# Ruminal VFA production rates, whole body metabolite kinetics and blood hormone concentrations in sheep fed high- and low-fibre diets*

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Ten Dohne merino wethers with an average mass of 50 kg, were used to study differences in ruminal VFA production rates, whole body kinetics of VFA, glucose and FFA as well as blood hormone concentrations when fed a high- or a low-fibre diet. Dietary carbohydrate was provided as either structural (HF treatment) or readily fermentable (LF treatment), while keeping energy and nitrogen intakes constant. The wethers were fitted with ruminal cannula and with an indwelling catheter in the abdominal aorta and a temporary catheter inserted into the jugular vein. Either (1-$^{14}$C)-acetate or (2-$^{14}$C)-propionate was continuously infused into the rumen concurrently with either a (9,10 N-$^{3}$H)-FFA or (6-$^{3}$H)-glucose, respectively, infusion into the jugular vein. Blood and digesta samples were drawn simultaneously after plateau specific radioactivity in all labelled markers had been achieved. Total ruminal VFA, acetate and butyrate production rates were not altered by diet, whereas propionate production rates were higher ($p < 0.05$) for the LF than the HF treatment, 2.86 and 2.45 ± 0.04 mol.day$^{-1}$, respectively. Arterial propionate concentrations were lower ($p< 0.05$) in the LF than the HF treatment (0.121 and 0.164 ± 0.005 mM, respectively) whereas the glucose concentrations were higher in the LF than the HF treatment (4.39, and 3.61 ± 0.177 mM, respectively). There were no differences in the arterial concentrations of acetate and propionate between the two treatments. The glucose irreversible loss rate was higher ($p < 0.05$) in the LF than the HF treatment (42.2 and 36.1 ± 1.98 mmol.h$^{-1}$, respectively), whereas the FFA entry rate was lower in the LF than the HF treatment (6.2 and 10.5 ± 0.541 mmol.h$^{-1}$, respectively). Insulin, glucagon and thyroxin concentrations were higher ($p < 0.05$) in the HF than the LF treatment. Triiodothyronine concentrations were similar for both treatments. The arterial oxygen and carbon dioxide concentrations were higher ($p < 0.05$) in the HF than the LF treatment (HF: 5.07 and 18.9 mM, respectively; LF: 4.58 ± 0.22 and 16.0 ± 0.875 mM, respectively).

Tien Dohne merino-hammels met 'n gemiddelde massa van 50 kg is gebruik om die verskil in ruminale vlugtige vetsuur- (VVS) produksietempos, heel liggaam kinetika van VVS, glukose en vrye vetsure (FVS) sowel as die bloedkonsentrasies van hormone, wanneer hoë of lae vesel diëte gevoer is, te bepaal. Die dieetkoolhidrate is as struktreel (HF-behandeling) of maklik fermenteerbaar (LF-behandeling) aangebied, terwyl die energie- en stikstofinnames konstant gebly het. 'n Kannula is

in die rumen, en 'n blywende kateter in die abdominale aorta en 'n tydelike kateter in die nekaar van die hammels ingesit. Die diëte is halfuurliks gevoer. Of (1-$C^{14}$)-asetaat of (2-$C^{14}$)-propionaat is deurentyd intra-ruminaal geinfuseer gelyktydig met óf (9,10-$^{3}$H)-FVS óf (6-$^{3}$H)-glukose, onderskeidelik in die nekaar geinfuseer is. Bloed- en digesta-monsters is op dieselfde tyd geneem nadat 'n plato van spesifieke radioaktiwiteit vir alle isotope behaal is. Totale ruminale VVS, asetaat- en butiraat- produksietempos is nie verander deur die verskillende diëte wat aangebied is nie, terwyl propionaat-produksietempos hoër ($p < 0.05$) was vir die LF- as die HF-behandeling, 2.86 en 2.45 ± 0.04 mmol.dag$^{-1}$, onderskeidelik. Arteriële propionaatkonsentrasies was laer ($p < 0.05$) in die LF- as in die HF-behandeling (0.121 en 0.164 ± 0.005 mM, onderskeidelik) terwyl die glukosekonsentrasies hoër was in die LF- as die HF-behandeling (4.39 en 3.61 ü 0.177 mM, onderskeidelik). Daar was geen verskille in die arteriële konsetrasies van die asetaat en propionaat tussen die twee behandelings nie. Die glukose onomkeerbare verdwyningstempo was hoër ($p < 0.05$) in die LF- as die HF-behandeling (42.2 en 36.1 ± 1.98 mmol.uur$^{-1}$,onderskeidelik), terwyl die FVS toegangstempo laer was in die LF- as die HF-behandeling (6.2 en 10.5 ± 0.541 mmol.uur,$^{-1}$, onderskeidelik). Insulien-, glukagon- en tiroksienkonsentrasies was hoër ($p < 0.05$) in die HF- as die LF-behandeling. Triiodotironien- konsentrasies was dieselfde vir albei behandelings. Die arteriële suurstof- en koolsuurgaskonsentrasies was hoër ($p < 0.05$) in die HF- as die LF-behandeling (HF: 5.07 en 18.9 mM, onderskeidelik; LF: 4.58 ± 0.22 en 16.0 ± 0.875 mM, onderskeidelik).

