Effect of imbalance between energy and nitrogen supplies on microbial protein synthesis and nitrogen metabolism in growing double-muscled Belgian Blue bulls

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Effect of imbalance between energy and nitrogen supplies on microbial protein synthesis and nitrogen metabolism in growing double-muscled Belgian Blue bulls[1]

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ABSTRACT: Six double-muscled Belgian Blue bulls (initial weight: 345 ± 16 kg) with cannulas in the rumen and proximal duodenum were used in two juxtaposed 3 × 3 Latin squares to study the effect of a lack of synchronization between energy and N in the rumen on microbial protein synthesis and N metabolism by giving the same diet according to three different feeding patterns. The feed ingredients of the diet were separated into two 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 (14.6 g of RDN/kg of FOM), and the second provided N (33.3 g of RDN/kg of FOM). These two groups were fed to the bulls simultaneously or alternately with the aim of creating three different time periods of imbalance (0, 12, or 24 h) between energy and N supplies in the rumen. The introduction of imbalance affected neither microbial-N flow at the duodenum (P = 0.65) nor efficiency of growth (P = 0.69), but decreased (P = 0.016) the NDF degradation in the rumen 12.2% for a 12-h period of imbalance. N retention was not affected by imbalance (P = 0.53) and reached 57.8, 58.5, and 54.7 g/d, respectively, for 0-, 12- and 24-h imbalance. It seems that the introduction of an imbalance of 12 or 24 h between energy and N supplies for the ruminal microbes by altering the feeding pattern of the same diet does not negatively influence microbial protein synthesis or N retention by the animal. Nitrogen recycling in the rumen plays a major role in regulating the amount of ruminally available N and allows for continuous synchronization of N- and energy-yielding substrates for the microorganisms in the rumen. Therefore, a lack of synchronization in the diet between the energy and N supplies for the ruminal microbes is not detrimental to their growth or for the animal as long as the nutrient supply is balanced on a 48-h basis. Thus, these dietary feeding patterns may be used under practical feeding conditions with minimal effect on the performance of ruminant animals.

Key Words: Bulls, Energy, Nitrogen, Nitrogen Balance, Ruminal Fermentation, Synchronization

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Introduction

Balancing the supply of N- and energy-yielding substrates to ruminal microbes has been proposed as a mechanism for maximizing the capture of ruminally degradable N (**RDN**) and optimizing microbial growth rate and efficiency in ruminants. For the past 10 yr, different trials have been conducted to test the "synchrony" hypothesis and different methods used to achieve synchrony or asynchrony. Synchrony may be altered by giving a specific form of energy and N directly into the rumen in complement of a basal diet (Kim et al., 1999a,b) or by changing dietary ingredients (Witt et al., 1999a,b). These last studies have produced conflicting results, which may confound the potential effect of synchrony with the effect due to the different feed ingredients used (Dewhurst et al., 2000). One alternative way of improving synchrony using the same feed ingredients is to change the feeding pattern, either by increasing feeding frequency or by feeding different combinations of the same ingredients in different meals (Dawson, 1999). In this type of experiment, scarce conclusive evidence has been presented that shows positive effects on microbial protein synthesis (Ludden et al., 2002) or N retention (Richardson et al., 2003). The absence of a detectable effect on microbial protein synthesis and the lack of clear animal benefits of diets balanc-

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ing the supply of N and energy-yielding substrates to ruminal microbes suggest that achieving an instantaneous synchrony is not that crucial to diet formulation (Sauvant and van Milgen, 1995) and that ruminants and their microbes have developed mechanisms to overcome the effects of a lack of synchrony (Dawson, 1999).

The objective of the present study was to examine the effects of various periods of imbalance between N and energy supply for the ruminal microbes on double-muscled Belgian Blue (**dm-BB**) digestion and N metabolism. This was done by giving the same feed ingredients according to three different feeding patterns.

Materials and Methods

Animals

Six dm-BB (initial weight = 345 ± 16 kg), each 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 University of Gembloux Animal Care and Use Committee (protocol No. 00/10) and followed the guidelines issued by the committee on care, handling, sampling of the animals.

