

## Phenotypic correlations of milk urea and the efficiency of crude protein utilization with milk yield traits and cow performance in two contrasting dairy systems in New Zealand

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### Abstract

The objectives of the present study were to investigate phenotypic correlations of milk urea (MU) and efficiency of crude protein utilization (ECPU) with milk production and cow parameters in two contrasting herds. Between July 2016 and May 2017, 210 cows were milked twice daily with high supplementary feed inclusion (HS) and 258 cows were milked once daily with low supplementary feed inclusion (LS). In early, mid and late lactation, milk samples were collected to measure MU. At every herd-test date, ECPU was calculated as protein yield (PY) divided by crude protein intake (CPI); this last variable derived from intake estimations of metabolizable energy requirements. Positive correlations between milk yield (MY) and both dry matter intake (DMI) and CPI were observed in LS and HS. The ECPU only correlated positively with MY traits in LS, and there was no correlation of CPI with MU in either herd. A moderate negative correlation of ECPU with live weight (LW) was observed in both herds, but it was stronger in LS. By suppressing MY due to the reduced milking frequency of cows fed LS, these cows gained LW, had higher body condition score and CPI, and lower PY, reducing the ECPU. No correlation between ECPU and MU was detected.

**Keywords:** phenotypic correlations; intensification; crude protein utilization; milk urea

### Introduction

In New Zealand, temperate climate and natural soil fertility enable pastoral farming all year round. Driven by increasing global food demand, farmers have steadily intensified milk production over the past several decades. Although these systems are perceived as having low inputs when compared to those of countries in Europe and North America, a range of intensification among New Zealand farmers is detected when considering levels of inclusion of supplementary feed and nitrogen (N) fertilization.

Inefficiencies in N fertilization (de Klein et al. 2010), extra N incorporated through supplementary feeds (Powell et al. 2015) and low efficiency of N utilization by ruminants (Ryan et al. 2012) are linked to N losses. Moreover, the high loading rate of N under the urine patch of cows is exacerbated by the application of N fertilizers and waste effluents (Di & Cameron 2002), aggravating surface and ground water pollution. Because of this, regional authorities have incorporated regulatory limits for N leached from farms. For example, in the Manawatu region the Regional Council have imposed limits on N leached according to Land Use Capability class (Horizons 2016). Exploring mitigating strategies will contribute to the development of sustainable production systems.

Nitrogen utilization can be estimated as the ratio of protein present in milk to the intake of crude protein (CP) from feed consumed by lactating cows (Castillo et al. 2000), and is expressed as the efficiency of CP utilization (ECPU). Milk urea (MU), as the main non-protein source of N in milk, has been proposed as an indicator of dietary level of CP because of its direct relationship with blood urea N, which refers directly to the natural releases of excess N from metabolism (Gustafsson & Palmquist 1993). Milk urea concentration has been proposed as an index to

identify inefficiencies of N utilization in dairy systems (Jonker et al. 1998).

The objectives of the present study were to compare phenotypic correlations of MU and the ECPU with milk production performance and cow parameters throughout one entire season in two contrasting dairy systems of low and high supplementary feed inclusion.

### Materials and methods

This study was conducted at Massey University in Palmerston North NZ, from June 2016 to May 2017 at Dairy 1 with 258 cows fed low levels of supplementary feed (LS) milked once-daily and Dairy 4 with a total of 210 cows fed higher levels of non-pasture inputs (HS) milked twice-daily. Planned start of calving commenced at both farms in the second half of July.

Notwithstanding differences in supplement inclusion, each herd had access to fresh ryegrass (*Lolium perenne*)/white clover (*Trifolium repens*) pasture after each milking and were contained in their allocated forage area through the use of temporary electric fences.

