Nitrogen Supplementation of Corn Silages.

1. Effects on Feed Intake and Milk Production of Dairy Cows

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ABSTRACT

Feed intake and milk production responses to N supplementation of corn silage-based diets were measured in three 3 × 3 Latin square experiments. In each experiment, 9 Holstein cows received total mixed rations (TMR), based on corn silage. In Exp. 1, midlactation cows were used to study effects of diets with different ratios of effective rumen-degradable protein (ERDP; g) to fermentable metabolizable energy (FME; MJ), providing a large deficiency (RL), a slight deficiency (RM), and a slight excess (RH) in relation to the target level of 11 g of ERDP/MJ of FME, respectively, for lactating cows. Diets were formulated to be isoenergetic, and to satisfy the metabolizable protein requirements. In Exp. 2, early-lactation cows were used to evaluate effects of different proportions of quickly and slowly rumen-degradable protein (RDP), achieved by replacing soybean meal with urea in the concentrates (0, 0.5, and 1% urea). Experiment 3 investigated effects of synchronizing the availability of FME and ERDP in the rumen. Midlactation cows received a diet containing, on a dry matter (DM) basis, 45% corn silage, 5% ryegrass hay, 35% energy-rich concentrate, and 15% protein-rich concentrate (crude protein: 38% of DM; urea: 2% of DM). The protein-rich concentrate was fed either once (D1) or twice (D2) per day before the meal, or included in the TMR (DU). Treatment RL led to lower DM intake and milk yield, but higher milk production efficiency; there were no significant differences between treatments RM and RH. There were no significant treatment effects on DM intake, milk yield, or milk composition

in Exp. 2. Manipulating rumen synchrony by altering the timing of feeding affected milk yields, with D1 cows producing significantly less than D2 and DU cows, which were similar. The amount of ERDP in the diet should be matched to the amount of fermentable energy available to maximize intake, milk yields, and the conversion of feed N into milk protein. However, this study showed only small benefits to altering the diurnal pattern of supply of RDP and FME, and only with extreme feeding strategies that would not be used in practice. Urine volume increased in response to increased or unbalanced protein supply. Analysis of the allantoin:creatinine ratio in spot samples of urine was not useful in identifying predicted differences in microbial protein yield from the rumen.

(**Key words:** corn silage, dairy cow, nitrogen supplementation, rumen synchrony)

Abbreviation key: A/c = allantoin/creatinine; **D1** = protein-rich concentrate fed once a d before the a.m. meal; **D2** = protein-rich concentrate fed twice a d before both meals; **DU** = protein-rich concentrate given as a TMR with the basal diet; **ERDP** = effective rumen-degradable protein; **FME** = fermentable metabolizable energy; **QDP** = quickly rumen-degradable protein; **RH** = diet with a slight excess of ERDP in relation to FME; **RL** = diet with a large deficiency of ERDP in relation to FME; **RM** = diet with a slight deficiency of ERDP in relation to FME; **SDP** = slowly rumen-degradable protein; **U0** = concentrate with 0% urea; **U5** = concentrate with 0.5% urea; **U10** = concentrate with 1% urea.

INTRODUCTION

Milk production systems in the coastal region of northern and central Portugal are normally based on corn silage, with protein requirements met mainly by

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using corn byproducts and soybean meal. Dairy producers tend to feed additional dietary CP to dairy cows to promote higher feed intake and milk production (Cressman et al., 1980; Christensen et al., 1993; Wu and Satter, 2000). However, excess dietary CP has also been related to N pollution of water resources (Tamminga, 1992), production of greenhouse gases (Dewhurst et al., 2001), and impaired fertility (McCormick et al., 1999). Nitrate pollution of water resources is a major concern in Portugal, with some important dairy areas located in delimited N pollution-vulnerable areas. Dairy farm effluents are responsible for a significant portion of the N excreted onto land and water resources. Furthermore, protein sources continue to be expensive feed ingredients, so improving the efficiency of use of dietary CP is important both for increasing profitability and reducing potential pollution of dairy farms.

Modern protein rationing systems for dairy cows (e.g., AFRC, 1993; NRC, 2001) involve feeding protein first to meet the requirements of rumen microbes (for RDP) and then supplying RUP to meet the metabolizable protein requirements of the cow. The U.K. system (AFRC, 1993) considers that quickly degraded protein (**QDP**), defined as CP that is washed out of Dacron bags immediately, is used less efficiently than slowly degraded protein (**SDP**). The U.K. system considers **QDP** to be used with 80% efficiency as RDP, and defines the term "effective RDP" (**ERDP**) as 80% of **QDP** plus 100% of **SDP**.

Maximizing the utilization of RDP and conversion into microbial protein is a key objective of protein feeding strategies. Increasing the efficiency of microbial protein synthesis in the rumen will increase the utilization of the nutrients supplied to the host animal and the supply of essential AA at a relatively lower cost than via an increase in dietary RUP. Earlier studies have shown the importance of balancing the supply of RDP to the availability of fermentable metabolizable energy (**FME**) (Mabjeesh et al., 1997; Casper et al., 1999). This study tested these relationships using the feeds that are commonly used in northern Portugal. Other workers have suggested that it may be important to optimize the diurnal patterns of supply of RDP and **FME** (the "synchrony concept"), in addition to balancing overall supply. The basic assumption of the synchrony concept is that a lack of synchrony between the rates at which energy and N become available to the microbes reduces the efficiency of microbial capture of N and results in inefficient use of ATP for microbial growth (Chamberlain and Choung, 1995).

It has been very difficult to evaluate the effects of rumen synchrony in feeding experiments because of confounding between diet ingredients and synchrony indices (Dewhurst et al., 2000). One approach is to ma-

nipulate rumen synchrony by feeding the same diet overall, but manipulating the supply pattern of energy- and protein-rich components within a day. The studies reported in this paper have evaluated effects of synchrony, both by manipulating diet ingredients (Exp. 2) and by manipulating the times at which different feed ingredients were given (Exp. 3).

