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Effect of various levels of imbalance between energy and nitrogen release in the rumen on microbial protein synthesis and nitrogen metabolism in growing double-muscled Belgian Blue bulls fed a corn silage-based diet¹

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ABSTRACT: Seven double-muscled Belgian Blue bulls (initial BW: 341 ± 21 kg) with cannulas in the rumen and proximal duodenum were used in an incomplete replicated Latin square. The study examined the effect of imbalance between energy and N in the rumen on microbial protein synthesis and N metabolism by giving the same diet according to 3 different feeding patterns. The feed ingredients of the diet were separated into 2 groups supplying the same amount of fermentable OM (FOM) but characterized by different levels of ruminally degradable N (RDN). The first group primarily provided energy for the ruminal microbes (12.5 g of RDN/kg of FOM), whereas the second provided greater N (33.3 g of RDN/kg of FOM). These 2 groups were fed to the bulls in different combinations with the aim of creating 3 levels of imbalance (0, 20, and 40 g/kg of DM) between energy and N supplies in the rumen. Imbalance was measured by the variation of the degradable protein balance (OEB value in the Dutch system) of the diet between the 2 meals each a day. Diurnal variations in ruminal NH₃-N concentrations and plasma urea concentrations were greatly influenced by the feeding patterns of the diet. Introduction of imbalance

affected neither microbial N flow at the duodenum ($P = 0.97$) nor efficiency of growth ($P = 0.54$). The feeding patterns of the diet had no negative impact on NDF degradation in the rumen ($P = 0.33$). Nitrogen retention was not affected by imbalance ($P = 0.74$) and reached 49.7, 52.0, and 51.3 g/d, respectively for 0, 20, and 40 g of OEB/kg of DM imbalance. It seems that introduction of an imbalance between energy and N supplies for the ruminal microbes by altering the feeding pattern of the same diet does not negatively influence the microbial activity in the rumen nor N retention of the animal. Nitrogen recycling in the rumen plays a major role in regulating the amount of ruminally available N and allows a continuous synchronization of N and energy-yielding substrates for the microorganisms in the rumen. Therefore, imbalance between dietary energy and N created over a 24-h interval was not detrimental to rumen microbial growth for the animal as long as the level of imbalance did not exceed 40 g of OEB/kg of DM. Thus, these feeding patterns of the diet can be used under practical feeding conditions with minimal impact on the performance of ruminant animals for meat production.

Key words: energy, imbalance, nitrogen, nitrogen balance, rumen fermentation

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INTRODUCTION

Recent efforts to enhance productive performance of ruminants through synchronization of carbohydrate

and N to improve fermentation in the rumen did not result in detectable benefits for the animals (Richardson et al., 2003). Furthermore, the introduction of a 12- or 24-h imbalance between N and energy supplies in the rumen did not negatively influence microbial protein synthesis nor N use in sheep (Ludden et al., 2002a,b) or growing bulls (Valkeners et al., 2004). Both the ruminal microbes and their host animal possess the means to compensate for variations in kinetics of feed degradation to ensure a relatively continuous supply of nutrients to places of biosynthesis to optimize microbial growth (Dawson, 1999).

The movements of N across the gut play a major role in regulating the amount of ruminally available N, and provide overall plasticity to allow a rapid response to any changes in metabolic status (Lapierre and Lobley,

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2001). During periods of N excess in the rumen, NH_3 is absorbed in portal blood circulation and detoxified to urea by the liver. This N may then be recycled back to the rumen during subsequent periods of N shortage. Nevertheless, N recycling probably has limitations dependent on the time and the level of the imbalance created between energy and N release in the rumen and cannot totally buffer important temporary N excess.

The objective of the current study was to examine the effect of the level of imbalance between energy and N release in the rumen on digestion and N metabolism of double-musled Belgian Blue bulls. This was achieved by feeding the same diet according to different feeding patterns, because it has been suggested that differences due to imbalance may be attributed to some factors associated with the manipulation of the level or type of ingredients (Dewhurst et al., 2000). Therefore, increasing levels of imbalance between ruminal supplies of energy and N were tested.

MATERIALS AND METHODS

Animals

Seven double-musled Belgian Blue bulls (initial BW = 341 ± 21 kg; final BW = 480 ± 32 kg), fitted with a ruminal cannula (67 mm i.d.) and a T-type cannula at the proximal duodenum, were individually penned (1.5×2.5 m). Fresh water was available at all times. The experimental protocol was approved by the Gembloux Agricultural University Animal Care and Use Committee, and followed the guidelines issued by the committee on care, handling, and sampling of the animals.