In LS, cows were milked once daily at 6.30 am. In August and from March to May, cows received 3.5 kg of dry matter (DM) of pasture silage per cow per day. From December to February, cows grazed a mixed herb crop comprising chicory (*Cichorium intybus*), red clover (*Trifolium pratense*) and plantain (*Plantago lanceolata*) for three hours per day, at an allowance of 3.5 kg DM per cow. In February, cows were allocated 2.6 kg DM per cow of turnip (*Brassica campestris ssp. rapifera*). Lucerne (*Medicago sativa*) was grazed directly from the paddock in March and May at an allowance of 3 kg DM per cow per day.

Cows in the HS herd were milked daily at 5.30 am and 2.30 pm. Maize silage and grain-based concentrate were fed during the lactation at 3.5 kg DM per cow per day before the afternoon milking and 2 kg DM per cow per day inside the parlour, respectively. In January, pasture silage was fed in the paddock at 3 kg DM per cow per day. In March, 0.75 kg DM per cow of dried distillers grains was fed during the morning milking, and cows were also allocated turnip crop at 2 kg DM per cow per day.

In the ryegrass/white clover pastures, herbage mass measurements were taken before and after each grazing event with a rising-plate meter following a 'W' pattern across the grazing area, using a global positioning system. Three quadrat cuts (0.1 m<sup>2</sup>) were taken both before and after grazing to quantify kg DM per ha of the grazing crops. These measurements enabled calculation of apparent pasture and crop utilization, and also the proportion of herbage allocated to cows before each herd test.

Samples (approximately 1,500 g of wet weight) of fresh pastures and crops were taken by hand-plucking, and these, along with samples of silage and concentrate, were freeze-dried and ground (Wiley mill) to pass through a 1.0 mm screen. All samples were then analysed by the near infrared reflectance spectroscopy technique (Corson et al. 1999) to evaluate metabolizable energy (ME) and CP content.

Daily live weight (LW) measurements were generated with an automatic race walkover scale and body condition scores (BCS) were assigned in synchrony with each herd test by a single research technician using a 10-point scale (Macdonald & Macmillan 1993). Yields of milk (MY), fat (FY) and protein (PY) and somatic cell count (SCC) were collected from herd-test records. The SCC were log-transformed to somatic cell score (SCS). Lactation curves for milk production traits and cow performance were obtained using Legendre polynomials of 3rd order generating daily records for each cow during the season.

Additionally, milk samples from each cow were taken in early (September), mid (December) and late (March) lactation using herd-test milk meters provided by Livestock Improvement Corporation. These samples were analysed by MilkTestNZ (Hamilton, NZ) using the CombiFoss technique (Arunvipas et al. 2003) for MU (mg/dL) content and lactose percentage. Each MU record was converted into MU yield (MUY) (g MU/cow/day) using daily MY.

Apparent DM intake (DMI) (kg DM/cow/day) was estimated based on total ME requirements for maintenance, pregnancy, production and daily LW change (LW<sub>c</sub>), divided by the ME content of any feed offered (López-Villalobos et al. 1999). Concentration of CP (%) from feed quality analysis was utilized to calculate CP intake (CPI). Since herbage quality assessment was undertaken only on herd-test days, daily ECPU was calculated by dividing PY by CPI.

Least-squares means of the variables were obtained using the general linear model procedure of SAS using a mixed model that included the fixed effects of dairy

system, lactation number and as co-variables deviation from median calving date, proportion of Holstein-Friesian (F) and heterosis effect between F and Jersey (J), and the random effects of cow. Partial correlations between the dependent traits were obtained using MANOVA to obtain the phenotypic correlations adjusted by the factors included in the model. Partial correlation coefficients between two traits for a herd were compared to the other correlation coefficients for the other herd using the Fisher r-to-z transformation.

## Results

Descriptive statistics of the dependent variables for each of the dairy system are presented in Table 1. The difference between LS and HS in kg milk yield per cow per day was 26% and this gap was reduced to an average of 17% in milksolids yield (MSY) between herds over the lactation (Figure 1). Values for MU were predominantly higher in LS. The larger cow size of HS was reflected in higher LW, however, BCS was higher in the LS cows during the season.

