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# Comparison of Hindquarter Metabolite Uptakes in Belgian Blue Double-Muscled Bulls at Maintenance or During Fattening<sup>1</sup>

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**ABSTRACT:** Metabolism of muscle growth in the hindquarter was investigated by the arterio-venous difference (AVD) technique in Belgian Blue double-muscled type bulls at maintenance or at fattening. The bulls were fitted with an aortic ultrasonic blood flow probe and with catheters in the aorta and vena cava. They were offered a diet allowing for maintenance (MP) during a period of 15 d, at the end of which measurements were made over 3 d. Bulls were then given a fattening diet (FP) and the measurements were repeated. Arterial blood flow was approximately 1 L/min greater when the bulls were standing than when lying. Blood flow was 2 L/min higher during FP than during MP. The AVD and uptake of glucose were maximal at 1400 and 1600. Uptake of  $\alpha$ -

amino nitrogen decreased immediately after a meal. The increase in glucose from MP to FP fitted very well with the calculated energy needs for muscle growth. The AVD and uptake of  $\alpha$ -amino nitrogen, total amino acids, and total nonessential amino acids were negative during MP and positive and significantly higher during FP. There was also a significant increase in AVD and uptake of essential and branched-chain amino acids when the bulls were changed from MP to FP. When changing from maintenance to fattening, the incremental glucose and amino acid hindquarter uptake provided energy and supply for muscle protein accretion, respectively. The level of alanine transamination was also sharply reduced.

Key Words: Belgian Blue, Catheterism, Growth Rate, Metabolites

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## Introduction

The approach of muscle metabolism by arterio-venous balance was first reported by Felig (1975) in humans during exercise. However, little is known about energy metabolism for muscle growth per se. Muscle is known to catabolize the branched-chain amino acids (BCAA) as energy substrate (Odessey and Goldberg, 1979), but in ruminants the phenomenon is less efficient (Coward and Buttery, 1979). In addition, the specific role of glucose in muscle growth is unclear, as well as that of other metabolites (Eisemann et al., 1996).

Double-muscled type breeds are characterized by an exceptional muscle development, thus making the hindquarter of these animals a convenient model to study muscle metabolism. During growth, metabolite uptake is expected to provide substrates for structural development rather than to fulfill energy expenses. Hindquarter metabolism of Belgian Blue animals at

moderate growth or at fattening has already been the subject of previous work (Hornick et al., 1996). However, to point out the contribution of energy to muscle growth, hindquarter metabolism should be compared at maintenance and at fattening. Therefore, the objective of this experiment was to study changes in blood flow, glucose,  $\alpha$ -amino nitrogen (AAN), and amino acid exchange across the hindquarters of Belgian Blue double-muscled bulls during maintenance and fattening.

## Material and Methods

### Animals and Management

Four Belgian Blue bulls, double-muscled type, were used. They weighed an average of  $288 \pm 22.5$  kg and were 12 mo old at the beginning of the experiment. They were initially fattened during a period of 1 mo with a high-protein, high-energy diet based on dried sugar beet pulp supplemented with cereals, wheat bran, soybean meal, linseed meal, and minerals (Table 1). The diet was available for ad libitum consumption (Figure 1). After this first period, the bulls were fitted by surgery with a probe for blood flow measurement and with catheters for blood sampling.

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Table 1. Composition of the diets offered to bulls

Item	Period	
	MP <sup>a</sup>	FP <sup>b</sup>
Dry matter, %	87.2	87.4
Ingredient, (% DM)		
Sugar beet pulp	7.8	39.0
Barley	4.9	8.0
Maize	—	8.1
Spelt	5.3	8.0
Middlings	5.0	8.1
Soybean meal	1.8	11.4
Linseed meal	—	3.7
Molasses	1.3	3.2
Dried lucerne	8.4	—
Pelleted straw	54.5	—
Straw	9.0	9.1
Mineral mixture	1.9	1.3
Chemical analysis, g/kg of DM		
Organic matter	916.1	932.1
Crude protein	73.8	146.2
Ether extract	17.0	18.9
Acid detergent fiber	419.1	242.5
Ca	6.6	9.5
P	3.1	4.3

<sup>a</sup>Maintenance period.<sup>b</sup>Fattening period.

Feed was withdrawn 48 h and water 24 h before surgery. Bulls received  $2 \times 10^6$  units of penicillin before being anesthetized and were maintained under anesthesia with thiobarbital in a myorelaxant solution (1 g/500 mL of solution Gujatal 10%, Aesculaap, Gent, Belgium). A 30-cm incision was made perpendicular to the spine, on the left flank of the bulls recumbent on their right side. Muscles were dissected as far as the peritoneum, which was punctured to access the peritoneal cavity. The abdominal aorta and vena cava were isolated by gentle manual dissection. Polyethylene catheters (Lectro-cath, Vigon, Ecoquen, France; i.d., 1.5 mm; o.d., 2.5 mm) were introduced with the Seldinger method (Clark and Kruse, 1992) in the aorta and retrograde in the vena cava, caudal to the renal vessels. The catheters were fixed on the wall of the vessels so that a 10-cm length was in the lumen of the vessels. An ultrasonic blood flow probe (Transonic System, Ithaca, NY) was then placed around the abdominal aorta, between the aortic catheter and the renal vessels, to obtain frequent estimates of the blood flow throughout the hindlimbs. The catheters and cable of the flow probe were exteriorized near the dorsal commissure of the incision, which was then sutured. A rigid plastic plate was sutured on the skin to protect the extremity of the catheters and the cable for the probe. After surgery, the bulls received 3 kg/d of a low-energy, low-protein diet based on pelleted straw and concentrate, and they had access to straw on an ad libitum basis. This diet was formulated to allow maintenance (maintenance period, **MP**). The

catheters were filled every day with 5 mL of a .9% NaCl solution containing 200 units of heparin/mL. Between each sampling, catheters were flushed with the same solution. The sampling period at steady state began after a 12-d recovery and lasted 3 d. The bulls were then offered, following a 5-d transition period, 6 kg/d of the initial high-protein high-energy diet to restore fattening (fattening period, **FP**). Diets were offered twice daily, at 0800 and 1400, during the whole experiment. Measurements during fattening were made 7 d after the end of the transition period and also lasted 3 d. The bulls were then slaughtered. The hindquarters were removed from two bulls to separate lean meat, connective and adipose tissue, bones, and skin.