Diet and Feeding Management

Bulls received the same diet according to 3 different feeding patterns at an intake level of 76 g of DM/kg^{0.75}. Offering feed at this level was roughly 97.5% of ad libitum consumption (Fiems et al., 1999). Adaptation to the diet lasted 20 d. Feed was offered twice a day in equal amounts at 0830 and 2030. The diet was made up of 53% concentrate and 47% corn silage on a DM basis. The diet supplied 83 g of intestinal digestible proteins (DVE) and 7.5 MJ of NE for fattening (NEF) per kg of DM according to the Dutch system (Van Es and Van der Honing, 1977; Tamminga et al., 1994). The degradable protein balance (OEB value in the Dutch system) of the diet amounted to 0.4 g/kg of DM and was close to zero, which corresponded to the optimum OEB value according to the Dutch system. This OEB value is equivalent to a ratio between ruminally degradable N (RDN) and fermentable OM (FOM) of 24 g/kg, which is close to the optimal ratio of 25 g of N/kg of OM truly digested in the rumen proposed by Czerwaski (1986). The OEB value estimates the (im)balance between ruminal degradable CP and energy extracted during anaerobic fermentation in the rumen for microbial protein synthesis (Tamminga et al., 1994). When the OEB value

Table 1. Ingredient and chemical composition and nutritive value of the 2 groups of feed ingredients

Item	Group of feed ingredients ¹	
	LRDN	HRDN
Ingredient composition, % of DM		
Corn silage	47.0	47.0
Dehydrated sugar beet pulp pellets	28.0	23.5
Coarsely ground corn	8.2	2.9
Steam-flaked corn	11.0	4.5
Coarsely ground peas	—	5.0
Soybean meal	3.5	—
Coarsely ground lupins	—	13.3
NaCl	0.3	0.3
Soybean oil	1.0	1.0
Urea	—	1.5
Mineral and vitamin mix ²	1.0	1.0
Chemical composition, g/kg of DM		
OM	946	946
CP	95	175
NDF	290	299
ADF	166	180
Ether extract	33	42
Starch	387	306
Sugars ³	43	44
Fermentable OM ³	609	609
Ruminally degradable N ³	7.6	20.3
Nutritive value of DM ³		
NEF, MJ/kg of DM	7.49	7.46
DVE, g/kg of DM	83.2	82.8
OEB, g/kg of DM	-39.4	40.2

¹LRDN = part of the feed ingredients of the experimental diet theoretically deficient in ruminally available N according to the Dutch system; HRDN = part of the feed ingredients of the experimental diet theoretically providing ruminally available N in excess according to the Dutch system.

²Formulated to provide the following per kilogram of total diet DM: 1.2 g of Ca, 0.8 g of P, 0.4 g of Na, 0.35 g of Mg, 0.5 mg of I, 0.5 mg of Co, 1.5 mg of vitamin E, 60 µg of Fe, 10 µg of Cu, 30 µg of Mn, 60 µg of Zn, 0.2 µg of Se, 6,000 IU of vitamin A, and 1,200 IU of vitamin D₃.

³Values derived from the Dutch nutritional tables (CVB, 2000). NEF = net energy for fattening, calculated according to the Dutch system (Van Es and Van der Honing, 1977); DVE = intestinal digestible proteins (Tamminga et al., 1994); OEB: degradable protein balance (Tamminga et al., 1994).

is positive for a diet, potential loss of N from the rumen occurs, whereas a negative value means a shortage of ruminally degradable N with a consequent impaired microbial activity.

The feed ingredients of the diet were separated in 2 groups supplying the same amounts of DVE, NEF, and FOM, but with different amount of RDN. Ingredient composition, actual chemical composition, and tabular nutritive value (CVB, 2000) of the 2 groups of feeds are presented in Table 1. No forage was fed separately. The first group (HRDN) provided all the major protein sources (coarsely ground peas, coarsely ground lupins, and urea) of the experimental diet, with a tabular OEB value of 40.2 g/kg of DM, which corresponded to an RDN:FOM ratio of 33.3 g/kg. This part of the diet supplied RDN in excess in comparison with the available FOM to the ruminal microbes. The second group

