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Determination of the methionine requirement of growing double-muscled Belgian Blue bulls with a three-step method[1]

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ABSTRACT: The three-step technique was used to determine the requirements of total amino acids (TAA) and the first-limiting amino acid (AA) in growing double-muscled Belgian Blue bulls (BBb). In Exp. 1, three double-muscled BBb weighing initially 306 ± 28 kg received a basal diet consisting of 30% meadow hay and 70% concentrate that was poor in digestible protein but had adequate NE because of continuous infusion of dextrose into the duodenum. The intestinal apparent digestibility of essential AA (EAA) was defined according to their duodenal and ileal flows. It averaged 72% but varied between 60% for Met and 79% for Arg. In Exp. 2, five double-muscled BBb (334 ± 22 kg) received the same diet supplemented with duodenal infusions of dextrose and four doses of Na-caseinate (28, 56, 84, and 112% of intestinal digestible dietary AA) in a 4 × 4 Latin square design with one additional animal. Nitrogen retention for the basal diet alone and the four increasing supplements of Na-caseinate reached 49, 61, 70, 80, and 86 g/d, respectively. Nitrogen utilization improved from 34.3% without Na-caseinate supplementation to a maximum of 40.6%, with the third dose supplying 788 g/d of apparently digestible AA. Based on patterns of plasma concentrations, Met, Phe, and Arg were probably the limiting AA when animals optimized N utilization. In Exp. 3, six double-muscled BBb (315 ± 25 kg) fed the basal diet received duodenal infusions of dextrose and AA, equivalent to the third dose in Exp. 2, except for digestible Met (9.3, 14.4, 18.4, 22.4, 26.4, and 30.4 g/d) in a 6 × 6 Latin square design. The Met requirement was close to 26.4 g/d on the basis of N retention.

Key Words: Ruminants, Amino Acids, Duodenum, Requirements, Plasma, Nitrogen

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Introduction

Knowledge of metabolizable protein and essential amino acid (**EAA**) requirements is more advanced for pig and poultry nutrition, for which the theoretical notion of "ideal protein" is well-defined (Henry, 1993), than for ruminant nutrition. To reduce protein supply and N excretion into the environment, one of the objectives of nutritional research is to improve the efficiency of amino acid (**AA**) utilization by defining for ruminants a similar concept of ideal protein.

Double-muscled Belgian Blue bulls (**BBb**) are distinguished from other beef cattle by their hypermuscular development. Dressing percentage in relation to live weight averages 68% (Minet et al., 1996). The unique quality of this breed, with a high growth potential (ADG of 1.5 kg/d with a maximum of 2.1 kg/d; Gengler et al., 1995) and a greater proportion of muscle measured in the half-carcass (78.1% muscle, 7.5% fat, and 13.4% bone; Michaux et al., 1983), is likely to influence its AA requirements.

Requirements of EAA, particularly of Lys and Met, have been more thoroughly studied in dairy cattle than in beef cattle. For methodological reasons, few studies emphasize these EAA in animals bred for meat production and high ADG. The aim of our experiments was to determine for double-muscled BBb the requirement of total amino acids (**TAA**) and the first-limiting AA during the growing period. The method described by Titgemeyer et al. (1990a,b) was used and comprises three stages. The aim of the first stage was to define a low-protein diet and to measure the apparent intestinal digestibility of individual AA under these conditions. The second stage consisted of supplementing the diet with duodenal infusions of Na-caseinate to determine the optimal amount of AA that animals can use and to identify the first-limiting AA. The third stage was designed to investigate the requirement of this AA when the animals received the other AA in optimal amounts.

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Table 1. Chemical composition of the basal diet

Ingredient	% of DM
Meadow hay	30.0
Coarsely ground corn	25.2
Rolled barley	16.1
Sugar beet pulp	14.7
Ground peas	7.0
Molasses	2.8
Urea	1.0
Na-bicarbonate	1.4
Minerals and vitamins mixture[a]	1.4
Chalk	.4
	g/kg DMI
Organic matter	895
Crude protein	130
Neutral detergent fiber	343
ME, kcal/kg DMI[b]	2,666

[a]Composition (%): 8 Ca, 11 P, 3.5 Na, 2.5 Mg; (mg/kg): 40 I, 40 Co, 500 Cu, 2,850 Fe, 2,500 Mn, 3,500 Zn, 12 Se, 500 vitamin E; (IU/kg): 500,000 vitamin A, 100,000 vitamin D_3.
[b]Calculated according to the Dutch system (Van Es and Van der Honing, 1977).

