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MUSCLE PROTEIN METABOLISM IN RELATION TO GROWTH RATE IN DOUBLE MUSCLED BELGIAN BLUE BULLS: AN INTEGRATED APPROACH

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ABSTRACT

Muscle protein (MP) metabolism in Belgian Blue double muscled (BBDM) bulls, was studied using two approaches: a long term fattening trial and a more physiological short term hindlimb catheterization experiment. In both experiments the bulls were subjected to two growth rates intensities: initially zero growth (maintenance period, MP) followed by a normal fattening period (FP) during which compensatory growth occurred.

In the fattening trial 4 control BBDM bulls (CG) were compared to 4 others maintained during about 60 days on a zero growth rate followed by a fast growth normal fattening period until slaughter. During both periods animal performances, N balance and urinary 3 methylhistidine (3MH) excretion, a non invasive marker of in vivo MP turnover, were measured.

In the physiological experiment 4 young bulls were fitted by surgery with an ultrasonic probe for measuring blood flow and with abdominal aorta and vena cava catheters for measuring arterio venous differences (AVD) and uptakes rates accross the hindlimb for glucose and individual amino acids (AA). After an initial fattening period of 1 month the animals were given a low protein low energy diet for 15 days and blood was sampled over a 12 hours period during the last 3 days of this period. After a 5 days transition period they were accustomed again to the fattening diet and the second measurements were made during the 3 final days of the fattening period which lasted 10 days.

In both experiments growth rate was close to zero during the maintenance period with some transient negative periods as N balance was slightly negative. Refeeding an adequate diet increased growth rate to 1.3-1.4 kg/d in the fattening trial and to about 1 kg/d in the catheterization experiment. N balance and MP turnover, hindlimb blood flow and uptakes of glucose and amino N increased upon changing to rapid growth. Uptakes of several individual amino acid were negative during MP and increased in compensatory growth. Apparently, a non negligible fraction of AA is used for energy requirements of muscle during MP, while in FP these requirements are largely met by glucose, leaving the AA for muscle protein synthesis.

INTRODUCTION

Beef meat production is essentially skeletal muscle protein (SMP) deposition in farm animals. The Belgian Blue breed, double muscled type (BBDM), is famous for its large muscle mass and high meat accretion yield. This skeletal muscle protein accretion is the net result of synthesis and degradation rates of muscle protein, the whole process being a continuous turnover. The mechanisms and the regulatory factors governing muscle protein turnover and hence SMP deposition are still incompletely understood.

The aim of this work was to study muscle protein (MP) metabolism in Belgian Blue double muscled (BBDM) bulls, using two approaches: a long term fattening trial, in which in vivo MP turnover was studied at whole animal level, and a more physiological short term experiment using hindlimb catheterization, whereby MP metabolism was examined at muscle tissue level. In both experiments the bulls were subjected to two very different growth rate intensities: initially zero growth

(maintenance period, MP) followed by a normal fattening period (FP) during which rapid compensatory growth (CoG) occurred.

MATERIAL AND METHODS

Animals and diets

In the *fattening trial*, 4 control BRDM bulls (CG), maintained all time on a conventional fattening diet (14.6 % crude protein, CP, and 12 MJ metabolisable energy, ME), based on dried sugar beet pulp and a protein concentrate, were compared to 4 others which were, after an initial fattening period maintained during about 60 days on a zero growth rate followed by a fast growth normal fattening period (compensatory growth, CoG) until slaughter. During the zero growth rate period (maintenance period) the bulls were given a low energy, low protein diet, rich in straw (7.4 % CP, 8 MJ ME).The whole experiment the bulls were kept on metabolic stalls.
In the *physiological experiment* 4 young bulls were fitted by surgery with an ultrasonic probe for measuring blood flow and with abdominal aorta and vena cava catheters for measuring arterio venous differences (AVD) and nutrient uptake rates accross the hindlimb. After an initial fattening period of 1 month the animals were given the low protein, low energy diet for 15 days. After a 5 days transition period, the bulls were accustomed again to the fattening diet for a fattening period of 10 days.

