations are the dietary variables mainly linked to milk urea formation, and showed how the effect of the CP percentage on MUN must be connected with feed energy availability. As regards this, mention must be made of how MUN was found highly and directly correlated to the ratio of CP to  $EN_L$  concentrations ( $121 \pm 17$  g/Mcal, range 82-166;  $r=0.70$ ,  $P<0.001$ ), and especially to the ratio between CP and NDF content ( $0.77 \pm 0.22$ , range 0.49-1.31;  $r=0.78$ ,  $P<0.001$ ) in the diet, in agreement with other authors (Jelinek *et al.*, 1996; Cannas *et al.*, 1998; Nousiainen *et al.*, 2004) who identified the dietary ratio of CP to energy as the most important nutritional factor affecting MUN.

#### *Dietary variables affecting MUN*

Results of stepwise selection using dietary factors and milk yield (Table 3) showed

Table 3. Individuation of dietary variables, including milk yield, which provide a significant contribution in regressions with milk urea nitrogen concentration using multiple stepwise regression models (n=205) (significance of entering variables was set at 0.15).

| Variable step entered | Intercept | Slope  | P-value | Partial R <sup>2</sup> |
|-----------------------|-----------|--------|---------|------------------------|
| Intercept             | 37.20     |        | 0.0001  |                        |
| CP % DM               |           | 1.00   | 0.0001  | 0.5554                 |
| NDF "                 |           | -1.16  | 0.0001  | 0.0684                 |
| <sup>1</sup> FCM g/d  |           | 0.002  | 0.0191  | 0.0126                 |
| DM intake "           |           | -0.027 | 0.0001  | 0.0053                 |
| NDF intake "          |           | 0.079  | 0.0013  | 0.0050                 |
| Total R <sup>2</sup>  |           |        |         | 0.6564                 |

<sup>1</sup>FCM=3.5% fat-corrected milk, according to: Milk (g) \* (0.634+0.1046\*Fat %) (Pulina *et al.*, 1991).

that the best variable to explain MUN variability was the dietary CP percentage, which alone absorbed 55.5% of MUN total variance. Cannas *et al.* (1998) observed a significant linear increase in MUN concentration as both CP concentration and CP intake of ewes increased, but the coefficient of determination was higher when CP concentration, rather than CP intake, was used as a regressor. Similarly, our MUN concentration data was better correlated with CP concentration of diet (0.76,  $P < 0.001$ , Table 2) than with CP intake (0.40,  $P < 0.001$ , Table 2). For this reason, only the CP concentration was taken into account in the stepwise analysis.

In the second step of stepwise selection, the NDF concentration of diet entered into the regression model, showing a partial  $R^2$  equal to 0.0684. The other variables entering into the model, until reaching a total  $R^2$  of 0.66, were FCM, DM intake and NDF intake, which supplied very low partial contributions; hence they could be considered scarcely significant variables.

In the stepwise selection, when ratios CP/EN<sub>L</sub> contents and CP/NDF of diet were also included among dietary factors (Table 4), the best variable explaining MUN

variability was the CP/NDF ratio, which absorbed 60.11% of the total MUN variance, rather than the CP/EN<sub>L</sub> ratio or CP content. Therefore, the dietary ratio of CP to NDF was the most important nutritional factor affecting MUN. It underlines the importance, also for goats, of balancing dietary protein to NDF and consequently to energy, being NDF and EN<sub>L</sub> closely related ( $r = -0.41$ ,  $P < 0.001$ , Table 2), in order to optimise fermentation activity and nitrogen utilization in the rumen, thus controlling the milk urea concentration.

In the subsequent steps of selection, barley proportion in the diet, FCM and diet CP content entered into the regression model, absorbing an extra 4.59% of MUN variability, thus determining only a slight increase in the total  $R^2$  for MUN ( $R^2 = 0.65$ ).

