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Original article

Propionate supplementation did not increase whole body glucose turnover in growing lambs fed rye grass

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Abstract — The objective of the present study was to investigate the effects of propionate supplementation on whole body glucose turnover in growing lambs fed frozen rye-grass at 1.5 × maintenance using [1-^{13}C]-glucose. Intraruminal infusion of propionate (0.55 and 0.91 mol·d^{-1}) increased the ruminal molar proportions of propionate from 25% with the control to 40% with the highest propionate treatment. It did not however modify glucose turnover (26 mmol·d^{-1}·kg^{-1}), nor the conversion of its carbon into L-lactate (21%) and alanine (21%), nor glucose recycling (9%). All of the results suggest that in the present conditions glucose turnover and metabolism were not influenced by the supply of propionate.

glucose turnover / propionate / growing lambs / [1-^{13}C]-glucose

1. INTRODUCTION

In the context of improvement of beef meat quality, in particular of tenderness, juiciness and flavour, it has been suggested that nutrition and in particular the ratio between gluconeogenic and ketogenic substrates can influence the orientation of muscle energy metabolism and therefore muscle characteristics involved in meat quality [1, 2]. An increased supply of glucose to muscle may favour the glycolytic metabolism and increase the intramuscular glycogen content and triglyceride synthesis [3–5].

In a recent study designed to tackle this issue in growing lambs fed rye-grass, hepatic production of glucose was not modified by propionate supplementation, despite a significant increase in hepatic propionate extraction and an unchanged potential contribution of other gluconeogenic substrates (lactate, glycerol) to glucose synthesis [6]. This effect was associated to a significant

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increase in insulin secretion and in its hepatic extraction. Despite the unchanged splanchnic release of glucose, Majdoub et al. [6] reported an increase in glucose uptake by the hind limb. They suggested that propionate, via insulin, inhibited any increase in the net hepatic release of glucose but probably increased whole body glucose turnover via an enhanced renal glucose production. Majdoub et al. [6] also reported modifications in lactate metabolism with propionate supplementation and suggested that these modifications were associated to modifications in glucose metabolism. Other authors [7, 8] had reported an increase in glucose turnover in growing steers supplemented with propionate. An increased supply of propionate to growing lambs or to lactating cows has not however been systematically associated to a higher net hepatic production of glucose [6, 9, 10].

Consequently, the objectives of the present paper were to test the hypotheses raised by [6]. They were aimed at investigating the effects of propionate supplementation on glucose turnover and on the conversion of glucose carbons into three-carbon substrates in growing lambs. [1-^{13}C]-glucose was used. The use of [1-^{13}C]-glucose, well developed for human studies, is innovative in ruminant studies. It specifically allows the determination of the recycling of glucose carbon, and of the possible carbon interconversions between glucose and L-lactate. The results were subsequently interpreted in the light of splanchnic and hindlimb metabolism data obtained in [6].

2. MATERIALS AND METHODS

2.1. Animals, diet and treatments

Six INRA 401, 3 month-old, male lambs of 26 (SD 1.0) kg were surgically equipped with a rumen cannula (12 mm internal diameter) and chronic blood catheters in the right jugular vein and carotid. During the whole experimental period, the catheters were rinsed with sterile saline and filled with diluted heparin (2500 IU heparin·mL^{-1} saline). During sampling, the catheters were filled with heparin diluted at 500 IU·mL^{-1} saline.

The animals were housed in individual stalls with ad libitum access to drinking water and salt lick, under continuous lighting. After a minimum 1-week recovery period from surgery, the lambs were adapted to the basal diet for 2 weeks. They were offered perennial rye-grass (first cut, fertilised at 80 kg nitrogen (N)·ha^{-1}, harvested at the grazing stage, chopped into 5 cm lengths, frozen at –35 °C and stored at –15 °C) at an estimated daily level of 690 kJ metabolizable energy intake (MEI)·kg body weight (BW)$^{-0.75}$, i.e. approximately 75% of the ad libitum level, in 24 daily equal meals. Rye-grass metabolizable energy (ME) content was estimated at 2.7 Mcal·kg^{-1} dry matter (DM) [11].