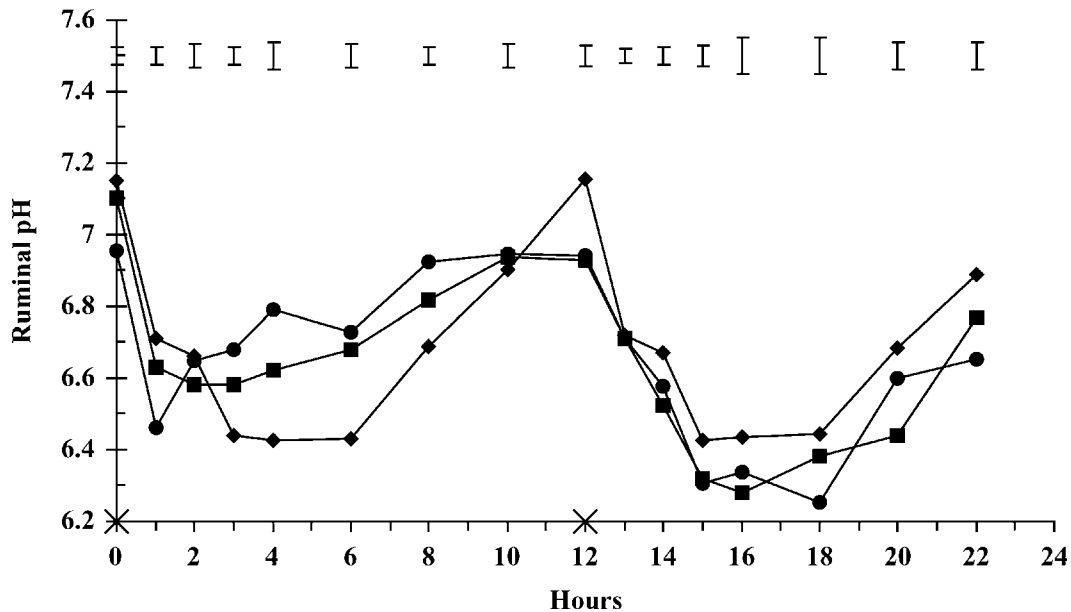


Figure 1. Ruminal pH in bulls fed the same diet but offered in 3 feeding patterns to induce different levels of imbalance between energy and nitrogen supplies for ruminal microbes measured by the variation of the degradable protein balance (OEB) between the 2 meals of a day, with 0 (◆), 20 (■), or 40 (●) g of OEB/kg of DM. Feeding times are indicated on the x-axis by ×. Error bars indicate SEM for n = 6.

(LRDN) was characterized by an OEB value of -39.4 g/kg of DM, which corresponded to an RDN:FOM ratio of 12.5 g/kg. According to the Dutch system, this part of the diet had a low RDN content relative to the available FOM.

The diet was fed to the bulls according to 3 different feeding patterns so that 3 levels of imbalance between energy and N supplies for the ruminal microbes were created. The level of imbalance is measured by the variation of the OEB value between the 2 meals of a day while, on a daily basis, the OEB value was close to zero. The first feeding pattern of the diet (L0) consisted of LRDN and HRDN in equal amounts at each meal. Therefore, the energy and N-yielding substrates to the ruminal microorganisms were considered balanced because the OEB value was close to zero at each meal. The second pattern (L20) consisted of feeding LRDN and HRDN at a ratio of 3:1 in the morning and 1:3 in the evening. The RDN:FOM ratios averaged 17.7 and 28.1 g/kg. With this second feeding pattern, an imbalance between the energy and N supplies for the ruminal microbes of an OEB variation of 20 g/kg of DM was created experimentally. However, the OEB value per day was close to zero because the same feed ingredients were ingested on a 24-h basis. The last feeding pattern (L40) consisted of increasing the level of imbalance to an OEB variation of 40 g/kg of DM by feeding alternately LRDN at the morning meal and HRDN at the evening meal. On a 24-h basis, the OEB value was close to zero because the same amounts of LRDN and HRDN were ingested compared with L0 and L20.

Digestibility was measured using Cr_2O_3 as an indigestible flow marker. From 5 d before sample collection

and continually thereafter, 3.5 g of Cr_2O_3 /kg of DMI was administered to each bull twice daily (just before each meal) intraruminally via gelatin capsules (Milton et al., 1997). Bulls were weighed at the beginning of each treatment period before the 0830 feeding and feed offered was adjusted to the metabolic weight of each animal and fixed during the period.

Experimental Protocol and Sample Collection

The 7 bulls were allocated to 3 treatment periods in an incomplete replicated Latin square, separated by a 20-d rest period. Each treatment period lasted 18 d and included collection of digesta (duodenal and fecal), urine, blood, and ruminal fluid. Refusals were collected daily before the morning feeding, weighed, and dried at 60°C . Samples of each group of feed ingredients were obtained each time LRDN and HRDN were mixed, ground to pass a 1-mm screen (Cyclotec 1.093, Foss Tecator AB, Höganäs, Sweden), and pooled by period on an equal weight basis.

Urine was collected during the first 10 d of each treatment period with an adaptation of the apparatus of Veenhuizen et al. (1984). During collection, urine was acidified with 4 N H_2SO_4 to pH 3 at 0900, 1700, and 2100 to avoid N losses. Total urine collection was weighed daily at 0900, sampled, and frozen at -20°C . The majority of the fecal output was collected simultaneously into containers placed underneath each animal, sampled daily at 0830, and frozen at -20°C .

Samples of duodenal digesta were collected on d 11 to 13 to cover a 24-h period in 1.5-h steps. After removal of the cap of the duodenal cannula, accumulated digesta

were discarded, and when the flow seemed normal, 400 mL of duodenal contents was collected. For each collection, a 100-g sample of duodenal contents was immediately used to extract liquid-associated bacteria according to the method described by Poncet and Rémond (2002). Moreover, the solid-adherent bacteria were extracted according to the method developed by Legay-Carmier and Bauchart (1989) for rumen samples. The remainder of each duodenal sample was frozen at -20°C .