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## Introduction

The VFA are produced in large quantities in the rumen of sheep and cattle. The proportions of VFA produced are influenced by numerous factors including pH, substrate composition and availability of substrate (Dijkstra, 1994). Thus diets rich in glucose favour propionate production, while more fibrous diets favour acetate production. The molar proportions of VFA in the rumen determine the subsequent uptake and metabolism of the VFA for the energy economy of the host (Webster, 1980).

All the VFA are readily used as energy sources by the host for maintenance, growth and fattening and are estimated to contribute 70–80% towards the animal's energy requirements (Annison & Armstrong, 1970). Although the VFA have a high theoretical efficiency of utilisation (78 to 80%), early work (Armstrong *et al.* 1957; 1958; 1961; Tyrell *et al.*, 1979) suggested that, *in vivo*, acetate may not be efficiently utilised. The average overall efficiency of VFA utilisation was calculated as between 60–70%, and the utilisation of each acid for energy retention was modified by the presence of other VFA. Gill *et al.* (1984) and Black *et al.* (1987) suggested that the balance between dietary acetate, propionate and glucose, and not the quantity of an individual nutrient (e.g. acetate) absorbed affects the energetic efficiency of both protein and acetate utilization. Gill *et al.*, (1989) predicted, via mathematical modeling, that the energetic efficiency of acetate utilization would be 58–70% at maintenance and would decrease to between 25–60% as the energy intake increases.

It is therefore clear that factors other than the availability of propionate (i.e. glucogenic potential of the diet) are implicated in the overall control of energy metabolism. Although there is substantial theoretical support for this hypothesis, there are, unfortunately, few *in vivo* results to confirm it. Most studies have focused on either VFA, lipid, or glucose metabolism, ignoring their interdependence and thus, have failed to adequately explain the control of nutrient intake and metabolism

(Gill *et al.*, 1984; MacRae *et al.*, 1985). As a result of the differences in energetic efficiency observed in a previous growth study with lambs (Linington *et al.*, 1996) and a lack of differences observed in a previous digestion study with sheep (Linington *et al.*, 1997), it was decided to investigate the effect of varying the form of carbohydrate and thus the pattern of the ruminal VFA production rates on the metabolism of ruminal acetate and propionate to FFA and glucose, respectively, in the whole body of sheep fed the high- and low-fibre diets at constant energy and nitrogen intakes.

## Methods

### Animals

Ten Dohne Merino wethers (47–53 kg), aged 1–2 years were used. The sheep had each been fitted with silastic ruminal cannula and were housed indoors in individual metabolism crates under continuous lighting. The sheep were randomly divided into two groups and assigned to treatments HF and LF, and fed either a high-fibre (HF) or low-fibre (LF) diet, respectively (Linington *et al.*, 1996).

The HF sheep were fed at 90% of their previously determined *ad libitum* intake, and the LF sheep an amount of the LF diet equal in energy to the amount offered to the HF sheep i.e. 1500 $g.day^{-1}$ and 1260 $g.day^{-1}$ respectively, in equal portions at half-hourly intervals. Feed refusals were determined daily. The nitrogen (N) and metabolizable energy (ME) intakes were thus the same for both groups of sheep, viz. 138 $gN.day^{-1}$ and 13.18 MJ $ME.day^{-1}$, respectively. Water was freely available.