### Measurements

Feed intake was recorded daily, and live weight was measured at the beginning of the maintenance and the transition periods and at the end of the transition and the fattening periods. Measurements were taken at 0800 and subsequently every 2 h until 2000. Arterial blood flow was determined every 3 s during 10 min each time, the bull being left in a quiet environment. Blood samples (10 mL) were then withdrawn from the aorta and vena cava and collected in tubes containing fluoride oxalate. The 0800 and 1400 samples were obtained before the meal. Tubes were centrifuged immediately at  $1,550 \times g$  and  $4^\circ\text{C}$  for 20 min. Plasma was aliquoted and stored at  $-20^\circ\text{C}$ . On one occasion during MP and FP, blood flow was also recorded every second over a 10-min period on the four bulls either lying or standing, to estimate the effect of the position of the bull on blood flow. Concentrations of glucose and AAN were measured with a Technicon Autoanalyser method using the o-toluidine (Henry et al., 1974) and the trinitrobenzene sulfonate methods (Palmer and Peters, 1969), respectively. Plasma amino acids were measured on pooled samples by

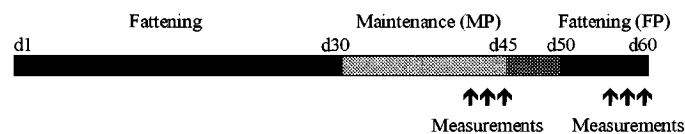


Figure 1. Illustration of the experimental design. Belgian Blue bulls were initially fattened during 1 mo. They were then implanted with catheters in the aorta and vena cava and with an ultrasonic blood flow probe. They received during 15 d a limited amount of low-energy and low-protein diet calculated to allow maintenance. The measurements were made the last 3 days of the maintenance period. After a 5-d transition period, they were accustomed again to the fattening diet, and a second set of measurements was made during the last 3 days of the fattening period, which lasted 10 d.

fused silica capillary gas chromatography using a modified method of Degrès et al. (1979). The changes to this original method were as follows: 1) the glass column was replaced with a fused silica capillary column, 25 m × .32 μm, coated with DB1 dimethylpolysiloxane, chemically bonded, providing higher inertness and stability; 2) esterification of the carboxyl group was carried out using isobutanol + acetylchloride instead of isobutanol saturated with gaseous HCl; the former technique, whereby HCl is generated in situ, was more convenient; 3) only one internal standard was used (cycloleucine), and it was added to the samples before cleanup. Because the amidic amino acids glutamine and asparagine were hydrolyzed in the acidic derivatization conditions, the concentrations of glutamate and aspartate represented the sum of the amidic and the corresponding amino acids (Glx and Asx).

Hindlimb uptake (concentration unit/minute) was calculated using the relationship  $U = ([A] - [V]) \times F \times (1 - Ht/100)$ , where  $U$  = hindquarter uptake,  $[A]$  = arterial concentration of substrate,  $[V]$  = venous concentration of substrate,  $F$  = hindquarter blood flow (L/min), and  $Ht$  = hematocrit. According to previous measurements, hematocrit was assumed to be 25% in all animals and experiments (Hornick et al., 1996). Glucose, AAN, and individual amino acids were, respectively, expressed as micromoles/liter, micromoles of nitrogen/liter, and micromoles/liter of plasma. For glucose and AAN, data relative to each sampling time were averaged over 3 d by treatment and by bulls. The averages obtained at 1000, 1200, or 1400 were compared with the corresponding data before feeding at 0800 by paired Student *t*-test. A similar procedure was used to compare data at 1600, 1800, or 2000 with values at 1400 obtained before the second meal. Overall means related to glucose, AAN, and individual amino acids were also calculated per treatment and per bulls and compared with the paired Student *t*-test.

## Results

After surgery, there was no interruption in feed intake, suggesting a quick recovery of the bulls. During MP, the bulls received a restricted amount of their particular diet. Similar amounts were previously offered to other bulls with equal live weight and were found to allow maintenance of these animals. The intake of the fattening diet during FP was limited to 6 kg/d to prevent rumen disturbances. The bulls weighed an average of 328 ± 27.8 kg at the beginning of the MP. Weight gain averaged  $-.01 \pm .14$  and  $1.05 \pm .39$  kg/d during MP and FP, respectively. The weight of the hindquarter was 27% (± .6) of the animal weight. Muscle accounted for 74.2% (± .7), bones and connective-adipose tissue for 17.4% (± .9), and skin

for 8.3% (± .3) of the hindquarter weight.

Figure 2 illustrates the second-by-second changes in hindquarter blood flow in one bull, either lying down or standing up. It shows that when the bull was standing up the blood flow was approximately 1 L/min higher than when it was lying down. The difference between the maintenance and fattening periods was about 2 L/min.