Table 2 describes the diet composition in the present study, and indicates higher usage of supplementary feed in HS. Pasture allocation represented 93% and 65% of the total feed intake during the season for LS and HS cows, respectively. In the HS herd, the inclusion of more supplements, with lower CP, led to a reduction in the total CPI. Conversely, in the LS herd, due to a higher allowance of pasture with a greater CP content there was an overall greater dietary CPI and higher MU.

Table 3 presents least-squares means adjusted for lactation number, deviation from median calving date, proportion of F and F×J heterosis effects for milk production and cow performance per herd. Milk yield and MSY were both greater (P<0.05) for HS cows, with an additional 22% in MY and an additional 14% in MSY. Adjusted means of LW and BCS records resulted in higher LW (P< 0.013) and in greater BCS (P<0.0001) for the LS herd. Apparent DMI was 12% greater for HS (P<0.0001), but CPI was 9% higher for LS (P<0.0001). In HS, ECPU was higher (P<0.0001) and MU was lower (P<0.0001), but no differences were found in MUY between herds (Table 3).

Partial correlation coefficients between milk production traits and cow performance across scenarios are detailed in Table 4. There was a strong correlation between DMI and milk production traits. Milk yield traits correlated positively with ECPU in LS but not HS cows. Lastly, there were no significant correlations between MU and ECPU across both herds.

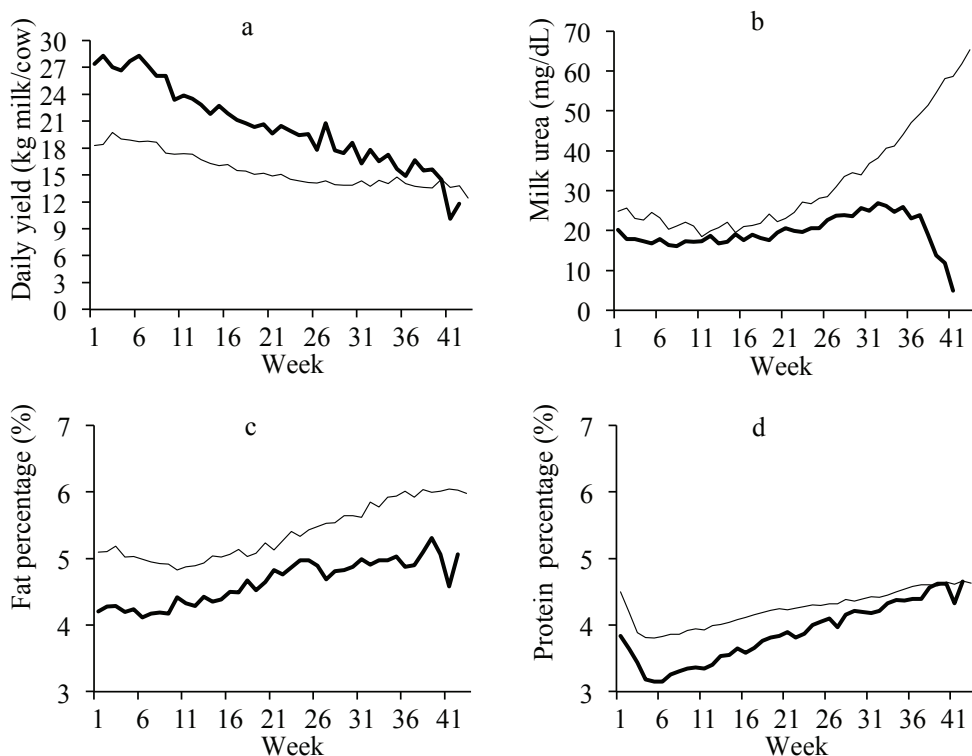
## Discussion

Once-a-day milking and low levels of supplements fed to LS cows led to lower MY compared to HS cows. Moreover, the lower dietary CP in HS cows resulted in lower levels of MU and higher ECPU (Table 2 and 3). The lower MY of cows milked once daily was expected (Phyn et al. 2012), and this lower MY resulted in more nutrients

**Table 1** Descriptive statistics for milk production traits, milk urea, live weight and body condition score in grazing cows at Massey University Dairy 1 (Low supplement) and Massey University Dairy 4 (High supplement) during season 2016-17.