On d 14, central venous catheters (polyurethane aliphatic, No. 67262J17, Plastimed, Le Plessis Bouchard, France) were inserted into one of the external jugular veins of each bull after an intravenous injection of analgesic (xylazine 2%, 0.08 mL/100 kg of BW). On d 15, blood samples (20 mL) were slowly collected into 2 heparinized (143 IU/10 mL) tubes every 2 h for 24 h. On the last 3 d of each treatment period, rumen digesta were sampled 0, 1, 2, 3, 4, 6, 8, and 10 h after each meal to cover a 24-h period.

Laboratory Analyses

Concentrate, corn silage, and feed refusals pooled per animal at each treatment period were ground to pass a 1-mm screen (Cyclotec 1.093, Foss Tecator AB) before DM, OM, ether extract, Kjeldahl N (AOAC, 1990), NDF, and ADF (Van Soest et al., 1991) analyses were conducted. The NDF was determined without use of sodium sulfite but with the addition of heat-stable amylase (Thermamyl 120, NOVO Nordisk, Bagsvaerd, Denmark) for the last 30 min of boiling. A small part of these samples was ground to pass a 0.5-mm screen for starch determination (Faisant et al., 1995). After freeze-drying and grinding (1 mm), DM, OM, N, NDF, and Cr_2O_3 (François et al., 1978) were measured in feces pooled per animal and per period. Urinary N was also analyzed (AOAC, 1990).

Blood was centrifuged at $1,200 \times g$ for 15 min immediately after sampling. Plasma was removed and frozen at -20°C until further analyses. After thawing, plasma urea N (PUN) was analyzed using the coupled urea-glutamate dehydrogenase enzyme system (Talke and Schubert, 1965). This analysis was automated by using a Vitalab 200 and 200R (Vital Scientific NV, AC Dieren, The Netherlands).

The pH of ruminal fluid was measured (Microprocessor pH-meter, pH 320/SET, WTW, Weilheim, Germany) immediately after sampling and filtration (250 μm). Supernatant from $1,200 \times g$ centrifugation for 12 min was frozen at -20°C after H_2SO_4 acidification to pH 3 until $\text{NH}_3\text{-N}$ determination. The $\text{NH}_3\text{-N}$ concentration was determined by steam distillation with MgO using a 2,300 Kjeltex Analyser Unit (Foss Tecator AB).

Duodenal samples were freeze-dried, ground to pass a 1-mm screen, and pooled per animal and period before DM, OM, N, $\text{NH}_3\text{-N}$, NDF, and Cr_2O_3 determinations on the whole digesta. A small part was ground to pass a 0.5-mm screen for starch and purine determinations.

Purine content was measured following the method of Zinn and Owens (1986) adapted for hydrolysis of nucleotides by perchloric acid (Makkar and Becker, 1999), and for wash solution used for washing the precipitate of free purines with silver nitrate (Obispo and Dehority, 1999). Duodenal bacteria samples were freeze-dried, ground with a coffee grinder, and pooled per animal and period before N, OM, and purine content determinations.

Calculations and Statistical Analyses

Nutrient flows at the duodenum and fecal output were calculated by reference to Cr_2O_3 . The proportion of microbial N flowing at the duodenum was calculated by dividing the duodenal purine:N ratio by the corresponding purine:N ratio in duodenal microbes. An endogenous N flow of 2.98 g/kg of OM to duodenum (Kim et al., 2001) was applied for the fractionation of the estimated duodenal nonmicrobial N into endogenous and undegraded dietary N.

Statistical analyses on N balance, total tract and ruminal digestibility, and microbial protein synthesis were conducted using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Data were analyzed by ANOVA for an incomplete replicated Latin square design. Model sums of squares were separated into bull, period, and treatment. Least squares means were separated into significant main effects by the PDIF option of SAS. Because of animal illness, the last experimental period could not be completed and the statistical analysis was done with missing values in place of the actual data. Therefore, the SEM for $n = 6$ are presented. For data collected over time within each sampling period (ruminal pH, ruminal $\text{NH}_3\text{-N}$ concentration, and PUN), a repeated-measures analysis was performed using the REPEATED statement within the GLM procedure of SAS. Effects in the model included bull, period, treatment, time, and treatment \times time interaction. When treatment \times time interactions were significant, variables were analyzed within time periods. Differences among treatments were considered significant when $P < 0.05$, whereas when $P > 0.05$ but $P < 0.10$, differences were considered to indicate a trend toward a significant effect.

RESULTS

Ruminal Fermentation Characteristics and Plasma Urea N

A level of imbalance \times time interaction ($P < 0.001$) was observed in ruminal pH (Figure 1). However, this interaction was largely due to differences in ruminal pH at specific time points and probably not biologically significant, because a pH of 6.2 is generally considered the pH threshold below which the growth of fiber-utilizing microbes and digestibility of fiber and OM are decreased (Pitt et al., 1996).

