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Ryegrass-based diet and barley supplementation: Partition of energy-yielding nutrients among splanchnic tissues and hind limbs in finishing lambs¹

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ABSTRACT: Splanchnic metabolism of energy-yielding nutrients and their uptake by the hind limb were studied in finishing lambs receiving ryegrass harvested at grazing stage (ear at 10 cm) with or without barley supplementation. Six ruminally cannulated and multi-catherized lambs (40.2 ± 1.5 kg) were fed with frozen ryegrass (RG) at 690 kJ of metabolizable energy intake ($\text{MEI} \cdot \text{d}^{-1} \cdot \text{BW}^{-0.75}$) successively with and without barley supplementation (RG + B), according to a triplicated Latin square design. Barley supplementation represented 21% of DM intake and increased the MEI by 32% ($P < 0.002$). In ruminal fluid, barley supplementation increased the acetate and butyrate concentrations by 21.2 and 49.6%, respectively ($P < 0.04$), without modifying those of propionate. Thus, molar proportions of acetate and butyrate were not modified, and those of propionate tended ($P < 0.06$) to decrease from 26 to 23%. As a result, the net portal appearance of propionate was not modified. Net portal appearance of butyrate and β -hydroxybutyrate increased ($P < 0.03$), and that of acetate was not modified. Consequently, hepatic up-

take of butyrate increased and probably spared acetate from hepatic metabolism. The hepatic fractional extraction of propionate decreased ($P < 0.03$), whereas the net flux of lactate switched from a net release to a net uptake, suggesting an alteration in the contribution of gluconeogenic substrates to glucose synthesis without modification in net hepatic glucose release. As a consequence, barley supplementation increased net splanchnic release of acetate ($P < 0.02$), propionate ($P < 0.001$), and β -hydroxybutyrate ($P < 0.01$) by 60, 157, and 78%, respectively. In addition, the net splanchnic release of insulin increased ($P < 0.03$) because of a decrease ($P < 0.02$) in its hepatic extraction. Despite those changes, the net uptake of nutrients by the hind limb was not modified and even decreased in the case of glucose ($P < 0.02$), suggesting a stimulation of lipogenesis in adipose tissues. Results from the present study suggested that supplementation of a ryegrass-based diet would likely have little effect on the orientation of muscle energy metabolism and on meat quality because the net uptake of nutrients by the hind limb was unchanged.

Key Words: Barley, Energy Intake, Liver, Lolium, Nutrients, Portal Vein

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Introduction

In European countries, consumers show a growing interest for meat produced from grazing ruminants, which is considered to be healthier and more natural than that obtained from grain-fed animals (Geay et al., 2001). Grass-finished ruminants have lower growth rates (Murphy et al., 1994), lighter carcasses, and insufficient marbling (McCaughey and Cliplef, 1996) compared with those finished on grain. Concentrate

supplementation is often applied in the finishing period to improve growth rate. It also improves marbling (McCaughey and Cliplef, 1996) and tenderness with increases in glycogen and intramuscular lipid contents (Vestergaard et al., 2000a,b). Recently, it was suggested that muscle characteristics involved in meat quality may partly depend on muscle energy metabolism, which may itself be influenced by the balance between energy-yielding nutrients (Hocquette et al., 1998). Grass feeding seems to favor the availability of acetogenic rather than glucogenic nutrients (Ortigues-Marty et al., 2002), which are more favorable to flavor and color development in meat. We hypothesized that a concentrate supplement, besides elevating energy intake, would improve the supply and use of glucogenic nutrients by muscle and thereby contribute to improving meat tenderness and marbling.

However, no direct relationship exists between the balance of digestion end products and the orientation

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of muscle energy metabolism because the supply of energy-yielding nutrients to muscle is greatly influenced by splanchnic metabolism and hormonal status. The objectives of the present study were thus to characterize the effects of grain supplementation on the profile of absorbed blood nutrients, splanchnic nutrient release, and muscle metabolism. Preliminary results have been published elsewhere (Majdoub et al., 2002; Vernet et al., 2001).

Materials and Methods

Animals, Diet, and Treatments

Five INRA 401 intact male lambs were surgically equipped with a ruminal cannula (12 mm i.d.) and chronic blood catheters in the portal, hepatic, and external iliac veins and a mesenteric artery, as described by Majdoub et al. (2003). They were also fitted with two ultrasonic blood flow probes (Transonic Systems, Ithaca, NY) in the portal vein (16 A) and in an external iliac artery (3 R). Animals were housed in individual stalls with ad libitum access to drinking water and trace-mineralized lick salt (0.75% Mn, 0.15% Cu, 0.90% Zn), and continuous lighting. During the experimental period, catheters were rinsed with physiological sterile saline and filled with diluted heparin (2,500 IU of heparin/mL of physiological saline). The day before and the day of sampling, catheters were filled with saline-sodium citrate buffer in order to avoid the effects of heparin on the metabolism of triglycerides (TG) and NEFA.

After an adaptation period of 2 wk, lambs (7 mo old, at an average experimental weight of 40.2 ± 1.5 kg) received two treatments, ryegrass (RG) and ryegrass + barley (RG + B) according to a Latin square design with three repetitions. Lambs received each treatment diet during 2 wk. For the RG treatment, animals were offered perennial ryegrass (first cutting, fertilized at 80 kg of N/ha), which was harvested at grazing stage (ear at 10 cm), chopped 5 cm in length, frozen at -35°C , and stored at -15°C , at an estimated level of 690 kJ of ME/kg BW^{0.75}, which represented approximately 75% of the ad libitum level, in 12 equal daily meals. For the RG + B treatment, lambs were supplemented with 19 g/BW^{0.75} of whole barley, which represented 26% of total estimated MEI. Ryegrass and barley ME contents were estimated at 11.59 and 13.25 MJ/kg of DM, respectively (INRA, 1978).