Experimental Procedures

Experiment 1

Animals and Diet. Double-muscled purebred BBb (n = 3; 306 ± 28 kg), fitted with one ruminal cannula (67-mm i.d.) and two T-type cannulas at the proximal duodenum and at the terminal ileum, were individually penned (1.5 × 2.5 m) and received a low-protein diet (Table 1) at an intake level of 85 g DM/kg$^{.75}$. The diet supplied 87 g of intestinal digestible proteins and 1,762 kcal of NE per kilogram of DMI according to the Dutch system (Van Es and Van der Honing, 1977; Tamminga et al., 1994). Fresh water was available at all times. Adaptation to the diet lasted 21 d. Feed was offered twice a day in equal amounts at 0830 and 1630. A dextrose solution (360 g/d, supplying 1.5 Mcal of NE) was continuously infused through polyvinyl chloride tubing into the duodenum with peristaltic pumps. Pumps were adjusted to finish the infusions at 1030, and the solutions were changed every morning at 1100. Warm water was infused to clean the tubing for 30 min. Duodenal infusion of dextrose began 7 d before sampling. Chromic sesquioxide (3 g/kg DMI) included in alfalfa pellets was fed just before the meal to determine the flows of nutrients in the digestive tract. It was fed 7 d before sample collection and continually thereafter. Refusals were collected daily before the morning feeding.

Experimental Protocol and Sample Collection. The experiment consisted of two 13-d periods separated by 4 wk and included digesta (duodenal, ileal, and fecal) and ruminal fluid collections. Ingestion of DM was adjusted to the metabolic weight at the start of each period. Animals weighed, respectively, 281 ± 22 kg and 331 ± 30 kg at the beginning of the first and the second periods.

Urine was collected during the first 9 d of each period with the apparatus of Veenhuizen et al. (1984). During the collection, urine was acidified with 2 *N* H_2SO_4 to pH 3 at 0900, 1300, 1700, 2100, and 2400 to avoid N losses. Total urine collection was weighed every morning at 0900, sampled (approximately 100 mL), and frozen. Feces were simultaneously collected, sampled (approximately 400 g of DM), and frozen.

Duodenal and ileal samples (approximately 400 mL) were collected every 6 h on d 10 to 12 to cover 24 h by steps of 2 h. The digestible supply of N and each EAA was determined by the difference between duodenal and ileal flows. The last day of each period was devoted to ruminal fluid collection. The rumen was sampled every 2 h between 4 h before and 10 h after the morning meal.

Experiment 2

Animals and Treatments. Double-muscled purebred BBb (n = 5; 334 ± 22 kg) fitted with a T-type cannula at the proximal duodenum received the basal diet and were held in conditions similar to those in Exp. 1. Animals were weighed at the beginning of the experiment, and DMI was adjusted to their metabolic weight (85 g DM/kg$^{.75}$). Four doses of Na-caseinate were added to the dextrose infusion described in Exp. 1 to increase the supply of apparently digestible AA by 28, 56, 84, and 112% in comparison with the basal diet. They corresponded on average to duodenal infusion of 122, 244, 366, and 488 g/d of Na-caseinate. This procedure allowed weight variations of the bulls to be taken into account for the determination of protein and AA requirements. The AA composition of Na-caseinate is given in Table 2. Alfalfa pellets containing chromic sesquioxide (3 g/kg DMI) were fed 7 d before and during

Table 2. Amino acid composition of Na-caseinate

Amino acid	% of DM
Arg	3.7
His	2.6
Ile	5.5
Leu	9.7
Lys	8.3
Met	2.9
Cys	.3
Phe	5.3
Thr	4.0
Trp	1.3[a]
Val	6.8
Σ EAA[b]	50.1
Σ NEAA[c]	49.2
Σ TAA[d]	99.3

[a]Estimated from tabulated values. Armor Protéines—Lacto Bretagne Associés S.A. (Saint Brice en Cogles, France).
[b]EAA: essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).
[c]NEAA: nonessential amino acids (Ala, Asp, Cys, Glu, Pro, Ser, and Tyr).
[d]TAA: total amino acids (EAA + NEAA).

duodenal samples and feces collection. Refusals were collected daily before the morning feeding.

Experimental Protocol and Sample Collection. The experiment was a 4 × 4 Latin square design with one additional animal. Before the first and after the last period of the Latin square design, two control periods were devoted to sampling duodenal contents to measure AA flows originating from the rumen when the animals did not receive Na-caseinate. Basal diet was fed for 21 d, and dextrose was infused 7 d before the first control period to adapt the animals. Each of control periods lasted 10 d, and the periods of protein supplementation lasted 7 d.