Variable <sup>1</sup>	Dairy system									
	Low supplement					High supplement				
	N	Mean	SD	Min	Max	N	Mean	SD	Min	Max
MY, kg/day	2,286	15.7	6.0	0.9	36.6	1,218	21.2	5.5	3.0	37.7
FY, kg/day	2,286	0.8	0.3	0.1	2.5	1,218	1.0	0.2	0.2	1.5
PY, kg/day	2,286	0.6	0.2	0.1	1.3	1,218	0.8	0.2	0.2	1.4
LY, kg/day	728	0.8	0.3	0.1	1.9	558	1.1	0.3	0.5	1.9
FP, %	2,286	5.34	0.90	1.77	8.8	1,218	4.64	0.87	2.46	8.86
PP, %	2,286	4.19	0.53	2.88	6.51	1,218	3.85	0.55	2.75	5.77
LP, %	728	4.99	0.16	4.37	5.36	558	5.16	0.15	4.74	5.57
SCS	2,278	5.8	1.6	0.0	12.4	1,218	5.1	1.5	1.6	12.0
MU <sub>am</sub> (mg/dL)	727	28.05	10.06	9.10	61.70	587	20.67	7.61	2.30	46.60
MU <sub>pm</sub> (mg/dL)	-	-	-	-	-	578	21.06	5.67	0.30	38.70
MUY <sub>am</sub> (g/day)	727	4.44	1.76	0.71	10.67	587	2.95	1.04	0.21	6.65
MUY <sub>pm</sub> (g/day)	-	-	-	-	-	574	1.74	0.62	0.01	3.88
LW (kg)	2,361	487	70	320	684	1,998	502	61	352	770
BCS	2,065	4.62	0.44	2.50	6.50	1,408	4.16	0.42	3.00	5.50

<sup>1</sup> MY = milk yield, FY = fat yield, PY = protein yield, LY = lactose yield, FP = fat percentage, PP = protein percentage, LP = lactose percentage, SCS = somatic cell score calculated as  $SCS = \log_2(\text{somatic cell count})$ , MUY = milk urea yield, LW = live weight, BCS = body condition score.

**Figure 1** Predicted lactation curves of (a) milk yield (MY), (b) milk urea (MU), (c) fat percentage and (d) protein percentage in a low (LS) (—) and high (HS) (—) supplementary feed inclusion dairy system during season 2016-17.

(energy and protein) available for superior BCS and increases in LW (Table 3). This is why ECPU correlates positively with DMI and CPI, and negatively with BCS, LW and LW<sub>c</sub> (Table 4). Holmes et al. (1992) observed similar increases in LW and BCS in herds milked once a day. A diet higher in CP, along with lower MY, resulted in LS cows having simultaneously higher CPI and lower PY, which reduced the ECPU.

In this study, the CP percentage of the diet was between 18.3% and 21.1% and between 14.5% and 16.2% for LS and HS herds, respectively. An increase in CP percentage of the diet was accompanied by higher MU concentration throughout the lactation. Cosgrove et al. (2014) measured significant increases in MU in cows when forage CP percentage was above 19% along with reduced rumen degradable protein in both once- and twice-daily milking

**Table 2** Feed quality (mean  $\pm$  standard deviation) allocated to cows before each milk sampling, expressed in MJ ME/kg DM feed and in percentage of crude protein (CP) and milk urea (MU) concentration (mg/dL) in early, mid- and late-lactation stage in grazing cows at Massey University Dairy 1 (Low supplement) and Massey University Dairy 4 (High supplement) during season 2016-17.