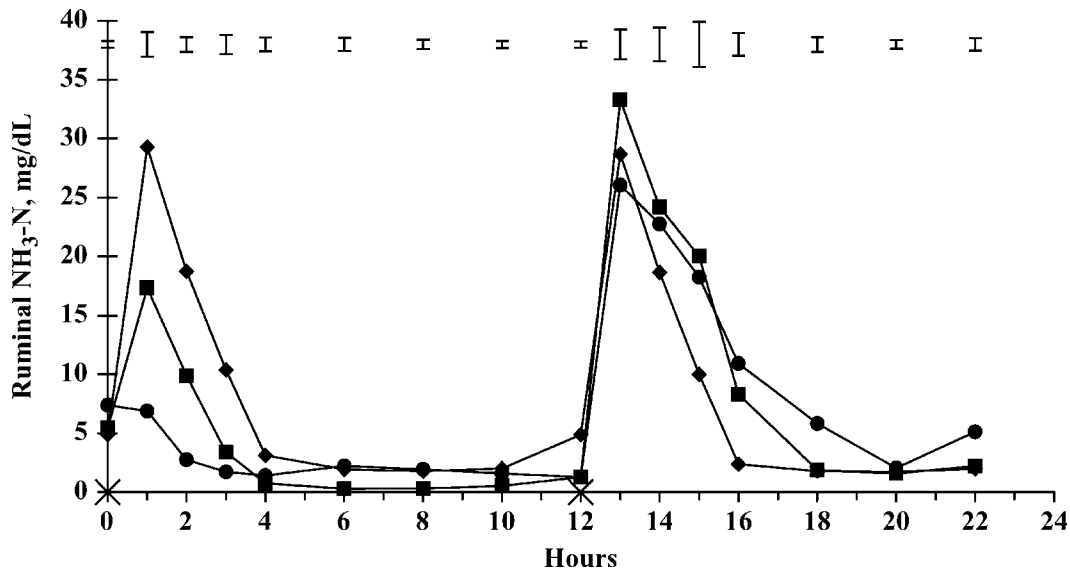


Figure 2. Ruminal NH₃-N concentrations in bulls fed the same diet but offered in 3 feeding patterns to induce different levels of imbalance between energy and nitrogen supplies for ruminal microbes measured by the variation of the degradable protein balance (OEB) between the 2 meals of a day, with 0 (◆), 20 (■), or 40 (●) g of OEB/kg of DM. Feeding times are indicated on the x-axis by ×. Error bars indicate SEM for n = 6.

There was an effect of time and interaction between time and level of imbalance ($P < 0.001$) on ruminal NH₃-N (Figure 2). The temporal pattern of ruminal NH₃-N was similar, but peak concentrations during the day were influenced by the pattern of feeding the diet and the nature of the group of feed ingredients ingested. At 1, 2, and 3 h after the morning meal, ruminal NH₃-N levels were significantly different among the levels of imbalance. Ruminal NH₃-N concentrations were lower when the proportion of LRDN in the meal was high. At 4 h after the evening meal, ruminal NH₃-N measured in animals on L0 were lower ($P = 0.03$) than those on L20 and L40. Ruminal NH₃-N concentrations were greater when the proportion of HRDN in the meal was high. Daily mean concentrations of ruminal NH₃-N were not different among the treatments ($P = 0.456$) and amounted to 6.91, 6.31, and 6.04 mg/dL, respectively for L0, L20, and L40.

There were a time effect and level of imbalance × time interaction ($P < 0.001$) on PUN (Figure 3). At 4, 10, and 12 h after the morning meal, PUN measured on animals on L40 were significantly lower ($P < 0.01$) than those measured on L0 and L20, whereas at 14 and 16 h, bulls on the L20 and L40 feeding patterns had lower ($P < 0.05$) PUN than those on the L0 feeding pattern. The hourly evolution of PUN was influenced by the nature of the group of feed ingredients ingested. Daily mean concentrations of PUN tended to be influenced ($P = 0.086$) by the feeding pattern of the diet. The daily PUN in bulls fed the diet according to the feeding pattern L40 was lower (6.2 vs. 7.5 mg/dL) than those obtained in bulls fed the diet according to the feeding patterns L0 and L20.

Intake and Digestibility

Intakes of OM, NDF, and starch were not different among treatments (Table 2). Total tract digestibility of OM and NDF were greater ($P < 0.048$ to 0.049) in bulls on L40 compared with those on L0, with L20 being intermediate (Table 2). Ruminal digestibility of OM, NDF, and starch were not influenced by the feeding patterns. The ratio of ruminal:total tract digestibility of NDF were high and not significantly different between the feeding patterns. The feeding patterns of the diet did not affect intake or digestibility of N (Table 3). Moreover, apparent ruminal digestibility of N may be considered equal to zero according to a conformity test of the mean ($t_{\text{observed}} < t_{0.975}$).

Flow of Nitrogenous Components at the Duodenum

Level of imbalance between energy and N supplies for the ruminal microbes had no effect on NH₃-N, non-NH₃-N, and microbial N flows at the duodenum (Table 3). Efficiencies of microbial protein synthesis reached 29.2, 25.8, and 24.6 g of N/kg of OM apparently digested in the rumen and 21.1, 19.4, and 18.6 g of N/kg of OM truly digested in the rumen, respectively for L0, L20, and L40, and were not significantly affected by the level of imbalance.