Analysis of allantoin and creatinine in spot urine samples was used to investigate effects on microbial protein yield from the rumen. This technique assumes that purines leaving the rumen are essentially of microbial origin (McAllan, 1982) and that there is a positive relationship between purines leaving the rumen and excretion of their derivatives (mainly allantoin in cattle) in urine (Chen et al., 1990). Creatinine has been used as an internal marker of urinary output, based on the assumption of a relatively constant daily output in relation to body mass (De Groot and Aafjes, 1960). Dewhurst et al. (1996) suggested that the purine derivatives:creatinine ratio in spot urine samples has potential as a on-farm diagnostic of microbial CP supply.

MATERIALS AND METHODS

This series of experiments was conducted at the Dairy Unit of the Direção Regional de Agricultura do Entre-Douro e Minho of the Ministry of Agriculture (Paços de Ferreira, Portugal). This unit has approximately 50 milking cows with a mean 305-d lactation in 2000 of 8961 kg.

Experiment 1

Six multiparous and three primiparous Holstein cows averaging 564 kg of BW (SD = 55.8), 149 DIM (SD = 13.0), and 29 kg/d of milk (SD = 4.6) were blocked according to milk production and DIM into three groups of three cows each. Cows were randomly assigned to dietary treatment sequences in three 3 × 3 Latin squares. Each experimental period lasted for 4 wk, with measurements performed in the last 7 d. The cows were kept in tie stalls with individual feed bins in an animal house, which was well ventilated and had continuous access to water. Diets contained (DM basis) approximately 40% corn silage, 5% coarsely chopped ryegrass hay, and 55% concentrate. The whole-crop corn silage was prepared during late September 1999 without the use of a silage additive. The three treatments were formulated, according to AFRC (1993), to be isoenergetic, satisfy metabolizable protein requirements, and to differ in the ratio of **ERDP**:**FME**, providing a large deficiency (**RL**), a slight deficiency (**RM**), and a slight excess (**RH**) of **ERDP** in relation to the target level (11 g of **ERDP**/MJ of **FME**) suggested in the U.K. system

Table 1. Ingredient composition (% as mixed) of the concentrates.¹

Item	Experiment 1			Experiment 2			Experiment 3	
	RL ²	RM ²	RH ²	U0 ³	U5 ³	U10 ³	CEn ⁴	CPr ⁴
Wheat grain	20.0	17.0	23.0	23.0	21.0	21.0	35.0	...
Corn gluten feed	14.1	31.8	5.5	38.0	45.0	47.0	40.0	...
Corn gluten meal	7.0	...	...	...	...	...	...	...
Citrus pulp	33.0	5.5	4.4	4.0	4.5	6.0	13.5	...
Rapeseed meal	4.0	18.0	25.0	...	...	...	...	...
Soybean meal, 44% CP	7.5	5.0	12.5	25.0	19.0	15.0	...	38.0
Sunflower meal	7.5	13.0	19.0	...	...	...	...	57.0
Molasses	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Hydrogenated fat ⁵	0.6	3.0	4.0	3.0	3.0	3.0	3.0	...
Urea	...	...	...	...	0.5	1.0	...	2.0
CaCO ₃ powder	...	1.5	1.4	1.5	1.5	1.5	1.7	...
Dicalcium phosphate	1.1	...	...	0.3	0.3	0.3	0.8	...
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.7	...
Sodium bicarbonate	0.85	0.85	0.85	0.85	0.85	0.85	1.20	...
Magnesium oxide	0.65	0.65	0.65	0.65	0.65	0.65	0.80	...
Mineral and vitamin premix ⁶	0.2	0.2	0.2	0.2	0.2	0.2	0.3	...

¹For each experiment, concentrates were prepared in a single batch by SODOL, Sociedade Descascadora Ovarense, Ltd., Ovar, Portugal.

²Concentrates are named according to the effective RDP:fermentable metabolizable energy ratio of the diet: RL, RM, and RH provided a large deficiency, a slight deficiency, and a slight excess, respectively.

³Concentrates are named according to their urea content: U0 = 0%, U5 = 0.5%, and U10 = 1.0%.

⁴Concentrates are named according to the main nutrients supplied: CEn = energy-rich nutrients and CPr = N-rich nutrients.

⁵Hydrogenated palm fatty acids (Vetagrimalimentar SA, Cantanhede, Portugal).

⁶Contained 7,500,000 IU/kg of vitamin A; 1,250,000 IU/kg of vitamin D₃; 20,000 mg/kg of vitamin E; 200 mg/kg of Co; 1000 mg/kg of Cu; 25,000 mg/kg of Fe; 385 mg/kg of I; 50,000 mg/kg of Mg; 30,000 mg/kg of Mn; 65 mg/kg of Se; and 20,000 mg/kg of Zn.

(AFRC, 1993) for lactating cows. The different ERDP:FME ratios were achieved by altering the composition of the concentrates (Table 1). Diets were fed as TMR for ad libitum intake, with fresh feed offered twice each day (0830 and 1500 h). The troughs were cleaned out each morning and orts were collected and weighed. Feed offered was adjusted each week to produce weigh backs of approximately 10% of amounts fed. Cows were milked twice daily at 0800 and 1730 h.

Milk production was measured throughout the experimental period. Milk was sampled at both milkings on 2 consecutive days during the last week of each experimental period, and proportional composites were analyzed for fat, protein, and lactose (AOAC, 1990; Milkoscan 133, Foss Electric, Hillerød, Denmark). Samples of feces and urine were collected from each cow at 8-h intervals (0800, 1600, and 2400 h), on the same 2 consecutive days, respectively, for estimation of diet digestibility using acid-insoluble ash as an indigestible internal marker, and for urinary allantoin and creatinine concentrations analysis. When cows did not voluntarily urinate, even after stimulation of the vulva, urine collection was performed using a catheter. Blood samples were collected into heparinized tubes from the jugular vein of each cow at 3 h after the morning feeding. Samples were immediately centrifuged at 3500 rpm for 10

min and the plasma stored at -15°C prior to analysis for glucose, urea, albumin, and total proteins. Cows were weighed at the same time on the final day of each period. Maximal and minimal daily barn temperatures were recorded throughout the experiment.