The experiment was conducted in a manner compatible with national legislation on animal care (Certificate of Authorization to Experiment on Living Animals, No. 004495, Ministry of Agriculture).

Measurements

Lambs were weighed twice a week during the experimental period. Feed samples were taken daily and pooled for each animal and treatment period in order

to determine chemical composition (DM, OM, CP, soluble N, crude fiber, and soluble sugar). CP contents were analyzed according to Kjeldahl method (NF V18-100). The solubility of N was determined according to the Durand method (Vérité and Demarquilly, 1978). Crude fiber content was determined according to the Weende method (NF V03-040), and soluble sugar content was determined using the Bertrand method (Halbwachs-Strich, 1969).

On the last day of treatment, blood flows in the portal vein and in the external iliac artery were continuously recorded over 4 h (over two feeding cycles, between 1100 and 1500). Hepatic arterial blood flow was estimated at 5.3% of the portal blood flow (PBF) (Barnes et al., 1986; Milano et al., 2000). A 5% contribution of hepatic arterial blood flow to the hepatic blood flow had been measured by Barnes et al. (1986) in ovine with the microsphere method. To facilitate the interpretation of net iliac flux, visual observations were made of the animals' behavior during this period, and blood sampling was carried out in quietly standing animals only. Eight sets of blood samples were taken from the portal, hepatic, and external iliac veins and from the mesenteric artery 30 min apart, starting 15 min postprandially for individual chemical analysis. For each sample, 2 mL of blood was taken using airtight syringes containing 50 μL of diluted heparin (500 IU of heparin/mL of physiological saline). One milliliter was immediately deproteinized with 2 mL of perchloric acid (0.6 M), centrifuged at -4°C , and the supernatant stored at -20°C for further analyses of glucose (Bergmeyer et al., 1974), L-lactate (Gutmann and Wahlefeld, 1974), and β -hydroxybutyrate (Williamson et al., 1962). The other 1 mL was pooled and stored at -80°C for later determination of VFA using 2-ethylbutyrate as internal standard (Reynolds et al., 1986). Additionally, 2.5 mL of blood was taken using EDTA-K (25 μL) as anticoagulant and aprotinine (1/10 vol/vol) and used for hematocrit determination prior to centrifugation at -4°C . The resultant plasma was frozen at -20°C for further analyses of NEFA (Kit NEFA C Wako, Unipath SA, Nuess, Germany), TG (Kit TG PAP 1000, Bio Mérieux, Marcy l'Etoile, France), and insulin (Kit Insulin-CT, Cis Bio Int., Gif sur Yvette, France).

A total of three ruminal fluid samples were taken, one at each 30-min interval over one feeding cycle (between 1500 and 1700). For each sample, pH was immediately measured and 10 mL of filtered ruminal fluid was acidified with 1 mL of metaphosphoric acid (5%, vol/vol) and frozen at -20°C for subsequent analysis of VFA by GLC (Jouany, 1982) and ammonia (Van Eenaeme et al., 1969). Each sample was individually analyzed, and values were subsequently averaged per animal and per treatment.

Calculations and Statistical Analyses

Iliac blood flows of lambs in quietly standing state were calculated after elimination of values corres-

Table 1. Dietary intake and ruminal fluid measurements in lambs fed frozen ryegrass supplemented with barley

	n	Treatments ^a		SEM ^b	Probability
		RG	RG + B		
BW, kg	5	40.1	40.3	0.47	0.32
Intake					
DM, g/d	5	926	1177	46.6	0.003
ME, MJ/d	5	10.7	14.1	0.54	0.002
N, g/d	5	20.8	25.8	0.95	0.002
Ruminal fluid variables					
pH	5	6.80	6.56	0.056	0.05
NH ₃ , mM	5	6.97	7.75	0.633	0.39
VFA, mM					
Total VFA	5	73.63	88.18	4.965	0.02
Acetate	5	44.92	54.45	3.420	0.03
Propionate	5	19.03	20.09	1.117	0.59
Butyrate	5	7.21	10.79	0.748	0.04
Isobutyrate	5	0.76	0.75	0.031	0.67
Valerate	5	0.57	0.89	0.073	0.03
Isovalerate	5	0.91	0.85	0.040	0.58
Caproate	5	0.22	0.38	0.033	0.01

^aRG = ryegrass, RG + B = ryegrass + barley.

^bSEM = residual standard error of treatment means.

ponding to agitated and lying state according to Iserty and Ortigues (1994). Net nutrient fluxes through the portal drained viscera (PDV), the liver, and the hind limb were calculated as described by Katz and Bergman (1969) and Milano et al. (2000), with the average concentrations of metabolites calculated from the eight samples per vessel and the blood flow values averaged over time. A positive net flux indicates a net release, whereas a negative net flux indicates a net uptake. Fractional extraction was calculated as the ratio of the net flux to the inflow of nutrient to the tissue. Net tissue energy flux was calculated using heats of combustion (MJ/mol) of the acetate (0.875), propionate (1.535), butyrate (2.191), glucose (2.813), lactate (1.36), β -hydroxybutyrate (2.055), NEFA (11.106), and TG (34.945). Maximal potential contribution of substrates to hepatic glucose production was calculated assuming all carbons in substrates were converted into glucose carbon.