The lambs (at an average experimental weight of 30 ± 0.6 kg) successively received three treatments according to a 3 × 3 Latin Square design, with two independent repetitions. The treatments consisted of 8 d of continuous intraruminal infusion of a salt solution for the control treatment (C), or of a propionic solution at 0.23 M for the Propionate 1 (P1) and at 0.41 M for the Propionate 2 (P2) treatments. The details of the treatments are reported in [6].

On the last day of intraruminal infusion, a solution of [1-^{13}C] glucose (99 atom % excess ^{13}C, Eurisotop, St Aubin, France) at a concentration of 12.6 mmol·L^{-1} was infused in the jugular vein during 4 h (11.00–15.00 h). [1-^{13}C] glucose was diluted in saline and sterilised. A priming dose (627.8 mmol [1-^{13}C] glucose diluted in 20 mL of saline) was injected before the continuous infusion of a solution of [1-^{13}C] glucose at a rate of 0.5 mL·min^{-1} (0.21 µmol·min^{-1}·kg BW^{-1}).

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

2.2. Measurements and chemical analyses

The animals were weighed just before the start and at the end of each treatment period. Feed samples were taken daily, pooled for each animal and treatment period, prior to analysing their chemical composition (dry matter, organic matter, crude protein, soluble nitrogen, crude fibre and soluble sugar).

The day of [1-^{13}C] glucose infusion, eight blood samples were taken from the carotid (between 10.00 h and 15.00 h). Two samples were taken 30 and 50 min before the start of [1-^{13}C] glucose infusion (10.10 h and 10.30 h). The other six samples were taken in the last 2 h of the [1-^{13}C] glucose infusion, every 20 min. On four sampling days (two with C and two with P2), additional samples were taken at 20 min intervals over the whole duration of the [1-^{13}C] glucose infusion in order to describe the changes in ^{13}C enrichment of the different molecules with time. For each sample, 3 mL of blood were taken using EDTA-K as the anticoagulant (25 µL) and aprotinine as the antipeptidase (1/10 v/v) and used for hematocrit determination prior to centrifugation at –4 °C. Plasma was frozen at –20 °C for further analyses of insulin (Kit Insulin-CT, Cis Bio international, Gif-sur-Yvette, France, kit validated with goat insulin and parallelism of standard curves checked between goat and sheep insulin). Additionally, 5 mL of blood were taken using diluted heparin (500 IU heparin·mL^{-1} saline), immediately deproteinized with 10 mL of perchloric acid (0.6 N) and centrifuged at –4 °C. The supernatant was either (5 mL) neutralised with K_2CO_3 (3.2 M) for the subsequent determination of the ^{13}C isotopic enrichment of glucose, L-lactate and alanine or stored at –20 °C for further analyses of glucose [12] and L-lactate [13] concentrations. For the determination of the isotopic enrichment of glucose, the neutral supernatant was purified by sequential anion-cation exchange chromatography (AG-1-X8-formate and AG-50-WX4-H$^+$ ion exchange resins) in order to obtain the glucose fraction. The elute was freeze dried before being derived as aldonitrile pentacetate, using the method of [14]. This type of derivation was chosen in order to obtain two ion fragments (C_1-C_4 and C_4-C_6) required for the determination of the glucose turnover and the recycling of the glucose carbons (R) [15]. For the determination of the isotopic enrichment of alanine, the neutral supernatant was acidified with HCL and purified using AG-50-WX4-H$^+$ ion exchange resin. The alanine fraction was freeze dried and subsequently derived as t-butyl-dimethyl silyl, using acetonitrile and N-methy-N-(tert-butyl-dimethylsilyl)-trifluoro-acetamide (MTBSTFA) [16]. For L-lactate, neutral supernatant was extracted three times with 3 mL of ethylacetate. The extract was dried with anhydrous sodium sulphate and the ethylacetate was then evaporated under nitrogen flow [17]. The L-lactate fraction was then derived as t-butyldimethylsilyl, using pyridine and MTBSTFA [17].