Diet and Feeding Regimen

The bulls received the same diet according to three different feeding patterns at an intake level of 85 g of DM/kg$^{0.75}$. Adaptation to the diet lasted 21 d before the first period and 15 d between the other periods. Feed was offered twice a day in equal amounts at 0830 and 2030. The diet was made up of 91% concentrates and 9% of wheat straw. The diet supplied 97 g of intestinal digestible proteins (**DVE**) and 1,842 kcal of NE for fattening (**NEF**) per kilogram 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 experimental diet amounted to 0 g/kg of DM and corresponds to the optimal OEB value for a diet according to the Dutch system. This OEB value is equivalent to a ratio between RDN and fermentable OM (**FOM**) equal to 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 shows the (im)balance between microbial protein synthesis potentially possible from ruminal degradable CP and from the energy extracted during anaerobic fermentation in the rumen (Tamminga et al., 1994). When the OEB value is positive for a diet, potential loss of N from the rumen occurs, whereas a negative value means a shortage of ruminal degradable CP and the microbial activity may be consequently impaired.

The feed ingredients of the experimental diet were separated in two groups supplying the same amounts of DVE, NEF, and FOM, but different amounts of RDN.

Table 1. Ingredient and chemical compositions and nutritive value of the two groups of feed ingredients

	Group of feed ingredients	
Item	LRDN[a]	HRDN[b]
Ingredient composition, % of DM		
Spelt	11.7	20.4
Dehydrated sugarbeet pulp pellets	35.5	24.5
Coarsely ground maize	18.6	10.1
Coarsely ground peas	2.0	10.2
Soybean meal	0.8	3.4
Coconut meal	10.2	1.2
Rapeseed meal	1.5	10.2
Alfalfa pellets	8.0	6.6
Soybean oil	1.7	2.5
Urea	—	0.9
Minerals and vitamins mixture[c]	1.0	1.0
Wheat straw	9.0	9.0
Chemical composition, g/kg of DM		
OM	933	939
CP	113	187
NDF	381	336
ADF	193	175
Ether extract	46	46
Starch[d]	195	220
Sugars[d]	49	47
NFC[e]	408	396
FOM[d]	575	575
RDN[d]	8.4	19.1
Nutritive value of DM[d]		
NEF, kcal/kg of DM[f]	1,842	1,842
DVE, g/kg of DM[g]	97	97
OEB, g/kg of DM[h]	–30	31

[a]LRDN = part of the feed ingredients of the experimental diet theoretically deficient in ruminally available N according to the Dutch system.

[b]HRDN = part of the feed ingredients of the experimental diet theoretically providing ruminally available N in excess according to the Dutch system.

[c]Composition, %: Ca, 8; P, 12; Na, 4.0; Mg, 2.5; (mg/kg): I, 50; Co, 50; vitamin E, 150; (ppm): Fe, 6,000; Cu, 1,000; Mn, 3,000; Zn, 6,000; Se, 20; (IU/kg): vitamin A, 600,000; vitamin D_3, 120,000.

[d]Values derived from the Dutch nutritional tables (CVB, 2000).

[e]NFC = nonfiber carbohydrates (g/kg DM), calculated as 1,000 – (CP + NDF + ether extract + ash).

[f]NEF = net energy for fattening, calculated according to the Dutch system (Van Es and Van der Honing, 1977).

[g]DVE = intestinal digestible proteins in the Dutch system (Tamminga et al., 1994).

[h]OEB = degradable protein balance in the Dutch system (Tamminga et al., 1994).

Ingredient composition, actual chemical composition and tabular nutritive value (CVB, 2000) of the two groups of feeds are presented in Table 1. No forage was fed separately. The first group (**HRDN**) provided the bulls with all the major protein sources (coarsely ground peas, soybean meal, rapeseed meal, and urea) of the experimental diet and was characterized by a tabular OEB value of 31 g/kg of DM, which corresponded to a RDN:FOM ratio of 33.3 g/kg. This part of the diet was supplied to the ruminal microbes RDN in excess compared with the available FOM. On the other hand, the second group (**LRDN**) was characterized by an OEB value of –30 g/kg of DM, which corresponded

Table 2. Illustration of the three different feeding patterns of the experimental diet fed to bulls to induce different time periods of imbalance between energy and N supplies for ruminal microbes either of 0 h (D0), 12 h (D12), or 24 h (D24)