Experiment 2

Six multiparous and three primiparous Holstein cows averaging 550 kg of BW (SD = 49.7), 63 DIM (SD = 31.3), and 34 kg/d of milk (SD = 5.0) were used. This experiment also used three 3 × 3 Latin squares with 4-wk periods and measurements performed in the last 7 d. Three isoenergetic and iso-ERDP diets, based on corn silage, were formulated to differ in the ratio of QDP:SDP. The different QDP:SDP ratios were achieved by replacing soybean meal with urea in the concentrates (0, 0.5, and 1.0% urea for treatments U0, U5, and U10, respectively) (Table 1). The whole-crop corn silage was prepared during late September 2000 without the use of a silage additive. Assignment of cows to treatments and management were the same as in Exp. 1. The TMR differed in that 60% of the DM was from concentrate, 35% from corn silage, and 5% from ryegrass hay, and that fresh feed was offered at 0800 and 1530 h each day. Cows were milked twice daily at 0700

and 1630 h. Feces and urine were collected from each cow at 0600, 1400, and 2200 h over 2 consecutive days. Blood samples were taken 4 h after the morning feeding.

Experiment 3

This experiment was carried out simultaneously with Exp. 2, using the same forages. This experiment also used three 3×3 Latin squares with 4-wk periods. Three multiparous and six primiparous Holstein cows averaging 542 kg of BW (SD = 37.4), 127 DIM (SD = 31.0), and 29 kg/d of milk (SD = 5.9) were used. To avoid confounding between rumen synchrony and diet ingredients, all treatments were based on a single diet, which contained (on a DM basis) corn silage (45%), ryegrass hay (5%), energy-rich concentrate (35%), and protein-rich concentrate (15%). Details of the concentrate composition are given in Table 1. Feed was offered at the same times as Exp. 2, with the protein-rich concentrate either fed once (before the morning meal; **D1**) or twice (before both meals; **D2**) a day before the meal, or as a TMR given with the basal diet (**DU**). Assignment of cows to treatments and management was as described for Exp. 2, except that blood samples were collected immediately before and 4 h after the morning feeding.

In Sacco Feed Degradability

Two nonlactating Holstein cows (480 and 575 kg of BW) fitted with rumen cannulae (10 cm diameter; Bar Diamond Inc., Parma, ID) were used to measure rumen degradability of feeds. The cows were fed a diet comprising (DM basis) 45% corn silage (DM: 28%; NDF: 53% of DM; starch: 24% of DM), 5% ryegrass hay (CP: 8% of DM; NDF: 58% of DM), and 50% commercial concentrate (CP: 24% of DM; starch: 22% of DM; urea: 0.9% of DM) at 1.2 $\times$ maintenance (AFRC, 1993). The cows were kept in individual tie stalls with individual feed bins in an animal house, which was well ventilated and had continuous access to water. Diets were fed as TMR with fresh feed offered twice each day (0930 and 1730 h). The nylon bag technique (Ørskov et al., 1980) was used to measure the DM and N degradation of feeds in the rumen. Nylon bags (10 $\times$ 20 cm; Bar Diamond Inc.) containing 4 g of forages that had been ground through a 4-mm screen or the concentrates as-fed were incubated in the rumen of each cow for 12 h immediately after the morning feed on two nonconsecutive days. In total, there were 8 replicates for each feed sample (2 cows $\times$ 2 d $\times$ 2 bags). Immediately after removal from the rumen, the bags were washed in cold water and frozen at -15°C . At the end of the collections, they were

unfrozen and washed together with the time-zero bags (not incubated in the rumen) in a washing machine for 40 min at 40°C and then dried at 65°C for 24 h. Dried residues were analyzed for N content according to the Kjeldahl method (AOAC, 1990).

Chemical Analysis

Samples of corn silage, ryegrass hay, concentrates, and Orts were sampled daily, and after oven DM determination (65°C , 48 h), were composited over the recording week of each experimental period and submitted for chemical analysis. Ground samples (1 mm) were analyzed for ash and Kjeldahl N (AOAC, 1990). Crude protein was calculated as Kjeldahl N $\times$ 6.25. Neutral detergent fiber, ADF, and acid detergent lignin were determined as described by Robertson and Van Soest (1981). Ether extract was determined by extracting the sample with petroleum ether using a Gerhardt Soxhtherm 2000 Automatic (AOAC, 1990). Total sugars, P, Ca, and concentrates urea were determined by official Portuguese standard methods (Norma Portuguesa-1785, 1986; Norma Portuguesa-873, 1997; Norma Portuguesa-1786, 1985; and Norma Portuguesa-3255, 1986, respectively). Modified ADF content of corn silages and ryegrass hays was determined by the method described by Ministry of Agriculture, Fisheries and Food (1986). Metabolizable energy content of corn silages and ryegrass hays was estimated from modified ADF content according to Givens (1990) and Moss and Givens (1990), respectively. The metabolizable energy content of concentrates was estimated according to Eq. [E3] from Thomas et al. (1988). Acid-insoluble ash was determined as described by the official standard method (Norma Portuguesa-2971, 1985). Starch was determined from 0.5-mm samples by the method described by Solomonsson et al. (1984). Urinary allantoin and creatinine concentrations were determined by HPLC as described by Dewhurst et al. (1996). Jugular plasma was analyzed for glucose, urea, albumin, and total proteins (Automated chemistry analyzer, Olympus, AUG40, Melville, NY) by enzymatic (glucose-oxidase and urease) and colorimetric (bromocresol and biuret) methods as described by Bauer (1982).

Statistical Analysis

One cow from Exp. 1 had a displaced abomasum; therefore, all related results were excluded from the analysis. Results were excluded from two cow periods in Exp. 2 (one cow had a digestive upset in period 1 and one cow was injured in period 3). One cow was removed from Exp. 3 because of chronic mastitis. In

Table 2. Chemical analysis (% of DM, unless stated otherwise), metabolizable energy (ME; MJ/kg DM) content, and 0- and 12-h CP degradability in sacco (CPDeg; %CP) of the feeds used in this work.