### Surgery

The rumen-cannulated sheep were fitted with indwelling catheters in the abdominal aorta. A temporary catheter in the jugular vein was inserted when required. Single lumen, medical grade, polyethylene (I.D. 1.00 mm, O.D. 1.45 mm, Portex, U.K.) was used to catheterise the jugular vein. Medical grade polyvinylchloride (PVC) tubing (I.D. 1.00 mm, O.D. 2.00 mm, Dural Plastics, Australia) was used to catheterise the abdominal aorta via the femoral artery aorta as described by Katz & Bergman (1969). Locating cuffs were secured 200 mm from the tip of the aortic catheter with cyclohexanone. After insertion the catheters were filled with saline containing heparin (750 $U.ml^{-1}$, Glaxo, U.K.) and plugged with a blocked hypodermic needle of suitable gauge. Catheters were serviced every 2 to 3 days. The aortic catheters remained patent for 5 months.

### Experimental Design

There were two parts to this study, separated by two weeks, to minimise blood loss and tracer build up. In Phase 1, the metabolism of acetate and FFA was traced by infusing $^{14}C$-acetate into the rumen and $^{3}H$-FFA into the jugular vein. In Phase 2, propionate and glucose metabolism were estimated using an intraruminal $^{14}C$-propionate infusion and an intrajugular infusion of $^{3}H$-glucose. The protocols for Phases 1 and 2 were identical. Two sheep from each group ($2 \times 2$) were used for a set of infusions and the procedure repeated with another four sheep a week later.

#### *Infusions*

*Phase 1*: (1-$^{14}C$)-acetate (Amersham, U.K) was made up in sterile water and was continuously infused (19.12 $KBq.l^{-1}$, 0.5 $ml.min^{-1}$) into the anterior section of the rumen, starting 16 h before sampling commenced to ensure that steady state tracer kinetics were achieved in the rumen and blood. No priming dose was administered. The $^{3}H$-FFA infusate (30 $KBq^{-1}$, 0.8 $ml.min^{-1}$) was made up of equal mixtures of (9,10(N)$^{3}H$)-oleic and (9,10(N)$^{3}H$)-palmitic acids (New England

Nuclear, U.S.A.), and prepared individually for each sheep since endogenous plasma albumin was used to adsorb the FFA.

*Phase 2:* (2-$^{14}$C)-propionate (Amersham, U.K.) was dissolved in sterile water and continuously infused (19.12 KBq.l$^{-1}$, 0.5 ml.min$^{-1}$) into the rumen starting 16 h before sampling. No priming dose was administered. D-(6-$^{3}$H)-glucose (Amersham, U.K.) containing 20 mg.l$^{-1}$ unlabelled glucose as inert carrier was infused (30 KBq.l$^{-1}$, 0.8 ml.min$^{-1}$) continuously via the jugular vein after a priming dose of 75 ml was administered 5 h before sampling commenced.

*Sampling*

Rumen and blood samples (4 sets of each) were drawn simultaneously at half-hourly intervals. The rumen sample was strained through double-layered cheesecloth and a 10 ml aliquot of the fluid was stored cold (4°C) after the addition of 100 µl of mercuric chloride as preservative. Blood samples were withdrawn from the arterial catheter into heparinized syringes and 3 ml immediately used for blood gas, haemoglobin and haematocrit determinations, as well as for $CO_2$ specific activity estimation. A further 3 ml whole blood was added to tubes prepared for VFA analysis. Finally, a 10 ml heparinized blood sample was used to obtain plasma, which was stored cold (4°C) and used for the remainder of the analyses.

## Analyses

Total VFA were extracted from clarified rumen fluid and blood by ether distillation (Neish, 1952; Van der Walt & Briel, 1976), and the samples were dried to salts. The VFA were separated on a reverse-phase, C-18 column (Bush *et al.* 1979) after being reconstituted in 2 N phosphoric acid. Peaks were detected via a refractive index detector (Du Pont, USA). VFA concentrations were determined from peak areas, while radioactivity was measured in a liquid scintillation spectrometer (LKB, Sweden).