	Feeding time			
	d_i		d_{i+1}	
Treatment	0830	2030	0830	2030
D0				
Type of meal[a]	½ LRDN + ½ HRDN	½ LRDN + ½ HRDN	½ LRDN + ½ HRDN	½ LRDN + ½ HRDN
OEB, g/kg of DM	0	0	0	0
D12				
Type of meal	LRDN	HRDN	LRDN	HRDN
OEB, g/kg of DM	–30	31	–30	31
D24				
Type of meal	LRDN	LRDN	HRDN	HRDN
OEB, g/kg of DM	–30	–30	31	31

[a]LRDN = 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. See Table 1 for other definitions.

to a RDN:FOM ratio of 14.6 g/kg. According to the Dutch system, this part of the diet had a low RDN content in comparison with the available FOM.

The experimental diet is fed to the bulls according to three different feeding patterns, so that three different time periods of imbalance between energy and N supplies for the ruminal microbes were created. The three feeding patterns of the diet are illustrated in Table 2. The first feeding pattern of the diet (**D0**) consisted of giving the two groups of feed ingredients (LRDN and HRDN) in equal amounts at each meal. Therefore, the energy- and N-yielding substrates to the ruminal microorganisms were considered synchronized, as the OEB value was zero at each meal. The second pattern (**D12**) consisted of giving only LRDN at the morning meal and only HRDN at the evening meal. An imbalance of a time period of 12 h between the energy and N supplies for the ruminal microbes was thus experimentally created. However, the OEB value per day was zero, and the same feed ingredients were ingested on a 24-h basis. The last feeding pattern (**D24**) consisted of increasing the imbalance period to 24 h by alternately feeding LRDN for two meals one day, and HRDN at two meals the day after. On a 48-h basis, the OEB value was zero and the same amounts of LRDN and HRDN were ingested compared with D0 and D12.

Digestion was measured using chromic oxide (Cr_2O_3) as an indigestible flow marker. From 5 d before the sample collection and continually thereafter, 3 g of Cr_2O_3/kg of DMI were administered to each bull twice daily, just before the meal, intraruminally via gelatin capsules (Milton et al., 1997). Bulls were weighed at the beginning of each treatment period before the 0830 feeding, and ingestion of DM was adjusted to the metabolic weight for each animal.

Experimental Protocol and Sample Collection

The six bulls were allocated to three treatment periods in two juxtaposed 3 × 3 Latin squares separated by a 15-d rest period. The treatment period lasted 23 d and included digesta (duodenal and fecal), blood, and ruminal fluid collections. 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 as described by Veenhuizen et al. (1984). During the 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 every morning at 0900, sampled, and frozen at –20°C. The majority of the fecal outputs were simultaneously collected into a container placed under each animal, sampled daily at 0830, and frozen at –20°C.

Duodenal samples (approximately 400 mL) were collected on d 11 to 15 to cover 48 h by 3-h steps. For each collection, 100 g 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 rest of each duodenal sample was frozen at –20°C.

On d 16, central venous catheters (polyurethane aliphatic, Plastimed, Le Plessis Bouchard, France, No. 67262J17) were inserted into one of the external jugular veins of each bull after an i.v. injection of analgesic (xylazine 2%; 0.08 mL/100 kg of live weight). On d 17 and 18, blood samples (20 mL) were slowly collected into two heparinized tubes (143 IU/10 mL) every 2 h during 48 h.

The last 5 d of each treatment period were devoted to ruminal fluid collection. The rumen was sampled at 0, 1, 2, 3, 4, 6, 8, and 10 h after each meal to cover 48 h.

Laboratory Analyses

Concentrates, wheat straw, and orts 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 200 μL of amylase (Thermamyl 120, Novo Nordisk; Bagsvaerd, Denmark)

for the last 30 min of boiling. 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 × *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 measured (Sigma kit No. 16-11). This procedure was automated with a Vitalab 200 and 200R (Vital Scientific NV, AC Dieren, The Netherlands).