During control periods, duodenal contents were sampled for the first 3 d, and urine and feces were collected the last 7 d as described for Exp. 1. The first day of each period of protein supplementation served to adapt the animals to the dose of Na-caseinate infused. Urine and feces were sampled for the next 6 d to measure the N retained by the bulls. Only a short adaptation time was necessary because ruminants adapt rapidly to changes in postruminal supply (Hovell et al., 1983). Blood samples were collected by jugular venipuncture into heparinized tubes (250 IU/60 mL of blood) on the last day of each period, 2 and 6 h after the morning meal. The apparently digestible supply of one AA was determined by multiplying its duodenal flow measured on each animal during the two control periods by its intestinal apparent digestibility measured during Exp. 1. Infused AA were considered entirely absorbed. The marginal net utilizations of N infused in the duodenum were obtained with the following equation:

$$\text{Net utilization (\%)} = \frac{(\text{N retained dose}_{\,i} - \text{N retained dose}_{\,i-1})}{(\text{N infused dose}_{\,i} - \text{N infused dose}_{\,i-1})} \times 100.$$

Preparation of Solutions. Infused solutions were prepared daily. Dextrose was first dissolved in 7 kg of warm water. The Na-caseinate was added slowly and mixed until a homogenous solution was obtained. The weight of each solution was accurately adjusted to 8 kg with water.

Experiment 3

Animals and Treatments. Double-muscled purebred BBb (n = 6; 315 ± 25 kg) fitted with a T-type cannula at the proximal duodenum received the same basal diet and were held in conditions similar to those in previous experiments. Animals were weighed at the beginning of the experiment, and DMI was adjusted to their metabolic weight (85 g DM/kg$^{.75}$). Six solutions were infused into the duodenum to supply dextrose (360 g/d) and a supplement equivalent to 88.0% of digestible AA with an EAA profile similar to Na-caseinate, except for Met (Table 3). An identical amount of Glu and Gly replaced all the nonessential AA (**NEAA**), except Cys, in the mixture of free AA on an equal weight basis. Alfalfa pellets containing chromic sesquioxide (3 g/kg DMI) were fed 7 d before and during duodenal samples and feces collection. Refusals were collected daily before the morning feeding.

Experimental Protocol and Sample Collection. The experiment was in a 6 × 6 Latin square design. The periods lasted 6 d and included 1 d for adaptation to treatment and 5 d for urine sampling. Feces were only collected during Treatment 1. Before the first and after the last period of the Latin square design, two extra 3-d periods were devoted to sampling duodenal contents as described in Exp. 1 to measure EAA flows originating from the rumen when the animals did not receive AA supplement. The supplies of digestible AA were calculated as in Exp. 2. Basal diet was fed for 21 d, and dextrose was infused for 7 d before the beginning of sampling to adapt animals.

Preparation of Solutions. A first mixture, containing the branched-chain AA (Val, Ile, and Leu), was dissolved in 2 L of warm water acidified with 100 mL of 6 *N* HCl. A second one, composed of Arg, Cys, Gly, His, Lys, Phe, Thr, and Trp, was added afterward. The Glu was dissolved separately in 300 mL of water containing 20.2 g of NaOH and was incorporated into the solution. The Met was then added. Acidity of the solutions was adjusted to pH 5.5 before dissolving dextrose and Na-caseinate. Weight of solutions was accurately adjusted to 8 kg. The solutions were prepared daily.

Laboratory Analyses

Concentrate, hay, alfalfa pellets, and refusals pooled per animal were ground through a 1-mm screen (Pulverisette 14; Fritsch, Idar-Oberstein, Germany) before DM, NDF, ash, and N (AOAC, 1984) analyses were conducted. Chromic sesquioxide was measured in alfalfa pellets (François et al., 1978). After freeze-drying and grinding (1 mm), DM, ash, N, and chromic sesquioxide were measured in feces. Urinary N was also analyzed (AOAC, 1984).

The pH of ruminal fluid was immediately measured (Titroprocessor 686; Metrohm, Herisau, Switzerland) after sampling and filtration (250 μm). Supernatant from 1,200 × *g* centrifugation for 10 min was frozen after H_2SO_4 acidification to pH 3 until NH_3 N determination.