The specific activity of carbon dioxide in blood was determined according to the method of Hinks *et al.* (1966). Radioactivity was measured using a liquid scintillation spectrometer (LKB, Sweden). Glucose concentrations in plasma were colorimetrically determined (Boehringer-Mannheim, Germany). The specific activity of both $^{3}$H- and $^{14}$C-glucose was determined, in duplicate, as glucose penta-acetate, isolated from plasma according to the method of Jones (1965). Radioactivity was measured using a liquid scintillation spectrometer (LKB, Sweden). The FFA were determined colorimetrically according to the method of de Villiers *et al.* (1977). The $^{3}$H- and $^{14}$C-FFA specific activities were determined after extracting the FFA from plasma (Dole, 1965) after which the FFA were bound to resin according to the method of Kelly (1968). Activity bound to resin was estimated using liquid scintillation spectrophotometry (Packard Tricarb, USA).

The concentrations of insulin, free thyroxin ($T_4$) and triiodothyronine ($T_3$) in plasma were determined using commercially available radio-immunoassay kits (Amersham, U.K.). The glucagon samples were stored with a proteolytic inhibitor, aprotinin and plasma concentrations were determined using a commercially available radio-immunoassay kit (Serona Diagnostics, Italy). The haemoglobin content of blood was determined according to the cyanmethemoglobin method (A.O.A.C., 1990). The $pO_2$, $pCO_2$ and pH of blood was determined within 30 min of sampling using a blood gas analyser (Radiometer, Denmark).

## Calculations

The values of the various metabolites in the arterial samples were used for calculating whole body variables and rates. The production rates of VFA in the rumen were determined according to the

method of Leng & Leonard (1965) and corrected for interconversion between acids according to the method of Bergman *et al.* (1965). The glucose irreversible loss (GIL) rate and FFA entry rates were determined from the ratio of the infusion rate and plateau specific activity according to the method of Bergman *et al.* (1966) and Bergman & Wolff, (1971). The proportion of $CO_2$ from ruminal acetate or propionate was determined from the ratio of the mean arterial carbon dioxide specific activity and the plateau acetate or propionate specific activity. The ruminal quantities of acetate or propionate converted to FFA or glucose, respectively, were calculated according to Leng *et al.* (1967).

Oxygen concentration was calculated from the $pO_2$, pH and haemoglobin concentration after a correction of $pO_2$ for the Bohr effect. Carbon dioxide concentrations (mM) were calculated from the Henderson-Hasselbach equation (Oddy *et al.*, 1984). The interconversion of metabolites was calculated from the entry rates and the specific activities of $^{14}C$ in glucose, FFA and $CO_2$.

### Statistics

The statistical significance of differences between diets was calculated using Statgraphics 6.0 (Manugistics, Inc., Maryland, U.S.A.) personal computer package in which the two-sample test with pooled variance provided a *t*-statistic for unpaired samples with unequal variances. The blood metabolite concentration levels obtained during the whole body, tissue energy use and organ experiments were the mean results for four sheep and for each sheep the mean values for four blood samples were considered.

## Results

### Diets

The composition of the diets is identical to those used in an experiment previously reported (Linington *et al.*, 1996). Feed refusals were small and infrequent and the sheep ate 99% of the feed offered.

### Ruminal Volatile Fatty Acid Production Rates

The concentrations of VFA in the rumen and the molar percentages of the major VFA are given in Table 1. The low-fibre content of the LF diet led to ruminal proportions of acetate that were signifi-

**Table 1** Ruminal VFA concentrations and molar proportions of the individual acids expressed as a percentage of total VFA concentration in wethers fed the HF and LF diets ($n$ = 4 per group; mean ± s.e.m.)