The isotopic enrichment of the different molecules (molar percent excess, MPE) was determined by Gas chromatography-Mass Spectrometry (GC-MS). A Hewlett-Packard 5890II gas chromatograph equipped with a 25-m fused silica capillary column (OV 1701, Chrompack, brudegewater, NJ, USA), interfaced with a mass-selective detector HP5971 was used [13]. The chromatographic conditions used for the determination of the isotopic enrichment of glucose have already been described by Beylot et al. [15]. For alanine, the injector temperature was 260 °C and the oven temperature was 250 °C, with a temperature programme (140 °C, 20 °C·min^{-1} until 250 °C). For L-lactate, the conditions were described

by [17]. Fragmentation of the different molecules was performed by electron impact and the ions with a mass to charge ratio (m/z) of 217–218 (glucose C_4-C_6), 242–243 (glucose C_1-C_4), 261–262 (L-lactate) and 260–261 (alanine) were monitored. Standard solutions of known enrichments of glucose, alanine and L-lactate, were used to determine the sample enrichments.

Four rumen fluid samples were taken every 30 min over two feeding cycles, starting 10 min postprandially (between 15.00 and 17.00 h). pH was immediately measured and 10 mL of filtered rumen fluid were acidified with 1 mL of metaphosphoric acid (5%, v/v) and frozen at –20 °C for subsequent analysis of volatile fatty acids by gas liquid chromatography [18] and ammonia [19]. Another 10 mL of filtered rumen fluid were frozen directly, without acid, for subsequent measurements of osmolality and L-lactic acid concentrations (Kit Boehringer Mannheim, Diffchamb France SARL, Lyon, France).

2.3. Calculations and statistical analyses

Isotopic enrichment of the different molecules (E) was expressed as molar percent excess (MPE), which corresponded to the ratio of labelled over total molecules (labelled and unlabelled molecules).

The apparent glucose turnover (AGT, $\mu mol \cdot min^{-1} \cdot kg^{1}$) was calculated as described by [20] by the equation:

$$AGT = (F / E_{C1\text{-}C4}) - F$$

where F is the [1-^{13}C] glucose infusion rate ($\mu mol \cdot min^{-1} \cdot kg^{-1}$) and $E_{C1\text{-}C4}$ is the isotopic enrichment of the C1-C4 fragment of glucose.

Following glycolysis, [1-^{13}C] glucose is expected to be converted into pyruvate, and then into L-lactate and alanine, especially in the peripheral tissues. The proportion of glucose carbon that was transferred to L-lactate was calculated as twice the ratio of the ^{13}C enrichment of L-lactate to $E_{C1\text{-}C4}$ [21]. Similarly, the labelled carbon transfer of glucose to alanine was calculated as twice the ratio of the ^{13}C enrichment of alanine to $E_{C1\text{-}C4}$.

The labelled 3-carbon compounds ([3-^{13}C]-lactate, [3-^{13}C]-alanine, [3-^{13}C]-pyruvate) reaching the liver can be reincorporated into newly synthesised glucose. ^{13}C can be incorporated in the (carbons 1-3) fragment or in the (carbons 4-6) fragment [21]. This incorporation is identical whether it occurs in the upper or in the lower part of the glucose molecule. Recycling (R, %) represents the reincorporation of ^{13}C in the newly synthesised glucose and was determined by the equation:

$$R = (E_{C4\text{-}C6} / E_{C1\text{-}C4}) \times 100$$

where $E_{C4\text{-}C6}$ is the enrichment of the C4-C6 fragment of glucose.

This recycling does not account for the labelling on carbon 4 of the C_1-C_4 fragment of glucose, but this labelling is much lower than the labelling on carbons 5 and 6 and the resulting error is minimal.

In terms of statistical analysis, because of a lack of some catheter patency, data were analysed according to a simple analysis of variance using the treatment, animal and period as the main factors. Analyses were carried out using the GLM procedure of Statistica version 5.5 (1984–2000, StatSoft, Tulsa, OK74104, USA). Means were compared by orthogonal contrasts in order to test responses to the presence of propionate (Contrast 1: comparison between C and the propionate treatments (P1 + P2)), and to the level of propionate (Contrast 2: P1 vs. P2). Differences were declared significant at $P < 0.05$. LSMeans were reported.

3. RESULTS

The correct positioning of catheters was checked at necropsy. A lack of catheter

patency occurred on one animal shortly after surgery but the animal could not be replaced. For another animal, the carotid catheter lost patency for the P2 treatment only.