Measurements

In the *fattening experiment*, animal performances, N balance, urinary 3 methylhistidine (3MH) excretion, a non invasive marker of in vivo MP turnover, were measured during both periods. At slaughter, slaughter and carcass weights as well as dressing rates were recorded. Carcass composition was obtained using the three rib cut technique (Martin and Torreele, 1962).
In the short term *physiological experiment* arterio venous differences (AVD) and uptake rates accross the hindlimb for glucose and individual amino acids were measured in blood samples obtained over a 12 hours period during the last 3 days of the zero growth period. The second series of measurements was made on the last 3 days of the compensatory fattening period.
During each collection period blood flow was measured every 3 seconds during 10 minutes.

Assumptions

In the fattening experiment, in vivo MP turnover was estimated from N balance and urinary 3 methylhistidine (3MH) excretion assuming:

1) MP Accretion (MPA) = retained N (in g/d) * 6.25
2) MP Degradation (MPD) = urinary 3MH excretion (µmol/d)/3.51
3) MP Synthesis (MPS) = MPA + MPD

Analysis:

3MH was measured by GC2 and N by an automated Kjeldahl method (Autoanalyzer)

RESULTS AND DISCUSSION

Zootechnical performances

Zootechnical performances are reported in Table 1.
In both experiments growth rate was around zero during the maintenance period with some transient negative periods, as N balance was slightly negative. Refeeding an adequate diet increased growth rate to 1.3-1.4 kg/d in the fattening trial, although the difference with CG was not significant. In the catheterization experiment daily growth increased only to 1 kg/d. However, in this experiment, intake was lowered to 6 kg/d in order to avoid rumen disturbances. The higher growth rate in CoG, however, was obtained at the expense of a non proportional increase in feed intake as the feed conversion ratio rose from 6.18 for GC to 7.63 kg/kg growth in CoG.

Table 1 *Zootechnical Performances observed during maintenance and compensatory growth periods in Belgian blue double muscled bulls during a fattening and a catheterization experiment*

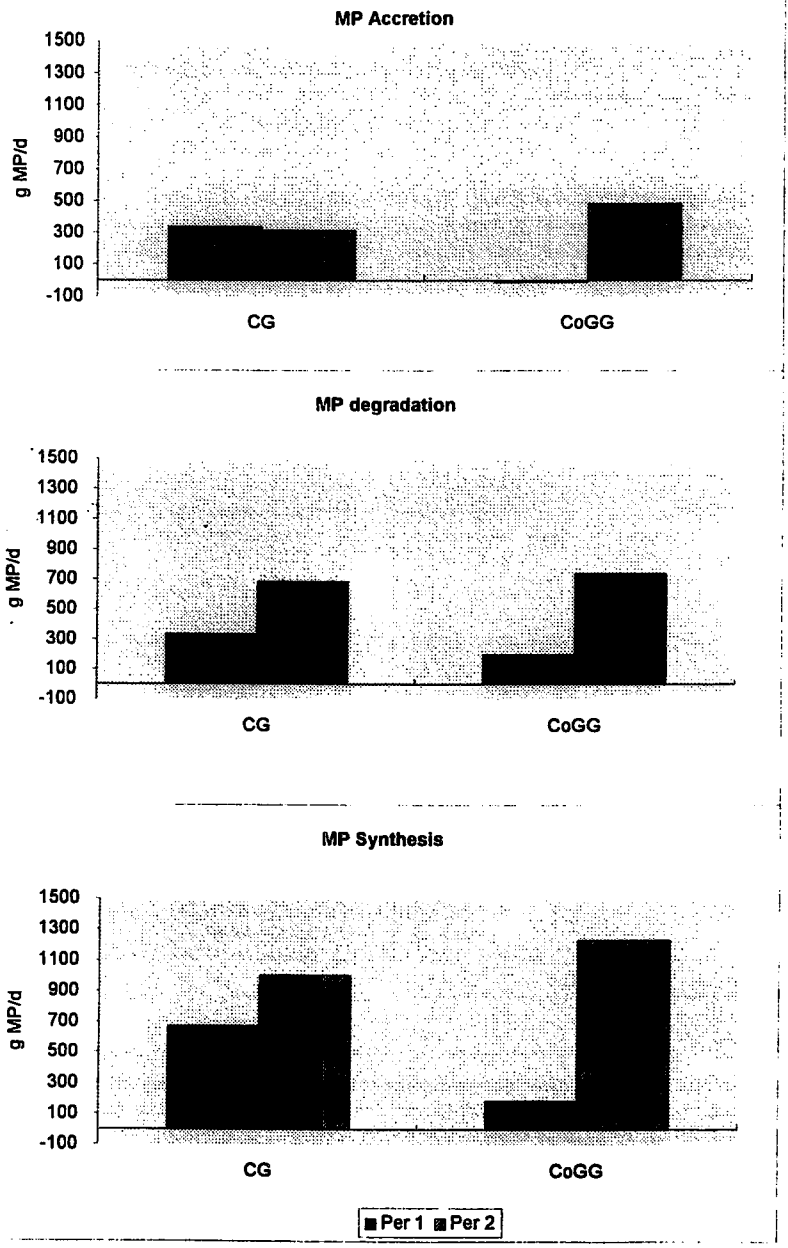
Parameter	*Fattening Experiment*				*Catheterization Experiment*	
	CG		CoG			
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Number of bulls	4		4		4	
Maintenance Period						1.
Initial weight kg			413.5	10.3	328	13.9
Final weight kg			400.2	11.0	328	13.7
Weight gain kg			-13.3	2.1	-	
Duration d			64		15	
Daily growth kg/d			-.2	0.03	-0.01	0.070
Feed intake kg			250.5	0	45	
Feed conversion kg/kg			-21.2	2.79		
Fattening period						
Initial weight kg	295	17.0	400.2	11.0	328	13.7
Final weight kg	589.7	10.25	587.3	28.9	343	9.3
Weight gain kg	294.7	24.7	187.1	21.6	15.7	2.9
Duration d	237		143.8	3.2	15	
Daily growth kg/d	1.24	0.1	1.34	0.18	1.05	0.19
Feed intake kg	1790.9	107	1381.3	14.6	90	0
Feed conversion kg/kg	6.18	0.51	7.63	1.07	6.7	1.02
Slaughter data						
Dressing rate	62.7	1.3	62.9	0.8		
% Muscle	74.05	0.7	74.67	1.04		
% Fat	13.59	1.09	13.04	0.92		
% Bone	12.37	0.29	12.29	0.31		