| | VFA Concentration | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | mM | | | | $mmol.100mmol^{-1}$ | | | |
| | HF | LF | s.e.m. | $p$ | HF | LF | s.e.m. | $p$ |
| Total | 91.5 | 91.3 | ±4.54 | NS | – | – | | |
| Acetate | 64.3 | 55.5 | ±1.86 | 0.05 | 70.3 | 60.2 | ±2.46 | 0.05 |
| Propionate | 19.5 | 23.3 | ±0.43 | 0.05 | 21.3 | 25.2 | ±0.87 | NS |
| Butyrate | 6.6 | 11.3 | ±0.49 | 0.05 | 7.2 | 12.4 | ±0.50 | 0.05 |
| A : P ratio | 3.29 | 2.38 | ±0.24 | 0.05 | | | | |
| NGR | 3.97 | 3.35 | ±0.47 | 0.05 | | | | |

s.e.m. = standard error of the mean; A : P = acetate : propionate; NGR = non-glucogenic ratio

cantly lower ($p < 0.05$) than those of the HF diet. The concentrations of propionate and butyrate were significantly higher ($p < 0.05$) in the LF than in the HF group. This resulted in the acetate : propionate ratio being 27.8% higher in the HF compared to the LF group, (3.29 vs. 2.38 ± 0.243; $p < 0.05$, respectively) and the non-glucogenic ratio was 16% higher (3.97 vs. 3.35 ± 0.473 for the HF and LF group, respectively).

The effective production rates of ruminal VFA after being corrected for interconversions between acids are shown in Table 2. When expressed as moles VFA produced per kg DM intake, total production rates were significantly lower on the LF than the HF diet (6.43 and 8.02 ± 0.985 moles.kg DM intake$^{-1}$, respectively) although they were similar when expressed as moles produced per day per MJ ME intake (0.67 and 0.65 ± 0.07 moles.MJ ME intake$^{-1}$, for the HF and LF diets, respectively). Only propionate production rates differed significantly ($p < 0.05$) between groups, the rate in the LF group being 42% higher than in the HF group. This agrees with results of Bauman *et al.* (1971) who found that increasing the amount of concentrates fed did not affect the acetate production rate but increased the ruminal propionate production rate.

The specific activities of the individual VFAs in the rumen during the two infusions are shown in Table 3 and these values were used to calculate the interconversions of the various acids in the rumen which are given in Table 4. It is clear from the table that there is considerable interconversion of acids in the rumen, especially butyrate from acetate.

The rate at which acetate was converted to butyrate (mmol.min$^{-1}$) was 56% greater in the HF than in the LF group ($p < 0.05$). Although the amount of propionate converted to acetate was greater in the LF than in the HF group, this was so small as to be of no consequence. The percent-

**Table 2** Effective VFA production rates in the rumen measured simultaneously in wethers fed either the HF or LF diet ($n$ = 4 per group; mean ± s.e.m.)

| | VFA Production rates (mmol.min$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| when | (1-$^{14}$C)-Acetate | | | or (1-$^{14}$C)-Propionate | | | was infused |
| | HF | LF | s.e.m. | HF | LF | s.e.m. | $p$* |
| Total | 5.87 | 6.16 | ±0.338 | 6.17 | 6.44 | ±0.35 | NS |
| Acetate | 3.78 | 3.54 | ±0.25 | 3.62 | 3.69 | ±0.28 | NS |
| Propionate | 1.67 | 1.94 | ±0.04 | 1.73 | 2.02 | ±0.05 | 0.05 |
| Butyrate | 0.59 | 0.61 | ±0.03 | 0.59 | 0.62 | ±0.04 | NS |

*Significant difference in production rate between diets, for both infusions; s.e.m. = standard error of the mean

**Table 3** Specific radioactivities (Bq.gC$^{-1}$) of the individual VFA in the rumen of wethers fed either the HF or LF diet during the (1-$^{14}$C)-acetate or (1-$^{14}$C)-propionate infusions ($n$ = 4 per group; mean ± s.e.m.)

| | Specific activity (Bq.gC$^{-1}$) of VFA | | | | | | |
|---|---|---|---|---|---|---|---|
| when | (1-$^{14}$C)-Acetate | | | or (2-$^{14}$C)-Propionate | | | was infused |
| | HF | LF | s.e.m. | HF | LF | s.e.m. | |
| Acetate | 184 | 196 | ±6.86 | 319 | 1.31 | ±0.59 | |
| Propionate | 2.11 | 3.24 | ±0.48 | 329 | 239 | ±18.61 | |
| Butyrate | 68.1 | 44.2 | ±5.94 | 3.24 | 5.16 | ±3.55 | |

s.e.m. = standard error of the mean

**Table 4** Interconversion percentages and rates (mmol.min$^{-1}$) of one acid to another within the rumen of wethers fed either the HF or LF diet ($n$ = 4 per group; mean ± s.e.m.)