3.1. Intake and ruminal fermentation parameters

The organic matter, crude protein, crude fibre and soluble sugars were 88.2 (SD 7.36), 12.9 (SD 0.26), 23.9 (SD 0.67) and 16.3 (SD 1.01) % on a DM basis, respectively. Soluble nitrogen represented 25.0 (SD 1.37) % of total nitrogen. The DM content of the rye grass offered was measured daily and averaged 15.9 (SD 0.62) %. Propionate infusion had no effects on grass DM intake ($P < 0.43$). The amounts of propionate infused daily averaged 0.54 and 0.91 moles for P1 and P2 respectively (Tab. I). Consequently, the total estimated ME intake for C, P1 and P2 averaged 8.82, 9.32 and 9.95 $MJ \cdot d^{-1}$ of which 0, 8.9 and 14.0%, respectively, originated from propionate supplementation.

In rumen fluid, propionate infusion resulted in 50% (P1) and 95% (P2) increases ($P < 0.001$) in propionate concentrations, leading to significant increases in total volatile fatty acid (VFA) concentrations (Tab. I). Ruminal fluid concentrations of acetate, butyrate and caproate remained unchanged while isoacid concentrations decreased and those of valerate increased ($P < 0.007$). Consequently, the molar proportions of acetate dropped from 61.3 to 55.2 and 49.4% (SEM = 0.014; $P < 0.006$) while those of propionate rose from 24.7 to 33.3 and 39.6% (SEM = 0.013; $P < 0.001$) with the C, P1 and P2 treatments respectively. Rumen fluid pH was not significantly modified whereas the osmolality tended to increase ($P < 0.10$) from 248 mosm with C to 262 and 285 mosm with P1 and P2, respectively. Ammonia and L-lactic acid concentrations averaged 6.24 and 0.70 mM with C and decreased by 6 and 11% with P1 and by 34 and 49% with P2, respectively (Tab. I).

3.2. Arterial concentrations

Hematocrit was stable during the sampling period and was not modified by treatments averaging 0.31 ($P < 0.49$; Tab. II). Arterial concentrations of glucose and L-lactate averaged 3.17 and 0.65 mM with C and were not significantly modified with propionate infusion. Similarly, plasma insulin concentrations were not modified ($P < 0.28$) and averaged 21.2 $mIU \cdot L^{-1}$ (Tab. II).

3.3. Whole body metabolism of glucose

The kinetics of enrichment measured on four sampling days (in the presence and the absence of propionate) showed that the $E_{C1\text{-}C4}$ became stable 40 min after the beginning of [1-^{13}C] glucose infusion and averaged 1.3% (SD 0.07) (Fig. 1). A high enrichment was observed just after the beginning of [1-^{13}C] glucose infusion and was probably associated to the priming-dose injection. On the contrary, the isotopic enrichments of alanine, L-lactate and the $E_{C4\text{-}C6}$ were low (generally below 0.2) and were not stable before 200 min after the beginning of [1-^{13}C] infusion (Fig. 2). For this reason the AGT, carbon transfer and R were subsequently calculated on the basis of the last three samplings only.

Generally, whole body glucose metabolism was not altered by propionate infusion. AGT averaged 17.8 $mmol \cdot min^{-1} \cdot kg^{-1}$with C and was not modified ($P < 0.3$) by the treatment, despite a numerical increase which was due to one animal (Tab. II). The transfers of labelled carbon from glucose to L-lactate and to alanine amounted to 21.0 and 20.8% for C and were not significantly modified by the treatments. The recycling of the glucose carbon was also not modified and averaged 9.4% across all three treatments.

Table I. Dietary intake and rumen fluid parameters in lambs fed frozen rye-grass supplemented with intraruminal infusion of propionate.