Because of the death of one lamb just at the beginning of the experiment, results were analyzed by ANOVA with animal, treatment, and period as main factors ($\text{Var} = \mu + \alpha \text{ animal} + \beta \text{ treatment} + \delta \text{ period} + \varepsilon$). Analysis was carried out using the GLM procedure of Statistica (version 5.5, StatSoft, Tulsa, OK). Differences were declared significant at $P < 0.05$ and tendencies were declared for $0.05 < P < 0.1$. Linear regression procedures of Statistica were used to determine relationships between DMI or MEI and blood flows or net nutrient fluxes across the different tissues.

Results

Correct positioning of catheters and probes was checked at necropsy. At the splanchnic level, catheters

in five animals had remained functional during the experimental period. At the hind limb level, catheters were functional in three animals.

Intake and Ruminal Fermentation Parameters

The contents of ryegrass in OM, CP, crude fiber, and soluble sugars were 89.8 ± 0.41 , 14.1 ± 0.75 , 26.1 ± 1.01 , and $10.5 \pm 1.09\%$ on a DM basis, respectively. Soluble N accounted for 26.3% of total N. The ryegrass DM content was measured daily and averaged $15.2 \pm 1.04\%$. Total DMI, ME intake (MEI), and nitrogen intake (NI) for RG averaged 926.4 g/d, 10.7 MJ/d, and 20.8 g/d, respectively (Table 1). Barley supplementation increased DMI, MEI, and NI by 27, 32, and 24%, respectively.

In ruminal fluid, total VFA concentration increased ($P < 0.02$) from 73.6 to 88.2 mM with barley as a result of a significant rise in ruminal acetate and butyrate concentrations by 21.2 and 49.6%, respectively (Table 1). Ruminal valerate and caproate concentrations also increased ($P < 0.03$). However, concentrations of propionate and the isoacids were not significantly modified. Consequently, molar proportions of acetate, propionate, and butyrate averaged 60.7, 25.8, and 9.9% for RG, and 61.6 (SEM = 0.660; NS), 22.8 (SEM = 0.656; $P < 0.06$), and 12.4% (SEM = 0.667; NS) for RG + B, respectively. Acetate:propionate ratio increased from 2.4 to 2.7 (SEM = 0.077; $P < 0.02$). Ruminal fluid pH decreased ($P < 0.06$) from 6.8 to 6.6. Ammonia concentrations were not statistically modified by barley supplementation and averaged 7.36 mM.

Blood Flow and Nutrient Concentrations

Blood flow in the portal vein and in the external iliac artery were 113.0 and 8.5 L/h, respectively, with

Table 2. Blood flows and blood nutrient concentrations in lambs fed frozen ryegrass supplemented with barley

Item	n	Treatments ^a		SEM ^b	Probability
		RG	RG + B		
Blood flow, L/h					
Portal vein	5	113.02	125.62	3.210	0.05
External Iliac artery	3	8.50	9.78	0.556	0.20
Arterial blood, mM					
Acetate	5	1.94	2.16	0.095	0.20
Propionate	5	0.11	0.12	0.036	0.87
Butyrate	5	0.02	0.03	0.003	0.06
Glucose	5	2.95	3.04	0.085	0.39
L-Lactate	5	0.77	0.62	0.054	0.21
β -Hydroxybutyrate	5	0.38	0.45	0.038	0.21
Arterial plasma, mM					
NEFA	5	0.11	0.09	0.001	0.22
TG ^c	5	0.022	0.018	0.002	0.19
Portal blood, mM					
Acetate	5	3.35	3.28	0.130	0.28
Propionate	5	0.67	0.63	0.029	0.46
Butyrate	5	0.08	0.13	0.011	0.03
Glucose	5	2.84	2.98	0.090	0.17
L-Lactate	5	0.83	0.70	0.055	0.25
β -Hydroxybutyrate	5	0.43	0.55	0.042	0.11
Portal plasma, mM					
NEFA	5	0.11	0.10	0.014	0.43
TG	5	0.020	0.018	0.002	0.30
Hepatic blood, mM					
Acetate	5	2.84	3.44	0.166	0.02
Propionate	5	0.14	0.16	0.007	0.06
Butyrate	5	0.04	0.05	0.005	0.05
Glucose	5	3.10	3.24	0.092	0.12
L-Lactate	5	0.84	0.68	0.056	0.21
β -Hydroxybutyrate	5	0.48	0.63	0.049	0.07
Hepatic plasma, mM					
NEFA	5	0.10	0.09	0.001	0.29
TG	5	0.020	0.017	0.002	0.39
Iliac blood					
Acetate	3	0.87	1.27	0.150	0.07
Propionate	3	0.09	0.09	0.003	0.39
Butyrate	3	0.01	0.02	0.003	0.25
Glucose	3	2.77	2.84	0.086	0.80
L-Lactate	3	0.66	0.60	0.048	0.53
β -Hydroxybutyrate	3	0.21	0.29	0.048	0.49
Iliac plasma, mM					
NEFA	3	0.12	0.11	0.002	0.52
TG	3	0.022	0.016	0.003	0.75

^aRG = ryegrass, RG + B = ryegrass + barley.

^bSEM = residual standard error of treatment means.

^cTG = triglycerides.

RG. Barley supplementation ($P = 0.05$) increased PBF by 15% and did not significantly modify iliac blood flow (Table 2). Hematocrit was stable across the sampling period and was not modified by treatment averaging 0.27.