Duodenal and ileal samples were freeze-dried, ground through a 1-mm screen, and pooled per animal and period before DM, ash, N, NH_3 N, and chromic sesquioxide analyses were performed. A small part was ground through a .5-mm screen for AA analysis (Cohen and Strydom, 1988). After hydrolysis (25 mg of protein sample in 15 mL of 6 *N* HCl at 110°C for 21 h) and phenylisothiocyanate derivatization, AA were separated with the Pico-Tag HPLC procedure (Waters, Milford, MA) using a C_{18} column (3.9 × 300 mm). Aminobutyric acid was used as an internal standard. Sulfur AA are partially destroyed by 6 *N* HCl hydrolysis. The Met and Cys were, therefore, separated as cysteic acid

Table 3. Composition of solutions infused during Experiment 3 and digestible Met supplement for each treatment

Treatment	Na-caseinate	Free AA	Free Met	Met supplement[b]
	— % Digestible AA[a] supplied by the diet —		——— g/d ———	
1	0	88	0	0
2	44	44	0	5.1
3	44	44	4	9.1
4	44	44	8	13.1
5	44	44	12	17.1
6	44	44	16	21.1

[a]AA: amino acid.
[b]Met contained in the Na-caseinate + free Met.

and methionine sulfone following protection with performic acid oxidation (Cohen and Strydom, 1988).

Blood was centrifuged at 2,600 × *g* for 15 min immediately after collection. Plasma was removed and frozen until further analyses. After thawing, urea was measured (Sigma kit No. 640; Sigma Chemical Co., St. Louis, MO). Plasma samples were then deproteinized using 3,000-Da ultrafiltration (Amicon Micropartition method, kit MPS No. 4010; Amicon, Beverly, MA) before the determination of free AA by HPLC (Cohen and Strydom, 1988).

Statistics

Statistical analyses of intestinal digestibility, fecal and urinary N excretion, N retention, and plasma AA concentrations were conducted with the GLM procedure of SAS (1985).

In Exp. 1, data were analyzed with ANOVA. Two effects (period and animal) were studied for apparent intestinal digestibility and N balance.

In Exp. 2, the effect of the Na-caseinate dose on N balance and plasma AA was analyzed with ANOVA for a Latin square design. Model effects were animal, treatment, and period. Least squares means were used to compensate for the unbalanced Latin square design (SAS, 1985). Differences between means were compared using the Newman-Keuls test (Dagnelie, 1986). Results for the control periods were analyzed separately with ANOVA. Model effects were animal and period.

In Exp. 3, data were analyzed with ANOVA for a Latin square design with animal, period, and treatment as model effects. Differences between means were compared using the Newman-Keuls test (Dagnelie, 1986).

Results and Discussion

Experiment 1

Optimal ruminal protein synthesis requires a pH of the liquid phase ranging between 6.0 and 7.0 and an NH_3 N concentration constantly higher than 5 mg/dL (Satter and Slyter, 1974; Dehareng and Ndibualonji, 1994a). Incorporation of Na-bicarbonate into the diet buffered the ruminal fluid and maintained its pH (6.64 ± .34) constantly higher than 6.0. On average, the NH_3 N concentration reached 12.9 mg/dL. It rose to a maximum of 35.6 ± 7.1 mg/dL 2 h after the morning meal. This content is not excessive and does not affect reticulorumen motility (Dehareng and Ndibualonji, 1994b). However, NH_3 N concentrations were lower than 5 mg/dL 6 and 8 h after the morning meal despite incorporation of ground peas into the diet, generating NH_3 N in a more continuous way in the rumen than urea. During those critical hours, N could be limiting for protein synthesis and may have led to a net transfer of N from blood to the rumen. Urea transfer is negatively correlated to NH_3 N content of the rumen and positively correlated to plasma urea concentration when the content is less than 30 mg/dL (Dehareng and Ndibualonji, 1994b). Our results show that plasma urea concentration was low and ranged between 5.3 and 7.2 mg/dL during the day, which assumes that daily N recycling in the rumen was not excessive.

The supply of apparently digestible TAA reached 67 g/kg of DMI (Table 4). In the ileal digesta and in plasma, Cys could not be measured correctly. Its digestibility varied largely following variable ileal flows, and, we think it was too low compared with other AA. Apparent intestinal digestibility of most EAA were above 70% but lower for His, Met, and Val. For His, the high content in endogenous secretions could constitute an explanation (Lobley, 1986). Values in the literature show that Met can be one of the best (Cecava and Parker, 1993) or the least (Coomer et al., 1993) digested AA.