Item	Experiment 1					Experiments 2 and 3		Experiment 2			Experiment 3	
	CS ¹	RH ²	RL ³	RM ³	RH ³	CS ¹	RH ²	U0 ⁴	U5 ⁴	U10 ⁴	CEn ⁵	CPr ⁵
DM, %	32.7	88.6	89.1	89.9	89.8	32.6	83.7	90.5	90.7	90.9	90.8	90.6
Ash	4.3	4.7	8.6	10.0	9.7	3.6	6.0	9.4	9.8	9.9	10.0	7.7
CP	7.2	6.5	19.5	22.3	25.3	7.4	6.0	23.0	22.8	24.2	15.1	37.7
EE ⁶	3.4	1.2	3.4	4.3	4.4	3.2	1.4	4.5	4.6	4.5	4.9	2.7
NDF ⁷	46.5	70.0	24.7	29.9	26.1	42.7	66.4	21.5	23.5	23.3	22.5	30.0
ADF	26.5	39.3	11.0	12.8	13.9	23.2	40.8	8.8	8.4	8.9	9.1	20.4
ADL ⁸	3.9	7.4	4.2	4.8	5.2	3.4	6.3	2.5	2.3	2.8	2.4	5.9
Sugars	0.8	8.4	13.6	8.8	9.4	0.9	13.8	7.4	7.5	6.7	8.3	9.5
Starch	28.3	ND ⁹	16.0	15.4	14.3	33.8	ND	24.3	23.1	24.3	26.2	4.3
Urea	ND	ND	ND	ND	ND	ND	ND	ND	0.5	1.0	ND	2.0
P	0.20	0.14	0.67	0.74	0.72	0.25	0.18	0.74	0.79	0.76	0.71	0.81
Ca	0.21	0.32	1.41	1.45	1.48	0.21	0.39	1.18	1.28	1.24	1.59	0.62
ME	10.4	7.7	12.6	12.1	12.1	10.7	8.4	12.9	12.7	12.7	12.7	11.4
0-h CPDeg	55.6	20.8	43.9	57.8	57.4	55.0	25.0	57.8	61.5	65.0	69.4	63.2
12-h CPDeg	56.6	28.1	58.4	78.8	78.2	60.5	25.9	76.4	77.4	81.4	81.2	81.6

¹Corn silage.²Ryegrass hay.³Concentrates are named according to the effective RDP:fermentable metabolizable energy ratio of the diet: RL, RM, and RH provided a large deficiency, a slight deficiency, and a slight excess, respectively.⁴Concentrates are named according to their urea content: U0 = 0%, U5 = 0.5%, and U10 = 1.0%.⁵Concentrates are named according to the main nutrients supplied: CEn = energy-rich nutrients and CPr = N-rich nutrients.⁶EE = ether extract.⁷Ash free. Assayed without sodium sulfite. Alpha amylase was not used for ryegrass hay.⁸ADL = acid detergent lignin.⁹ND = Not determined.

addition, data from three cow-periods (one in each period) were excluded for reasons that were not directly related to treatments (two cases of mastitis; one cow consuming the wrong diet).

All data were subjected to least squares ANOVA for three 3 × 3 Latin squares (Steel and Torrie, 1980) using the GLM procedure of SAS (Version 8e, SAS Inst. Inc., Cary, NC). The model included square, cow within square, period within square, dietary treatment, and residual error. For the analysis of urinary allantoin and creatinine concentrations (all experiments) and of plasma metabolites (Exp. 3), the model also initially included sampling time and the dietary treatment × sampling time interaction. The dietary treatment × sampling time interaction was never significant ($P > 0.05$) for urinary measurements and was removed from the model. When differences were significant the least squares difference test (Steel and Torrie, 1980) was used to compare means.

RESULTS

Mean maximal and minimal daily barn temperatures were 27.1°C (SD = 5.41) and 14.1°C (SD = 2.38) for Exp. 1, and 13.7°C (SD = 2.10) and 9.3°C (SD = 1.88) for Exp. 2 and 3, respectively.

The chemical composition of the corn silages, ryegrass hays, and concentrates are given in Table 2. The corn silage used in Exp. 2 and 3 had a higher starch content than that used in Exp. 1. The CP contents of the concentrates used in Exp. 1 closely matched the values from the formulation, and concentrate RL had a high total sugar content as a result of the high inclusion of citrus pulp. The concentrates used in Exp. 2 showed urea contents as formulated. In Exp. 3, the energy-rich concentrate contained (percentage of DM) 15.1% CP and 26.2% starch, and the protein-rich concentrate had 37.7% CP and 2% urea. Table 3 shows the composition of diets as consumed. The dietary CP level in Exp. 1 increased with the increase in ERDP:FME ratio. The composition of the diets used in Exp. 2 was very similar; all of these diets and also the diet used in Exp. 3 had a higher starch content than the diets used in Exp. 1. Table 4 presents the calculated metabolizable energy, FME, ERDP and RUP contents, and ERDP:FME ratio of each diet. The ERDP:FME ratios, calculated according to AFRC (1993) and based on the determined CP degradability in sacco values of feeds for Exp. 1, were 6.7, 10.1, and 11.2 g of ERDP/MJ of FME for diets RL, RM, and RH, respectively; and for Exp. 2 were 10.0, 10.1, and 11.1 g of ERDP/MJ of FME

Table 3. Composition of diets as consumed after correction for orts (% of DM) from different dietary treatments. SD is given in parentheses.