The pH of ruminal fluid was immediately measured (microprocessor pH-meter, pH 320/SET, WTW, Weilheim, Germany) after sampling and filtration (250 μm). Supernatant from 1,200 × *g* centrifugation for 12 min was frozen at –20°C after H_2SO_4 acidification to pH 3 until NH_3 N determination. The NH_3 N concentration was determined by steam distillation using a Kjeltec 2300 analyzer 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, NH_3 N, NDF, and Cr_2O_3 determinations on the whole digesta. A small part was ground to pass a .5-mm screen for the purine determination. Purine content was measured with the method proposed by Zinn and Owens (1986) adapted for the hydrolysis of nucleotides by perchloric acid (Makkar and Becker, 1999) and for the 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 of N balance, fecal 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 two juxtaposed 3 × 3 Latin squares. Model sums of squares were separated into animal, period, and treatment. Differences between means were compared using Dunnett's *t*-test (Dagnelie, 1986). For the fermentation data collected and PUN data, an additional analysis was performed using the REPEATED statement within the GLM procedure of SAS. Therefore, differences between means were compared using Dunnett's *t*-test (Dagnelie, 1986).

Table 3. Ruminal fermentation parameters and plasma urea N of bulls fed the same diet offered in three feeding patterns to induce different time periods of imbalance between energy and N supplies for ruminal microbes either of 0 h (D0), 12 h (D12), or 24 h (D24)

	Treatment[a]					Dunnett's *t*-Test	
Item	D0	D12	D24	SEM[b]	*P*	D0 vs. D12	D0 vs. D24
pH	6.42	6.37	6.45	0.03	0.209	0.245	0.467
Ammonia N, mg/dL	8.8	9.9	10.0	0.91	0.944	0.328	0.279
PUN, mg/dL[c]	8.4	8.3	8.4	0.37	0.948	0.805	0.976

[a]Treatment means calculated over a 48-h period.
[b]n = 6.
[c]PUN = plasma urea N.

Results

Ruminal Fermentation Characteristics and Plasma Urea N

The effects of the feeding patterns on ruminal fermentation characteristics and PUN are shown in Table 3. The mean pH calculated on a 48-h period (Table 3) and the kinetics of pH values (data not shown) were not different ($P > 0.05$) between the three feeding patterns of the diet.

The means of ruminal ammonia concentration calculated on a 48-h period were not different ($P > 0.05$). Kinetics of ruminal ammonia concentration exhibited a cyclical trend between meals, but maximum concentrations during the 2 d, which were reached 1 h after meal ingestion, were influenced by the group of feed ingredients ingested (Figure 1). The ingestion by group HRDN led to higher ($P < 0.001$) maximum concentra-

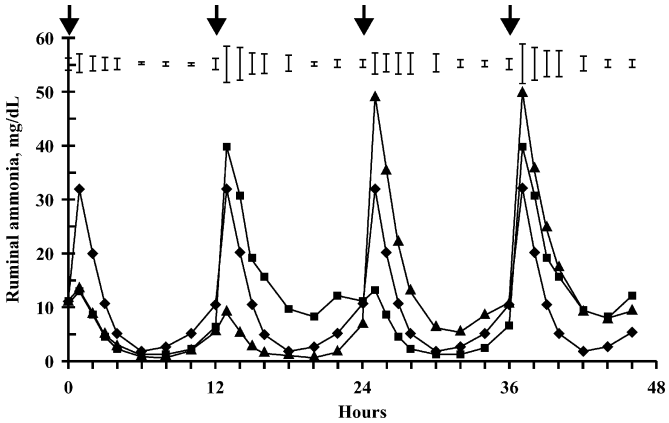

Figure 1. Ruminal ammonia concentration in bulls fed the same diet offered in three feeding patterns to induce different time periods of imbalance between energy and N supplies for ruminal microbes either of 0 h (♦), 12 h (■), or 24 h (▲). Six observations per treatment mean. Arrows indicate feeding times. Error bars indicate SEM.

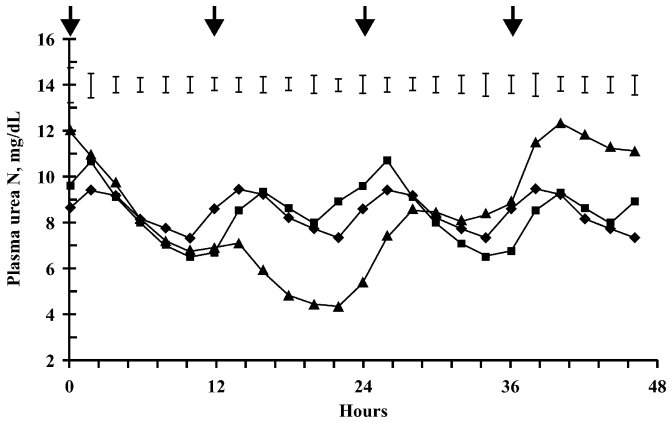

Figure 2. Plasma urea N in bulls fed the same diet offered in three feeding patterns to induce different time periods of imbalance between energy and N supplies for ruminal microbes either of 0 h (♦), 12 h (■), or 24 h (▲). Six observations per treatment mean. Arrows indicate feeding times. Error bars indicate SEM.