| Intercon- | Proportional change (%) | | | Rate of change (mmol.h$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| version | HF | LF | s.e.m. | HF | LF | s.e.m. | $p$(rate) |
| B from A | 36.95 | 22.57 | ±1.92 | 0.218 | 0.138 | ±0.009 | 0.05 |
| P from A | 0.79 | 1.61 | ±0.355 | 0.014 | 0.031 | ±0.001 | 0.05 |
| B from P | 1.00 | 2.42 | ±0.079 | 0.006 | 0.015 | ±0.001 | 0.05 |
| A from P | 0.75 | 1.93 | ±0.050 | 0.029 | 0.070 | ±0.004 | 0.05 |
| A to B | 11.7 | 4.50 | ±0.49 | 0.44 | 0.28 | ±0.021 | 0.05 |
| A to P | 0.51 | 0.57 | ±0.02 | 0.03 | 0.02 | ±0.001 | NS |
| P to B | 0.69 | 1.49 | ±0.012 | 0.012 | 0.030 | ±0.003 | NS |
| P to A | 1.68 | 3.65 | ±0.039 | 0.028 | 0.071 | ±0.002 | 0.05 |

A = acetate, P = propionate, B = butyrate. NS = not significant; s.e.m. = standard error of the mean

age of butyrate derived from acetate varies considerably in the literature, from 16% to 61% (Vanhoutert, 1993) and appears to be dependent on the bacterial population in the rumen. Thus the differences reported here appear to reflect the effect of diet on the bacterial population in the rumen.

The contribution of the VFA to the energy balance of the sheep is given in Table 5. The production rates determined in this experiment were 994.4 and 1139 ± 117.21 mmol.100g DOM (digestible organic matter) intake$^{-1}$, and are within the range (650–1200 mmol.100 g DOM intake$^{-1}$) reported by other authors (Van der Walt & Briel, 1976; McAllan *et al.*, 1994). When VFA production was expressed as a percentage of digestible energy (DE) intake, it was higher in the LF than in the HF group owing to the lower DE intake by the LF compared to the HF group (15.2 vs. 16.3 ± 0.88 MJ.day$^{-1}$, NS; Linington, *et al.*, 1997). These values agree with the average values found in the literature which estimate the energy derived from VFA production in the rumen to be between 54–62% of the DE intake (Vanhoutert, 1993). Propionate production contributed significantly more ($p < 0.05$) to energy metabolism in the LF diet (4.39 vs. 3.74 ± 0.06 MJ.day$^{-1}$) than in the HF diet. The VFA production in the rumen contributed to 42.3% and 52.7% of the GE intake of the HF and LF diets, respectively. When expressed in terms of ME intake the VFA contributed 77.4% and 82.0% of the ME for the HF and LF diets, respectively.

## Propionate and glucose kinetics

The arterial concentrations and specific activities of propionate, glucose and carbon dioxide are given in Table 6. The concentration of propionate was lower ($p < 0.05$) in the LF than in the HF group although the ruminal concentration was higher. The concentrations of propionate are low in arterial blood (< 0.2 mM) since the major proportion of absorbed propionate is removed by the liver and only small amounts are found in peripheral blood (Remond *et al.*, 1993). The circulating glucose concentrations were 22% ($p < 0.05$) higher in the LF than in the HF group i.e. 4.39 and 3.61 ± 0.177 nM respectively. The relatively high concentrations in both groups reflect the high ME intake

**Table 5** Corrected daily VFA production rates and estimates of daily energy provision from the VFA, in MJ.day$^{-1}$* also expressed as a percentage of the digestible energy intake (DEI)** in the rumen of wethers fed either the HF or LF diets. ($n$ = 8 per group; mean ± s.e.m.)