Figure 3 shows arterial concentration, venous concentration, arterio-venous difference (AVD), and uptake of glucose and AAN at the different sampling times. During MP, hindquarter blood flow remained close to 3 L/min, although it showed a decrease after the first meal ( $P < .05$ ). During FP, blood flow was higher and close to 4.5 L/min. It increased 4 h after the first meal ( $P < .1$ ) and showed a marked increase after the second meal ( $P < .05$ ). The mean cardiac frequency was 1.74 times higher during FP than during MP (105.3 vs 60.2 pulses/min,  $P < .001$ ). Even though they were small, changes were observed in the course of the day for arterial or venous concentrations of glucose at maintenance or during fattening. During MP, plasma glucose concentration was higher at 1400 than at 0800 ( $P < .1$ ). During FP, plasma glucose concentration tended to decrease 2 h after the second meal ( $P < .13$ ). Plasma glucose concentration was numerically higher in FP than in MP. Significance was reached in venous plasma at 1800 and 2000. During FP, the AVD and uptake of glucose showed a higher value at 1400 and 1600, and then they decreased ( $P < .1$ ). No treatment effect was observed for AVD, but during FP the uptake was higher at 1400 and 1600 compared to corresponding values during MP ( $P < .1$ ).

The arterial and venous AAN concentrations showed large between-bull variability. They were relatively stable during the day but decreased generally after meals, particularly in arterial blood ( $P < .1$ ;  $P < .001$ ). No differences were observed between the two periods at the different sampling times. However, arterial concentration during FP was numerically higher than during MP by contrast to observations made for venous concentration. At each sampling time, the AVD and uptake of AAN was higher in FP than during MP. Differences were significant at 1200, 1600, and 1800. The AVD and uptake decreased after the first meal ( $P < .01$  in MP and  $P < .1$  in FP). During MP, AVD and uptake were lower at 1400 than at 0800 ( $P < .05$ ).

Tables 2, 3, and 4 compare overall means related to hindquarter blood flow, arterial concentration, venous concentration, AVD and uptake of glucose, AAN, and individual amino acids during MP and FP. Hindquarter blood flow increased during FP ( $P < .001$ ). Arterial and venous plasma glucose were higher during FP than during MP ( $P < .1$ ). The AVD showed no differences, but uptake was higher during FP ( $P < .1$ ). As opposed to glucose, plasma concentrations of

Table 2. Comparison of hindquarter blood flow (HQBF), arterial (A) and venous (V) concentrations, arterio-venous difference (AVD), and uptake (U) of glucose, alpha-amino nitrogen (AAN), total amino acids (TAA), total branched-chain amino acids (BCAA), total essential amino acids (EAA), and total nonessential amino acids (NEAA) in Belgian Blue bulls, double-muscle type, at maintenance (MP) or during fattening (FP)

Item	Group		SED <sup>a</sup>	<i>P</i> < <i>F</i> <sup>b</sup>
	MP	FP		
HQBF, L/min	2.81	4.73	.09	.000
Glucose				
A <sup>c</sup>	4,298.79	4,691.69	138.55	.073
V <sup>d</sup>	4,129.85	4,540.11	145.06	.059
AVD <sup>e</sup>	168.94	151.58	26.65	.561
U <sup>f</sup>	362.45	537.19	76.85	.100
AAN				
A	3,790.59	4,049.53	405.59	.569
V	3,837.78	3,656.82	355.62	.646
AVD	-47.19	392.72	61.91	.006
U	-110.41	1,393.39	190.76	.004
TAA				
A	2,213.01	2,377.88	116.91	.253
V	2,297.08	2,126.13	132.34	.287
AVD	-84.07	251.75	46.48	.006
U	-180.36	896.53	171.05	.008
EAA				
A	868.57	839.82	64.51	.686
V	863.38	740.89	63.74	.150
AVD	5.19	98.93	10.52	.003
U	10.11	350.59	32.51	.002
BCAA				
A	469.74	510.54	19.53	.128
V	449.01	436.81	18.66	.560
AVD	20.74	73.73	17.47	.056
U	44.57	255.77	41.19	.014
NEAA				
A	1,317.88	1,502.60	81.12	.107
V	1,406.63	1,349.52	82.13	.537
AVD	-88.75	153.08	42.25	.011
U	-189.85	545.85	138.39	.013

<sup>a</sup>SED: standard error of the difference between groups.

<sup>b</sup>*P* < *F*: probability of a nonsignificant difference.

<sup>c</sup>μmol/L for glucose or amino acids and μmol N/L for AAN.

<sup>d</sup>μmol/L for glucose or amino acids and μmol N/L for AAN.

<sup>e</sup>μmol/L for glucose or amino acids and μmol N/L for AAN.

<sup>f</sup>μmol/min for glucose or amino acids and μmol N/min for AAN.

AAN did not differ between the two treatment periods, but AVD and uptake were higher during fattening than during maintenance (*P* < .01).

As observed for AAN, there was no difference in plasma total individual amino acid (TAA) concentration between MP and FP, but AVD and uptake differed (*P* < .01). Similar conclusions were found for essential amino acids (EAA), BCAA, or nonessential amino acids (NEAA). The AVD and uptake of TAA was negative during MP and positive during FP (*P* < .01). Similar results were observed with NEAA. The AVD and uptake of EAA and BCAA were positive in both periods, but during MP statistical significance was not reached for EAA.