		LSMeans					Orthogonal contrasts†	
	ddl	C	P1	P2	SEM*	Treatment effect	Propionate	Level
Body weight (BW) kg	14	30.0	29.2	30.5	0.48	0.13	0.75	0.06
Intake, $g \cdot d^{-1}$								
Dry matter (DM)	14	784	757	759	16.3	0.43	0.22	0.94
Crude protein (CP)	14	101	97	98	2.5	0.44	0.23	0.89
Infused propionate, $mol \cdot d^{-1}$	14	0	0.54	0.91	0.032	0.001	0.001	0.001
Rumen fluid parameters								
pH	14	6.4	6.3	6.2	0.11	0.46	0.27	0.52
Osmolarity (mosm)	14	248	262	285	8.9	0.10	0.06	0.18
NH_3, mM	14	6.24	5.87	4.14	0.500	0.09	0.08	0.08
L-lactate	14	0.70	0.62	0.49	0.043	0.05	0.03	0.12
Volatile fatty acids (VFA), mM								
Total VFA	14	78.29	86.44	94.44	3.164	0.04	0.02	0.17
Acetate	14	48.10	47.85	46.84	2.796	0.95	0.82	0.83
Propionate	14	19.17	28.70	37.40	1.973	0.004	0.001	0.04
Butyrate	14	8.64	7.81	8.46	1.123	0.85	0.71	0.73
Isobutyrate	14	0.66	0.56	0.44	0.031	0.01	0.006	0.05
Valerate	14	0.51	0.59	0.55	0.042	0.05	0.03	0.23
Isovalerate	14	1.01	0.74	0.53	0.085	0.02	0.01	0.18
Caproate	14	0.20	0.18	0.12	0.098	0.28	0.20	0.26

* SEM $\sqrt{}$ (Residual mean square/*n*).
C: control treatment; P1: infusion of propionate at 0.23 M; P2: infusion of propionate at 0.41 M.
† Orthogonal contrasts: Propionate = C vs. (P1 + P2), level = P1 vs. P2.

Table II. Arterial metabolite and insulin concentrations and whole body glucose metabolism in lambs fed frozen rye-grass supplemented with intraruminal infusion of propionate.

		LSMeans					Orthogonal contrasts†	
	ddl	C	P1	P2	SEM*	Treatment effect	Propionate	Level
Hematocrit	14	32	31	31	0.9	0.49	0.26	0.99
Arterial concentrations								
Blood (mM)								
Glucose	14	3.17	3.22	3.05	0.068	0.36	0.62	0.18
L-lactate	14	0.65	0.73	0.59	0.044	0.22	0.85	0.11
Plasma ($mIU \cdot L^{-1}$)								
Insulin	14	24.7	20.7	18.21	2.61	0.28	0.14	0.57
Whole body metabolism of glucose								
Infused glu ^{13}C ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	14	0.216	0.211	0.211	0.005	0.85	0.58	0.97
AGT ($\mu mol \cdot min^{-1} \cdot kg^{-1}$)	14	17.83	19.35	21.26	1.343	0.30	0.17	0.41
C transfer to L-lactate (%)	14	21.02	21.80	19.94	0.010	0.95	0.85	0.80
C transfer to alanine (%)	14	20.80	20.10	23.92	0.010	0.47	0.60	0.26
Recycling of C (%)	14	8.90	9.90	9.37	0.003	0.51	0.27	0.86

* SEM $\sqrt{}$ (Residual mean square/*n*).
C: control treatment; P1: infusion of propionate at 0.23 M; P2: infusion of propionate at 0.41 M.
† Orthogonal contrasts: Propionate = C vs. (P1 + P2), level = P1 vs. P2.

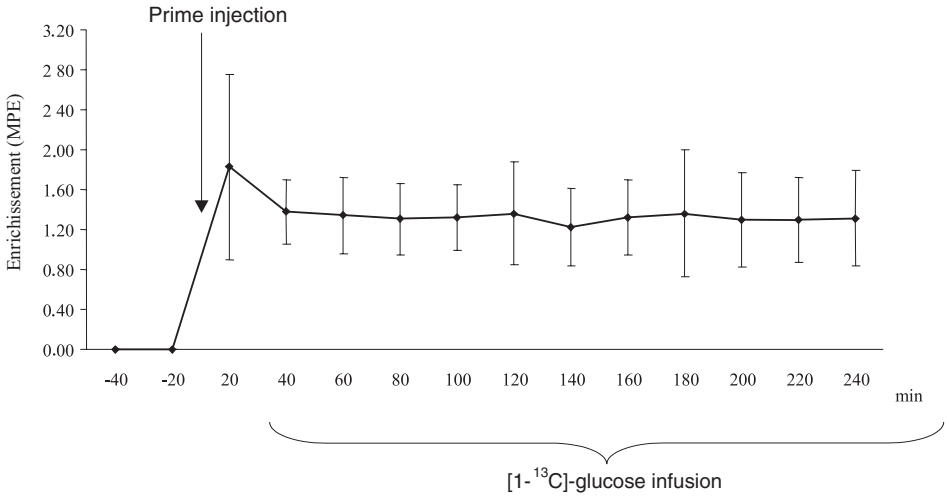

Figure 1. The enrichment kinetics of the C1-C4 fragment of glucose in ^{13}C in growing lambs following a prime injection and the continuous infusion of [1-^{13}C]-glucose in the jugular blood. The results represent the mean of 4 sampling days (in the presence or in the absence of propionate).