Nitrogen Balance

Fecal and urinary outputs of N, as well as absolute and relative amounts of N retained did not differ significantly due the feeding pattern (Table 3).

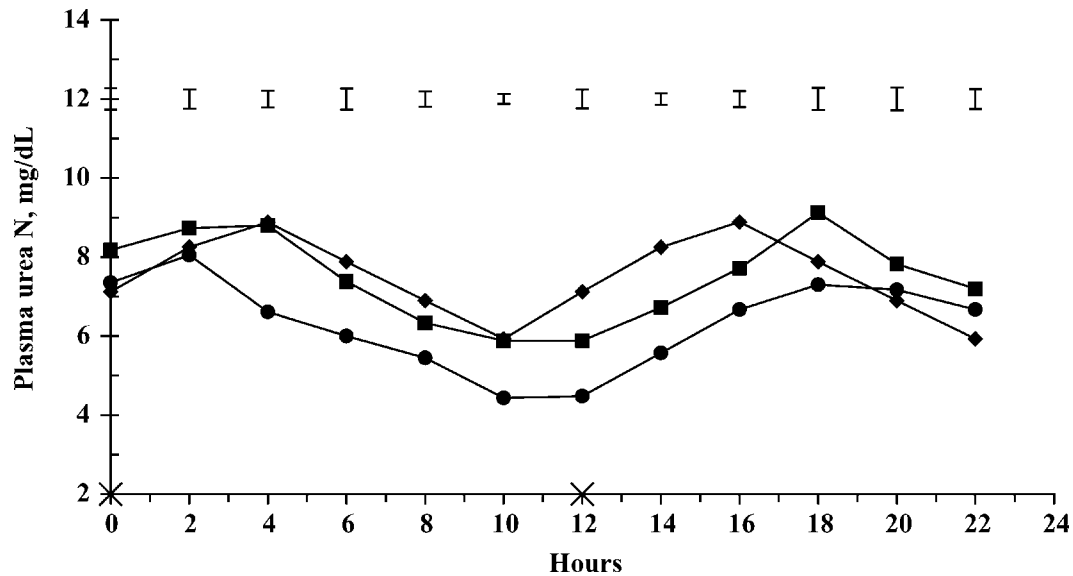


Figure 3. Plasma urea N in bulls fed the same diet but offered in 3 feeding patterns to induce different time periods of imbalance between energy and nitrogen supplies for ruminal microbes measured by the variation of the degradable protein balance (OEB) between the 2 meals of a day, with 0 (◆), 20 (■), or 40 (●) g of OEB/kg of DM. Feeding times are indicated on the x-axis by ×. Error bars indicate SEM for $n = 6$.

DISCUSSION

According to the Dutch system (Tamminga et al., 1994), the feeding pattern L0 reaches the target of a good balance of energy and N release for the microorganisms in the rumen. In practice, because the apparent ruminal digestibility of N (-9.3%) may be considered equal to zero, the amount of feed N degraded in the rumen was roughly equal to the amount used by the microorganisms. Thus, the recycling of urea N in the rumen balanced the amount of ruminal NH_3 absorbed through the rumen wall and transferred to the blood.

The total tract and ruminal digestibility of OM and NDF measured with L0 were consistent with those generally measured on double-musled Belgian Blue bulls (Fiems et al., 1997; Valkeners et al., 2004). The theoretical ruminal N degradability (in sacco values), estimated from the Dutch tabular values (CVB, 2000) averaged 50.2 and 72.4%, respectively, for LRDN and HRDN. For the feeding pattern L0, the estimated in sacco ruminal N degradability was 64.5%, which was close to the true ruminal digestibility of N measured with L0 (59.6%). Efficiency of microbial protein synthesis obtained with L0 (29.2 g of microbial N/kg of OM appar-

Table 2. Least squares means for intake, and ruminal and total tract digestibility measured in bulls fed the same diet but offered in 3 feeding patterns to induce different levels of imbalance between energy and N supplies for ruminal microbes¹

Item	Feeding pattern			SEM	P-value
	L0	L20	L40		
OM					
Intake, g/d	6,095	6,149	6,051	47.0	0.384
Apparent ruminal digestibility, %	42.6	46.7	49.7	2.3	0.845
True ruminal digestibility, %	57.8	61.2	64.9	2.1	0.063
Apparent total tract digestibility, %	72.2 ^a	73.4 ^{ab}	74.0 ^b	0.4	0.049
NDF					
Intake, g/d	1,874	1,891	1,863	15.0	0.422
Ruminal digestibility, %	54.2	55.7	59.5	2.3	0.332
Total tract digestibility, %	60.7 ^a	62.5 ^{ab}	63.7 ^b	0.6	0.048
Ruminal:total tract digestibility ratio, %	89.3	89.7	94.4	3.2	0.510
Starch					
Intake, g/d	1,559	1,578	1,542	14.0	0.261
Ruminal digestibility, %	50.0	54.7	58.6	4.0	0.423

^{a,b}Within a row, least squares means without a common superscript letter differ, $P < 0.05$.