Table 3. Comparison of arterial (A) and venous (V) concentrations, arterio-venous difference (AVD) and uptake (U) of individual essential amino acids in Belgian Blue bulls, double-muscle type, at maintenance (MP) or during fattening (FP)

Amino acid	Group		SED <sup>a</sup>	<i>P</i> < <i>F</i> <sup>b</sup>
	MP	FP		
Val				
A <sup>c</sup>	235.35	260.78	11.21	.108
V <sup>d</sup>	217.36	230.39	9.79	.276
AVD <sup>e</sup>	17.99	30.39	12.62	.398
U <sup>f</sup>	38.61	104.31	28.38	.104
Thr				
A	99.16	85.22	10.32	.270
V	101.87	73.04	13.2	.117
AVD	-2.72	12.18	4.92	.056
U	-6.17	43.17	17.09	.063
Leu				
A	127.23	127.94	6.80	.923
V	137.30	102.21	13.42	.079
AVD	-10.08	25.73	7.82	.020
U	-22.10	90.72	22.34	.015
Ile				
A	107.16	121.83	6.57	.112
V	94.34	104.21	11.52	.455
AVD	12.82	17.61	11.27	.700
U	28.06	60.74	32.68	.391
Met				
A	26.56	35.46	5.46	.201
V	27.07	35.72	5.39	.207
AVD	-.51	-.26	.91	.800
U	-.63	.09	3.93	.867
Phe				
A	94.36	86.20	13.14	.579
V	94.52	72.83	10.77	.138
AVD	-.17	13.37	11.55	.326
U	-2.01	47.42	25.91	.153
Lys				
A	120.61	90.13	11.40	.076
V	127.06	81.71	22.60	.138
AVD	-6.45	8.42	11.96	.302
U	-13.75	30.92	42.62	.372
Arg				
A	84.71	67.74	17.41	.402
V	90.91	76.50	15.41	.419
AVD	-6.20	-8.77	6.20	.707
U	-12.52	-26.69	23.76	.593

<sup>a</sup>SED: standard error of the difference between groups.

<sup>b</sup>*P* < *F*: probability of a difference.

<sup>c</sup>μmol/L.

<sup>d</sup>μmol/L.

<sup>e</sup>μmol/L.

<sup>f</sup>μmol/min.

The treatment effects were different with regard to individual amino acids (Tables 3 and 4). Higher venous plasma concentration of Ala and Leu and higher arterial plasma concentration of Lys were found during MP (*P* < .05; *P* < .1; *P* < .1). Other amino acids showed higher concentrations during FP: Gly, Ser, Asx, Glx, and Tyr in arterial plasma and Asx and Glx in venous plasma. Individual BCAA Ile and Val had positive AVD and uptake in both periods. The AVD and uptake of Leu were negative during MP and

Table 4. Comparison of arterial (A) and venous (V) concentrations, arterio-venous difference (AVD) and uptake (U) of individual nonessential amino acids in Belgian Blue bulls, double-muscle type, at maintenance (MP) or during fattening (FP)

Amino acid	Group		SED <sup>a</sup>	<i>P</i> < <i>F</i> <sup>b</sup>
	MP	FP		
Ala				
A <sup>c</sup>	222.83	183.90	26.91	.244
V <sup>d</sup>	271.57	137.52	28.78	.019
AVD <sup>e</sup>	-48.74	46.38	10.30	.003
U <sup>f</sup>	-104.35	165.13	27.15	.002
Gly				
A	339.31	474.11	41.45	.047
V	381.52	413.09	36.19	.447
AVD	-42.21	61.02	8.23	.001
U	-90.60	216.51	22.09	.000
Ser				
A	91.19	121.48	7.71	.029
V	88.26	92.57	7.09	.586
AVD	2.93	28.91	2.95	.003
U	5.95	103.43	12.02	.004
Pro				
A	77.48	78.50	6.23	.881
V	90.70	68.44	13.02	.190
AVD	-13.22	10.06	9.13	.084
U	-28.85	36.15	22.94	.066
Hpr				
A	58.84	59.54	5.11	.900
V	65.04	70.21	6.36	.476
AVD	-6.20	-10.67	4.88	.428
U	-13.28	-36.28	12.18	.156
Asx <sup>g</sup>				
A	50.48	69.13	2.44	.005
V	48.69	56.29	2.58	.060
AVD	1.79	12.85	4.32	.083
U	3.98	45.11	8.99	.020
Orn				
A	90.97	90.03	1.40	.549
V	84.02	92.03	11.97	.551
AVD	6.95	-2.00	11.33	.487
U	15.25	-4.82	41.22	.660
Glx <sup>h</sup>				
A	331.17	357.87	9.43	.066
V	315.16	359.16	17.81	.090
AVD	16.01	-1.29	14.69	.324
U	35.12	-7.55	47.18	.433
Tyr				
A	55.61	68.04	4.69	.077
V	61.68	60.23	7.24	.854
AVD	-6.07	7.81	2.62	.013
U	-13.07	28.18	6.41	.008

<sup>a</sup>SED: standard error of the difference between groups.

<sup>b</sup>*P* < *F*: probability of a difference.

<sup>c</sup>μmol/L.

<sup>d</sup>μmol/L.

<sup>e</sup>μmol/L.

<sup>f</sup>μmol/min.

<sup>g</sup>Asx = Asp + Asn.

<sup>h</sup>Glx = Glu + Gln.

positive during MP. Only Leu showed a significantly higher uptake during FP than during MP. Most of the other amino acids had AVD and uptakes close to zero during MP, and they were significantly positive during

FP. Differences between periods were observed for Thr (*P* < .1), Ser (*P* < .01), Pro (*P* < .1), Asx (*P* < .05), and Tyr (*P* < .01). The AVD and uptake of Hpr and Arg were negative in both periods. During FP, Orn and Glx had AVD and uptake close to zero and numerically lower than during MP.

Figure 4 shows the differences of individual amino acid uptake between FP and MP, expressed on the concentration of the corresponding amino acids in muscle dry matter. Alanine and Gly showed the greatest values, close to .55 and .66 g DM/min, respectively. Among the EAA, Leu and Phe showed the highest significantly positive value at .21 and .22, respectively. Methionine showed a value close to 0 g DM/min, and Arg and Glu had nonsignificant negative values (-0.05 and -0.11 g MS/min, respectively).

