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Original article

Energy expenditure of splanchnic tissues and hindlimbs in growing lambs fed frozen rye-grass: effects of barley supplementation and changes between 2.5 and 7 months of age

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Abstract — In order to study the effects of barley supplementation (Exp. 1), six lambs of 40 kg of body weight were fed on either rye-grass or rye-grass supplemented with barley according to a triplicate Latin Square design at an estimated level of 1.5 and 2.0 fold the maintenance energy requirements. In order to study the effects of age (Exp. 2), 3, 6 and 5 lambs aged 2.5, 4 and 7 months old respectively were used. They were fed with rye-grass only at estimated levels of 1.8, 1.7 and 1.5 fold the maintenance energy requirements. All animals were equipped with chronic blood catheters in the mesenteric artery, portal, hepatic and external iliac veins and with ultrasonic blood flow probes around the portal vein and the external iliac artery. In Experiment 1, portal blood flows increased by 11.5% with supplementation ($P < 0.05$) while iliac blood flow tended to increase by 15% (NS). Consequently, O_2 consumption increased by 20% ($P < 0.03$), 35% ($P < 0.08$) and 19% (NS) in the portal drained viscera, liver and hindlimb, respectively, with barley supplementation. In Experiment 2, portal blood flow, expressed on a metabolic body weight basis, decreased by 17% from 2.5 to 4 months of age and did not change from 4 to 7 months ($P < 0.004$). Iliac blood flow did not increase significantly between 2.5 and 7 months of age due to large inter-individual variability. No significant effect of age was noted on portal drained viscera and liver energy expenditure expressed on a metabolic body weight basis, while the hindlimb energy expenditure tended to increase (NS). It was concluded that in supplementing ryegrass with barley, the increment in splanchnic and carcass energy expenditure accounted for 30 and 26% of the increment of ME intake in finishing lambs and experimental results on organ and tissue energy expenditure obtained in ruminant lambs below 7 months of age may be applied to growing and finishing lambs.

energy expenditure / splanchnic tissues / hindlimb / growing lamb

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Résumé — Dépenses énergétiques des tissus splanchniques et de la patte arrière chez des agneaux alimentés avec du ray-grass congelé : effets d'une supplémentation en orge et changements entre les âges de 2,5 et 7 mois. Pour étudier les effets d'une supplémentation en orge (Exp. 1), six agneaux de 40 kg de poids vif ont été alimentés avec du ray-grass ou du ray-grass + de l'orge suivant un plan en triple carré latin à un niveau estimé à 1,5 et 2,0 fois leurs besoins d'entretien. Pour étudier les effets de l'âge (Exp. 2), 3, 6 et 5 agneaux âgés de 2,5, 4 et 7 mois, respectivement, ont été utilisés. Ils étaient alimentés avec du ray-grass seul à des niveaux estimés à 1,8, 1,7 et 1,5 fois les besoins énergétiques d'entretien. Tous les animaux étaient équipés de cathéters dans l'artère mésentérique, les veines porte, hépatique et iliaque externe et de sondes ultrasoniques de débit sanguin autour de la veine porte et de l'artère iliaque externe. Dans l'Expérience 1, les débits sanguins porte ont augmenté de 11,5 % avec la supplémentation ($P < 0,05$) alors que le débit iliaque tendait à augmenter de 15 % (NS). En conséquence, la consommation d'O_2 a augmenté de 20 % ($P < 0,03$), 35 % ($P < 0,08$) et 19 % (NS) dans les tissus drainés par la veine porte, le foie et la patte arrière, respectivement, avec la supplémentation en orge. Dans l'Expérience 2, les débits sanguins porte, exprimés sur la base du poids métabolique, ont diminué de 17 % entre les âges de 2,5 et 4 mois et n'ont pas changé entre 4 et 7 mois ($P < 0,004$). Le débit sanguin iliaque n'a pas augmenté entre 2,5 et 7 mois d'âge en raison d'une importante variabilité inter-individuelle. Aucun effet significatif de l'âge n'a été noté sur les dépenses énergétiques des tissus drainés par la veine porte et du foie exprimés sur la base du poids métabolique, tandis que celles de la patte arrière ont eu tendance à augmenter (NS). En conclusion, avec la supplémentation en orge, les augmentations des dépenses énergétiques des tissus splanchniques et de la carcasse représentent 30 et 26 % de l'augmentation de l'EM ingérée chez l'agneau en finition et les résultats expérimentaux sur les dépenses énergétiques des tissus et organes obtenus chez les agneaux ruminants en dessous de l'âge de 7 mois peuvent être appliqués aux agneaux en croissance et en finition.

dépense énergétique / tissu splanchnique / patte arrière / agneau en croissance

1. INTRODUCTION

Supplementation is a normal practice in ruminant production systems, including in growing lambs fed grass. It allows an increased energy intake but also an improved efficiency of metabolisable energy (ME) utilisation [10] as a result of changes in the quantity and the nature of nutrients which are metabolised at the organ and tissue level.