At the arterial level, blood concentrations of acetate and propionate were of 1.94 and 0.11 mM with RG and were not significantly affected by treatment, whereas that of butyrate averaged 0.02 mM with RG and increased with RG + B ($P < 0.06$; Table 2). Arterial concentrations of all other measured metabolites re-

mained unmodified by treatment. Similarly, in portal blood, only blood butyrate concentrations were affected by treatment, increasing from 0.08 to 0.13 mM with RG + B ($P < 0.03$). In the hepatic vein, all blood VFA concentrations increased significantly with RG + B, from 2.84, 0.14, and 0.04 mM with RG to 3.44, 0.16, and 0.05 mM with RG + B, for acetate, propionate, and butyrate, respectively. Similar effects were noted for β -hydroxybutyrate ($P < 0.07$) and glucose (NS). Finally, at the hind limb level, only iliac venous blood acetate concentrations increased from 0.87 to 1.27 mM

Table 3. Net nutrient fluxes through the PDV, the liver, the splanchnic tissues and the hind limb in lambs fed frozen ryegrass supplemented with barley

Item	n	Treatments ^a		SEM ^b	Probability
		RG	RG + B		
PDV blood, mmol/h ^c					
Acetate	5	157.08	139.85	8.073	0.21
Propionate	5	61.94	63.92	2.960	0.63
Butyrate	5	6.49	12.48	1.245	0.03
Glucose	5	-13.39	-7.91	1.566	0.05
Lactate	5	6.64	9.12	0.681	0.02
β -Hydroxybutyrate	5	6.09	12.58	1.289	0.03
PDV plasma					
NEFA	5	0.46	1.08	0.157	0.02
TG ^c	5	-0.11	-0.05	0.018	0.09
Liver blood, mmol/h					
Acetate	5	-50.73	29.90	13.946	0.003
Propionate	5	-59.53	-57.96	2.817	0.73
Butyrate	5	-4.44	-9.34	1.026	0.04
Glucose	5	30.71	34.11	2.584	0.54
Lactate	5	1.28	-2.20	1.457	0.03
β -Hydroxybutyrate	5	6.93	10.59	1.171	0.001
Liver plasma, mmol/h					
NEFA	5	-0.83	-1.21	0.123	0.15
TG	5	-0.12	-0.06	0.027	0.05
Splanchnic tissues blood, mmol/h					
Acetate	5	106.34	169.75	13.280	0.02
Propionate	5	2.41	5.96	0.676	0.001
Butyrate	5	2.06	3.14	0.379	0.16
Glucose	5	17.32	26.19	2.752	0.15
Lactate	5	7.93	6.92	1.425	0.56
β -Hydroxybutyrate	5	13.02	23.17	2.182	0.01
Splanchnic tissues plasma, mmol/h					
NEFA	5	-0.36	-0.13	0.079	0.18
TG	5	-0.23	-0.10	0.029	0.02
Hind limb blood, mmol/h					
Acetate	3	-8.24	-7.62	0.614	0.94
Propionate	3	-0.20	-0.27	0.027	0.28
Butyrate	3	-0.04	-0.08	0.010	0.06
Glucose	3	-1.90	-1.66	0.264	0.02
Lactate	3	-0.48	-0.27	0.116	0.18
β -Hydroxybutyrate	3	-1.07	-1.35	0.206	0.74
Hind limb plasma, mmol/h					
NEFA	3	0.000	0.03	0.023	0.74
TG	3	-0.005	0.004	0.0025	0.13

^aRG = ryegrass, RG + B = ryegrass + barley.

^bSEM = residual standard error of treatment means.

^cPDV = portal-drained viscera, TG = triglycerides.

with RG + B ($P < 0.07$). All other metabolites remained statistically unaffected by treatment.

Net Splanchnic Metabolism

Net portal appearance (NPA) of acetate, propionate, and butyrate averaged 157.08, 61.94, and 6.49 mmol/h with RG (Table 3). Only NPA of butyrate was modified by treatments, rising by 92.3% with barley supplementation. Simultaneously, NPA of β -hydroxybutyrate increased by 106.5% ($P < 0.06$). Net portal appearance of NEFA was 0.46 mmol/h with RG and increased by 134.8% with barley supplementation ($P < 0.02$) due to an increase in net fractional PDV appearance (from 5.36 with RG to 13.80% with RG + B, SEM = 0.023,

$P < 0.01$). A slight net uptake of TG (0.11 mmol/h) was noted with RG (Table 3). This uptake decreased ($P < 0.09$) with barley supplementation. Concerning glucidic-type metabolites, net PDV flux of glucose was negative, representing a fractional extraction of 4.03% of arterial blood with RG (Table 3). The net PDV uptake of glucose decreased with RG + B by 40.9% ($P < 0.05$) as a result of the decrease in the fractional extraction (2.05%, SEM = 0.005, $P < 0.01$). Net appearance of L-lactate in the portal blood increased by 37.3% ($P < 0.02$) with barley supply.