*Dietary CP prediction by MUN*

On the whole, dietary CP concentration was demonstrated to be the best single predictor of MUN for grazing goats, in accordance with Cannas *et al.* (1998) and Nousiainen *et al.* (2004). This close relationship of MUN with dietary CP content suggested the possibility of using the measurement of MUN for estimating the CP concentration

Table 4. Individuation of dietary variables, including CP/NE<sub>L</sub> ratio, CP/NDF ratio and milk yield, which provide a significant contribution in regressions with milk urea nitrogen concentration using multiple stepwise regression models (n=205) (significance of entering variables was set at 0.15).

| Variable step entered | Intercept   | Slope | P-value | Partial R <sup>2</sup> |
|-----------------------|-------------|-------|---------|------------------------|
|                       | -10.42      |       | 0.0067  |                        |
| CP/NDF                |             | 5.28  | 0.0714  | 0.6011                 |
| Barley proportion     | % DM intake | 0.199 | 0.0001  | 0.0185                 |
| <sup>1</sup> FCM      | g/d         | 0.002 | 0.0048  | 0.0143                 |
| CP                    | % DM        | 0.883 | 0.0001  | 0.0131                 |
| Total R <sup>2</sup>  |             |       |         | 0.6470                 |

<sup>1</sup>FCM=3.5% fat-corrected milk, according to: Milk (g)\*(0.634+0.1046\*Fat %) (Pulina *et al.*, 1991).

Table 5. Predictions of dietary crude protein (CP, % of DM) according to simple and multiple regression models and mixed effects regression model, using individual data of goats (n=205) and mean data of goats receiving the same feeding treatment at the same time (n=28);  $Y=A+BX_1+CX_2+DX_3$ , where A is the intercept, B, C and D are regression variables and goat is the random effect used in mixed effects regression model.

|  | Individual data   |  |                          | Mean data         |  |
|--|-------------------|--|--------------------------|-------------------|--|
|  | Simple regression | Multiple regression                                  | Mixed effects regression | Simple regression | Multiple regression                                  |
| $X_1, X_2, X_3$  | <sup>1</sup> MUN  | <sup>1</sup> MUN, <sup>2</sup> PA, <sup>3</sup> MILK | <sup>1</sup> MUN, goat   | <sup>1</sup> MUN  | <sup>1</sup> MUN, <sup>2</sup> PA, <sup>3</sup> MILK |
| A  | 10.23             | 8.75   | 14.16                    | 6.91              | 6.67   |
| SE   | 0.67              | 0.70   | 1.15                     | 1.42              | 1.88   |
| P-value  | <0.001            | <0.001   | <0.001                   | <0.001            | 0.002  |
| B  | 0.46              | 0.39   | 0.30                     | 0.61              | 0.57   |
| SE   | 0.03              | 0.03   | 0.04                     | 0.06              | 0.07   |
| P-value  | <0.001            | <0.001   | < 0.001                  | < 0.001           | <0.001   |
| C  |                   | 0.018  |                          |                   | 0.01   |
| SE   |                   | 0.005  |                          |                   | 0.01   |
| P-value  |                   | <0.001   |                          |                   | 0.42   |
| D  |                   | 0.001  |                          |                   | 0.0004   |
| SE   |                   | 0.0004   |                          |                   | 0.002  |
| P-value  |                   | 0.001  |                          |                   | 0.85   |
| R <sup>2</sup>   | 0.56              | 0.62   | 0.59                     | 0.79              | 0.80   |
| Y (mean)   | 20.54             | 20.54  | 20.81                    | 20.72             | 20.72  |
| <sup>4</sup> Y <sup>^</sup> (mean)                     | 20.62             | 20.66  | 20.81                    | 20.77             | 20.86  |
| Pearson correlation (Y, Y <sup>^</sup> )               | 0.75              | 0.79   | 0.77                     | 0.89              | 0.90   |
| Rank correlation                                       | 0.73              | 0.77   | 0.87                     | 0.85              | 0.86   |
| <sup>5</sup> $\sigma$ Y <sup>^</sup>                   | 2.46              | 2.60   | 1.57                     | 2.80              | 2.83   |
| <sup>6</sup> $\sigma$ Y - $\sigma$ Y <sup>^</sup>      | 0.82              | 0.67   | 0.48                     | 0.34              | 0.31   |
| <sup>7</sup> Bias                                      | -0.079            | 0.112  | 0.000                    | -0.054            | -0.135   |
| <sup>8</sup> $\sigma$ (Y - Y <sup>^</sup> ) (MSEP)     | 2.18              | 2.02   | 1.32                     | 1.42              | 1.38   |
| <sup>9</sup> [ $\sigma$ (Y - Y <sup>^</sup> ) / Y]*100 | 10.62             | 9.81   | 6.34                     | 6.84              | 6.68   |