The contribution of splanchnic tissues to whole body energy expenditure (EE) varies with the feeding level and with the nature of the diet. It increases with the feeding level [3, 16, 20, 23] and 17 to 61% of the increment in whole body EE with intake could be attributed to the portal drained viscera (PDV) while 14 to 44% could be due to changes in hepatic metabolism [8, 9, 18, 19, 23]. In addition, as for the effects of the nature of the diet, the contributions of splanchnic tissues are lower with concentrate rich diets than with forage rich diets [18, 23]. Barley supplementation of grass diets implies both an increased energy intake and a change in diet composition, the combined effects of which on splanchnic EE are not known.

Regarding the contribution of muscle to whole body EE, little data are available on the effects of feeding level and the nature of the diet. In adult animals, the muscle mass contributes to only 5 to 7% to the increment in whole body EE with intake [19, 28] and muscle contribution to whole body EE decreases with intake [17, 19]. No data are available in ruminant lambs.

In growing animals age can also modify organ and tissue EE. In growing bovines, the splanchnic contribution to whole body EE decreases with increasing age, while that of the hindquarters does not seem to change [3]. No such data are available in ovines where body composition changes more rapidly with time, which restricts the extrapolation of research findings from one growth stage to another. Consequently, the objectives of the present paper were to study the effects of (1) barley supplementation and (2) age on the EE of splanchnic tissues and hindlimbs in lambs fed frozen grass. The effects of barley supplementation on tissue and organ EE were studied in a separate experiment, whose data on net nutrient fluxes are published elsewhere [13]. The effects of age were studied by combining the results obtained in different experiments which have been partly published and which address other issues [12–14].

2. MATERIALS AND METHODS

2.1. Experiment 1. Influence of barley supplementation on splanchnic and hindlimb energy expenditure

Six male cross-bred, early weaned lambs of 7 months of age and weighing 40.1 ±1.5 kg on average were used. The animals were equipped with chronic blood catheters in the mesenteric artery (A), portal (P), hepatic (H) and external iliac (eI) veins and with 2 ultrasonic blood flow probes around the portal vein (16A) and external iliac artery (3R), as described in detail by Majdoub et al. [14]. The duration of the post-surgical recovery period was a minimum of one week based on the end of medication, a return to normal behaviour and a stabilisation of intake level at pre-surgical levels without any digestive perturbations. This was followed by an adaptation period to the basal diet of two weeks. Subsequently, the lambs successively received two treatments according to a triplicate Latin Square design. For both treatments, they were offered perennial rye-grass (first cut, fertilised at 80 kg N·ha^{-1}, harvested at the grazing stage, chopped in 5 cm length, frozen at –35 °C and stored at –15 °C) at an estimated level of 690 kJ ME per average metabolic liveweight ($W^{0.75}$). The two treatments were: (1) the control treatment constituted of rye-grass only (RG) and (2) rye-grass + barley (RG+B). They were applied for 2 weeks each. In the RG+B treatment, the animals were supplemented daily with 210 kJ ME whole grain barley per kg $W^{0.75}$. The ME contents of rye-grass and barley were estimated respectively at 11.6 and 13.25 MJ per kg dry matter (DM) [10]. Feed allowances were distributed in 12 equal daily meals every 2 h.

Feed samples were taken at regular intervals and pooled per treatment for subsequent determination of Kjehldal N and crude fibre (CF) contents.

At the end of the experimental period, portal and iliac blood flows were recorded continuously over 2 feeding cycles. Hepatic arterial blood flows were estimated at 5.3% of the portal blood flow [14]. In order to account for changes in spontaneous physical activity and their influence on hindlimb blood flows, visual observations of animal behaviour were made and iliac blood flows were calculated for quietly standing animals according to Isserty and Ortigues [11].

Six sets of blood samples were collected in heparinised glass syringes for the immediate determination of total oxygen content according to [29]. Sampling was always carried out on quietly standing lambs. Oxygen consumption and fractional extraction were calculated as in [14]. Tissue or organ EE (kJ·h^{-1}) was calculated as 20.44 × O_2 consumption (L·h^{-1}) [15]. The experiment was conducted in a manner compatible with national legislation on animal care (Certificate of Authorisation to Experiment in Living Animals, No. 004495, Ministry of Agriculture).

Data were analysed by analysis of variance with animal, treatment and period as the main factors. In an attempt to dissociate the effects of ME intake (MEI) from those of other components of intake, a descriptive analysis of relations between PDV, liver and hindlimb EE, MEI, and nitrogen intake

(NI) was also performed using a principal component analysis [27]. Finally, the proportions of variance in organ and tissue EE explained by their contributors (blood flows and O_2 concentrations) or their factors of variation (MEI and NI) were calculated by a multiple ascending regression approach [27].