On average, daily N ingested and excreted in urine and feces reached 124.6, 43.6, and 39.9 g/d, respectively, and differed among periods ($P < .001$). Nitrogen retention (41.1 g/d) was only influenced by the animal effect ($P < .001$). During the experimental periods, animals attained an ADG of 1.1 ± .1 kg/d, which is considered low for double-muscled BBb at the live weight studied; this fact probably reflects the deficiency of digestible AA in the basal diet. Relative to ingested and digested N, N retention reached 34.1 and 52.1%, respectively, for the first period and 32.0 and 49.7% for the second one. The slight decreases between periods are significant ($P < .01$) and are probably due to the lower N utilization by heavier animals during the second period.

Table 4. Digestible supplies and small intestinal apparent digestibility of proteins and individual amino acids with the basal diet (Exp. 1)

AA[a]	Supply of digestible AA	SE	Apparent digestibility	SE
	g/kg DMI		%	
Arg	3.94	.5	79.0	4.5
His	1.17	.2	66.2	6.7
Ile	3.74	.6	75.3	4.3
Leu	6.19	.9	74.5	4.4
Lys	7.09	1.7	78.2	6.7
Met	1.63	.5	60.1	7.0
Phe	3.55	.5	73.8	4.2
Thr	3.00	.4	67.8	5.2
Val	4.10	.8	64.8	6.8
∑ EAA[b]	34.39	5.2	72.5	5.0
∑ NEAA[c]	32.85	4.5	72.3	5.2
∑ TAA[d]	67.24	9.6	72.4	5.0
N × 6.25	78.06	9.9	65.1	2.5

[a]AA: amino acid.
[b]EAA: essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).
[c]NEAA: nonessential amino acids (Ala, Asp, Cys, Glu, Pro, Ser, and Tyr).
[d]TAA: total amino acids (EAA + NEAA).

Experiment 2

Nitrogen Balance. Nitrogen intake, excretion, and retention of animals are presented in Table 5. Nitrogen intake represents N supplied by the basal diet and by the infusions minus refusals. Unlike fecal excretion, urinary N excretion varied markedly between the two control periods. Average weights of animals were 342 and 399 kg for these periods, respectively, but intake was kept constant during the experiment. Considering a maintenance requirement of 400 mg N/kg$^{.75}$ (Ørskov and MacLeod, 1982; Hovell et al., 1983; Ørskov, 1991), the increase in live weight (+ 57 kg) during the experiment, and consequently in maintenance requirements, accounts for the entire difference in urinary excretion between the control periods.

The dose of Na-caseinate infused into the duodenum did not influence fecal N excretion but increased urinary N excretion (Table 5). Nitrogen retention increased regularly with the first three doses of Na-caseinate and more slightly with the fourth dose. The effect of Na-caseinate infusion on the utilization of N ingested was less pronounced, and no significant effect was detected for the utilization of N digested (Table 5).

The marginal net utilizations of N reached 48, 55, and 28% for dose 2 vs dose 1, dose 3 vs dose 2, and dose 4 vs dose 3, respectively. Even though dose 4 maximized the absolute N retention, the supplement of N infused into the duodenum was poorly utilized by the animals compared to dose 3. If we consider that the animal's requirement is reached with an optimal utilization of digested nutrients, such results imply that the digestible AA supply by dose 4 moved away from the requirement of BBb kept under our experimental conditions, whereas dose 3 tended to approach their requirement.

Nitrogen retention measured in this experiment is markedly higher than values from comparable studies by Titgemeyer and Merchen (1990a) using Limousin-cross steers (43 g/d) and Robinson et al. (1995) using Holstein steers (62 g/d). Our results express all the ability of the double-muscled BBb to grow rapidly and confirm the high values of N retention measured for BBb by Hornick et al. (1998), ranging from 53 to 73 g/d for rapid growth periods, and by Bogaerts et al. (1995), from 63 to 71 g/d.

The third dose supplied on average 788 g/d of apparently digestible TAA (Table 6). By comparison, Wessels and Titgemeyer (1997) estimated the metabolizable protein requirement for an ADG of 1 kg/d to be at least 526 g/d. With crossbred yearling steers, Shain et al. (1998) measured an ADG of 1.5 kg/d with a supply of 720 g/d of metabolizable proteins. In our experiment, the supply of AA corresponded to an optimal apparently digestible AA:NE ratio of 60 g of AA/Mcal of NE. This ratio must be considered as maximal because most of the EAA were supplied in excess, and only the first-limiting one could correspond to the animal's require-

Table 5. Nitrogen intake, excretion, and retention in bulls supplemented with Na-caseinate infused into the duodenum (Exp. 2)