tions than the ingestion by group LRDN (44.5 vs. 12.1 mg/dL). The maximum concentration measured after the ingestion of the two groups simultaneously led to a value halfway between the two others. The time period that ruminal ammonia concentration was below 5 mg/dL depended on the nature of the meal ingested. When D12 and D24 ingested LRDN, this time period lasted for an average of 9 h over the 12-h period. Conversely, after the HRDN meals of D12 and D24, the ruminal ammonia concentration never decreased below 5 mg/dL. The time period obtained after the ingestion of the two groups simultaneously by D0 led to a value between the two others.

The means of PUN calculated on a 48-h period were not different between the three feeding patterns (Table 3). However, the hourly evolution of PUN was greatly influenced by the nature of the group of feed ingredients ingested only with D24 (Figure 2). The consecutive ingestion by D24 of two LRDN meals led to a decrease of PUN, whereas the consecutive ingestion of two HRDN meals led to an increase of PUN compared with the value obtained with D0 when LRDN and HRDN were fed simultaneously. Depending on the nature of the diet and the time after feeding, PUN measured with D24 could reach 60 to 150% of the PUN measured with D0.

Digestibility

Apparent ruminal and total-tract digestibility are given in Table 4. The total-tract digestibility of OM and NDF were not affected ($P > 0.05$) by the feeding patterns. For the rumen, results showed numerical digestibility differences between the three feeding patterns, but differences were only significant for NDF (Table 4). The feeding patterns did not affect ruminal apparent or total-tract digestibility of N. Moreover, ruminal digestibility of N may be considered equal to zero according to a conformity test of the mean ($t_{observed} < t_{0.975}$).

Flow of Nitrogenous Components at the Duodenum

Duration of imbalance between energy and N supplies for the ruminal microbes had no significant effect on ammonia N, nonammonia N, or microbial N flows at the duodenum. The partition of N at the duodenum is presented in Table 4. Efficiencies of microbial protein synthesis (**EMPS**) reached 27.9, 31.2 and 29.1 g of N/kg of OMADR for D0, D12, and D24, respectively, and were not affected by the duration of imbalance ($P > 0.05$). The numerical differences between the EMPS reflected the numerical differences between the OMADR as the microbial N flows to the duodenum were not found to differ ($P > 0.05$).

Nitrogen Balance

Results of the N balance are shown in Table 5. Nitrogen intakes and fecal and urinary outputs were not affected by the duration of imbalance ($P > 0.05$). Absolute amounts of N retained and relative amounts expressed as a percentage of N intake or N digested were not found to differ significantly because of the feeding pattern of the diet and reached on average 56.6 g/d, 33.2% of ingested N and 49.4% of digested N, respectively.

Discussion

This experiment compared three different feeding patterns of a diet to measure the potential impact of three different time periods (0, 12, and 24 h) of imbalance between energy and N supplies for the ruminal microbes on the digestion and the N metabolism of dm-BB. In this way, the opportunity for synchronization of energy and N release in the rumen is also tested.

According to the Dutch system, the feeding pattern D0 targets the synchrony of energy and N release for the microorganisms in the rumen. In practice, as the apparent ruminal digestibility of N (–2.1%) 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 and thus, the recycling of urea N in the rumen balanced the amount of ruminal ammonia absorbed through the rumen wall and transferred to the blood. The fecal and ruminal digestibility of OM and NDF measured with D0 were consistent with those generally measured on dm-BB fed with similar diets (Beckers et al., 1995; Fiems et al., 1997). The EMPS obtained with D0 (27.9 g of microbial N/kg of OMADR) is in agreement with the average value of 24 g of microbial N/kg of OMADR adopted for all diets by the Dutch system (Tamminga et al., 1994). From these results, it appears that the feeding pattern D0 created appropriate conditions in the rumen for the microorganisms