2.2. Experiment 2. The effect of age on splanchnic and hindlimb energy expenditure

A total of 14 male, cross-bred, early-weaned lambs of different ages and weights were used. Three age groups were represented: 2.5, 4 and 7 months of age with 3, 6 and 5 animals in each group, respectively. Animal weights averaged 25.8 ± 6.1 kg, 32.1 ± 2.3 kg and 40.1 ± 1.5 kg, respectively. The 7-month old age group corresponded to that used in Experiment 1. The surgical preparation of all animals was similar to that described in Experiment 1. All lambs received the basal diet of the same nature as in Experiment 1 (first cut perennial rye-grass, fertilised at 80 kg $N \cdot ha^{-1}$, harvested at the grazing stage, chopped in 5 cm length, frozen at –35 °C and stored at –15 °C). Two different batches of grass obtained from the same plot at a 1-year interval were used. The first batch was fed to the 2.5 and 4-month-old animals, the second batch to the 7-month-old lambs. For both batches, the ME content of rye-grass was estimated at 11.6 $MJ \cdot kg^{-1}$ DM [10]. The lambs were planned to be fed the same level of MEI per kg metabolic body weight, however, because of variations in the DM content of the grass between the 2 batches and within a batch, the lambs received the same diet at different levels of MEI. The lambs of the 2.5, 4 and 7 month old groups were fed, respectively, at 1.8, 1.7 and 1.5 fold the maintenance ME requirements estimated at 460 $kJ \cdot kg\ W^{-0.75} \cdot d^{-1}$ in 12 equal meals every 2 h. Feed distribution, sampling, measurements and calculations were as described for Experiment 1.

Because of the possible confounding effects between age, MEI, crude fibre intake (CFI) and NI, a descriptive analysis of relations between variables (expressed when necessary per kg metabolic live-weight; PDV, liver and hindlimb EE, MEI, CFI and NI) was initially performed according to a principal component analysis [27]. The relative impact of live-weight and MEI on tissue EE and their determinants (blood flow, arterial concentrations, arterio-venous concentration differences) was determined by a multiple regression approach as in Experiment 1. The effect of age on tissue EE was subsequently analysed according to a one-way factorial design using the GLM procedure [25] and using MEI (expressed by kg metabolic live-weight) as a covariable.

3. RESULTS

3.1. Experiment 1. The influence of barley supplementation on splanchnic and hindlimb energy expenditure

Correct positioning of catheters was checked at necropsy. The results were obtained on 5 animals for the splanchnic tissues and 3 animals only for the hindlimb due to the infection of one animal at the catheter level and a lack of patency for 2 iliac catheters, respectively. The dietary characteristics have already been described in [13]. Briefly, the N and CF contents averaged 2.25% and 24.1% on a DM basis for the RG diet and 2.20% and 18.1% for the RG+B diet. The daily DM intake (DMI), MEI and NI increased with barley supplementation by 28%, on average ($P < 0.003$) (Tab. I).

Portal blood flows increased by 11.5% with the RG+B treatment ($P < 0.05$, Tab. I) while the iliac blood flow increased numerically by 15% (NS). Arterial blood O_2 oncentrations increased numerically by 9% with barley supplementation (Tab. I).

Table I. Animal weight, dietary intake, blood flow, blood oxygen concentrations, fractional extraction and energy expenditure by the portal drained viscera (PDV), liver, splanchnic tissues and hindlimb in lambs fed frozen rye-grass supplemented with barley.

	n	Treatments		SEM	Treatment effect
		Rye-grass	Rye-grass + barley		
Live weight (kg)	5	40.1	40.3	0.47	NS
Intake					
– Dry matter ($g \cdot d^{-1}$)	5	926	1177	46.6	0.003
– Metabolisable energy ($MJ \cdot d^{-1}$)	5	10.7	14.1	0.54	0.002
– Crude fibre ($g \cdot d^{-1}$)	5	223.6	213.2	4.54	NS
– Nitrogen ($g \cdot d^{-1}$)	5	20.8	25.8	0.95	0.002
Blood flows					
– Portal vein ($L \cdot h^{-1}$)	5	113	126	3.2	0.05
– Iliac artery ($L \cdot h^{-1}$)	3	8.50	9.78	0.34	NS
Blood O_2 concentrations ($mmol \cdot L^{-1}$)					
– Artery	5	6.43	7.00	0.21	NS
Blood O_2 concentration differences ($mmol \cdot L^{-1}$)					
– Portal – arterial	5	–1.81	–1.99	0.070	NS
– Hepatic – portal	5	–1.07	–1.31	0.069	NS
– Hepatic – arterial	5	–2.88	–3.30	0.088	0.05
– Iliac – arterial	3	–3.61	–3.75	0.032	NS
O_2 Fractional extraction (%)					
– PDV	5	28.3	28.4	0.07	NS
– Liver	5	24.8	27.8	0.09	NS
– Splanchnic tissues	5	45.0	47.2	0.05	0.05
– Hindlimb	3	56.5	54.7	0.13	NS
Energy expenditure ($MJ \cdot d^{-1}$)					
– PDV	5	2.288	2.758	0.0718	0.03
– Liver	5	1.541	2.078	0.1378	0.08
– Splanchnic tissues	5	3.829	4.836	0.1778	0.03
– Hindlimb	3	0.340	0.406	0.0094	NS

SEM = √(error mean square / nb observations per treatment).