## Discussion

The animal performances during MP were in agreement with values expected from previous experiments (unpublished results). The 1.05 kg/d growth during FP was lower than the 1.45 kg/d reported by Minet et al. (1996) for 129 Belgian Blue fattening bulls. Limited amount of diet offered during fattening explained the lower performances in the present experiment. Nevertheless, there were large differences between MP and FP. Dissection of hindquarters showed a high proportion of muscle, indicating that the Belgian Blue bull of the double-muscle type is a good model for studies of hindquarter muscle metabolism (Hornick et al., 1996), as compared to other breeds (Bell et al., 1976; Boisclair et al., 1993). In such a model, the interference of the nonmuscular tissues on metabolite uptake measured in abdominal large vessels was strongly reduced.

Plasma is often used as a vector for measuring the concentration of metabolites in blood samples (Bergman and Heitmann, 1978). However, red cells have been recognized to be involved in transport of some amino acids (Heitmann and Bergman, 1980b; McCormick and Webb, 1982), leading to a possible bias in the interpretation of the AVD or uptakes.

Care must be taken when interpreting estimates of blood flow across the hindquarter, because not only dietary factors but also posture and time influence the results. Evolution of blood flow showed cyclic variations with shorter (about 30 s) and longer (about 5 min) periods, particularly during the fattening period. Short variations are probably related to the kinetics of rumen motility, but the reasons for longer periods of variation are unclear. Despite that, it is clear that the longer the measurement period, the more accurate the estimation of the blood flow.

The lower and more stable blood flow, at 2.8 L/min, during MP is explained by a reduced basal metabolism of the bulls during this period. At FP, blood flow

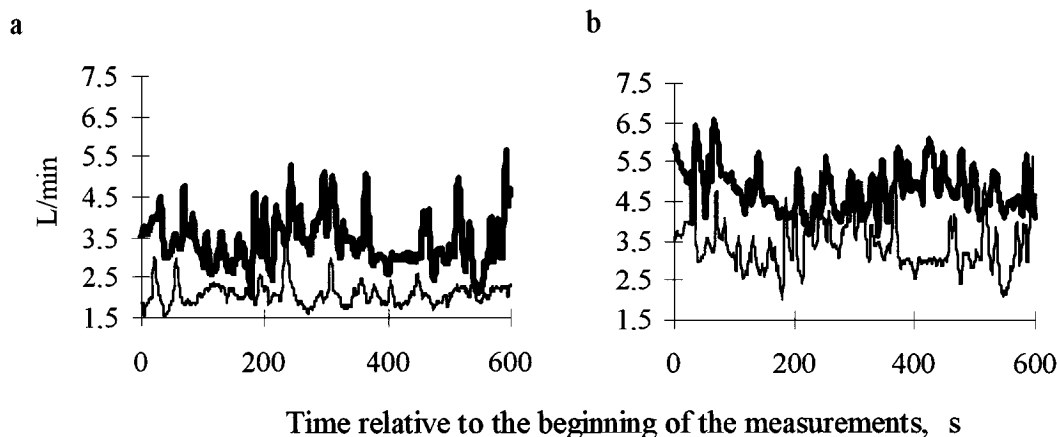


Figure 2. Changes in hindquarter blood flow in one bull. Measurements were made every second during 10 min at maintenance (thin lines) and fattening (thick lines) with the bull either lying (a) or standing (b).

increased at values close to 4.7 L/min, reflecting a higher metabolism and a higher cardiac output (Eisemann et al., 1988), as assessed by the higher cardiac frequency in FP than in MP. Blood flow also increased after meals, probably in response to higher requirements for metabolism of digestion. Similar effects of feeding on hindleg blood flow in steers or sheep have been reported by others (Early et al., 1987; Harris et al., 1992; Boisclair et al., 1993). These effects are not limited to muscle but are also seen in tissues such as skin (Harris et al., 1994).

The decrease of plasma glucose and, to some extent, of plasma AAN after the second meal reflects a response to insulin secretion, which is triggered, according to Istasse et al. (1987), by the effect of propionic acid on the pancreas. The reason why this decrease was not observed after the first meal was not clear, but it was probably related to the kinetics of rumen fermentation, which was more intense after the second meal (Mayombo et al., 1997). The higher overall mean glucose concentration during fattening resulted from the correspondingly larger energy intake. The highest AVD and uptake of glucose observed at 1400 and 1600 could also be ascribed to the secretion of insulin at this time and to the subsequent stimulation of the incorporation of nutrients by muscle. The higher blood flow during FP was probably responsible for the higher mean uptake during FP with respect to MP. These data suggest that the activity or the number of insulin-sensitive glucose transporters was increased during the fattening period (Hocquette and Balage, 1996). The absorption of other metabolites such as acetate, lactate, or  $\beta$ -hydroxybutyrate was probably also improved after refeeding because they provide a large part of energy to muscle (Coward and Buttery, 1982; Boisclair et al., 1993). From these results it was possible to estimate the requirements of glucose for the growth process, and particularly for protein deposition, because during