Item	n	Ash	CP	NDF	ADF	ADL ⁴	Starch
Experiment 1							
RL ¹	8	6.6 (0.20)	14.0 (0.46)	34.7 (1.55)	18.1 (0.48)	4.3 (0.26)	20.8 (1.90)
RM ¹	8	7.5 (0.15)	15.7 (0.37)	37.9 (0.71)	19.1 (0.44)	4.6 (0.13)	20.0 (1.58)
RH ¹	8	7.2 (0.17)	17.5 (0.12)	35.5 (1.13)	19.5 (0.32)	4.8 (0.23)	19.6 (1.72)
Experiment 2							
U0 ²	9	7.2 (0.04)	16.8 (0.44)	31.0 (0.24)	15.3 (0.98)	3.0 (0.64)	26.7 (0.25)
U5 ²	9	7.5 (0.12)	16.7 (0.33)	32.0 (0.50)	14.8 (0.62)	2.8 (0.30)	26.3 (0.56)
U10 ²	7	7.5 (0.17)	17.5 (0.64)	31.9 (0.56)	15.2 (0.52)	3.1 (0.29)	26.7 (0.40)
Experiment 3							
DU ³	7	6.5 (0.16)	14.5 (0.38)	34.2 (0.96)	18.2 (0.82)	3.4 (0.23)	25.9 (0.33)
D2 ³	7	6.5 (0.15)	14.4 (0.34)	34.4 (0.79)	18.3 (0.75)	3.5 (0.16)	25.8 (0.47)
D1 ³	7	7.2 (1.56)	14.5 (0.47)	34.2 (1.04)	18.0 (1.09)	3.4 (0.19)	26.1 (0.49)

¹Diets are named according to the effective RDP:fermentable metabolizable energy ratio of the diet: RL, RM, and RH provided a large deficiency, a slight deficiency, and a slight excess, respectively.

²Concentrates are named according to their urea content: U0 = 0%, U5 = 0.5%, and U10 = 1.0%.

³Treatments are named according to the mode of distribution of the protein-rich concentrate: DU = as a TMR with the basal diet; D2 = in two meals, immediately before the distribution of the basal diet; and D1 = in one meal, immediately before the distribution of the morning meal.

⁴ADL = acid detergent lignin.

for diets U0, U5, and U10, respectively. In Exp. 3, the diet had a ERDP:FME ratio of 9.3 g/MJ.

Effects on feed intake, diet digestibility, milk production, and milk composition are presented in Table 5. Diet RL led to significantly lower DMI and milk yield than RM and RH. The efficiency of utilization of dietary CP for milk protein synthesis was significantly higher with diet RL, with no differences between RM and RH. In Exp. 2, there were no treatment effects, except for

Table 4. Calculated metabolizable energy (ME; MJ/kg DM), fermentable metabolizable energy (FME; MJ/kg DM), effective RDP (ERDP; g/kg DM), and RUP (g/kg DM) contents, and ERDP:FME (g/MJ) ratio of diets from different dietary treatments.

Item	ME	FME ¹	ERDP ²	RUP ³	ERDP:FME
Experiment 1					
RL ⁴	11.4	9.9	66.3	59.2	6.7
RM ⁴	11.2	9.4	95.1	40.9	10.1
RH ⁴	11.2	9.4	105.3	45.2	11.2
Experiment 2					
U0 ⁵	11.9	10.1	101.2	45.4	10.0
U5 ⁵	11.8	10.0	101.3	43.2	10.1
U10 ⁵	11.7	10.0	111.3	39.5	11.1
Experiment 3	11.3	9.6	88.9	35.7	9.3

¹Calculated according to AFRC (1993).

²According to AFRC (1993), assuming quickly degradable protein (QDP) equals 0-h CP degradability × CP, and slowly degradable protein (SDP) equals (12-h CP degradability – 0-h CP degradability) × CP; calculated as $0.8 \times \text{QDP} + \text{SDP}$.

³Calculated as $\text{CP} - (\text{QDP} + \text{SDP})$.

⁴Diets are named according to the ERDP:FME ratio of the diet: RL, RM, and RH provided a large deficiency, a slight deficiency, and a slight excess, respectively.

⁵Diets are named according to the urea content of the concentrates: U0 = 0%, U5 = 0.5%, and U10 = 1.0%.

the efficiency of utilization of CP of diet for milk protein synthesis, which was significantly lower for diet U10. In Exp. 3, the cows that received all of their protein concentrate in a single meal (D1) produced less milk than the other cows (D2 and DU).

Allantoin and creatinine concentrations and allantoin/creatinine ratios (A/c) in spot urine samples, as well as estimated microbial CP supply according AFRC (1993), are given in Table 6. The concentrations of both allantoin and creatinine were significantly higher for the low-ERDP:FME diet in Exp. 1. However, urinary A/c was not significantly affected by diet. Sampling time did not affect any of the urine measurements in Exp. 1. Urinary concentrations of allantoin and the A/c ratio were significantly lower for diet U5 in Exp. 2, with no effect on creatinine concentration. Sampling time affected all of the urine measurements in Exp. 2 and 3, with the exception of urinary allantoin concentration. In Exp. 3, dietary treatment only significantly affected the urinary creatinine concentration, which was lower for diet D1.

Plasma concentrations of glucose, urea, albumin, and total proteins are given in Table 7. Plasma urea concentrations increased significantly with the increase in ERDP:FME ratio in Exp. 1. The treatments used in Exp. 2 and 3 had little overall effect on concentrations of plasma metabolites. However, concentrations of glucose and urea of Exp. 3 were higher and lower, respectively, in plasma sampled before the morning meal vs. plasma sampled after the morning meal (a significant treatment × sampling time interaction effect for plasma urea).

Table 5. Least squares means for feed intake, milk production, milk composition, and diet OM digestibility and BW from the different dietary treatments.