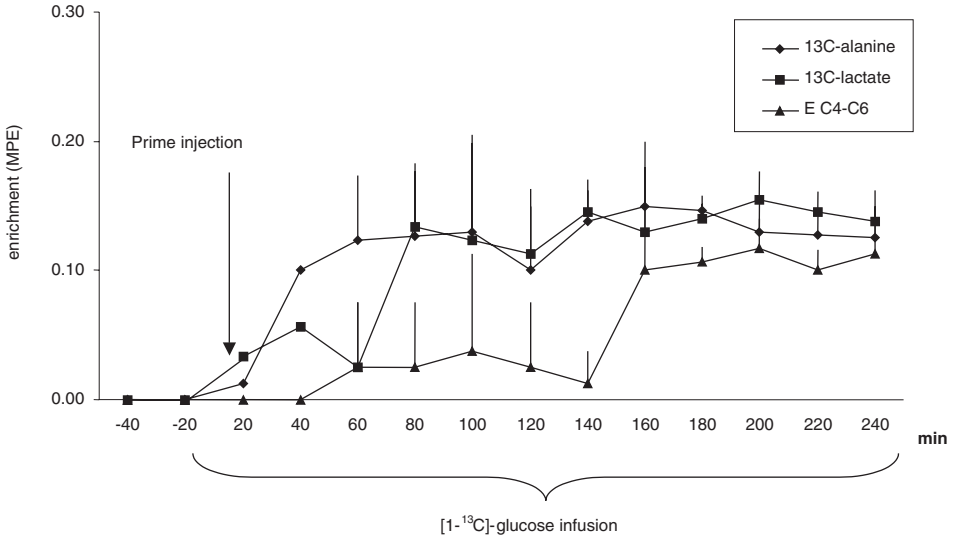

Figure 2. The enrichment kinetics of L-lactate, alanine and of the C4-C6 fragment of glucose in ^{13}C in growing lambs following a prime injection and the continuous infusion of [1-^{13}C]-glucose in the jugular blood. The results represent the mean of 4 sampling days (in the presence or in the absence of propionate).

4. DISCUSSION

4.1. Rumen parameters

Intraruminal propionate infusion aimed at modifying the VFA profile in the rumen in order to approach that obtained with cereal rich diets, without disturbing intake, the rumen function and the basal production of VFA. The basal diet being fed at 1.5 times the maintenance energy requirements, propionate infusion had no negative effects on the appetite of animals and the dry matter intake (DMI). As in a first study using the same diet and the same levels of propionate [6], propionate infusion, especially for P2, elevated the molar proportions of propionate above the levels frequently reported for ruminants receiving high-concentrate diets [22]. However, these proportions remained physiological. Indeed Moloney [23] reported similar proportions in steers or sheep receiving high-grain diets. Additionally, propionate infusion did not affect rumen fermentations since pH, acetate and butyrate concentrations remained stable. The osmolality increased significantly, but it remained physiological since it did not exceed 350 mosm [24]. The ammonia concentrations as well as the isoacid concentrations decreased and suggested a decrease in ruminal proteolysis [25, 26]. The increase in propionate concentrations in the rumen was also associated with a decrease in the rumen L-lactic acid concentrations, which disagreed with data reported by Overton et al. [27]. All these effects were similar to those observed with an addition of monensin which favours propionate producing bacteria [28, 29]. It has been reported that monensin increased the quantity of protein escaping ruminal degradation and inhibited the development of micro-organisms producing lactate [28].