A net uptake of the three major VFA by the liver was noted with RG (Table 3). Fractional extraction accounted for 13.09, 78.57, and 51.24% of the input flow for acetate, propionate, and butyrate, respectively

Table 4. Fractional extraction of nutrients through the liver and the hind limb in lambs fed frozen ryegrass supplemented with barley

	n	Treatments ^a		SEM ^b	Probability
		RG	RG + B		
Liver blood, %					
Acetate	5	13.09	-6.87	0.034	0.001
Propionate	5	78.57	73.02	0.030	0.03
Butyrate	5	51.24	57.52	0.030	0.25
Glucose	5	-9.15	-8.70	0.007	0.63
Lactate	5	-1.40	2.42	0.016	0.003
β -Hydroxybutyrate	5	-13.26	-14.77	0.014	0.69
Liver plasma, %					
NEFA	5	8.66	12.97	0.015	0.09
TG ^c	5	6.24	4.01	0.014	0.11
Hind limb blood, %					
Acetate	3	53.17	39.92	0.052	0.21
Propionate	3	21.00	23.35	0.016	0.59
Butyrate	3	27.73	29.84	0.025	0.62
Glucose	3	7.44	5.57	0.004	0.02
Lactate	3	8.17	3.87	0.018	0.17
β -Hydroxybutyrate	3	37.49	31.55	0.018	0.43
Hind limb plasma, %					
NEFA	3	-0.24	-2.65	0.030	0.88
TG	3	4.41	-4.06	0.022	0.31

^aRG = ryegrass, RG + B = ryegrass + barley.

^bSEM = residual standard error of treatment means.

^cTG = triglycerides.

(Table 4). The net hepatic uptake of acetate switched to a net release with RG + B ($P < 0.003$). Across both treatments, the net hepatic flux of acetate (**NHA**, mmol/h) was highly correlated to NPA of acetate (**NPAA**, mmol/h) ($\text{NHA} = [-0.793 \times \text{NPAA}] + 0.636$; $r = 0.96$; $P < 0.001$). Net hepatic uptake of butyrate (**NHB**, mmol/h) increased by 110% with RG + B as a result of the increase in NPA of butyrate (**NPAB** mmol/h) ($\text{NHB} = [0.102 \times \text{NPAB}] - 0.90$; $r = 0.999$, $P < 0.001$). Net hepatic fractional extraction of butyrate was not modified by barley supplementation, and 74.8% of its net portal appearance was extracted by the liver. The net hepatic uptake of propionate remained unchanged with barley supplementation (Table 3), whereas its fractional extraction decreased ($P < 0.03$) by 7%. Besides propionate, the other major hepatic glucose precursors reported here are L-lactate and TG. A net hepatic release of L-lactate was noted with RG (Table 3). It switched to a net uptake with RG + B ($P < 0.03$). Nevertheless, the hepatic fractional extraction of lactate was low (2.42%, Table 4). The net hepatic uptake of TG was slight (0.12 mmol/h) with RG and decreased by 50% with barley supplementation ($P < 0.05$). In parallel to the net hepatic fluxes of glucose precursors, a net hepatic release of glucose was measured which remained stable across the treatments (Table 3). The maximal contribution of propionate to hepatic glucose production decreased from 96.9% with RG to 85.0% with RG + B. The maximal contribution of L-lactate to glucose production represented only 3.2% with RG + B. Among the other metabolites of interest,

NEFA were taken up by the liver with RG (0.83 mmol/h). Its fractional extraction increased with barley supplementation (12.97% with RG + B vs. 8.66% with RG, $P < 0.09$, Table 4). Finally, the net hepatic release of β -hydroxybutyrate increased ($P < 0.001$) with barley supplementation as a result of the significant increase in its NPA (Table 3).

As a result of the aforementioned changes in net PDV and hepatic metabolism, barley supplementation increased the net splanchnic release of acetate and propionate by 63.41 ($P < 0.02$) and 3.55 mmol/h ($P < 0.001$), respectively, and that of β -hydroxybutyrate by 10.15 mmol/h ($P < 0.01$). Net glucose release toward peripheral tissues also increased numerically by 8.87 mmol/h ($P < 0.15$), whereas that of net L-lactate remained unchanged and averaged 7.42 mmol/h. On the other hand, the limited net splanchnic uptakes of NEFA and TG were slight and decreased by 0.23 ($P < 0.18$) and 0.13 mmol/h ($P < 0.02$), respectively.

Net Splanchnic Fluxes of Insulin

Plasma arterial concentration of insulin averaged 29.4 $\mu\text{IU/mL}$ with RG and was not affected by barley supplementation (Table 5, $P < 0.61$). Net insulin secretion by the PDV was 0.57 IU/h with RG and was not modified with RG + B ($P < 0.20$), whereas the net hepatic flux of insulin switched ($P < 0.03$) from a net extraction (0.64 IU/h) with RG to a net flux with RG + B, which was not significantly different from zero. As a result, net splanchnic flux of insulin increased

from -0.07 IU/h with RG to the net release of 0.60 IU/h with barley supplementation ($P < 0.03$).

Hind Limb Metabolism

In the hind limb, acetate was the major energy-yielding nutrient being taken up. Its net uptake was 8.24 mmol/h with RG and was not modified by barley supplementation (Table 3, $P < 0.94$). The uptake of propionate was slight (0.20 mmol/h) and was not altered by barley supplementation. Net uptake of butyrate was 0.04 mmol/h with RG and doubled with RG + B (Table 3, $P < 0.06$). Despite the trend for a higher net splanchnic release of glucose with barley supplementation, net glucose uptake by the hind limb decreased by 0.24 mmol/h (Table 3, $P < 0.02$). Its fractional extraction dropped from 7.44 with RG to 5.57% with RG + B ($P < 0.02$; Table 4). Simultaneously, the net uptake of L-lactate which averaged -0.48 mmol/h tended to decrease by 43.7% with RG + B (NS, Table 3). Its fractional extraction decreased from 8.17% with RG to 3.87% with RG + B (NS, Table 4). Finally, the net uptake of β -hydroxybutyrate was not significantly modified with barley supplementation. Its fractional extraction averaged 34.52% (Table 4). For NEFA and TG, very small net fluxes were measured and were not modified by barley supplementation. Insulin net flux could not be obtained because the analytical method used was not precise enough to detect small arterio-venous differences.