Item	Experiment 1					Experiment 2					Experiment 3				
	RL ¹	RM ¹	RH ¹	SEM	P	U0 ²	U5 ²	U10 ²	SEM	P	DU ³	D2 ³	D1 ³	SEM	P
n	8	8	8			9	9	7			7	7	7		
DMI, kg/d	19.3 ^a	21.3 ^b	20.7 ^b	0.28	**	23.7	24.3	24.1	0.27	NS	20.3	20.7	20.2	0.23	NS
Milk															
Yield, kg/d	27.6 ^a	30.1 ^b	29.2 ^b	0.46	*	37.3	36.9	36.3	0.71	NS	29.2 ^a	29.3 ^a	27.9 ^b	0.31	*
4% FCM, kg/d	26.4	27.5	28.6	0.65	NS	34.6	35.4	35.0	0.97	NS	27.1	27.5	25.6	0.53	NS
Fat, %	3.72	3.46	3.88	0.140	NS	3.49	3.72	3.73	0.135	NS	3.43	3.58	3.43	0.123	NS
Protein, %	3.34	3.27	3.34	0.043	NS	3.21	3.24	3.23	0.019	NS	3.51	3.43	3.45	0.052	NS
Lactose, %	5.16	5.05	5.05	0.038	NS	5.20	5.22	5.17	0.026	NS	5.18	5.15	5.18	0.032	NS
Fat, kg/d	1.02	1.03	1.13	0.039	NS	1.31	1.38	1.37	0.055	NS	1.02	1.05	0.96	0.035	NS
Protein, kg/d	0.92	0.98	0.97	0.017	†	1.20	1.19	1.17	0.023	NS	1.01	1.00	0.95	0.016	†
Lactose, kg/d	1.42 ^a	1.52 ^b	1.47 ^{ab}	0.017	*	1.94	1.93	1.89	0.042	NS	1.51	1.50	1.44	0.017	†
Yield/DMI	1.43	1.42	1.41	0.039	NS	1.58	1.54	1.52	0.030	NS	1.45	1.42	1.39	0.025	NS
4% FCM/DMI	1.36	1.29	1.37	0.045	NS	1.45	1.46	1.45	0.037	NS	1.32	1.32	1.27	0.018	NS
MP/CPI ⁴	0.34 ^a	0.29 ^b	0.27 ^b	0.010	**	0.30 ^a	0.30 ^a	0.28 ^b	0.005	*	0.35	0.34	0.33	0.008	NS
OMD, % ⁵	80.5	79.3	73.7	2.12	NS	79.7	77.5	79.1	1.72	NS	78.6	82.1	78.8	1.60	NS
BW, kg	596	588	587	3.1	NS	630	623	623	4.7	NS	604	618	608	5.7	NS

^{a,b,c}Values in the same row that share a common superscript are not statistically different ($P > 0.05$).

¹Diets are named according to the effective RDP:fermentable metabolizable energy ratio of the diet: RL, RM, and RH provided a large deficiency, a slight deficiency, and a slight excess, respectively.

²Diets are named according to the urea content of the concentrates: U0 = 0%, U5 = 0.5%, and U10 = 1.0%.

³Treatments are named according to the mode of distribution of the protein-rich concentrate: DU = as a TMR with the basal diet; D2 = in two meals, immediately before the distribution of the basal diet; and D1 = in one meal, immediately before the distribution of the morning meal.

⁴Milk protein/CP intake.

⁵OMD = OM digestibility.

* $P < 0.05$.

** $P < 0.01$.

† $P < 0.10$.

DISCUSSION

Feed Evaluation

The formulation objectives for Exp. 1 were to produce three isoenergetic diets that met metabolizable protein requirements, but which differed widely in ERDP:FME ratio. Application of U.K. standard equations to the results of feed analysis showed that diets were isoenergetic, in agreement with the application of NRC (2001) equations. The targets for ERDP:FME ratios were a large deficiency, a slight deficiency, and a slight excess of ERDP in relation to the AFRC (1993) target level of 11 g of ERDP/MJ of FME for lactating cows: 8, 10, and 12 g of ERDP/MJ of FME respectively. Results from the in sacco evaluation showed that although we did not achieve the ERDP:FME targets precisely, we produced diets that met the target of a slight excess, a slight deficiency, and a large deficiency. In fact, the large deficiency was usefully larger than we had predicted based on published values. The estimates of ERDP:FME ratios were further confirmed by plasma urea values, which increased with the increase on ERDP:FME ratio. In spite of the large difference in ERDP content of diets, they were all predicted to be adequate or in excess for

metabolizable protein balance (AFRC, 1993). This was achieved for diet RL because of its lower CP degradability.

In Exp. 2, although the objective of isoenergetic diets was almost achieved, the calculated ERDP:FME ratio for a fixed rumen retention time of 12 h for diet U10 was higher than that for U0 and U5. However, this apparent discrepancy was not confirmed by differences in plasma urea concentrations. Additionally, the relatively high values for plasma urea in this experiment suggest that microbial CP synthesis would have been limited by FME supply. The relatively low ERDP:FME ratio of diet used in Exp. 3 agreed with the formulation aim.

Feed Intake

The reduced feed intakes that result when cows are offered low-protein diets are well recognized (Cressman et al., 1980; Christensen et al., 1993; Wu and Satter, 2000). In the current study, a diet containing 10.1 g of ERDP/MJ of FME was sufficient to avoid intake depression. This level of ERDP feeding is below the level (11 g of ERDP/MJ of FME) suggested in the U.K. system

Table 6. Least squares means for allantoin and creatinine concentrations and allantoin/creatinine ratios (A/c) in the spot urine samples from the different dietary treatments and sampling time, and predicted microbial CP supply (MCP).

Item	Diets			SEM	P	Sampling time (h)			SEM	P
Experiment 1	RL ¹	RM ¹	RH ¹			8	16	24		
n	23	24	24			24	23	24		
Allantoin (mmol/L)	58.1 ^a	41.0 ^b	44.4 ^b	4.42	*	42.2	47.9	53.4	4.33	NS
Creatinine (mmol/L)	14.8 ^a	11.0 ^b	11.5 ^b	0.97	*	11.4	12.4	13.5	0.96	NS
A/c (mmol/mmol)	3.83	3.69	3.93	0.130	NS	3.79	3.81	3.85	0.128	NS
Predicted MCP (g/d) ²	1294 ^a	2020 ^b	2167 ^c	27.8	***					
Experiment 2	U0 ³	U5 ³	U10 ³			6	14	22		
n	27	27	21			25	25	25		
Allantoin (mmol/L)	47.9 ^a	38.6 ^b	49.4 ^a	2.53	**	43.6	48.2	44.1	2.47	NS
Creatinine (mmol/L)	11.4	10.5	11.2	0.50	NS	10.0 ^a	12.4 ^b	10.6 ^a	0.49	**
A/c (mmol/mmol)	4.14 ^a	3.70 ^b	4.40 ^a	0.118	***	4.28 ^a	3.84 ^b	4.12 ^{ab}	0.116	*
Predicted MCP (g/d) ²	2403 ^a	2459 ^a	2690 ^b	30.5	***					
Experiment 3	DU ⁴	D2 ⁴	D1 ⁴			6	14	22		
n	21	21	21			21	21	21		
Allantoin (mmol/L)	69.0	59.2	53.3	5.77	NS	54.7	64.8	62.1	5.11	NS
Creatinine (mmol/L)	18.7 ^a	14.6 ^{ab}	13.8 ^b	1.37	*	12.2 ^a	17.7 ^b	17.2 ^b	1.21	**
A/c (mmol/mmol)	3.98	4.00	3.91	0.213	NS	4.37 ^a	3.80 ^b	3.72 ^b	0.189	*
Predicted MCP (g/d) ²	1804	1838	1793	20.0	NS					