refeeding of young cattle tissue deposition is almost exclusively composed of protein (Wright and Russel, 1991). Indeed, 3 KJ are provided by the oxidation of 1 mmol of glucose in aerobic cellular respiration. Moreover, the energy cost for protein synthesis, especially for peptide bond formation, is approximately 4.48 KJ/g of protein synthesized (Buttery and Annison, 1973; Millward and Garlick, 1976; Webster et al., 1978). Assuming that energy consumption for postural needs was similar during the two periods and that efficiency of muscle protein deposition was close to 38%, as reported by Van Eenaeme et al. (1989) in Belgian Blue bulls, the 175  $\mu$ mol/min increase in uptake of glucose, observed between MP and FP, allowed a protein synthesis of 169 g, or a protein accretion of 64.2 g. Such a value corresponds to a hindquarter muscle deposition of about 292 g, on the basis of a 25% dry matter content in muscle and an 88% protein content in muscle dry matter in Belgian Blue bulls (Minet et al., 1996). Assuming a body development similar to that of the hindquarter, these 292 g corresponded to a whole-animal muscle ADG close to 1,081 g/d, the hindquarter representing 27% of the live weight, as reported above. In these conditions, the increase in glucose uptake between fattening and maintenance fitted very well with the energy needed for the incremental protein synthesis. However, glucose is not necessarily completely oxidized in muscle. Ortigues et al. (1996) reported that, in calves, about 50% of the glucose uptake may be converted into lactate and exported from muscle. Moreover, increased availability of plasma acetate following refeeding may interfere with oxidation of glucose (Pethick and Vernau, 1984). In contrast, recent work from Eisemann et al. (1996) showed that lactate release from the hindquarter in beef steers was negligible compared to glucose uptake. Some reports also emphasize the importance of glucose metabolism for growth in double-musled cattle (Picard et al., 1994; Hoc-

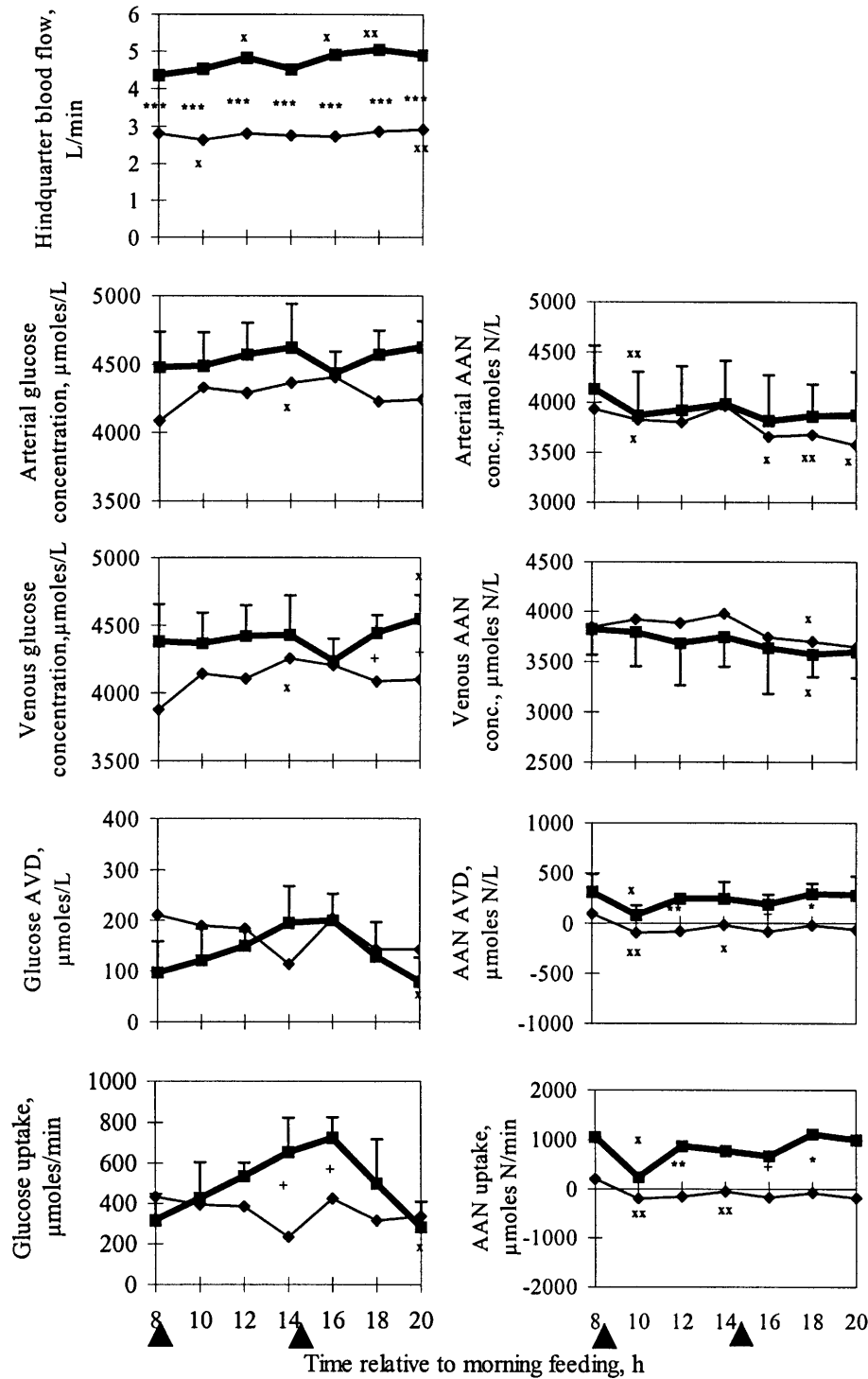


Figure 3. Changes in hindquarter blood flow, arterial and venous concentration, arterio-venous differences (AVD), and uptake of glucose and alpha-amino nitrogen (AAN) at 2-h intervals from 0800 to 2000 in Belgian Blue double-musced bulls at maintenance (♦) or during fattening (■). Arrows indicate the feeding time. +, \*, \*\*, \*\*\*: differences between periods ( $P < .1$ ,  $P < .05$ ,  $P < .01$ ,  $P < .001$ ); x, xx: differences with values at previous meal ( $P < .1$ ,  $P < .05$ ). Bars indicate the standard error of the difference between groups.

quette and Balage, 1996); muscles of these animals are characterized by a higher proportion of glycolytic fibers, which are dependent on metabolism of glucose as an energy source. In such conditions, increased glucose uptake following refeeding could be a major

source of energy for growth, even if glucose is not degraded by the aerobic pathway.