Organ and tissue energy expenditure was calculated from O_2 uptake, assuming that one mole of O_2 corresponded to 457.9 kJ.

P values higher than 0.10 were considered as non significant (NS).

The H–A O_2 concentration difference increased by 14.5% with the treatment ($P < 0.05$) while P–A, H–P and eI–A O_2 concentration differences increased non significantly by 10%, 22%, and 4%, respectively. Consequently, organ and tissue EE increased with barley supplementation by 20% ($P = 0.03$), 35% ($P = 0.08$) and 19% (NS) in the PDV, liver, and hindlimb, respectively (Tab. I).

The descriptive analysis of the relations between the main variables (PDV, liver and hindlimb EE, MEI and NI) was completed by a principal component analysis (Fig. 1). Sixty-four percent of the total variance among data was attributed to PDV and liver EE, MEI and NI which defined the first axis, while 20.5% of the total variance was attributed to hindlimb EE (second axis), showing an independence between splanchnic and peripheral tissue EE. The discrimination of animals between the 2 treatments was clear and mostly due to the first axis.

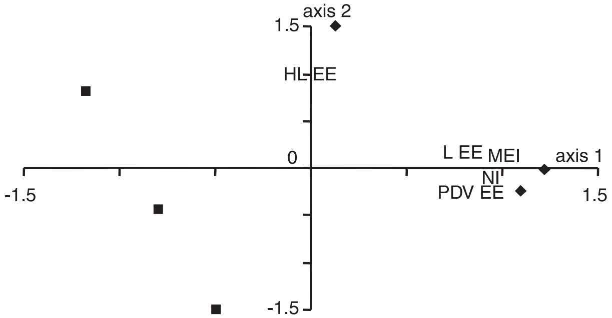

Figure 1. Biplot of the principal component analysis of data obtained on lambs fed frozen rye-grass supplemented with barley. The variables considered were: portal drained viscera, liver and hindlimb energy expenditure (PDV EE, L EE, HL EE), metabolisable energy intake (MEI) and dietary nitrogen intake (NI); projection of the observations identified by treatments (■: ray-grass diet, ◆: ray-grass + barkey diet).

The relative weight of the different contributors to tissue EE (blood flow and O_2 concentration differences) was quantified by an incremental regression approach. In this analysis, EE, blood flows, MEI and NI were expressed per kg metabolic weight. Before all, it should be noted that at the splanchnic level, quantitatively (but not statistically) the treatment effects were greater on O_2 concentration differences than on portal blood flow values. Consequently, the variance in PDV and liver EE was explained to a large extent (81% and 82%, respectively) by the variance in O_2 concentration differences and to a lower extent (19% and 17%, respectively) by that in portal blood flow. The portal blood flow itself was explained by 54% by the variance in MEI. The variance in P–A O_2 concentration differences was explained at 27% by arterial concentration and H–P O_2 concentration differences at 34% by portal concentrations. At the hindlimb level, the variance in EE was explained by 95% and 5% by the variance in iliac blood flow and eI–A O_2 concentration differences, respectively. Iliac blood flow itself did not depend on MEI or NI.

3.2. Experiment 2. Effect of age on splanchnic and hindlimb energy expenditure

The chemical composition of the grass offered averaged 20.8, 20.7 and 24.1% CF and 3.0, 3.2 and 2.2% N, DM basis, for the 2.5, 4 and 7 month old age groups, respectively. Expressed per kg metabolic weight, DMI, MEI, CFI and NI tended to decrease with age (NS) while NI decreased significantly with age ($P < 0.01$, Tab. II). The correct positioning of the catheters was checked at necropsy. Because of a lack of catheter patency in the iliac vein, hindlimb results were obtained on 3 animals of each age group only.

The principal component analysis showed that the variance among the following variables: PDV, liver and hindlimb EE, dietary intake (expressed per kg $W^{0.75}$), i.e. MEI, CFI and NI, could be explained at 86% by two axes (Fig. 2). The first axis which explained 65% of the total variance was described by PDV and liver EE, MEI, CFI and NI. These variables were highly and positively correlated to each other. The higher the MEI, the higher the splanchnic

Table II. Animal weight, dietary intake, blood flow, blood oxygen concentration, fractional extraction and energy expenditure by the portal-drained viscera (PDV), liver and hindlimb in lambs of different ages fed frozen rye-grass. Metabolisable energy intake (MEI) was taken as the covariable. Least square means are presented.