Dairy system	Lactation stage	Feed composition	ME (MJ ME/kg DM)	CP (%CP)	MU (mg/dL)
Low supplement	Early	100 % pasture	11.700 $\pm$ 0.004	18.300 $\pm$ 0.017	22.86 $\pm$ 0.35
	Mid	68 % pasture	11.083 $\pm$ 0.004	19.943 $\pm$ 0.017	22.37 $\pm$ 0.35
		32 % mixed herb crop			
Late	48 % pasture 17 % pasture silage 35 % grazing lucerne	9.457 $\pm$ 0.005	21.082 $\pm$ 0.018	40.08 $\pm$ 0.35	
High supplement	Early	65 % pasture 26 % maize silage 9 % concentrate	11.193 $\pm$ 0.005	14.512 $\pm$ 0.019	17.92 $\pm$ 0.45
	Mid	44 % pasture 20 % maize silage 20 % pasture silage 16 % concentrate	10.965 $\pm$ 0.005	14.893 $\pm$ 0.019	20.44 $\pm$ 0.46
		Late			

<sup>1</sup> Dried distillers grain

**Table 3** Least-squares means ( $\pm$  standard errors of mean) of lactation length, total yield of milk, fat and protein, somatic-cell score, live weight and live weight change accumulated along the lactation, BCS, DM intake (DMI), crude protein intake (CPI), efficiency of crude protein utilization (ECPU), milk urea (MU) measured in the morning (MU<sub>am</sub>) and in the evening (MU<sub>pm</sub>) and milk urea yield (MUY) at Massey University Dairy 1 (Low supplement) and Massey University Dairy 4 (High supplement) during season 2016-17.

	Dairy system		P-value
	Low supplement	High supplement	
N	258	210	
Breeding worth	89	67	
Lactation length (days)	270 $\pm$ 2	272 $\pm$ 3	0.6359
Milk yield (kg)	4205.9 $\pm$ 55.1	5387.5 $\pm$ 75.9	<0.0001
Milksolids yield (kg)	385.4 $\pm$ 4.5	448.1 $\pm$ 6.2	<0.0001
Fat yield (kg)	215.9 $\pm$ 2.6	246.6 $\pm$ 3.5	<0.0001
Protein yield (kg)	169.5 $\pm$ 2.0	201.8 $\pm$ 2.8	<0.0001
Lactose yield (kg)	211.3 $\pm$ 2.9	300.2 $\pm$ 4.1	<0.0001
SCS <sup>1</sup>	5.74 $\pm$ 0.07	4.94 $\pm$ 0.10	<0.0001
Live weight (kg)	487 $\pm$ 3	476 $\pm$ 4	0.0132
Live weight change (kg)	24.3 $\pm$ 2.8	15.8 $\pm$ 3.3	0.0503
BCS	4.61 $\pm$ 0.02	4.23 $\pm$ 0.02	<0.0001
DMI (kg/day)	12.90 $\pm$ 0.06	14.71 $\pm$ 0.11	<0.0001
CPI (kg/day)	2.47 $\pm$ 0.01	2.24 $\pm$ 0.02	<0.0001
ECPU (%)	25.29 $\pm$ 0.12	33.58 $\pm$ 0.31	<0.0001
MU <sub>am</sub> (mg/dL)	28.30 $\pm$ 0.36	21.42 $\pm$ 0.51	<0.0001
MU <sub>pm</sub> (mg/dL)	-	21.40 $\pm$ 0.36	-
MU (mg/dL)	28.29 $\pm$ 0.34	21.30 $\pm$ 0.48	<0.0001
MUY <sub>am</sub> (g)	1207.76 $\pm$ 20.04	831.85 $\pm$ 27.57	<0.0001
MUY <sub>pm</sub> (g)	-	473.04 $\pm$ 9.17	-
MUY (g)	1207.92 $\pm$ 22.56	1269.28 $\pm$ 31.04	0.1106

<sup>1</sup> The somatic cell count records were log-transformed to SCS. BCS on a 1-10 scale.