^{a,b,c}Values in the same row that share a common superscript are not statistically different ($P > 0.05$).

¹Diets are named according to the effective RDP:fermentable metabolizable energy ratio of the diet: RL, RM, and RH provided a large deficiency, a slight deficiency, and a slight excess, respectively.

²Calculated based on actual intake, according to AFRC (1993) for a mean rumen retention time of 12 h.

³Diets are named according to the urea content of the concentrates: U0 = 0%, U5 = 0.5%, and U10 = 1.0%.

⁴Treatments are named according to the mode of distribution of the protein-rich concentrate: DU = as a TMR with the basal diet; D2 = in two meals, immediately before the distribution of the basal diet; and D1 = in one meal, immediately before the distribution of the a.m. meal.

* $P < 0.05$.

** $P < 0.01$.

*** $P < 0.001$.

(AFRC, 1993) for lactating dairy cows. The CP content of diets used in Exp. 1 increased in line with increasing ERDP:FME, and it is often difficult to distinguish whether cows are responding to RDP (Roffler and Satter, 1975) or both RDP and RUP (Kalscheur et al., 1999). In this study, the marked decline in feed intake with diet RL suggests that the rumen microbes were restricted by lack of ERDP, in agreement with some earlier studies (Dhiman and Satter, 1993; Weigel et al., 1997). The CP content of the RM diet (15.7% of DM) was below levels that are often used in practice, but feeding additional protein (RH) did not result in any additional milk protein yield, so the efficiency of feed N declined. This result demonstrates the potential to develop feeding strategies that reduce N excretion in feces and urine without compromising milk yields.

The type and amount of energy available for microbial fermentation (Hoover and Stokes, 1991), as well types of protein (Erasmus et al., 1994) and the synchrony of energy and N supply to the rumen (Shabi et al., 1998), should also be considered in interpreting the responses to dietary CP content. Altering the synchrony of energy and N supply to rumen microbes by changing feeds (to alter the QDP:SDP ratio; Exp. 2) or by altering

meal patterns of protein-rich concentrate (Exp. 3) did not affect DMI. This may have been the consequence of the high levels of nonstructural carbohydrate in these diets (Jaquette et al., 1987).

Dawson (1999) postulated that ruminants could detect asynchrony in the rate of supply of nutrients in the rumen. She further speculated that cows have developed mechanisms to overcome or minimize its effects, by altering their pattern of feed intake to avoid an excessive load of ammonia, storing carbohydrates by rumen microbes during periods of N deficit, and by higher recycling of urea to the rumen. The highly significant treatment $\times$ sampling time interaction effect on plasma urea concentration (Table 7) confirms our success in generating different patterns of N intake between the three treatment groups. The cows were not successful in overcoming the imposed manipulations of synchrony. Diet D1 represent a much greater degree of asynchrony than might be observed in practice, at least with diets based on corn silage. The relatively low CP content of the diets used in Exp. 3 also provides the maximal opportunity for us to identify effects of rumen synchrony since rumen effects would not be masked by excess RUP. Whereas grass silage has an extremely

Table 7. Least squares means for plasma concentrations of glucose, urea, albumin, and total proteins from the different dietary treatments.

	n	Sampling time	Glucose, mg/dl	Urea, mg/dl	Albumin, g/dl	Total proteins, g/dl
Experiment 1						
RL ¹	8		53.5	23.3 ^a	3.20	8.13
RM ¹	8		51.7	30.0 ^b	3.28	7.80
RH ¹	8		55.1	36.5 ^c	3.22	8.05
SEM			1.20	1.06	0.073	0.118
P			NS	***	NS	NS
Experiment 2						
U0 ²	9		62.3	37.9	3.59	7.70
U5 ²	9		64.6	37.6	3.87	7.45
U10 ²	7		63.1	39.1	3.75	7.46
SEM			1.47	1.26	0.105	0.156
P			NS	NS	NS	NS
Experiment 3 ³						
DU ⁴	7	Before	68.0	28.6 ^a	3.69	7.47
	7	After	59.4	31.5 ^{ab}	3.61	7.27
D2 ⁴	7	Before	68.8	25.1 ^c	3.65	7.35
	7	After	57.8	33.5 ^{bd}	3.62	7.30
D1 ⁴	7	Before	65.5	23.4 ^c	3.67	7.58
	7	After	61.3	35.3 ^d	3.64	7.51
SEM			2.2	1.09	0.034	0.086
Effects						
Treatment			NS	NS	NS	†
Sampling time			***	***	†	NS
Treatment × sampling time			NS	***	NS	NS

a,b,c,d Values in the same column that share a common superscript are not statistically different ($P > 0.05$).

¹Diets are named according to the effective RDP:fermentable metabolizable energy ratio of the diet: RL, RM, and RH provided a large deficiency, a slight deficiency, and a slight excess, respectively.

²Diets are named according to the urea content of the concentrates: U0 = 0%, U5 = 0.5%, and U10 = 1.0%.

³Interaction of treatment × sampling time for urea ($P < 0.001$).

⁴Treatments are named according to the mode of distribution of the protein-rich concentrate: DU = as a TMR with the basal diet; D2 = in two meals, immediately before the distribution of the basal diet; and D1 = in one meal, immediately before the distribution of the morning meal.