CG : control group; CoG : compensatory growth group; S.E.: standard error

In vivo MP Turnover

Rates of MP accretion, degradation and synthesis, obtained under the assumptions mentioned above, on a g MP per day base ("absolute rates") are depicted in figure 1. The corresponding "fractional rates" calculated on a total MP pool base are reported and commented hereafter in the text.
In the control group MP accretion rate decreased in time as the animals grew older, slightly on absolute base but more pronounced on a fractional base, e.g.from 339 to 317 gMP per day or from 0.92 to .62 % as Kg. This decrease in MP deposition resulted from increases in both synthesis and degradation rates, the extent of the increase of the latter being more important than that of the former : degradation doubled from 335 to 684 ($P<.05$) while synthesis only increased from 674 to 1001 g MP (+ 50%) per day ($P<.05$).
During the period of restricted feed intake, growth rate and consequently also MP accretion rate was negative: -14 g MP/d , and lower than in the control group ($P<.001$). MP synthesis and degradation rates were also lower : 201 vs 335 and 187 vs 674 g MP/d ($P<.001$), respectively for degradation and synthesis rates. Upon refeeding adequately, accretion rate rose from -14 to 483 g MP/d during the compensatory growth period ($P<.001$). Both synthesis and degradation rates increased and were higher than in the control group. Synthesis however, increased more than degradation, both on an absolute (+ 1043 vs + 537 g MP/d, $P<.001$) as on a fractional rate base (+ 2.52 % for Ks, vs + 1.29 % for Kd) from periods 1 to 2. The ratio of fractional rates of accretion (Kg) and synthesis (Ks) could be regarded as a measure of the efficiency of MP deposition. This Kg/Ks ratio is increased significantly from -3.71 to 42% ($P<.05$) during the compensatory growth period and was higher than in the control animals (32%).
As between zero growth and compensatory growth periods diets were quite different, both in level and presumably also in quality of protein, it might be useful to express degradation data on N intake base. In these conditions MP degradation rose from about 40 to 50% of ingested N in CG while in the other group it was higher than in the control group and remained constant at about 60% during both restricted and compensatory growth periods. When protein degradation was estimated by urinary excretion rates of urea or total N relative to N intake, respective decreases from 15 to 9% and from 36 to 22 % are observed during compensatory growth, while in the control group both ratios rise in time. This could be an indication a better utilisation of the intracellular amino acids pool during compensatory growth.
Between the two measurement periods N intake rose by 86.9 and 134.5 g N per day (ΔN intake), respectively in the control and in the compensatory growth group. When the increase in degradation rate (ΔMPD, expressed as N) is related on ΔN intake (ΔNin), ΔMPD/ΔNin equals 64 %; both in CG and in CoG. However, during compensatory growth both Δurea N excretion/ΔNin as ΔN excretion/ΔNin are about 1/4th of the control group, e.g. resp 6.4 vs 24.5 and 16.2 vs 63.2%. This means that during compensatory growth an improvement in intracellular amino acid recycling into MP synthesis reduces hepatic oxydation to about 1/4th of the control group. It should be noted also that in CG, ΔMPD/ΔNin and ΔN excretion/ΔNin are rather proportional e.g., 64.5 and 63.2 % This could indicate that in the normal process of MP turnover urinary N excretion is proportional to MPD while during compensatory growth it is lower due to enhanced intracellular amino acid recycling.
A simple approximation of the quantitative relationship between MPD and urinary N excretion is given by the ratio ΔN excretion to ΔMPD which is 98.0 % for the control group and 25.3 % in the compensatory growth group (1/4th). Finally, if the Δ's of the three MP turnover components are expressed on ΔN intake, MPA decreases by about 4% in CG while it increases by 60.2% in compensatory growth. This improvement is obtained by a rise in MPS with 124.4 % relative to intake, as ΔMPD/ΔNin is, as already mentioned, similar in both groups, e.g. 64.5 and 64.2%
Consequently, the improvement in MP accretion and in growth rate, observed in compensatory growth after a 2 months zero to negative growth, should be ascribed to both a higher MP synthesis rate and an increase in deposition efficiency, brought about by a higher recycling rate of the intracellular amino acid pool. This contrasts to the increase in deposition obtained by growth promoters such as anabolic agents where a decrease in MP degradation is the determining factor (Vernon & Buttery, 1976; Van Eenaeme et al, 1983).