	Control period				Na-caseinate period[a]					
Nitrogen	Period 1	Period 2	SEM	*P*	Dose 1	Dose 2	Dose 3	Dose 4	SEM	*P*
Intake, g/d	143.1	143.1	0	NS[b]	161.3[c]	179.7[d]	198.3[e]	216.2[f]	.1	.001
Fecal, g/d	42.6	43.5	.4	NS	45.7	45.5	44.9	45.3	.4	NS
Urinary, g/d	46.7[x]	55.1[y]	.5	.001	54.3[c]	64.1[d]	73.0[e]	85.4[f]	.6	.001
Retained										
g/d	53.8[x]	44.5[y]	.5	.001	61.3[c]	70.1[d]	80.4[e]	85.5[f]	.6	.001
% N ingested	37.7[x]	31.1[y]	.4	.001	38.0[c]	39.0[cd]	40.6[d]	39.6[cd]	.3	.01
% N digested	53.7[x]	44.6[y]	.5	.001	53.0	52.1	52.4	50.1	.4	NS

[a]Doses 1, 2, 3, and 4 increased, resepctively, the supply of digestible amino acids by 28, 56, 84, and 112% in comparison with the basal diet.
[b]NS: not significant ($P > .05$).
[c,d,e,f]Within a row, means lacking a common superscript letter differ ($P < .01$).
[x,y]Within a row, means lacking a common superscript letter differ ($P < .001$).

Table 6. Average supplies of digestible proteins and amino acids with duodenal infusion of Na-caseinate (Exp. 2)

AA[a]	Avg of control periods		Na-caseinate period[b]			
			Dose 1	Dose 2	Dose 3	Dose 4
	g/kg DMI	g/d				
Arg	3.87	25.7	30.2	34.7	39.2	43.8
His	1.16	7.7	10.9	14.0	17.2	20.4
Ile	3.28	21.8	28.5	35.2	41.9	48.6
Leu	5.41	35.9	47.7	59.6	71.4	83.2
Lys	7.18	47.7	57.8	68.0	78.1	88.2
Met	1.80	11.9	15.4	19.0	22.5	26.1
Phe	3.27	21.7	28.2	34.6	41.1	47.6
Thr	3.07	20.4	25.3	30.2	35.0	39.9
Val	3.78	25.1	33.4	41.7	50.0	58.3
∑ EAA[c]	32.81	217.9	277.4	337.0	396.5	456.0
∑ NEAA[d]	31.77	211.0	271.0	331.0	391.1	451.1
∑ TAA[e]	64.58	428.9	548.5	668.0	787.6	907.1
N × 6.25	80.71	536	650	764	878	992

[a]AA: amino acid.
[b]Doses 1, 2, 3, and 4 increased, respectively, the supply of digestible amino acids by 28, 56, 84, and 112% in comparison with the basal diet.
[c]EAA: essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).
[d]NEAA: nonessential amino acids (Ala, Asp, Cys, Glu, Pro, Ser, and Tyr).
[e]TAA: total amino acids (EAA + NEAA).

ment. A better AA profile of the digested protein would probably result in a reduction of the optimal supply.

With the aim of defining an adequate N supply for growing ruminants, Titgemeyer and Merchen (1990b) fed Simmental steers with a semipurified diet containing urea and Na-caseinate as the sole N sources. Treatments consisted of duodenal infusions of three Na-caseinate solutions (100, 200, and 300 g/d) with or without 12 g of L-methionine. Even though the higher dose maximized N retention (58 vs 28 g/d without infusion), the second one with Met supplement gave similar growth performances and optimized N utilization. These results confirmed that Met is often limiting when diets contain little true protein and thus microbial protein is the major source of AA available for absorption from the small intestine (Richardson and Hatfield, 1978; Storm and Ørskov, 1984).

Plasma Amino Acids. Plasma free AA represent only a low fraction of the total AA pool but are often used to estimate the protein status of animals. Even if an important part of digested AA is carried in the plasma in the form of small peptides (Seal and Parker, 1991; Koeln et al., 1993), the free AA pool gives a reasonable assessment of the difference between supplies of digestible AA and their metabolic use. It seems that AA storage by animals from one period to the next is not likely because AA removal from the plasma is so fast that their concentrations return to their initial levels as soon as the infusion of Na-caseinate is stopped (Hogan et al., 1968).

Only the plasma concentration of Arg (73 vs 89 μM), Leu (72 vs 87 μM), and Val (125 vs 161 μM) increased between the two control periods ($P < .05$). Table 7 presents the average of plasma concentration measured during these periods. For control periods and Na-caseinate periods, no difference appeared between the hours of sampling ($P > .05$).