Discussion

Partition of Nutrient Utilization with a Frozen Ryegrass Diet

The effects of barley supplementation depend on the partition of nutrient utilization measured with the basal diet. Therefore, it is important to characterize the nutrient fluxes obtained with fresh grass harvested at the grazing stage. In this study, frozen RG

was used in order to have a homogenous grass for the entire experimental period (2 mo). VFA concentrations and molar VFA proportions (C2/C3/C4) in the rumen were similar to data obtained on fresh ryegrass by O'Mara et al. (1997) and that reported previously for frozen ryegrass subjected to the same N fertilization and harvested at the same period of the year (Majdoub et al., 2003). However, $\text{NH}_3\text{-N}$ concentrations in the rumen were lower (6.97 vs. 9.63 mM), probably because of a lower CP content of RG (14.1 vs. 20.6% , DM basis) and a slightly older growth stage as indicated by the crude fiber content (26.1 vs. 21.2% , DM basis; Majdoub et al., 2003).

VFA represented 90.8% of the energy recovered in the portal vein (50.7 , 34.8 , and 5.3% for acetate, propionate, and butyrate, respectively). These proportions were higher than those reported by Lindsay (1993) and Ortigues and Visseiche (1995) and were associated with the higher NPA of acetate, propionate, and butyrate than previously reported for hay or even concentrate-based diets (Krehbiel et al., 1992; Reynolds et al., 1992; Lozano et al., 2000). Biases associated with the chemical determination of VFA are unlikely since analytical recovery rates of acetate, propionate, and butyrate in aqueous standard solutions ($n = 16$) averaged 1.01 ± 0.055 (SD), 1.01 ± 0.065 , and 1.04 ± 0.114 , respectively, and in blood samples ($n = 16$) following standard VFA overloading averaged 1.08 ± 0.153 (SD), 1.07 ± 0.078 and 1.06 ± 0.28 , respectively. The high portal appearance of VFA was probably associated with the high digestibility of the frozen ryegrass.

In the liver, an unexpected and important fractional extraction of acetate (13.1%) was observed. This result did not agree with previous data concerning hepatic metabolism of acetate. Generally, a net hepatic release of acetate is reported that originates from the acetyl-CoA-acetate pathway (Knowles et al., 1974) or from the partial β -oxidation of medium- and long-chain fatty acids in peroxysomes (Hocquette and Bauchart,

Table 5. Plasma insulin arterial concentrations, net fluxes, and fractional extraction through the splanchnic tissues in lambs fed frozen ryegrass supplemented with barley

	n	Treatments ^a		SEM ^b	Probability
		RG	RG + B		
Arterial concentrations, μIU	5	29.4	33.4	3.27	0.61
Net fluxes, IU/h					
PDV ^c	5	0.57	0.51	0.042	0.20
Liver	5	-0.64	0.09	0.184	0.03
Splanchnic tissues	5	-0.07	0.60	0.154	0.03
Fractional extraction, %					
PDV	5	-25.24	-17.79	0.024	0.31
Liver	5	19.24	-2.27	0.045	0.02
Splanchnic tissues	5	-0.20	-19.60	0.054	0.03

^aRG = ryegrass, RG + B = ryegrass + barley.

^bSEM = residual standard error of treatment means.

^cPDV = portal-drained viscera.

1999). On the other hand, acetate is metabolized in the liver for oxidation and β -hydroxybutyrate and nonessential amino acid synthesis (Pethick et al., 1981; Armentano, 1992; Seal and Reynolds, 1993). In ruminants, the magnitude of this metabolism is low, as is reflected by the low enzymatic activity of the acetyl-CoA synthase (Knowles et al., 1974), except perhaps when, as in rats, portal blood acetate concentrations are elevated (Rémésy et al., 1995). In the present experiment, blood acetate concentrations were higher than most published values (Goetsch et al., 1994; Seal and Parker, 1994), and the net acetate uptake by the liver can be associated with a high NPA of acetate. Additionally, some interaction may exist between acetate NPA, and protein intake. In lambs, Scollan and Jessop (1995) reported that high arterial concentrations of acetate were associated with low levels of protein intake, which is consistent with present results. Conversely, in two ruminant studies where high arterial and portal concentrations of acetate were measured in association with net hepatic uptakes of acetate (7 and 9% of inflow; Goetsch et al., 1994; Reynolds et al., 1994), animals had been supplemented with ruminal undegradable protein or alanine. The reasons for these discrepancies are unclear. Protein supply may thus have an effect on the hepatic uptake of acetate, but there is presently no clear understanding of this effect.