**Table 4** Partial correlation coefficients between production traits, cow performance, efficiency of crude protein utilization and MU for low (above diagonal) and high (below the diagonal) supplementary feed inclusion in grazing dairy cows during season 2016-17.

Trait <sup>1</sup>	MY	FY	PY	LY	FP	PP	LP	SCS	BCS	LW	LW <sub>c</sub>	DMI	CPI	ECPU	MU	MUY
MY		0.71	0.93	0.99	-0.13	-0.14	0.08	-0.19	-0.09	0.10	-0.21	0.74	0.72	0.52	-0.03 <sup>ns</sup>	0.54
FY	0.74		0.71	0.70	0.48	-0.03 <sup>ns</sup>	0.17	-0.06 <sup>ns</sup>	-0.01 <sup>ns</sup>	0.08 <sup>ns</sup>	-0.15	0.64	0.62	0.24	0.01 <sup>ns</sup>	0.44
PY	0.94	0.72		0.91	-0.11	0.09	0.12	-0.10	-0.03 <sup>ns</sup>	0.11	-0.17	0.70	0.68	0.62	0.01 <sup>ns</sup>	0.52
LY	0.99	0.74	0.93		-0.14	-0.16	0.29	-0.23	-0.09	0.10	-0.20	0.73	0.71	0.51	-0.02 <sup>ns</sup>	0.55
FP	-0.13	0.49	-0.14	-0.12		0.33	0.00 <sup>ns</sup>	0.18	0.06 <sup>ns</sup>	0.00 <sup>ns</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>	-0.30	-0.09	-0.14
PP	-0.02 <sup>ns</sup>	-0.05 <sup>ns</sup>	0.24	-0.04 <sup>ns</sup>	0.00 <sup>ns</sup>		-0.25	0.27	0.09	0.21	0.08 <sup>ns</sup>	-0.02 <sup>ns</sup>	-0.01 <sup>ns</sup>	-0.04 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.23
LP	0.16	0.17	0.12	0.29	0.00 <sup>ns</sup>	-0.25		-0.33	-0.05 <sup>ns</sup>	-0.03 <sup>ns</sup>	0.11	0.08	0.07 <sup>ns</sup>	-0.01 <sup>ns</sup>	0.03 <sup>ns</sup>	0.14
SCS	-0.17	-0.07	-0.14	-0.18	0.05 <sup>ns</sup>	-0.01 <sup>ns</sup>	-0.14		0.12	0.04 <sup>ns</sup>	-0.01 <sup>ns</sup>	-0.16	-0.15	-0.09 <sup>ns</sup>	-0.11	-0.23
BCS	0.00 <sup>ns</sup>	0.10 <sup>ns</sup>	0.01 <sup>ns</sup>	0.01 <sup>ns</sup>	0.15	0.01 <sup>ns</sup>	0.06 <sup>ns</sup>	-0.05 <sup>ns</sup>		0.32	-0.17	-0.07 <sup>ns</sup>	-0.05 <sup>ns</sup>	-0.15	0.11	0.03 <sup>ns</sup>
LW	0.13	0.13	0.12	0.13	0.05 <sup>ns</sup>	0.08 <sup>ns</sup>	0.05 <sup>ns</sup>	0.01 <sup>ns</sup>	0.20		-0.07 <sup>ns</sup>	0.35	0.36	-0.26	0.07 <sup>ns</sup>	-0.01 <sup>ns</sup>
LW <sub>c</sub>	-0.06 <sup>ns</sup>	-0.04 <sup>ns</sup>	-0.04 <sup>ns</sup>	-0.06 <sup>ns</sup>	-0.02 <sup>ns</sup>	0.04 <sup>ns</sup>	-0.01 <sup>ns</sup>	0.02 <sup>ns</sup>	-0.10 <sup>ns</sup>	0.14		0.07 <sup>ns</sup>	0.05 <sup>ns</sup>	-0.34	0.01 <sup>ns</sup>	-0.12
DMI	0.49	0.40	0.46	0.49	0.06 <sup>ns</sup>	0.03 <sup>ns</sup>	0.14	-0.11	0.08 <sup>ns</sup>	0.29	0.22		0.99	0.28	0.00 <sup>ns</sup>	0.39
CPI	0.41	0.31	0.42	0.42	-0.01 <sup>ns</sup>	0.11 <sup>ns</sup>	0.19	-0.08 <sup>ns</sup>	0.01 <sup>ns</sup>	0.13	0.17	0.79		0.26	0.00 <sup>ns</sup>	0.39
ECPU	0.00 <sup>ns</sup>	-0.03 <sup>ns</sup>	0.00 <sup>ns</sup>	0.00 <sup>ns</sup>	-0.08 <sup>ns</sup>	-0.13	-0.06 <sup>ns</sup>	-0.05 <sup>ns</sup>	0.04 <sup>ns</sup>	-0.13	-0.26	-0.37	-0.73		0.00 <sup>ns</sup>	0.32
MU	0.11	0.07 <sup>ns</sup>	0.15	0.11	-0.08 <sup>ns</sup>	0.11	0.01 <sup>ns</sup>	-0.02 <sup>ns</sup>	0.03 <sup>ns</sup>	-0.02 <sup>ns</sup>	0.04 <sup>ns</sup>	-0.07 <sup>ns</sup>	0.07 <sup>ns</sup>	-0.04 <sup>ns</sup>		0.71
MUY	0.56	0.41	0.57	0.55	-0.15	0.05 <sup>ns</sup>	0.10	-0.09 <sup>ns</sup>	0.04 <sup>ns</sup>	0.04 <sup>ns</sup>	0.02 <sup>ns</sup>	0.16	0.23	0.00 <sup>ns</sup>	0.85	