Table 4. Intake, and ruminal and total-tract digestibility of bulls fed the same diet offered in three feeding patterns to induce different time periods of imbalance between energy and N supplies for ruminal microbes either of 0 h (D0), 12 h (D12), or 24 h (D24)

	Treatment					Dunnett's *t*-test	
Item	D0	D12	D24	SEM[b]	*P*	D0 vs. D12	D0 vs. D24
Organic matter							
Intake, g/d	6,458	6,650	6,651	110	0.402	0.251	0.250
Apparent ruminal digestibility, %	41.1	36.2	38.3	2.4	0.396	0.188	0.433
True ruminal digestibility, %	55.3	50.6	52.3	2.0	0.305	0.139	0.326
Apparent total tract digestibility, %	72.4	71.7	71.5	1.0	0.809	0.641	0.547
Neutral detergent fiber							
Intake, g/d	2,298	2,394	2,422	75	0.430	0.392	0.216
True ruminal digestibility, %	52.5	46.1	48.7	1.5	0.045	0.016	0.112
Apparent total tract digestibility, %	60.2	59.4	60.8	1.4	0.903	0.798	0.832
Ruminal:fecal digestibility ratio, %	87.2	77.6	81.2	2.6	0.080	0.097	0.390
Nitrogen							
Intake, g/d	168.7	172.4	171.4	2.0	0.439	0.226	0.372
Duodenal flow, g/d							
Total N	172.8	181.3	179.6	5.2	0.503	0.280	0.383
Ammonia N	4.3	4.4	4.8	0.3	0.528	0.819	0.297
Nonammonia N	168.5	176.9	174.8	4.9	0.489	0.263	0.396
Microbial N	70.1	73.5	72.6	2.6	0.646	0.381	0.516
Endogenous-N[a]	19.2	19.7	19.8	0.4	0.501	0.332	0.306
Undegraded-N	79.2	83.7	82.4	3.0	0.577	0.322	0.477
Apparent ruminal digestibility, %	–2.1	–5.2	–4.9	3.3	0.769	0.520	0.569
True ruminal digestibility, %	52.9	51.4	51.8	2.0	0.876	0.630	0.723
Apparent total tract digestibility, %	67.5	67.8	67.3	0.9	0.925	0.838	0.857

[a]Endogenous N flow to duodenum: calculated assuming 2.98 g of N/kg of OM intake (Kim et al., 2001).
[b]n = 6.

and their activities. The N retention obtained during this experiment with D0 (57.8 g of N/d) expresses the ability of the dm-BB to grow rapidly (Hornick et al., 1998; Froidmont et al., 2000). Assuming 22% CP in the gain (De Campeneere, 2000), N retention observed with D0 corresponded to an ADG of 1.64 kg/d., which is close to the average ADG of 1.5 kg/d observed with the bulls during the whole experiment.

Over a 48-h period, no differences ($P > 0.05$) were noted between D12 and D24 average concentrations of ruminal ammonia compared with D0, although the diurnal variation for this parameter was important. With D12 and D24, a succession of periods of large excess or deficiency in N supply for the microorganisms was created. The introduction of an imbalance between energy and N supplies for the ruminal microbes by altering the feeding pattern of the feed ingredients had a great influence on the ruminal fermentation characteristics. The ingestion by the HRDN group led to a large production of ammonia in the rumen following the degradation of coarsely ground peas and urea included in this group of feed. It is very likely that large amounts of ammonia were lost from the rumen fluid by absorption through the rumen wall and transferred by portal blood to the liver to produce urea. According to our results, the maximum ammonia concentration in the rumen was followed 2 h later by the maximum PUN. This delay between these two peaks was in agreement with those observed by Gustafsson and Palmquist (1993). Conversely, the ingestion of LRDN led to a long period during which ruminal NH_3 N was below 5 mg/dL, which is generally considered to be the minimum required for an optimal microorganisms growth in the rumen (Satter and Slyter, 1974).