According to Bergen (1979), an individual AA accumulates more slowly in the plasma when it is limiting that when it is supplied in excess. Compared with dose 1, plasma concentrations of Met, Phe, and Arg accumulated more slowly than other AA, with dose 3 considered as optimal on a N retention basis. This suggests that these AA were probably limiting under our experimental conditions. Many studies have mentioned that Met is the first-limiting AA when microbial protein constitutes the major source of proteins to the animal (Fenderson and Bergen, 1975; Richardson and Hatfield, 1978); Arg could also be limiting (Storm and Ørskov, 1984), but, to our knowledge, no study has indicated that Phe could be limiting in these conditions.

Plasma urea concentration was not different between control periods ($P > .05$) and increased linearly ($r^2 = .97$) with the level of protein supplementation (Table 7). Some AA were supplied in excess and were catabolized by the liver in amine groups and hydrocarbon chains. Amine groups were converted into urea and were released in the blood before urinary excretion. An inadequate AA supply contributes in this way to increased N pollution.

Ecological Aspect. Nitrogen excretion reached for the four doses of Na-caseinate, respectively, 1.63, 1.56, 1.47 and 1.53 g/g of N retained. From an ecological point of view, dose 3 was still the most beneficial and decreased the N losses by 10% in comparison with dose 1. Enhanced N utilization is due to a smaller proportion used for maintenance (relative to growth) and the capacity

Table 7. Plasma amino acids and urea concentrations with duodenal infusion of Na-caseinate (Exp. 2)

AA[a]	Avg of control periods	Na-caseinate period				SEM
		Dose 1	Dose 2	Dose 3	Dose 4	
				μM		
Arg	81	89[f]	96[fg]	94[fg]	111[g]	3.7
His	38	50[f]	60[fg]	61[g]	74[h]	2.5
Ile	64	73[f]	91[fg]	90[fg]	120[g]	3.6
Leu	80	100[f]	114[f]	124[f]	168[g]	4.8
Lys	50	62[f]	73[fg]	80[fg]	89[g]	3.5
Met	27	27[f]	36[fg]	29[fg]	38[g]	.9
Phe	42	43[f]	51[f]	46[f]	59[g]	1.5
Thr	111	91[f]	118[g]	123[g]	122[g]	5.0
Val	143	180[f]	205[fg]	233[g]	309[h]	7.8
∑ EAA[c]	636	715[f]	844[f]	880[f]	1,090[g]	27.6
∑ NEAA[d]	1,057	900	947	877	1,015	28.7
∑ TAA[e]	1,693	1,615[f]	1,792[f]	1,758[f]	2,106[g]	49.6
				mg/dL		
Urea	7.6	8.0[f]	8.6[f]	9.9[g]	11.4[h]	.2

[a]AA: amino acid.
[b]Doses 1, 2, 3, and 4 increased, respectively, the supply of digestible amino acids by 28, 56, 84, and 112% in comparison with the basal diet.
[c]EAA: essential amino acids (Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val).
[d]NEAA: nonessential amino aicds (Ala, Asp, Cys, Glu, Pro, Ser, and Tyr).
[e]TAA: total amino acids (EAA + NEAA).
[f,g,h]Within a row, means lacking a common superscript letter differ ($P < .05$).

of animals to use a well-balanced protein supplementation efficiently.

Experiment 3

Nitrogen Balance. Amounts of total digestible Met (originating from infusions, microbial, and dietary undegraded sources) reached 9.3, 14.4, 18.4, 22.4, 26.4, and 30.4 g/d for Treatments 1 to 6, respectively. The digestible supplies of other AA (g/d) were similar for all treatments and were as follows: Arg, 35.0; His, 17.2; Ile, 39.7; Leu, 71.0; Lys, 72.4; Phe, 36.7; Thr, 33.0; Val, 48.0; NEAA, 356.4. Fecal N excretion was only measured for Treatment 1 and reached 46.8 ± 3.4 g/d. It was considered independent of the Met complementation following the results obtained in the preceding stage. Nitrogen intake, urinary excretion, and retention are presented in Table 8. Treatment 1 did not contain Na-caseinate and provided a different proportion of NEAA than Treatments 2 to 6. The synthesis of all the NEAA starting from Glu and Gly may have reduced the efficacy of utilization of these AA. This could explain the important improvement in N retention between Treatments 1 and 2, together with the greater amount of digestible Met supplied between these treatments (Table 3). Urinary N excretion decreased by 20% between Treatments 1 and 5. Proportional to ingested N, urinary N losses reached 42.1, 39.5, 37.8, 36.7, 33.9, and 35.3%, respectively, for the six treatments. The supply of digestible Met, therefore, increased N utilization following a biological value improvement of the digestible protein. Treatment 5 optimized N utilization and maximized N retention. Compared with Treatment 1, the infusion of 17.1 g/d of digestible Met increased N retention by 17.1 g/d, corresponding to a better utilization of N ingested and digested of 8.5 and 11.1%, respec-