4.2. Whole body glucose turnover

4.2.1. Methodological aspects

The measurement of whole body glucose turnover requires the use of tracers. With the evolution of methodological approaches, analytical techniques and tracer costs, different types of tracers have been used in ruminant studies over the years. Because of an increasing concern for the risks associated with the use of radioactive elements on health and the environment, the use of stable isotopes has developed in human studies. In ruminants, their use is recent and not very frequent because of the high cost of stable isotopes and analytical equipment. Only a few studies dealing with glucose turnover in ruminants have been conducted using stable isotopes ([30–32] with [U-^{13}C]-glucose, [33–36] with [^{2}H]-glucose).

The originality of the present experimental work reported here also relies on the use of [1-^{13}C]-glucose in growing lambs. With this tracer, the determination of the carbon recycling of glucose only requires the use of selected-ion-monitoring GC-MS (SIM-GC-MS), without any infusion of [H]-glucose [37]. No data has been published so far in ruminants on the use of the stable isotope [1-^{13}C]-glucose to determine glucose recycling. Additionally, this approach coupled to the measurement of the enrichment in some glucose derived metabolites (L-lactate, alanine) addressed questions relative to the metabolic fate of glucose. Compared to [U-^{13}C], [1-^{13}C]-glucose is less expensive and determining glucose turnover in ruminants is more feasible, despite the fact that the measurement of parameters, such as the conversions into other metabolites and recycling, requires a relatively important amount of tracer in order to have detectable and precise data.

4.2.2. Whole body glucose turnover with the control diet

AGT (59 mmol·d^{-1}·kg BW$^{-0.75}$) was similar to data reported in growing ovines [38, 39]. It was closer to values predicted for growing bovines than to values predicted for adult ovines [40] given the plane of feeding applied. It was also closer to the values predicted for adult ovines fed concentrate

based diets than forage based diets [40]. Only one other study reported glucose turnover data with grass diets being fed to adult non producing ewes [41], the results were close to the values predicted for concentrate based diets [40]. The nature of the diet used (frozen rye-grass) and the high portal absorption of propionate observed in a previous study [6, 42] were also coherent with the high turnover of glucose measured. More precisely, the level of glucose turnover measured in the present experiment was totally coherent with the net hepatic glucose production measured by Majdoub et al. [6, 42] in similar animals, fed the same diet at the same energy level. When combining the results of the present experiment with those of [6], hepatic glucose production was calculated to represent 90% of the AGT, which agreed with the data of [43], who reported a contribution of 85 to 90%. On the same basis, it could be calculated that 20% of the AGT were used by the portal-drained viscera, which is in the range of other published results [43, 44]. In those growing lambs, the estimated contribution of muscle mass to AGT amounted to 55% which was higher than the results reported for adult ewes, but which was totally compatible with the high contribution of muscle mass to total energy expenditure measured in growing animals [6]. These results support the hypothesis that glucose turnover may be higher in growing than in adult non producing ruminants at the same intake level considering the higher proportion of muscles in the body mass [40]. The transfer of the glucose carbon to L-lactate associated with glycolysis in peripheral tissues and in the portal drained viscera, represented 98% of the total production of L-lactate by the carcass (estimations from hindlimb data) and the portal drained viscera reported by [6]. The extent of recycling of glucose carbon measured in this present study (9%) was in the range of recycling reported for ruminants (4–33% [45]). Twenty-one per cent of the transfer of the glucose carbon to L-lactate and alanine was recycled into glucose. The remainder of L-lactate and alanine was probably used as energy sources.

4.2.3. Whole body glucose turnover and propionate supplementation

The influence of an increased supply of a gluconeogenic precursor, propionate, on the glucose turnover was specifically tested in this study. Growing lambs fed at a restricted intake were used. Based on previous conclusions, the lambs are supposed to have a high requirement in glucose [40]. A previous study [6] carried out in the same experimental conditions as the present one showed no differences in net hepatic glucose output with propionate supplementation while L-lactate metabolism was affected in the portal-drained viscera and the hindlimb. Consequently, the present study was aimed at confirming these results and specifically addressed the issue of the recycling of glucose C as L-lactate or alanine.