Fig 1: Muscle Protein Turnover



Hindlimb catheterization experiment

Measurement of arterio venous differences (AVD) and uptake (U) rates across a well defined muscle mass is a means of studying MP metabolism at muscle tissue level. This approach allows to measure nutrient (glucose, amino acids) uptake rates and metabolite release rates. Measurements at maintenance and rapid growth should give information about use of substrates for energetic or structural development purposes.
Table 2 summarizes the main results of the catheterization experiment.

Table 2: *Hindquarter blood flow (l/min), arterial (A) and venous (V) plasma concentrations (µmol/l), arterio-venous difference (AVD,µmol/l) and uptake (U, µmol/min) of glucose, total amino acids (TAA), total essential (EAA) and non essential (NEAA) amino acids and branched chain amino acids (BCAA) in double muscled Belgian Blue bulls at maintenance (MP) or during fattening (FP) : daily means.*

Parameter		*MP*		*FP*	
		Mean	S.E.	Mean	S.E.
Hind quarter blood flow (l/min)		2.81	0.02	4.73	0.09
Glucose	A	4298.79	76.5	4691.69	95.4
	V	4129.85	73.1	4540.11	101.1
	AVD	168.94	18.3	151.58	13.1
	U	362.45	9.3	537.19	12.7
TAA	A	2213.01	163.8	2377.88	103.4
	V	2297.08	182.7	2126.13	79.7
	AVD	-84.07	34.1	251.75	50.3
	U	-180.36	74.6	896.53	189.2
EAA	A	868.57	71.5	839.82	26.9
	V	863.68	69.6	740.89	39.2
	AVD	5.19	25.3	98.93	19.6
	U	10.11	55.0	350.59	73.5
BCAA	A	469.74	41.1	510.54	25.4
	V	449.01	38.1	436.81	21.4.
	AVD	20.74	13.2	73.73	6.4
	U	44.57	28.2	255.77	17.1
NEAA	A	1317.88	96.2	1502.6	89.7
	V	1406.63	114.3	1349.52	64.5
	AVD	-88.75	18.9	153.08	31.2
	U	-189.85	41.7	545.85	116.4

S.E. : standard error

Hindquarter blood flow was higher during compensatory growth than during maintenance: 4.73 vs 2.81 l/min, reflecting both a higher metabolic rate and an increase in cardiac output. Arterial and venous concentrations of glucose were systematically higher during FP and showed a decrease 2 hours after the second meal. This could be related to an insulin maximum induced by high ruminal propionate concentrations. Glucose uptake was maximal at the same moment. Averaged over the whole day it amounted to 537 µmol/min , which was different from the value for MP e.g. 362 µmol/min.($P<.1$).This increase in glucose uptake is almost entirely due to the higher blood flow during FP as AVD's were similar. This indicates that the intrinsic capacity of muscle to take up

glucose remained the same in FP as in MP, presumably because of non saturation of the muscle cell glucose transporter.