Table 8. Nitrogen intake, urinary excretion, and retention in bulls supplemented with different amounts of digestible Met (Exp. 3)

Nitrogen	Treatment[a]						SEM
	1	2	3	4	5	6	
Intake, g/d	189.1	191.2	191.6	191.8	191.5	192.0	.5
Urinary, g/d	79.3[b]	75.3[bc]	72.9[c]	70.4[c]	64.6[d]	67.3[cd]	.9
Retained							
g/d	63.0[b]	69.1[c]	71.9[cd]	74.6[cd]	80.1[d]	77.9[d]	.9
% N ingested	33.3[b]	36.1[c]	37.5[cd]	38.9[cde]	41.8[e]	40.5[de]	.5
% N digested	44.3[b]	47.9[c]	49.6[cd]	51.4[cd]	55.4[e]	53.6[de]	.6

[a]Treatments 1 to 6 supplied, respectively, 9.3, 14.4, 18.4, 22.4, 26.4, and 30.4 g/d of digestible Met.
[b,c,d,e]Within a row, means lacking a common superscript letter differ ($P < .01$).

tively. This experiment also confirms that digestible AA complements can have biological values higher than 1.0 (Storm and Ørskov, 1984; Ragland-Gray et al., 1997). Here, the infusion of 1.6 g/d of N (in Met form) improved N retention 11-fold. The explanation lies in the positive interaction between the supply of a limiting AA, Met in this case, and the utilization of whole available AA to synthesize proteins. The requirement of digestible Met is close to 26.4 ± 2.5 g/d in this study. Considering that animals weighed on average 353 kg during the experiment, the Met requirement reached 324 ± 19 mg/kg$^{.75}$. The majority of the studies quoted by Williams (1994) assessed the daily Met requirement of beef steers between 209 and 271 mg/kg$^{.75}$. The difference probably arises from the high growth rate and the hypermuscular development of double-muscled BBb. Treatment 6 supplied too much Met and did not increase N retention. From an ecological point of view, total N excretion into the environment in relation to N retention decreased by 30% with the optimal Met supplement.

For Campbell et al. (1997), Met may be used for several functions other than protein accretion, in particular as donor of methyl groups or as precursor of Cys. Even if it is an NEAA, Cys may influence the Met requirement. This could have occurred in this experiment because the duodenal flow of Cys reached 6.2 ± .7 g/d with the basal diet and the infusions supplied only 1.1 g/d because Na-caseinate is a low Cys source. If the apparent intestinal digestibility of Cys is supposed to be equivalent to that of TAA, the digestible supply of Cys would reach 5.6 g/d. If the digestibility of Cys is assessed to 59% (Campbell et al., 1997), the supply of this AA would reach 4.7 g/d and could be too low to minimize Met transulfuration. Campbell et al. (1997) reported, however, that Met requirement was near 8.4 g/d when the Cys supply reached 2.1 g/d and that supplementation of Cys did not spare Met. The proportion of Met and Cys provided in this study is, therefore, very close to that of Campbell et al. (1997).

Considering that Met represents roughly 2% of the body protein (Gabel et al., 1988), the efficacy of deposition of the infused Met reached 12.5% in this study, whereas values obtained by Campbell et al. (1997) and Titgemeyer et Merchen (1990a) were, respectively, 24 and 33%. These values confirm that Met is probably used to ends other than protein accretion. The high amounts of digestible Met supplied in this study caused, however, a reduction in its marginal utilization. For Campbell et al. (1997), animals needed 7.9 g/d of Met to retain 35 g/d of N. These values reached, respectively, 10.9 and 39 g/d for Titgemeyer and Merchen (1990a). The requirement of Met measured in their studies reached, respectively, 226 and 279 mg/g of N retained and are lower than the value observed in this study (330 mg/g of N retained), probably following higher N retention and growth potential of BBb.

Implications

Based on the digestible methionine requirement of double-muscled Belgian Blue bulls, methionine seemed to be the first-limiting amino acid when animals optimized nitrogen utilization in our experimental conditions. In the future, it would be interesting to define the requirements of more essential amino acids that animals need to attain growth performances close to practical ones (1.5 to 2.0 kg/d). Requirements of amino acids would, thereby, be more closely satisfied in order to optimized their utilization and to reduce nitrogen excretion in livestock effluents.

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