As opposed to glucose, the arterial and venous plasma AAN concentrations were similar between periods, indicating a homeostasis of the concentration

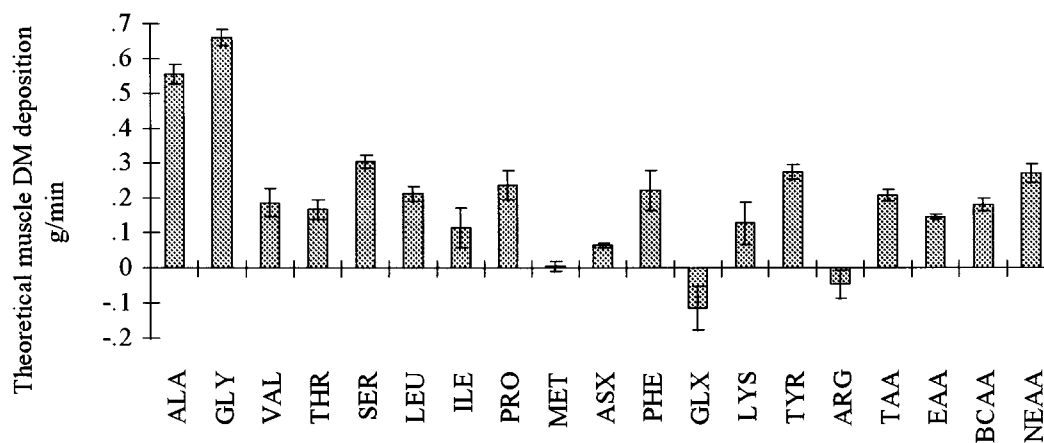


Figure 4. Changes in individual amino acid uptake between the fattening and the maintenance period, related to the concentration of the corresponding amino acids in muscle dry matter of fattening bulls. Bars indicate the standard error of the mean.

of these metabolites in cattle. A decline or trend for a decline in AAN plasma concentration observed after the first and second meal in arterial plasma and after the second meal in venous plasma is attributed to an input of AAN into cells. After the first meal, the trend for an increase in venous plasma AAN concentration during MP may be related to a muscle-liver nitrogen transfer of amino acids such as alanine or glutamine for urea and glucose synthesis by the liver. This hypothesis was supported by the decrease of AVD and uptake of AAN during this period. It indicates that amino acids were deaminated to supply energy, when dietary energy was not yet available for muscle metabolism. During the MP and FP, significant decreases of AVD and uptake were observed after the first meal, but not after the second. The period following the second meal was thus characterized by improved anabolic processes. This assumption was in agreement with higher glucose uptake observed around the second feeding.

Alanine and Gly are implicated in nitrogen transport from muscle to liver (Felig, 1975; Heitman and Bergman, 1980b; Coward and Buttery, 1982). The Ala concentration was significantly higher in venous plasma during MP. Such an increase could thus be related to the transamination of amino acids in muscle with subsequent secretion of Ala. In a previous experiment (Hornick et al., 1996), we failed to find a difference in Ala plasma levels between animals maintained under a low growth rate and fattening animals. This was probably due to a lower difference in growth rate than in the present experiment. Surprisingly, McCormick and Webb (1982) reported a decrease in plasma arterial Ala of feed-deprived Holstein steers compared with steers in steady state. A possible explanation might be that arterial plasma concentration is partly the reflection of the activity of the liver, which removes glucogenic amino acids from

blood. The higher plasma Gly concentration during FP was in line with our previous results (Hornick et al., 1996) but not with results of Bergman and Heitmann (1978) or McCormick and Webb (1982). However, their results compared fed and 3-d feed-deprived ruminants in a catabolic state. In our experiment, the increase in plasma Gly at fattening stemmed partly from higher Gly levels in the diet. The role of Ala and Gly as transporters of nitrogen was supported by the negative AVD and uptake during MP. By contrast, the important positive uptake during FP has to be associated with increased requirements for muscle protein synthesis at fattening. The large increase in Gly uptake must also be related to requirements for collagen synthesis. Similarly, most of the amino acids showed higher uptakes at fattening than at maintenance, so that uptake of TAA was largely positive at fattening but negative at maintenance. This latter value, similar to that of AAN, was weak and fitted well with the observed ADG, close to 0 kg/d. The negative uptake during MP was almost completely associated with NEAA, especially Ala and Gly, and EAA had an uptake close to 0. So, during maintenance, most of the amino acid secretion was related to Ala and Gly, and EAA were absorbed or secreted in equal amounts. The BCAA represent a significant source of nitrogen for muscle Ala or Gly synthesis during maintenance (Lindsay and Buttery, 1980); this was in agreement with the largest uptake of BCAA at this period. The only NEAA to be significantly absorbed during MP was Glx. Glutamine is known to be potential receptor of nitrogen in muscle (Goldberg and Chang, 1978). However, in this experiment, no speculation can be made because Glu could not be differentiated from Gln. During FP, the AVD or uptake of BCAA was about 3/4 of the uptake of EAA, which is higher than the BCAA:EAA ratio found in muscle. This indicates that the transamination

process was carried on but was not detectable by Ala or Gly excretion because these amino acids were also needed for muscle synthesis. It was previously reported that BCAA hindlimb uptake in Belgian Blue bulls accounted for the largest part of the EAA uptake during fattening and was even higher during low growth (Hornick et al., 1996). Such observations would imply that double-muscled animals of the Belgian Blue breed are able to transaminate BCAA more extensively than other breeds (Early et al., 1987). However, the fate of the BCAA could not be ascertained in this experiment because neither branched-chain keto acids (**BCKA**) nor their products of metabolism were measured. Weijs et al. (1993) reported in rats that growth decreased the catabolism of Leu. When offered high levels of protein in diet, the catabolism of Leu increased in absolute value but decreased as a percentage when related to protein supply. In this experiment, Leu uptake increased between MP and FP. Fattening increased absolute Leu oxidation, although the relative rate of oxidation was decreased, leaving more Leu available for muscle protein synthesis.