| | mol.day$^{-1}$ | | MJ.day$^{-1}$ | | % DEI | |
|---|---|---|---|---|---|---|
| | HF | LF | HF | LF | HF | LF |
| Total | 8.63 | 8.81 | 10.26 | 10.78 | 63.0 | 71.0 |
| s.e.m. | ±0.34 | | ±0.39 | | | |
| p | 0.05 | | 0.05 | | | |
| Acetate | 5.33 | 5.21 | 4.64 | 4.51 | 28.5 | 29.7 |
| s.e.m. | ±0.26 | | ±0.23 | | | |
| p | 0.05 | | 0.05 | | | |
| Propionate | 2.45 | 2.86 | 3.74 | 4.39 | 23.0 | 28.9 |
| s.e.m. | ±0.04 | | ±0.06 | | | |
| p | 0.05 | | 0.05 | | | |
| Butyrate | 0.86 | 0.87 | 1.88 | 1.88 | 11.5 | 12.4 |
| s.e.m. | ±0.03 | | ±0.05 | | | |
| $p$ | NS | | NS | | | |

* MJ energy calculated from the theoretical gross energy of VFA (CRC, 1984); ** DEI (DE intake.day$^{-1}$) determined previously (HF, LF = 16.28 , 15.18 $^{-1}$; NS; Linington *et al.*, 1997); NS = not significant; s.e.m. = standard error of the mean

of the sheep on both diets. The arterial $CO_2$ concentrations in the LF group were significantly lower ($p < 0.05$) than those in the HF group reflecting the low ruminal pH resulting from the rapid fermentation of the LF diet in the rumen.

The glucose irreversible loss (GIL) rate, the rate of conversion of ruminal propionate to glucose and the percentage glucose and $CO_2$ derived from ruminal propionate are also given in Table 6. The percentage of $CO_2$ derived from propionate was significantly higher in the LF group, indicating that at least some of the extra propionate produced in the rumen may have been channelled into catabolic reactions. The GIL rate in the LF group was 42.2 ± 2.3 mmol.h$^{-1}$, 17% higher than the 36.1 ± 5.1 mmol.h$^{-1}$ in the HF group. Glucose use was 147 mmol per day more in the LF than in the HF group. However, this value has not been corrected for portal appearance and hepatic tracer utilisation, which is low but may differ between diets.

The rate at which glucose was formed from ruminal propionate and the percentage of glucose from propionate, did not differ between diets. This is contrary to results of Waghorn *et al.* (1987) but similar to Teleni *et al.* (1989) and accounted for approximately 27–30% of glucose production. Similarly, the rate at which propionate was converted to glucose did not differ between groups. The percentage of ruminal propionate converted to glucose was similar (19–21%) for both the HF and LF groups.

Extrapolating the glucose production rate to a 24-h rate gives 135 and 182 ± 7.6 g.day$^{-1}$ ($p <$ 0.05) of glucose on the HF and LF diets, respectively. Although glucose disappearing from the

**Table 6** Arterial concentrations and specific activities of propionate, glucose and $CO_2$ and variables of glucose kinetics in the whole body (n=4 per group; mean ± s.e.m.)

| | HF | LF | s.e.m. | *p* |
|---|---|---|---|---|
| Propionate (mM) | 0.164 | 0.121 | ±0.005 | 0.05 |
| Glucose (mM) | 3.61 | 4.39 | ±0.177 | 0.05 |
| $CO_2$ (mM) | 18.9 | 15.6 | ±0.857 | 0.05 |
| [$^{14}$C]-Propionate (KBq.mmol$^{-1}$) | 11.64 | 9.75 | ±0.533 | NS |
| [$^{14}$C]-Glucose (KBq.mmol$^{-1}$) | 6.93 | 5.42 | ±0.273 | 0.05 |
| [$^{3}$H]-Glucose (KBq.mmol$^{-1}$) | 14.1 | 12.1 | ±0.828 | NS |
| [$^{14}$C]-$CO_2$ (KBq.mmol$^{-1}$) | 0.85 | 0.82 | ±0.068 | NS |
| *% $CO_2$ from propionate | 21.8 | 25.2 | ±1.41 | NS |
| FGIL rate: mmol.h$^{-1}$ | 36.1 | 42.2 | ±1.98 | 0.05 |
| **GIL rate: g.day$^{-1}$ | 135.2 | 182.6 | ü7.59 | 0.05 |
| Glucose from propionate (mmol.h$^{-1}$) | 10.7 | 11.7 | ±0.802 | NS |
| % Glucose from propionate | 29.7 | 27.8 | ±0.602 | NS |
| Propionate to glucose (mmol.h$^{-1}$) | 21.4 | 23.5 | ±0.851 | NS |
| % Propionate converted to glucose | 21.0 | 19.7 | ±0.629 | NS |
| #Glucose disappearance from s.intestine (g.day$^{-1}$) | 31.5 | 67.1 | ±4.26 | 0.05 |
| Glucose from propionate (g.day$^{-1}$) | 46.4 | 54.9 | ±3.38 | NS |
| % GIL rate from #glucose from s.intestine | 23.3 | 36.8 | ±2.43 | NS |
| % GIL rate from propionate and #glucose from s. intestine | 58.5 | 66.9 | ±3.86 | NS |