<sup>1</sup> MY = milk yield, FY = fat yield, PY = protein yield, LY = lactose yield, FP = fat percentage, PP = crude protein percentage, LP = lactose percentage, SCS = somatic cell score calculated as  $SCS = \log_2(\text{somatic cell count})$ , BCS = body condition score, LW = live weight, LW<sub>c</sub> = live weight change, DMI = dry matter intake, CPI = crude protein intake, ECPU = efficiency of crude protein utilization, MU = milk urea, MUY = milk urea yield. <sup>ns</sup> Correlation between variables not different from zero ( $P > 0.05$ ) within production system.

frequencies. In Table 4, no correlations between MU and ECPU were detected. Moreover, Aizimu et al. (2013) found a negative relationship between MU and ECPU only when dietary CP percentage did not exceed 19%. However, results in the present study were confounded, since high MU levels and lower MY of LS cows, and lower MU levels and higher MY of HS cows, resulted in similar accumulated MUY (Table 3).

Increasing levels of MU towards the end of the lactation in both herds (Table 2) may be explained by reduced MY along with increasing dietary CP during this period. Trevaskis et al. (1999) detected lower levels of MU in early lactation because of increasing tissue mobilisation using more of the N available as a mechanism to deliver more nutrients for increasing MY in peak lactation.

To conclude, the ECPU of LS cows was reduced by limiting MY and by offering a diet higher in CP, compared to HS cows. Milk urea failed to predict the inefficiency (or otherwise) of N use, because of a compensation between MY and MU levels, which resulted in similar MUY in both herds. At the industry level, a benchmark of ECPU may be useful to compare nutrition management practices between farms (Gourley et al. 2012). From an environmental perspective, unless ECPU is modified to include N captured in tissues and body reserves, the use of this indicator as a tool to assess N use efficiency and N losses in grazing cows may not be effective, considering that animals may be storing part of this nutrient rather than discharging it in the form of excreta. Additional measurements of N losses from grazing cows along with an enhanced ECPU will help on a better understanding N cycle of these systems.

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