	Age (months)			SEM	Probability	
	2.5	4	7		Treatment	MEI covariable
Live weight (kg)	27.0	32.5	38.9	1.16	0.0005	0.02
Intake						
– Dry matter ($g \cdot d^{-1} \cdot kg\ W^{-0.75}$)	73.22	68.22	58.23	6.37	NS	–
– Metabolisable energy ($kJ \cdot d^{-1} \cdot kg\ W^{-0.75}$)	848	789	674	74	NS	–
– Crude fibre ($g \cdot d^{-1} \cdot kg\ W^{-0.75}$)	15.20	14.13	14.05	0.04	NS	–
– Nitrogen ($g \cdot d^{-1} \cdot kg\ W^{-0.75}$)	2.20^b	2.18^b	1.31^a	0.08	0.01	–
Blood flow ($L \cdot h^{-1} \cdot kg\ W^{-0.75}$)						
– Portal	9.40^a	7.76^b	7.07^b	0.26	0.004	0.001
– Iliac	0.36	0.46	0.61	0.07	NS	0.06
Arterial O_2 concentration ($mmol \cdot L^{-1}$)	5.87	6.52	6.39	0.25	NS	NS
Blood O_2 concentration differences ($mmol \cdot L^{-1}$)						
– Portal – arterial	–1.63	–1.84	–1.84	0.101	NS	NS
– Hepatic – arterial	–2.62	–3.03	–2.94	0.123	NS	NS
– Hepatic – portal	–0.99	–1.19	–1.10	0.060	NS	NS
– Iliac – arterial	–3.02	–3.33	–3.62	0.305	NS	NS
O_2 fractional extraction (%)						
– PDV	27.8	28.3	29.0	1.80	NS	NS
– Liver	24.8	27.1	25.8	1.25	NS	0.05
– Hindlimb	51.7^a	53.1^a	56.0^b	4.79	0.001	NS
Energy expenditure ($kJ \cdot kg\ W^{-0.75} \cdot d^{-1}$)						
– PDV	169	158	142	13.0	NS	0.06
– Liver	117	115	95	5.0	NS	0.001
– Hindlimb	12	20	24	3.2	NS	NS

For results on the splanchnic tissues, there were 3, 6 and 5 observations for the 2.5, 4 and 7 month old animals, respectively. For results on the hindlimb, there were 3, 3 and 3 observations for the 2.5, 4 and 7 month old animals, respectively.

Organ and tissue energy expenditure was calculated from O_2 uptake, assuming that one mole of O_2 corresponded to 457.9 kJ.

P values higher than 0.10 were considered as non significant (NS); $^{a, b}$: values with different superscripts were different at $P < 0.05$.

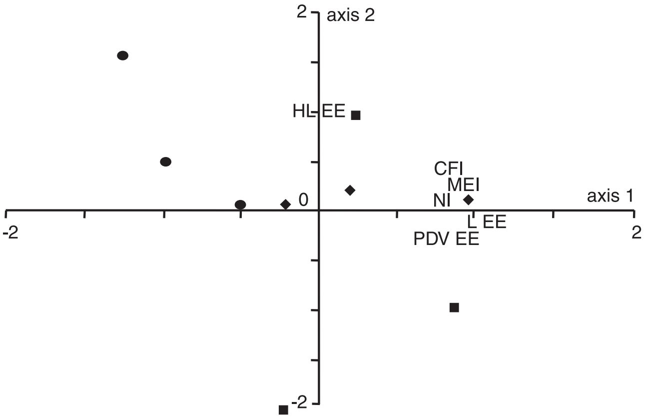

Figure 2. Biplot of the principal component analysis of data obtained on lambs aged of 2.5, 4 and 7 months fed frozen rye grass. The variables considered were: portal drained viscera, liver and hindlimb energy expenditure (PDV EE, L EE, HL EE), metabolisable energy intake (MEI), crude fibre and nitrogen intakes (CFI, Ni); projection of the observations identified by age (■: 2.5, ◆: 4, ●: 7 months).

tissue EE, and high MEI were generally associated with higher dietary CFI and NI. The second axis, which explained 21% of the total variance, was only described by hindlimb EE. Moreover, the respective positions of splanchnic EE, MEI, CFI and NI on axis 1 and hindlimb EE on axis 2 depicted the lack of correlation between these two groups of variables. Finally, when plotting individual animals along those two axes, the different age groups could not be clearly discriminated.

Subsequently, a multiple regression approach was applied in order to identify the major contributors of tissue EE and to quantify the impact of live-weight and MEI on their variance. All variables were expressed on a metabolic body weight basis. The majority of the variance in splanchnic tissue EE was explained by the variance in portal blood flow (65% for the PDV and 61% for the liver), arterio-venous O_2 concentration differences were smaller contributors (35% for the PDV and 39% for the liver). The variance in portal blood flow itself was explained for 63% by the variance in MEI and for 9% by the variance in CFI. No clear relationships could be drawn between changes in arterio-venous O_2 concentration differences, live weight and dietary intake, except between H–P O_2 concentration differences and live weight ($r^2 = 0.32$). Similarly at the hindlimb level, iliac blood flow explained 77% of the variance in hindlimb EE, the contribution of eI-A O_2 concentration differences was minor ($r^2 = 0.20$). The variance in the iliac blood flow itself was explained by 33% by the variance in CFI and 13% by the variance in MEI.