Between MP and FP there were no significant differences in arterial and venous concentrations of total (TAA), essential (EAA), branched chain (BCAA) or non essential amino acids (NEAA). However, AVD and uptake of TAA was negative during MP and largely positive during FP ($P<0.01$). Similar results were observed for NEAA. For EAA and BCAA, AVD and uptake were in MP slightly and in FP strongly positive.

Uptakes of individual EAA were either low (valine, isoleucine) or slightly negative (threonine, leucine, methionine, lysine, arginine) during MP. With the exception of arginine, all rose to positive values during FP. Of the total EAA uptake (351 ùmol/min), about 75% is accounted for by BCAA (256 µmol/min). Arginine uptake was negative in both periods and decreased even during FP. This release of arginine from hindlimb has already been observed in a former experiment (Hornick et al, 1996). Arginine is considered to be an essential AA during growth (Mehler, 1992) and could thus be limiting during periods of rapid growth. The net release could be hypothesized to be due to an increased hepatic demand for keeping the urea cycle going on. Transport of arginine between portal drained viscera, kidneys or muscle and liver has been reported by Heitmann and Bergman (1980), Windmueller (1982) and Wolff and Bergmann (1980).

The negative uptake of NEAA by the hindlimb during MP has to be accounted for to a large extent to alanine (-104 µmol/min) and glycine (-91 µmol/min), which have been known for some time as N shuttle between muscle tissue and liver.

During the maintenance period, when MPS and MPD are equal, amino acids are utilised for energy production, and the amino N resulting from transamination is transported out of muscle to the liver by alanine and glycine. The amino acids degraded in muscle are the BCAA, although we suspect that tyrosine and phenylalanine, which are described as being non degraded in muscle, might also, to some extent, be catabolized, as estimates of MPS based on uptake of these AA are too high. However, it should be noted that these, (and other) amino acids may possibly be exported from muscle as a non measured fraction, e.g. small peptides. During subsequent compensatory growth, both uptakes become largely positive (respectively 165 and 216 µmol/min), indicating a decrease in transamination rate.This is in agreement with the observations on MP turnover and intracellular AA recycling into MPS. So, in FP, alanine and glycine are mainly used for protein synthesis.

Finally, it might be interesting to verify to what extent the amino acid uptake pattern is optimal for maximal muscle protein synthesis. When relating, for the different amino acids, uptake to AA composition of muscle protein, an estimate of this matching between supply and demand could be attempted. This approach has already be described (Van Eenaeme et al, 1995). Application to the present data will be dealth with in more detail elsewhere. However, in the context of the present paper, a comparison of this ratios when changing between maintenance and fattening periods as a Δ relative uptake could be made.Results are reported in Table 3

Table 3: *Ratio of uptake of amino acids for MP and FP and Δ uptake reported on the concentration of the corresponding amino acids in muscle dry matter (g DM uptake/min).*

Amino acid	MP		FP		Δ	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Alanine	-0.215	0.039	0.340	0.034	0.554	0.055
Glycine	-0.195	0.033	0.465	0.018	0.660	0.047
EAA	0.0043	0.023	0.149	0.031	0.145	0.014
BCAA	0.038	0.024	0.218	0.015	0.180	0.035
NEAA	-0.070	0.015	0.200	0.043	0.270	0.051

S.E. :standard error

These values are by far the largest for alanine and glycine, confirming the change from AA catabolism during the maintenance period to muscle protein synthesis in FP.

In conclusion, during feed restriction and zero growth, a non negligible part of the intracellular amino acid fraction, especially the BCAA, is used to satisfy the energy requirements of muscle. In the subsequent compensatory growth period, these energy requirements are, to a large extent, covered by glucose absorption. The increase in amino acid uptake is then mainly utilised for the synthesis of muscle protein.

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