<sup>1</sup>MUN=milk urea nitrogen, mg/dl.

<sup>2</sup>PA=pasture allowance, kg DM/goat.

<sup>3</sup>MILK =milk yield, g/d.

<sup>4</sup> Mean of predicted values.

<sup>5</sup> Standard deviation of predicted values.

<sup>6</sup> Difference between standard deviations of actual and predicted values.

<sup>7</sup> Bias: mean of the differences.

<sup>8</sup> Standard deviation of differences between actual and predicted values (MSEP=mean square error predicted).

<sup>9</sup> Wilkink test: 100 times the ratio between the standard deviation of differences between actual and predicted values and the mean value (Macciotta et al., 2000).

of the diet, with the aim of providing the goats with an adequate energy supplementation in order to balance the forage protein content at pasture, considering that, from a practical point of view, milk is easily collected and can be accurately analysed for urea.

Thus, initially a simple linear regression was fitted using diet CP and MUN values from each goat. With the aim of improving the forecasting ability of regression to estimate the dietary CP, a multiple regression was also developed introducing variables other than MUN. These were chosen from among those that could be easily determined on-farm, such as PA and daily milk yield (Table 5). On the whole, results of forecasting cannot be considered satisfactory. The simple regression between CP and MUN showed a  $R^2$  of 0.56, lower than the 0.65 reported by Cannas *et al.* (1998) using individual data from dairy ewes. However, the difference between the real mean and forecasted mean was low (-0.079), as the MSEF. The correlation between actual and predicted data was 0.75, and rank correlation (0.73) was a little lower. Nevertheless, the Wilmlink test value, measuring the validity of the prediction, was higher than the range 8.0-8.5, according to which the forecasting ability of the model can be considered sound (Macciotta *et al.*, 2000). The inclusion of the other variables (PA and milk yield) in the regression model resulted in only a marginally better prediction of CP percentage of diet (slightly higher  $R^2$  and correlation between actual and predicted data), moreover Wilmlink test values did not improve. In every case, the improvement in forecasting power did not seem to justify the utilization of the other two variables.

When the random effect of goat was considered in a mixed effects regression fitting individual data (Table 5), the coefficient of determination of the relationship between

adjusted CP values and MUN only slightly increased ( $R^2=0.59$ ), even though the criteria assessing the adequacy of fit improved, especially the Wilmlink test value, as it was below the range 8.0-8.5 considered as optimal.