The above analyses clearly indicated that differences in MEI across age groups introduced biases when considering the potential effect of age on tissue and organ EE. For this reason a covariable was included in the analysis of variance. MEI was the most obvious covariable to use. No other covariable was used, CFI being correlated to MEI ($r = 0.97$), and when NI was used as another covariable it had a low significance and did not improve the statistical significance of the model.

The analysis of variance showed that portal blood flows ($L \cdot h^{-1} \cdot kg\ W^{-0.75}$) decreased by 17.4% from 2.5 to 4 months of age and did not change from 4 to 7 months of age (Tab. II, $P < 0.004$). On the contrary, iliac blood flows tended to increase strongly (+69%) and linearly from 2.5 to 7 months of age (NS). No effect of age could be detected on arterial O_2 concentrations. H–A and H–P O_2 concentration differences tended to be lower in 2.5 month old lambs (NS) but were not associated with significant differences in fractional extraction. On the contrary, the fractional extraction of O_2 at the hindlimb level increased with age ($P < 0.001$). No significant effect of age was noted on PDV and liver EE expressed on a metabolic weight basis, while hindlimb EE tended to increase with age (NS) (Tab. II).

4. DISCUSSION

4.1. Effect of supplementation

The barley supplementation applied here involved both an increase in MEI and NI. Generally, a higher intake induces an increase in organ and tissue EE [2, 18, 20, 23], as measured here with barley supplementation. Qualitatively, as shown by the principal component analysis, the relationships between MEI and PDV and liver EE were strong. The increment in PDV and liver EE represented 14 and 16% of the increment in MEI with barley supplementation. At the PDV level, these effects are of the same order of magnitude as those reported by Goetsch [5] and Seal and Reynolds [26] (11 to 27% of the increment in MEI), even if the rate of increase in PDV EE seems to decline towards ad libitum [5]. At the hepatic level, the increase in EE was higher than that predicted from Ortigues and Visseiche equations [16] using similar levels of intake (+35% vs. +24%) but remained within the limits of other published data (see review by Ortigues and Doreau [18]). These effects suggest that with barley supplementation the influence of the MEI increase was preponderant over that of a change in energy density of the diet. The results on the quantity and the pattern of nutrients in the portal vein of those animals confirm this interpretation; since the profile of nutrients appearing in the portal vein was little affected by barley supplementation [13]. Indeed with the basal diet considered here (frozen rye grass) which allowed a propionic–type ruminal fermentation profile, barley supplementation at the limited chopped (21% of total DMI) was shown to favour ruminal acetate and butyrate production with little impact on the ratio of gluco–to cetogenic nutrients at the portal level [13].

At the hindlimb level, barley supplementation did not significantly increase EE. An effect of feeding level on hindlimb EE has been shown by Harris [7], however, the increase in EE is generally of much lower magnitude than that of splanchnic tissues [16] and therefore difficult to detect. When extrapolating the non significant increase in hindlimb EE to the whole musculature [14], it could be calculated that with barley supplementation 26% of the increment of MEI would be lost as EE in muscles.

4.2. Effect of age

Before applying the above mentioned research findings, it was important to check that the growth stage of the lambs would not greatly modify the partition of energy utilisation among organs and tissues. Goetsch [5] suggested that the effects of dietary intake on splanchnic tissue EE may differ with the stage of maturity of the animal. The present data were the first attempt to address this issue in lambs which have a shorter growth period than cattle. Unfortunately some confounding effect was present in those data with a strong correlation between MEI and age. When using MEI as a covariable, PDV and liver EE (expressed relative to metabolic body weight) were shown to be unaffected by age (between 2.5 and 7 months of age) even if a numerical trend for a decrease in EE with age could be calculated (–12%). This trend was the same as that found by Eisemann et al. [3] in bovines with a larger number of experimental animals at the same estimated maturity stage (ratio of W to mature W) as our 2.5 and 7 month old lambs, i.e. 0.4 and 0.6. This trend is coherent with the known decrease in the contribution of splanchnic tissues to body weight with age in ruminants [1, 4, 24].

In the hindlimb, EE (expressed per metabolic body weight) increased non significantly with age. The estimated contribution of total musculature to whole body EE (calculated as described by Majdoub et al. [14]) increased with body weight (Fig. 3). This tendency was the opposite to that noted by Eisemann et al. [4] who found a light decrease in EE. These differences could not be explained by changes in body composition with age between ovine and bovine species since the percentage of muscles in the empty body weight is not greatly different between species (33% in ovines vs. 30% in bovines, on average) and is quite stable between the 0.4 to 0.6 maturity stages in both species [21, 24]. The effect is mainly attributed to lambs of the first group (2.5 months of age which had just been weaned). A more likely hypothesis is that in those animals, the growth rate of the carcass, and thus of the hindlimb, had been temporarily slowed down to the benefit of the splanchnic tissues. Furthermore, the number of animals we used was limited and the effects noted at the hindlimb level are always proportionally lower than those noted for the splanchnic tissues [4].