Barley Supplementation and Ruminal Parameters

Barley supplementation is often associated with a higher ruminal production of propionate (Journet et al., 1995; Moloney, 1998; Hristov et al., 2001). A concentrate diet composed of 60% barley enhanced the development of amylolytic microorganisms and the production of propionate compared with a forage diet (Martin et al., 1999). In the present study, propionate concentrations in the rumen that were already elevated with RG were not modified by barley supplementation offered at 21% of total DMI. Conversely, acetate and butyrate concentrations in the rumen increased. Molar proportions of VFA (C2/C3/C4) obtained with RG + B were comparable to those reported in cows receiving fresh ryegrass supplemented with sugared beet pulp at 19% of total DMI (O'Mara et al., 1997). Increases in the molar proportions of butyrate have also been reported by Moloney et al. (1992) in steers receiving grass silage supplemented with 25% of total DMI as barley or sugar-cane molasses and by Eadie et al. (1970) in heifers receiving a pelleted hay:barley ration. The effects of barley supplementation on ruminal VFA profile probably depend on the specificities of the frozen ryegrass based diet, the feeding frequency chosen (Majdoub et al., 2003), and the limited level of supplementation. In the present experiment, the high molar proportions of propionate measured with the basal diet, which arise from an elevated soluble sugar content and a forage that was probably highly digest-

ible, could have inhibited the development of bacteria implicated in the fermentation of carbohydrates into propionate or in the conversion of lactate into propionate (J. P. Jouany, personal communication). Additionally, a possible increase in the development of protozoa in the rumen with increasing ratios of concentrate (<60% DM) to roughage in diets (Eadie et al., 1970; Franzolin and Dehority, 1996; Martin et al., 1999) can favor acetate and butyrate production in the rumen at the expense of propionate (Kreikemeier et al., 1990; Wallace, 1995).

Consequently, from these ruminal fermentation data, it can be considered that barley supplementation of a frozen ryegrass diet resulted in a general increase in the production of energy-yielding end products of digestion (as noted with any general increase in the plane of feeding) with a slight modification in the balance among those end products toward more acetate and butyrate.

Barley Supplementation and Net Nutrient Portal Appearance

The 11% increase in PBF with barley supplementation reflects the now well-known rise in PBF with increasing MEI (Reynolds et al., 1992; Lapierre et al., 2000). The significant correlation between PBF ($\text{mL} \cdot \text{min}^{-1} \cdot \text{kg BW}^{-0.75}$) and MEI ($\text{kJ} \cdot \text{d}^{-1} \cdot \text{kg BW}^{-0.75}$) ($\text{PBF} = 0.097 \times \text{MEI} + 50.299$; $r = 0.77$; $P < 0.008$) was similar to that reported by Rémond et al. (1998) in sheep. Barley supplementation was especially associated with alterations in the NPA of lipid-type nutrients rather than glucidic-type nutrients. In coherence with the increased ruminal concentrations of acetate and butyrate, the NPA of butyrate and β -hydroxybutyrate increased, whereas that of acetate was not statistically modified. This suggests a rise in the net synthesis of β -hydroxybutyrate from both butyrate and acetate (Harmon et al., 1991; Kristensen, 2001), and probably an increase in the PDV metabolism of arterial (Kristensen et al., 2000) or absorbed acetate as CO_2 or de novo synthesized fatty acids. An increased oxidation of acetate, which reached maximally 75% of the increment in the PDV energy expenditure, can spare butyrate (Baldwin and Jesse, 1996), NEFA, and probably glucose (Harmon, 1986; Seal and Parker, 1994; Baldwin and McLeod, 2000), which agrees with the reduction in the net utilization of glucose measured with barley supplementation. With increasing intake, a sparing of glucose in the ruminal epithelium has also been associated with a reduced conversion of glucose into L-lactate (Harmon et al., 1991). In the present study, barley supplementation increased net portal appearance of L-lactate. Apart from PDV glucose metabolism, portal L-lactate can originate from the absorption of the ruminally produced L-lactate, and to a limited extent, from propionate metabolism in PDV tissues (Kristensen et al., 2000). Increased ruminal L-lactate production with barley supplement-

tation is plausible (Owens et al., 1998) considering the significant drop in ruminal pH. Finally, the significant albeit small increase in net portal appearance of NEFA and decrease in net arterial TG utilization do not suggest an enhanced fat synthesis and depot in the PDV, in particular from acetate.

Barley Supplementation and Hepatic Metabolism of Energy-Yielding Nutrients

The most notable influence of barley supplementation on net hepatic metabolism appeared to stem from the significant increase in the supply of butyrate and β -hydroxybutyrate to the liver (with increments of 5.99 and 6.49 mmol/h, respectively). With a fractional hepatic extraction of butyrate averaging 54%, 82% of the incremental net portal appearance of butyrate was extracted by the liver, which is of the same order of magnitude as that reported by Krehbiel et al. (1992) and Reynolds et al. (1992).

Specific effects of butyrate on hepatic metabolism in ruminants have been studied by Krehbiel et al. (1992), Reynolds et al. (1992), and Berthelot et al. (2002) following intraruminal or intramesenteric infusions of butyrate. The first effect of butyrate dealt with the metabolism of lipid-type nutrients. The net release of β -hydroxybutyrate increased. This increment could potentially explain 75% of the increment in net butyrate uptake as previously noted by Reynolds et al. (1992). An increase in butyrate metabolism in the liver may also favor the supply of acetyl-CoA (Madsen, 1983). Butyrate contributed maximally 48% to the increment in liver energy expenditure measured in the present experiment (Vernet et al., 2001), thereby sparing acetate and resulting in the measured increase in net hepatic release of acetate, as also noted by Berthelot et al. (2002).