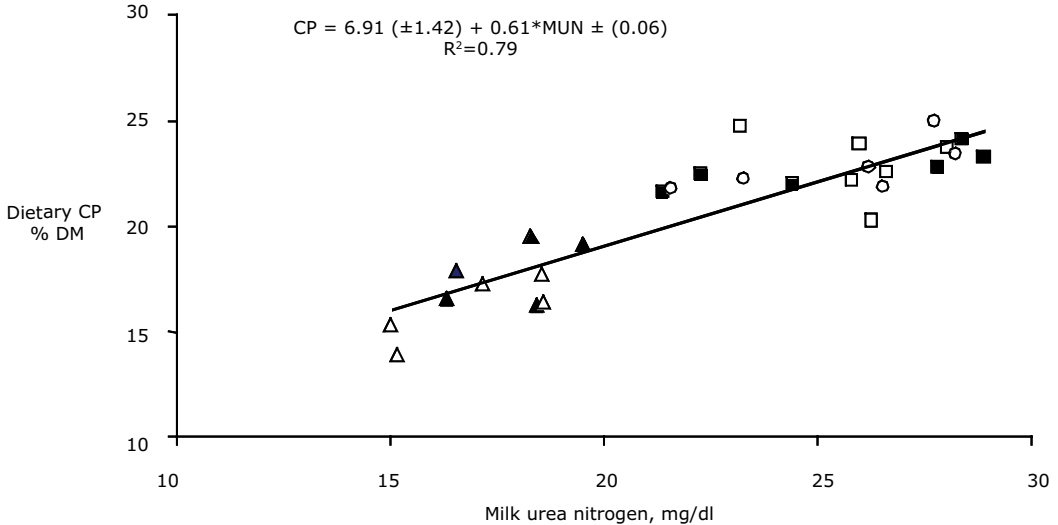
Subsequently, mean values of goats receiving the same feeding treatment at the same time were fitted by both simple and multiple regression, instead of individual goat data (Table 5). The aim was to limit the individual MUN variability, in accordance with Cannas *et al.* (1998), and to adapt the relationships to an on-farm condition, where urea is normally determined on bulk milk. In this case, the proportion of variation explained by the relationship between CP and MUN greatly increased up to 0.79 (Figure 1). All the evaluation criteria demonstrated the adequacy of the fit and the Wilmlink test value could be considered as optimal. Also using mean values, the inclusion of other factors, together with MUN in multiple regression model, did not explain more of the variation of MUN in better terms than only dietary CP content.

## Conclusions

In this study, wide variability emerged in the dietary variables of grazing goats, which was conditional on their response, in terms of selective behaviour, to the changes in pasture availability and quality. In particular, CP content was directly related to PA and diet energy, whereas a negative correlation resulted with NDF.

The MUN concentration observed in grazing goats was seen to be closely related to dietary factors, mainly CP, NDF and  $EN_L$  concentration, confirming the importance of CP and its ratio to NDF or energy in determining urea formation, which is widely recognised for the ruminant species. Particularly, the CP percentage and CP/NDF

Figure 1. Relationship between mean data of milk urea nitrogen (MUN) and dietary crude protein (CP) content obtained from goats receiving the same feeding treatment at the same time (n=28).



Numbers in parentheses are standard error of the coefficients. ▲ ryegrass grazed in the morning by 64 goats/ha (n=5); △ ryegrass grazed in the afternoon by 64 goats/ha (n=5); ■ ryegrass and berseem clover grazed by 72 goats/ha (n=6); □ ryegrass and berseem clover grazed by 48 goats/ha (n=6); ○ ryegrass and berseem clover grazed by 36 goats/ha (n=6).

ratio of diet resulted the best variables explaining the MUN variability. Weaker relationships were found linking MUN to milk yield, fat and casein content.

The linear relation found between mean values of dietary CP concentration and MUN suggests the possibility of using MUN to estimate the CP content of a diet, as a useful tool for developing appropriate feeding strategies aimed at balancing the rations of goats grazing at pasture. Nevertheless, as this predictive relationship is based on data of goats reared in specific environmental and nutritional conditions, its application could not be generalized for goats reared in other productive ambits. Therefore, further experiments in differ-

ent environments are required in order to broaden available data regarding grazing goats and thereby develop a more robust prediction of diet CP based on MUN. In addition, a more physiological approach might make it possible to define relationships of MUN to dietary variables and reproductive performance, and to provide reliable MUN reference values for goats.

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