Despite the increase in MEI and the higher gluconeogenic potential associated with propionate supplementation, AGT was not modified with propionate supplementation, which was coherent with the unchanged hepatic glucose production reported in the previous study [6]. However, it disagreed with the measured increase in glucose uptake by the hindlimb and the assumed increase in glucose production by the kidneys [6]. It cannot be excluded that the drop in enrichment which could have resulted from the above mentioned mechanisms (0.12) was too small to be reliably detected. The influence of raising the supply of glucose precursors on glucose turnover probably depends on the basal ME level. Lapierre et al. [46] showed a curvilinear relationship between net hepatic glucose release and the level of feed intake. Indeed, Seal and Parker [7] and Peiris et al. [47] measured only slight increases in the turnover of glucose (+13 and +15%) in steers fed approximately at 920 kJ MEl$\cdot$d$^{-1}\cdot$kg$^{-0.75}$ and supplemented with propionate (7.8 and 2.9 mmol$\cdot$d$^{-1}\cdot$kg BW^{-1}, respectively). In those experiments, the basal

glucose turnover (75 and 62 mmol·d^{-1}·kg BW$^{-0.75}$, respectively) was similar to the values measured in this present study. On the other hand, Veenhuizen et al. [8] fed steers at similar MEI and still reported a 59% increase in glucose turnover following propionate supplementation, at the same level as in this present study (30 mmol·d^{-1}·kg BW^{-1}). However, the steers presented a lower basal glucose turnover (42 mmol·d^{-1}·kg BW$^{-0.75}$) than that measured in the present study (60 mmol·d^{-1}·kg BW$^{-0.75}$), or by [14] and [48].

On the basis of the results obtained by [8] and [27] when glucose requirements are experimentally increased by enhancing urinary glucose excretion, it may be hypothesised that the effect of propionate supplementation on glucose turnover is influenced by the balance between the basal glucose turnover of the animals and their glucose requirements. A key notion is that of glucose requirements which are probably determined by the growth rate of the animals relative to their growth potential and by their nitrogen (N) intake [40]. The impact of the ratio between nitrogen (N) and ME intake on glucose turnover is probably important and could explain the discrepancies noted between responses to the nature of the diet and to the supply of the gluconeogenic precursors. Direct experimental evidence on this aspect is lacking. However, Seal et al. [49] hypothesised that the increase in the glucose turnover observed with a mixed diet is probably associated to a higher supply in nitrogen. On the contrary, Peiris et al. [47] reported that nitrogen retention is positively correlated to glucose turnover and suggested that glucose can be limiting for nitrogen retention.

Propionate supplementation did not significantly affect the interconversions between glucose and L-lactate or alanine. Majdoub et al. [6] reported an increase in the net portal appearance of L-lactate with intraruminal infusion of propionate in growing lambs, and assumed that L-lactate arises from glucose metabolism in the tissues of the portal-drained viscera. The transfer of glucose carbon to L-lactate measured here represented 101% of the lactate released by the portal-drained viscera during propionate supplementation [6]. The present measurements in the rumen indicate that the increased net portal appearance of L-lactate did not result from an increased absorption of L-lactate from the rumen. Consequently, it is probable that all of the glucose transfer to L-lactate occurred in the portal-drained viscera (assuming that all the net release of L-lactate originates from glucose). In the study of [6], propionate did not modify the hepatic glucose production nor the potential contribution of alanine and L-lactate to gluconeogenesis. This is coherent with the unchanged recycling of glucose observed in this present study.

Consequently, in ruminants, recycling of glucose in particular via the Cori cycle is probably more important in cases of glucose deficit, as in fasting animals or in early lactation [43, 50] than in situations when the supply of glucose or its precursors is plentiful.

5. CONCLUSIONS

The present study showed that glucose turnover cannot be strictly manipulated by the supply of gluconeogenic substrates and raised the assumption that it probably depends on the supply of other nutrients such as nitrogen. The notion of glucose requirements for growing animals would also warrant further investigation. In the present experiment, glucose requirements of lambs were probably already met by the basal gluconeogenesis allowed by the intake of frozen rye-grass. In situations of an excess supply of gluconeogenic precursors, the key regulating step of glucose metabolism probably lies in hepatic gluconeogenesis rather than in glucose recycling. Finally, the increased utilisation of glucose by the hindlimb observed with propionate supplementation in

a previous study [6] was not associated with measurable increases in glucose turnover. This suggests that at similar glucose supply, the partition of glucose utilisation among the different peripheral tissues may be altered.

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