*** $P < 0.001$.

† $P < 0.10$.

asynchronous supply of RDP and energy, it has a much higher CP content than corn silage; therefore, it is difficult to envisage practical diets and feeding situations in which rumen synchrony is an important factor affecting performance. Despite the extreme nature of diet D1, there was only a small and nonsignificant reduction in N use efficiency.

Urinary Allantoin and Creatinine Excretion

Several studies have shown that water intake (Silanikove et al., 1997; Dinn et al., 1998) and urine output (Holter and Urban, 1992; Moscardini et al., 1998) increase as the amount of excess N that has to be excreted increases. This effect results from the diuretic effect of increased protein intake (Broderick et al., 1974) and from a progressive increase in N that exceeds the capacity of the kidneys to concentrate urea that is mediated by changes in the glomerular filtration rate (Eriksson and Valtonen, 1982). In the present study, the highest

mean allantoin and creatinine concentrations were observed with the low-CP diets (RL, DU, D2, and D1). Overall, there was a significant negative correlation between allantoin and creatinine concentrations in urine and diet CP ($r = -0.346$, $P < 0.01$ and $r = -0.441$, $P < 0.001$ for allantoin and creatinine concentrations, respectively). Allantoin and creatinine concentrations also increased with increasing rumen synchrony in Exp. 3, perhaps as a result of the reduced requirement to eliminate urea. The effects of protein supply and synchrony on water intakes, and consequent rumen outflow rates, will complicate the interpretation of factors affecting N utilization.

The urinary A/c technique did not identify any consistent differences in microbial protein yield, predicted according to AFRC (1993), within the experiments, despite the observed differences in DMI and milk yields. Although these experiments were Latin square designs, and urine was collected from each cow three times daily over 2 consecutive days, the A/c ratio in urine spot

samples did not reflect the predicted effect of diets on microbial protein supply (Table 6). In fact, earlier results indicate that microbial N flow must be altered by approximately 84 g/d before the amount of allantoin excreted in the urine reflects changes in microbial N flow (Johnson et al., 1998). There are a number of possible reasons for imprecision in the urinary A/c technique, including effects of tissue catabolism (Nsahlai et al., 2000), variation in creatinine excretion with diet and between cow, and diurnal variations in allantoin and creatinine excretion (Shingfield and Offer, 1998). In the experiments reported here, we also found significant diurnal variation in urinary creatinine concentration (Table 6), and it is likely that diet could have had a significant effect on creatinine excretion. Therefore, the data presented are consistent with the statement of Shingfield and Offer (1998) that total urine collection appears necessary to assess accurately urinary purine derivative excretion in dairy cows. In their studies, even an intensive urine-sampling regimen (every 2 h) did not allow an acceptable prediction of daily mean A/c ratio.

Milk Production and Composition

As expected, the lower DMI with RL diet reduced milk production. However, this low-protein diet also resulted in the most efficient conversion of dietary CP intake into milk protein. Wu and Satter (2000) also obtained the highest efficiency for converting dietary N into milk N with the lowest protein treatment (15.4 to 16% CP), which was clearly deficient in protein.

Altering dietary protein degradability (Exp. 2) did not affect milk production. These results are consistent with those obtained by Christensen et al. (1993) who fed dairy cows a TMR containing either 16.4 or 19.4% CP with a ruminal CP degradability of 55 or 70%. The efficiency of conversion of CP intake into milk protein was significantly affected by dietary treatment, with the lowest value for cows that received the high-urea diet (U10), reflecting the highest CP content of diet U10 (Table 3).

Studies have shown that diets formulated to have more synchronized release of N and energy-yielding nutrients in the rumen significantly increase microbial protein synthesis and efficiency (Herrera-Saldana et al., 1990; Aldrich et al., 1993). In order to avoid confounding effects of different ingredient feeds, only the feeding pattern of the protein concentrate was altered in Exp. 3, and although dietary treatments did not significantly affect DMI, milk production was significantly lower with the less synchronized diet. Conversely, Kolver et al. (1998), who simply altered the time of feeding concentrates, demonstrated that the synchro-

nous diet promoted consistently lower ruminal ammonia concentrations but had no significant effect on milk production. However, if the frequency of feeding the whole diet or of the readily fermentable carbohydrate is altered, there are often pronounced effects on ruminal pH and the composition of VFA. Since these factors may influence microbial growth, it is suggested that altering the pattern of feeding the high-protein component of diets, while all other components of the diet are kept constant, is the fairest test of the rumen synchrony hypothesis (Chamberlain and Choung, 1995). Robinson et al. (1997; 2002) highlighted an additional factor that must be considered when manipulating meal patterns. They showed that rumen conditions and rumen degradation varied according to whether a protein supplement was given at night or during the day. These effects were attributed to diurnal variation in rumen fill and passage rates. Overall, the results reported here are in agreement with the statement of Chamberlain and Choung (1995) that a close matching of energy and N release will bring only a minor (if any) benefit in most practical conditions.

CONCLUSIONS

The results of the present study demonstrate that diets with low RDP:FME ratios restrict DMI and milk yield. Conversely, supplying RDP in excess of the amount necessary to match the amount of FME supplied by the diet and utilizable by rumen microbes did not increase DMI or milk yield in midlactation dairy cows and increased blood urea, leading to RDP waste and potential environmental pollution. It was also demonstrated that NPN could replace part of the vegetable protein in the diet without adverse effects on early-lactation dairy cow productivity as long as the supply of readily available carbohydrate was guaranteed. Providing more synchronized N and energy supply for the rumen microbes to midlactation dairy cows led to higher milk yields, due perhaps to increased microbial protein synthesis. However, these effects were only observed with extreme diets and feeding strategies, and it is concluded that attempts to match energy and N release in the rumen will not bring additional benefits in practical feeding conditions. Urinary output increased in response to increased or unbalanced protein supply. Analysis of the A/c ratio in spot samples of urine was not useful in identifying differences in microbial protein yield from the rumen.

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