¹As measured by the variation of the degradable protein balance (OEB) between the 2 meals of a day, with 0 (L0), 20 (L20), or 40 (L40) g of OEB/kg of DM.

Table 3. Least squares means for intake, ruminal and total tract digestibility, and balance of nitrogen in bulls fed the same diet but offered in 3 feeding patterns to induce different levels of imbalance between energy and N supplies for ruminal microbes¹

Item	Feeding pattern ¹			SEM	P-value
	L0	L20	L40		
N intake, g/d	140.1	141.5	138.9	1.3	0.398
Duodenal flow, g/d					
Total N	153.4	148.1	144.3	4.5	0.414
NH ₃ -N	4.9	4.3	4.3	0.3	0.322
Non-NH ₃ -N	148.5	143.8	140.0	4.4	0.437
Microbial N	73.5	72.1	72.5	3.9	0.972
Endogenous N ²	17.9	18.3	17.8	0.3	0.267
Undegraded N	56.9	53.4	49.7	4.5	0.579
Apparent ruminal digestibility, %	-9.3	-4.6	-3.9	3.8	0.639
True ruminal digestibility, %	59.6	62.3	64.4	3.6	0.702
Apparent total tract digestibility, %	65.0	66.5	66.4	0.5	0.165
Fecal N, g/d	49.1	47.4	46.6	0.7	0.111
Urinary N, g/d	41.3	42.2	41.1	1.3	0.820
Retained N					
g/d	49.7	52.0	51.3	1.9	0.740
% of ingested N	35.7	36.8	36.8	1.2	0.802
% of digested N	54.8	55.5	55.4	1.6	0.966

¹As measured by the variation of the degradable protein balance (OEB) between the 2 meals of a day, with 0 (L0), 20 (L20), or 40 (L40) g of OEB/kg of DM.

²Endogenous-N flow to duodenum; calculated assuming 2.98 g of N/kg of OM intake (Kim et al., 2001).

ently digested in the rumen) is in agreement with the average value of 24 g of microbial N/kg of OM apparently digested in the rumen adopted for all diets by the Dutch system (Tamminga et al., 1994). From these results, it seems that the feeding pattern L0 created appropriate conditions in the rumen for the microorganisms and their activities. The N retention obtained during this experiment with L0 (49.7 g of N/d) was indicative of the ability of the double-musled Belgian Blue bulls to grow rapidly (Hornick et al., 1998; Froidmont et al., 2000). Assuming 20.6% of CP in the gain (De Campeneere et al., 2001), N retention observed with L0 corresponded to an ADG of 1.51 kg/d., which is near the average ADG of 1.29 kg/d observed with the bulls on the whole experiment. The use of the N digested by L0 was high and attested that the supplies of NE for fattening and DVE were well balanced.

Over a 24-h period, no significant differences were observed between L20 and L40 average concentrations of ruminal NH₃-N compared with L0, although diurnal variation for this response was important. With L20 and L40, a succession of periods of excess or deficiency in N supply for the microorganisms was created. The group LRDN had a low CP content (95 g/kg of DM) and a theoretically low ruminal degradability of N (50.2%). The ingestion of LRDN at the morning meal by bulls fed L40 did not induce a postfeeding increase of ruminal NH₃-N concentration and led to a prolonged period (11 h over the 12 h after the morning meal; Figure 2) during which ruminal NH₃-N was below the concentration (5 mg/dL) generally considered as the minimum required for optimal microorganism growth in the rumen (Satter and Slyter, 1974). The average ruminal NH₃-N concen-

tration over the 12 h after LRDN feeding amounted to 2.8 mg/dL, and concentrations of PUN decreased from 8.1 to 4.4 mg/dL (Figure 3), probably indicating N transfer from the blood into the rumen. In contrast, the group HRDN had a high CP content (175 g/kg of DM) and contained feedstuffs with high ruminal N degradability. Ingestion of L40 (evening meal) led to a large production of NH₃ in the rumen. Average ruminal NH₃-N concentration over the 12 h after HRDN feeding was 9.2 mg/dL. It was very likely that large amounts of NH₃ were lost from the rumen fluid by absorption through the rumen wall and transferred by portal blood to the liver to produce urea because PUN increased for 12 h after HRDN ingestion. With L20, the groups LRDN and HRDN were fed in different combinations (3:1 and 1:3, respectively, for 0830 and 2030 feedings), which led to intermediate ruminal NH₃-N and PUN evolutions. Although each animal received the same diet on a 24-h basis, the feeding pattern of the diet and the nature of the meal ingested had a large influence on the diurnal evolution of the ruminal NH₃-N concentration during this period. Ruminal bacteria require NH₃ for microbial growth and activity. Low ruminal NH₃ has the potential to inhibit microbial activity and decrease rate of fiber digestion.