Although each animal received the same diet on a 48-h basis, the feeding pattern of the diet and the nature of the group of feed ingredients ingested (LRDN, HRDN, or both simultaneously) had a large influence on the diurnal evolution of the ruminal ammonia concentration during this period, which could eventually

Table 5. Nitrogen balance of bulls fed the same diet offered in three feeding patterns to induce different time periods of imbalance between energy and N supplies for ruminal microbes either of 0 h (D0), 12 h (D12), or 24 h (D24)

	Treatment[a]					Dunnet's *t*-Test	
Item	D0	D12	D24	SEM[b]	*P*	D0 vs. D12	D0 vs. D24
Nitrogen							
Intake, g/d	168.7	172.4	171.4	2.0	0.439	0.226	0.372
Fecal, g/d	54.7	55.2	56.1	2.0	0.878	0.855	0.625
Urinary, g/d	56.2	58.8	60.6	2.2	0.427	0.447	0.208
Retained, g/d	57.8	58.5	54.7	2.4	0.531	0.846	0.395
Retained, % N intake	34.4	33.9	32.0	1.1	0.332	0.759	0.169
Retained, % N digested	51.0	50.1	47.7	1.9	0.486	0.748	0.258

[a]n = 6.

impair microorganism growth and degradation activities. Duration of imbalance had no significant effect on nonammonia and microbial N flows at the duodenum. The EMPS obtained with D12 and D24 were not significantly different compared with D0. The lack of response to increased duration of imbalance between energy and N supplies for the ruminal microbes was in agreement with results obtained in vitro (Newbold and Rust, 1992) and in vivo (Casper et al., 1999). Henning et al. (1993) postulated that, provided the overall balance between ruminally degradable N and ruminally fermentable OM in the daily intake is sufficient, there is no further advantage in synchronizing the release of energy and N in the rumen over the short-term. Furthermore, our results tend to show that there were no negative impacts on the microbial protein synthesis when a 12 or 24-h imbalance between N and energy supplies in the rumen was experimentally created.

For D12, the digestibility results showed a decrease (P = 0.016) in the ruminal digestibility of NDF compared with D0. For D24, the numerical digestibility difference observed was not significant (P = 0.112). Conversely, total-tract digestibility of NDF was not affected by the imbalance created. The NDF ruminal:fecal digestibility ratios were high and tended to be lower with D12 than D0 (P = 0.097). Because there were small numeric increases in duodenal microbial N flows for D12 and D24 in comparison with D0, this might suggest that the differences among the treatments were the result of differences induced by random variations in marker recovery, which would lead to the simultaneous decreases in OM digestion and increases in microbial protein flow.

Because the N retention obtained with D12 and D24 did not differ from D0, it appeared that animal growth was not affected by a lack of synchrony. The fecal N digestibility was not influenced by the feeding patterns. Feeding the diet with an imbalance of 12 or 24 h has no significant impact on the utilization of the N digested by the animal, and therefore the overall balance of nutrient for the tissues was not modified by the imbalance of nutrient for the rumen.

A large extent of NH_3 N excess in the rumen may be excreted 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 was not lost in the urine in bulls and the same amount of N could return in the rumen during temporary N shortages. The ruminal recycling of endogenous urea helps the animal and its ruminal microbes to overcome deficiencies in the N supply and could minimize the effect of a lack of synchronization between energy and N supplies in the rumen (Dawson, 1999). As suggested by Huntington and Archibeque (2000), that means that both ruminal ammonia and body urea act together to smooth the short-term variation in N supply from the diet. Nevertheless, N-recycling mechanisms probably have limitations and might not completely buffer higher ammonia excess created in the rumen. A better comprehension of this mechanism and an accurate idea of its limitations may be useful in ruminant nutrition in order to minimize N excretion in the environment and maximize N retention by animals.

Implications

The introduction of an imbalance of 12 or 24 h between energy and nitrogen supplies for ruminal microbes by altering the feeding pattern of the same diet did not influence microbial protein synthesis nor the nitrogen retention of the animal in a negative manner. Nitrogen recycling in the rumen plays a major role in regulating the amount of nitrogen ruminally available for the microorganisms and allows for a continuous synchronization of nitrogen and energy-yielding substrates. Therefore, a lack of synchronization in the diet between the energy and the nitrogen supplies for the ruminal microbes should not be prejudicial for their growth or for the animal, as long as the nutrient supply is balanced on a 48-h basis. Thus, these dietary feeding patterns may be used under practical feeding conditions with minimal effect on the performance of ruminant animals.

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