The second major effect of butyrate on hepatic metabolism dealt with gluconeogenesis. Generally, increased butyrate supply modified the contribution of the different glucose precursors without clearly altering net hepatic glucose release. More specifically, butyrate may stimulate the activity of hepatic pyruvate carboxylase and therefore the synthesis of glucose from precursors such as pyruvate, L-lactate, or alanine at the expense of propionate (Faulkner and Pollock, 1986; Reynolds et al., 1992). Indeed, in steers, intramesenteric infusion of butyrate (25 mmol/h) over 3 d increased the net hepatic uptake of L-lactate and alanine by 56.7 and 44.4%, respectively (Reynolds et al., 1992). Decreases in the fractional extraction of propionate were also observed with butyrate in ovine, goat, and bovine hepatocytes by Faulkner and Pollock (1986) and Aiello et al. (1989), and in vivo by Krehbiel et al. (1992) and Reynolds et al. (1992). In the present study, the net hepatic flux of L-lactate switched from a net release to a net uptake, the net hepatic uptake of alanine increased (Our unpublished observations), the hepatic fractional extraction of propionate de-

creased while net glucose release remained unchanged. The maximal potential contribution of propionate dropped from 96.9% with RG to 85.0% with RG + B, whereas that of L-lactate increased from 0 to 3.2%, and that of alanine from 4.8 to 6.3% (preliminary results).

The mechanisms implicated in the inhibition of the hepatic propionate metabolism by butyrate are unclear. In the present case, barley supplementation did not modify insulin secretion, but significantly reduced its hepatic extraction. Consequences on arterial concentrations were, however, not statistically measurable. No significant changes in hepatic insulin extraction were noted by Reynolds et al. (1992) and Krehbiel et al. (1992) following butyrate infusion. The hepatic extraction of insulin is probably regulated by nutrient availability, although limited information is available (Grizard et al., 1986). The drop in hepatic insulin extraction supports a possible effect of glucagon on hepatic gluconeogenesis, although in the present experiment, no information has been obtained on glucagon. It has been reported that the main hepatic effect of insulin, in dogs as well as in bovine, was to inhibit the effect of glucagon (Donkin et al., 1997; Giacca et al., 1997). Glucagon is known to induce changes in the contribution of precursors to gluconeogenesis (Brockman and Manns, 1974; Donkin et al., 1997; She et al., 1999). A direct influence of butyrate supply on glucagon secretion or concentrations is not clear. After butyrate infusion at supraphysiological doses, increases in the arterial concentrations of glucagon were observed (De Jong, 1982). Conversely, at low levels of butyrate, neither the peripheral concentration of glucagon, nor its secretion were modified (Harmon, 1992; Reynolds et al., 1992; Sano et al., 1995).

As a consequence to splanchnic metabolism, barley supplementation significantly increased the net splanchnic release of acetate, propionate, and β -hydroxybutyrate by 63.41, 3.55, and 10.15 mmol/h, respectively, with tendencies for higher net releases of butyrate and glucose by 1.08 and 8.87 mmol/h. As a result, the splanchnic release of energy increased by 2.72 MJ/d and represented 80% of the increment in MEI. This was accompanied by an elevated net splanchnic insulin release, which could contribute to changes in the metabolic utilization of nutrients by peripheral tissues.

Barley Supplementation and Hind Limb Metabolism

Despite the significant increase in the splanchnic release of energy-yielding nutrients with RG + B as discussed above, their net uptake by the hind limb generally either was not modified (acetate, propionate, β -hydroxybutyrate) or even decreased (glucose and L-lactate), and increased only in the case of butyrate. Overall, energy taken up by the hind limb as energy-yielding nutrients was not modified and averaged 15.8 kJ/h. This supposes an unchanged metabolism in the

hind limb, which is coherent with the unmodified hind limb energy expenditure measured in those animals (Vernet et al., 2001). This apparent contradiction suggests an increase in the net uptake of the energy-yielding nutrients by other peripheral tissues, in particular adipose tissues that are not drained by the present surgical preparation. Grain supplementation is known to modify carcass composition of grazing ruminants toward a greater adiposity (Murphy et al., 1994) as a result of an increase in the level of intake (Smith et al., 1992; Greathead et al., 2001) and an alteration in the profile of nutrients available to peripheral tissues (Scollan and Jessop, 1995). McGrattan et al. (2000) reported a higher receptor affinity for insulin in adipose tissues than in the muscle of lambs, which is coherent with an increase in the uptake of energy-yielding nutrients by peripheral adipose tissues other than those included in the hind limbs. These results should be confirmed with a higher number of animals.

Conclusions

The present study contributed to characterizing the amount and balance of absorbed nutrients, as well as the partition of energy-yielding nutrients among splanchnic tissues and hind limbs in finishing lambs offered frozen ryegrass. This diet was characterized by a high proportion of energy absorbed as VFA, probably because of a high digestibility and soluble sugar content. The second aim of the present study was to look at the influence of barley supplementation. The characteristics of the basal diet could be responsible for the fact that barley supplementation in this case increased the ruminal levels of acetate and butyrate. This orientation of the VFA pattern and amounts absorbed increased splanchnic release of energy-yielding nutrients without modifying hind limb energy metabolism, suggesting an enhancement of lipogenesis in peripheral adipose tissues outside the hind limb.

Implications

The general issue addressed by this research was to evaluate whether limited grain supplementation to pasture-finished ruminants raised for meat production could modify the orientation of muscle energy metabolism and ultimately affect some aspects of meat quality. The present study showed that such supplementation could probably modify the composition of carcass, especially its adiposity. However, its effect on the orientation of muscle energy metabolism and on meat quality was unlikely because the net uptake of the different energy-yielding nutrients by the hind limb was unchanged. These results would likely not apply to higher levels of supplementation or to ad libitum ryegrass-fed animals because of possible feed substitution interactions.

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