Consequently, within the range of age considered, the changes in PDV, liver and hindlimb EE with age, when expressed relative to metabolic body weight, appeared to be marginal in lambs.

4.3. Determinants of energy expenditure

It is generally admitted that organ and tissue EE depend strongly on blood flow [2, 5, 18, 22, 23]. According to Ortigues and Doreau [18], changes in blood flows could explain 40%, 50% and 50% of the changes in O_2 consumption in the PDV, liver and hindlimb respectively. Our results on splanchnic tissues differed between the 2 experiments. In Experiment 1, when the animals had similar W and received diets of different energy density, PDV and liver EE were highly correlated to O_2 concentration differences (r^2 = 0.81 and 0.82, respectively) and to a lower extent to blood flow. In Experiment 2 on the contrary, when the animals had widely different W and DM and ME allowances, blood flow was the most important determinant of the splanchnic tissue which explained 0.65 and 0.61 of PDV and liver EE variation, respectively. So, the changes in the portal blood

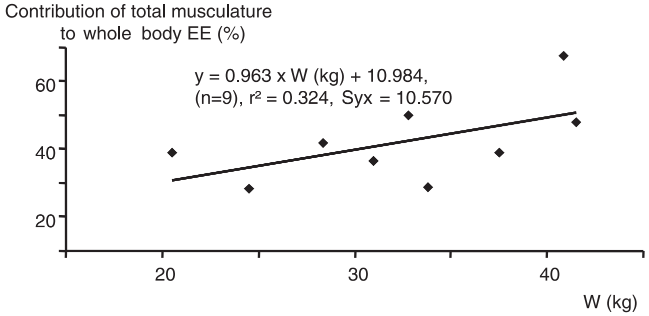

Figure 3. Estimated contribution of total musculature to whole body EE (%) as a function of live weight (W, kg) in lambs fed frozen rye-grass.

flow with animal weight are probably an important factor. Additionally, according to recent results by Han et al. [6], O_2 consumption by splanchnic tissues would be mainly related to changes in blood flow above maintenance and to changes in fractional O_2 extraction below maintenance. Also portal blood flow would be related to dietary bulk and O_2 fractional extraction to nutrient supply. Our results suggest that O_2 fractional extraction can also be modulated above maintenance by dietary density.

At the hindlimb level, iliac blood flow was the most important determinant of EE which explained 0.95 and 0.77 of the variation in Experiment 1 and Experiment 2, respectively.

4.4. Relation between tissues and organs

PDV and liver EE were highly correlated ($r^2 = 0.52$ in Exp. 1 and 0.55 in Exp. 2). The results in the literature also showed concomitant increases of PDV and liver EE in response to a rise in the feeding level [2, 3, 16, 20, 23]. However, all the results concerning the relation between splanchnic tissues and hindlimb EE were not completely coherent. Splanchnic tissues and hindlimb EE were independent as confirmed by the principal component analyses, the analysis of variance of Experiment 2 and the low correlations between them ($r^2 = 0.0$ and 0.0 in Exp. 1 and Exp. 2, respectively). However, the analysis of variance in Experiment 1 showed an increase in splanchnic tissue EE and a smaller increase in hindlimb EE, which was in agreement with the results obtained from the equations of Ortigues and Visseiche [16].

5. CONCLUSION

When supplementing a grass diet with barley in lambs, 56% of the increment in MEI would be lost as EE in splanchnic tissues and in the whole musculature while the rest (44%) would be available for energy deposition and energy requirements of other tissues. These results probably apply to lambs ranging in age from 2.5 to 7 months since the EE of splanchnic tissues and hindlimbs expressed relative to metabolic body weight were little affected by age.

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REFERENCES

[1] Brody S., Bioenergetics and growth, Reinhold Publishing corporation, New York, 1945.

[2] Burrin D.G., Ferrell C.L., Eisemann J.H., Britton R.A., Nienaber J.A., Effect of level of nutrition on splanchnic blood flow and oxygen consumption in sheep, Brit. J. Nutr. 62 (1989) 23–34.

[3] Eisemann J.H., Nienaber J.A., Tissue and whole-body oxygen uptake in fed and fasted steers, Brit. J. Nutr. 64 (1990) 399–411.

[4] Eisemann J.H., Huntington G.B., Catherman D.R., Patterns of nutrient interchange and oxygen use among portal drained viscera, liver, and hindquarters of beef steers from 235 to 525 kg body weight, J. Anim. Sci. 74 (1996) 1812–1831.

[5] Goetsch A.L., Splanchnic tissue energy use in ruminants that consume forage-based diets ad libitum, J. Anim. Sci. 76 (1998) 2737–2746.

[6] Han X.T., Nozière P., Rémond D., Chabrot J., Doreau M., Effects of nutrient supply and dietary bulk on O_2 uptake and nutrient net fluxes across rumen, mesenteric– and portal-drained viscera in ewes, J. Anim. Sci. 80 (2002) 1362–1375.