The increase in amino acid uptake between treatment periods expressed on the corresponding amino acid concentration in muscle does not take into account the requirements for maintenance, thus allowing a suitable comparison of the individual amino acid requirements for growth. Methionine had the lowest ratio, indicating that this amino acid could be the rate-limiting step for protein synthesis. However, these results must be taken with care, because the accuracy of the measurement of the S-containing amino acids was not optimal by our technique. The negative uptake of Arg during both periods and the negative ratio were surprising because this essential amino acid is well represented in muscle protein but are in agreement with previous reports of Van Eenaeme et al. (1995). Arginine is not considered strictly indispensable in mammals and could be synthesized in muscle and released in plasma; this would support the pathway of urea synthesis in liver (Heitmann and Bergman, 1980a). The twice-higher positive ratios for Ala and Gly compared to other amino acids are of interest because they indicate that not only the transamination process observed during maintenance decreased drastically during the FP but also that Ala and Gly were incorporated into muscle for protein accretion, as assessed by their positive uptake at FP. Thus, the shift from a net amino acid catabolism to protein accretion had to be matched by a direct recycling of amino acids released by muscle protein turnover. However, the higher ratio related to NEAA than to EAA indicated that the requirements for the former amino acids for growth are higher than those of the latter. It is suggested also that amino acids were still partly used as energy substrate for incremental growth.

Tyrosine and Phe are neither synthesized nor degraded in skeletal muscle and connective tissue (Coward and Buttery, 1982; Harris et al., 1992; Boisclair et al., 1993), and thus their uptake should reflect net protein deposition. Based on this assumption, the 47.42 and 28.18  $\mu\text{mol}/\text{min}$  uptake, respectively, for Phe and Tyr during FP gave similar estimates of hindquarter muscle deposition (respectively, 1.22 and 1.07 kg/d), assuming a 25% DM content in muscle and concentrations of 223 and 151 mmol/kg muscle DM, respectively, for Phe and Tyr. Estimates of protein deposition from AAN and TAA uptake were more in agreement with the observed body weight gains. They assumed a conversion factor of nitrogen to protein equal to 6.25, or the conversion of TAA uptake in proteins from the individual molecular weight of the amino acids. In such conditions, and on the basis of muscle composition reported above, the 1,393.39  $\mu\text{mol}/\text{min}$  uptake of AAN and the 896.5  $\mu\text{moles}/\text{min}$  uptake of TAA during FP corresponded to 176 and 133 g/d hindquarter protein deposition, or 798 and 605 g/d in terms of muscle deposition. The latter value was probably lower because all amino acids have not been measured by chromatography. Furthermore, the colorimetric method for AAN measurement was less accurate and may have overestimated the real uptake of AAN. The large difference between estimates from Phe and Tyr uptake and from TAA and AAN uptake suggests a catabolism of these amino acids in muscle; however, this hypothesis does not agree with the previous assumption. Experiments that led to the conclusion that these amino acids were not degraded in muscle were conducted *in vitro* with muscle from rats (Chang and Goldberg, 1978). Muscles of fattening bulls may behave differently, but this needs confirmation. Nevertheless, all calculated values of protein deposition were higher than what was expected from the observed ADG. The difference may be explained by an overestimation of hindquarter blood flow, by the exportation of a non- $\alpha$ -amino source of nitrogen, or by the contribution of vectors other than plasma. For example, red cells or peptides are also subject to amino acid flux (McCormick and Webb, 1982) and may bias results. Moreover, bone marrow is still active in young bulls and produces red cells continuously, which are exported from hindquarter and further degraded in the liver and in the spleen, thus leading to an unmeasured exportation of nitrogen, which might be important. If so, sources of energy for this "extra" protein synthesis are needed, because glucose uptake was found to provide energy to ensure only growth. Energy required for protein synthesis is provided by oxidation of other substrates such as acetate, lactate, nonesterified fatty acids, or 3-hydroxybutyrate (Coward and Buttery, 1982; Boisclair et al., 1993).

During maintenance, glucose was probably removed from blood for energy maintenance and also to support

the transamination process, as a source of pyruvate. It thus allowed the export of glucogenic amino acids and BCKA, which were further removed from blood by the liver. During fattening, the increased glucose uptake probably accounted to a large extent for energy needed for growth. Transamination was carried on, but all processes were mainly directed toward muscle growth.

### Implications

Study of hinqarter metabolism in Belgian blue bulls showed the importance of long-term measurement periods of blood flow through limbs. During maintenance, branched-chain amino acids are largely used for energy purposes, and during fattening, amino acids are recycled for muscle protein synthesis and glucose is used as an energy substrate. Methionine could be the most limiting amino acid for growth. When two meals are offered daily, nutrient uptake is optimized at the second meal. Further investigations are needed to elaborate an energetic model and the respective contribution of other nutrients to muscle growth in double-musled cattle.

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