Intake was not affected by the feeding patterns. Average DMI was 73.2 g of DM/kg^{0.75} and was near ad libitum, which is comparable to voluntary DMI of 78.2 g of DM/kg^{0.75} reported by Fiems et al. (1999) for double-musled Belgian Blue bulls fed a corn silage-based diet. Total tract digestibility of OM and NDF were not negatively influenced by the introduction of an imbalance between energy and N supplies for ruminal microbes

and were surprisingly greater ($P = 0.049$ and $P = 0.048$, respectively, for OM and NDF) for L40 than L0. The ruminal digestibility of OM, NDF, and starch were not found to differ among treatments. Across all treatments, the NDF ruminal:total tract digestibility ratios were high (Table 2) and not influenced by the level of imbalance. The fiber degradation activities of the ruminal microbes were not affected by the perturbations experimentally created in terms of energy and N supplies for the ruminal microbes when an imbalance of 20 or 40 g of OEB/kg of DM was induced. Although the periods of low ruminal $\text{NH}_3\text{-N}$ concentration were longer after the L20 and L40 morning feeding than after the L0 morning meal, it seemed that the N supplies in the rumen for the cellulolytic bacteria and the nonstructural carbohydrate-fermenting bacteria were satisfied even if the diet was fed with a level of imbalance of 20 or 40 g of OEB/kg of DM. Russell and Strobel (1987) found that in *in vitro* studies, NH_3 could be transported into microbes, reaching high intracellular NH_3 concentrations despite the fact that the $\text{NH}_3\text{-N}$ concentration used in the media was very close to zero. This mechanism of active NH_3 transport is likely to be present *in vivo*, allowing ruminal cellulolytic microbes to obtain NH_3 from the medium at low ruminal NH_3 concentration.

Level of imbalance had no significant effect on non- NH_3 and microbial N flows at the duodenum. The efficiency of microbial protein synthesis obtained with L20 and L40 were not significantly different compared with L0. The *in vitro* studies of Newbold and Rust (1992) and the *in vivo* studies of Henning et al. (1993), Sinclair et al. (1993, 1995), and Richardson et al. (2003) with sheep have focused on the synchronization of ruminal N and energy to prevent periods of N deficiency during the day. According to their results, there is some disagreement as to whether synchronizing dietary energy and N supply to the rumen improves microbial protein growth. Newbold and Rust (1992), Henning et al. (1993), and Richardson et al. (2003) found no effect of dietary synchrony on microbial growth either *in vitro* or *in vivo*. However, Sinclair et al. (1993, 1995) have found microbial growth rates or efficiency to be improved by dietary synchrony. In the current experiment, there was no significant effect on the microbial protein synthesis when a 12-h imbalance of a daily OEB variation of 20 or 40 g of OEB/kg of DM was created. This lack of response to an increased level of imbalance between energy and N supplies for the ruminal microbes is in agreement with results obtained in a previous study (Valkeners et al., 2004), which demonstrated that a lack of imbalance in the diet between the energy and N supplies for ruminal microbes was not detrimental to their growth as long as nutrient supply was balanced on a 48-h basis and the level of imbalance did not exceed 30 g of OEB/kg of DM.

Because N retention obtained with L20 and L40 did not differ from L0, it seemed that animal growth was not affected by a lack of synchrony. The total tract N

digestibility was not influenced by the feeding patterns. Feeding the diet with an imbalance of 20 or 40 g of OEB/kg of DM had no significant impact on use of N digested by the animal and therefore, the overall balance of nutrients for the tissues was not modified by the imbalance of nutrients for the rumen.

A large extent of $\text{NH}_3\text{-N}$ excess in the rumen may be excreted as urea in the urine by growing ruminants (Dehareng and Ndibualonji, 1994). In this experiment, urinary excretion of N was not affected by the feeding patterns. Our results tended to show that the temporary excess of ruminal degradable N created after ingestion of the evening meal by L20 and L40 were not lost in urine by the bulls but could return in the rumen during temporary N shortages. The ruminal recycling of urea helps the animal and its ruminal microbes to overcome a deficiency in N supply and could minimize the effect of a lack of synchronization between energy and N supplies in the rumen (Dawson, 1999). The N recycling might be an adaptive mechanism to retain N within the system and might be needed to provide NH_3 continuously for microbial protein synthesis (Marini and Van Amburgh, 2003). In the current study, this mechanism has minimized the potential negative impact of feeding a diet with a level of imbalance between energy and N supplies in the rumen of 20 or 40 g of OEB/kg of DM on digestion and N metabolism of double-muscled Belgian Blue bulls.

IMPLICATIONS

Provided the overall balance between ruminally degradable nitrogen and ruminally fermentable organic matter in the daily intake is sufficient, there is no further advantage for the double-muscled Belgian Blue bulls in balancing the release of energy and nitrogen in the rumen over the short term. Thus, these feeding patterns of the diet can be used under practical feeding conditions with minimal impact on the performance of ruminant animals for meat production.

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