[7] Harris P.M., Garlick P.J., Lobley G.E., Interactions between energy and protein metabolism in the whole body and hindlimb of sheep in response to intake, in: van der Honing Y., Close W. H. (Eds.), Energy metabolism of farm animals, EAAP Publication No. 43, Proceedings of the

2nd symposium, Lunteren, Netherlands, 1989, pp. 167–170.

[8] Huntington G.B., Energy metabolism in the digestive tract and liver of cattle: influence of physiological state and nutrition, Reprod. Nutr. Dev. 30 (1990) 35–47.

[9] Huntington G.B., Eisemann J.H., Whitt J.M., Portal blood flow in beef steers: comparison of techniques and relation to hepatic blood flow, cardiac output and oxygen uptake, J. Anim. Sci. 68 (1990) 1666–1673.

[10] INRA, Alimentation des Ruminants, INRA Publications, Versailles, 1978.

[11] Isserty A., Ortigues I., Méthodes d'exploitation des données concernant les débits sanguins mesurés au niveau des viscères et du train-arrière chez la brebis, Reprod. Nutr. Dev. 34 (1994) 399–413.

[12] Majdoub L., Savary I., Bayle G., Grizard J., Vermorel M., Ortigues-Marty I., Répartition des nutriments azotés et des dépenses énergétiques entre le tube digestif, le foie et les muscles chez des agneaux recevant du ray-grass, stade pâturage, Renc. Rech. Ruminants, 1999, pp. 119–122.

[13] Majdoub L., Vermorel M., Ortigues-Marty I., Ryegrass based diet and barley supplementation: partition of energy-yielding nutrients among splanchnic tissues and hindlimb in finishing lambs, J. Anim. Sci. 81 (2003) 1068–1079.

[14] Majdoub L., Vermorel M., Ortigues-Marty I., Intraruminal propionate supplementation modifies hindlimb energy metabolism without changing the splanchnic release of glucose in growing lambs, Brit. J. Nutr. 89 (2003) 39–50.

[15] Mc Lean J.A., The significance of carbon dioxide and methane measurements in the estimation of heat production in cattle, Brit. J. Nutr. 55 (1986) 631–633.

[16] Ortigues I., Visseiche A.L., Whole body fuel selection in ruminants: nutrient supply and utilization by major tissues, Proc. Nutr. Soc. 54 (1995) 235–251.

[17] Ortigues I., Adaptation du métabolisme énergétique des ruminants à la sous-alimentation. Quantification au niveau de l'animal entier et des tissus corporels, Reprod. Nutr. Dev. 31 (1991) 593–616.

[18] Ortigues I., Doreau M., Responses of the splanchnic tissues of ruminants to changes in intake: absorption of digestion end products, tissue mass, metabolic activity and implications to whole animal energy metabolism, Ann. Zootech. 44 (1995) 321–346.

[19] Ortigues I., Durand D., Adaptation of energy metabolism to under-nutrition in ewes. Contribution of portal-drained viscera, liver and hindquarters, Brit. J. Nutr. 73 (1995) 209–226.

[20] Patil A.R., Goetsch A.L., Park K.K., Kouakou B., Galloway D.L. Sr., Johnson Z.B., Influence of grass source and legume level on net flux of nutrients across splanchnic tissues in sheep, Small Rum. Res. 22 (1996) 111–122.

[21] Prud'hon M., La croissance globale de l'agneau : ses caractéristiques et ses lois, in: INRA, ITOVIC (Eds.), 2^{es} Journées de la Recherche Ovine et Caprine, Paris, 1976, pp. 9–26.

[22] Reynolds C.K., Lapierre H., Tyrrell H.F., Elsasser T.H., Staples R.C., Gaudreau P., Brazeau P., Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: net nutrient metabolism by portal-drained viscera and liver, J. Anim. Sci. 70 (1992) 752–763.

[23] Reynolds C.K., Tyrrell H.F., Reynolds P.J., Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: whole body energy and nitrogen balance and visceral heat production, J. Nutr. 121 (1991) 994–1003.

[24] Robelin J., Composition corporelle des bovins: évolution au cours du développement et différences entre races, Thèse de doctorat d'état, série E, n^o d'ordre 368, Université de Clermont-Ferrand II, France, 1986.

[25] SAS Institute Inc., SAS /STAT User's Guide, Version 6, Fourth Edition, Volume 2, SAS Institute Inc., Cary, NC, USA, 1990.

[26] Seal C.J., Reynolds C.K., Nutritional implications of gastrointestinal and liver metabolism in ruminants, Nutr. Res. Rev. 6 (1993) 185–208.

[27] Statoft, Statistica, version 5.5, Tulsa, OK 74104, USA, 1984–2000.

[28] Symonds M.E., Lomax M.A., Effect of chronic cold exposure and underfeeding on hind-limb tissue metabolism in pregnant sheep, J. Agric. Sci. 115 (1990) 421–428.

[29] Tucker V.A., Method for oxygen content and dissociation curves on microliter blood samples, J. Appl. Physiol. 23 (1967) 410–414.