s.e.m. = standard error of the mean; NS = not significant; * Conversions are based on the assumption that rumen production rate = whole body entry rate; **Not corrected for hepatic utilisation; # Value obtained during partial digestion trial (Linington *et al.*, 1997)

small intestine is not an indication of glucose appearing in the portal system owing to extensive metabolism of glucose by the gastro-intestinal tract tissues, it may be used as an estimate of the maximum amount available to the sheep. The glucose disappearing from the tract was estimated in a previous study (Linington *et al.*, 1997). This glucose could have accounted for 23 and 37 ± 2.4% of the GIL rate ($p < 0.05$) in the HF and LF groups, respectively. When the amount of glucose from propionate is extrapolated to daily rates and added to the glucose disappearing from the small intestine, it can be calculated that glucose derived from these two dietary sources may account for a maximum 59 and 67 ± 3.9% of the GIL rate for the HF and LF diets, respectively.

It is clear that none of the extra GIL associated with feeding the LF diet is derived from the small increase in propionate production. Although some of this increased GIL may be ascribed to the direct absorption of glucose from the small intestine, there remains a considerable fraction (maximum 11.8 g.day$^{-1}$) unaccounted for.

## Acetate and FFA kinetics

The arterial concentrations and specific activities of acetate, FFA and carbon dioxide are given in Table 7. There were no significant differences in the circulating concentrations of either acetate or FFA between groups. The arterial $CO_2$ values were similar to those found during the propionate/

**Table 7** Arterial concentrations and specific activities of acetate, FFA and $CO_2$ and variables of FFA kinetics in the whole body, (n=4 per group; mean s.e.m.)

| | HF | LF | s.e.m. | $p$ |
|---|---|---|---|---|
| Acetate (mM) | 1.41 | 1.20 | ±0.082 | NS |
| FFA (mM) | 0.288 | 0.322 | ±0.043 | NS |
| $CO_2$ (mM) | 18.9 | 15.6 | ±0.925 | 0.05 |
| [$^{14}$C]-Acetate (KBq.mmol$^{-1}$) | 2.83 | 3.28 | ±0.205 | 0.05 |
| [$^{14}$C]-FFA (KBq.mmol$^{-1}$) | 8.79 | 4.63 | ±0.668 | 0.05 |
| [$^{3}$H]-FFA (KBq.mmol$^{-1}$) | 177.2 | 280.3 | ±3.59 | 0.05 |
| [$^{14}$C]-$CO_2$ (KBq.mmol$^{-1}$) | 0.234 | 0.271 | ±0.25 | NS |
| *% $CO_2$ from acetate | 16.4 | 16.5 | ±0.834 | NS |
| **FFA entry rate (mmol.h$^{-1}$) | 10.5 | 6.2 | ±0.541 | 0.05 |
| % FFA from acetate | 36.4 | 16.5 | ±1.44 | 0.05 |
| FFA from acetate (mmol.h$^{-1}$) | 3.80 | 1.02 | ±0.165 | 0.05 |
| Acetate to FFA (mmol.h$^{-1}$) | 32.37 | 8.67 | ±2.88 | 0.05 |
| % Acetate to FFA | 14.6 | 4.0 | ±0.781 | 0.05 |

s.e.m. = standard error of the mean; NS = not significant; *Conversions are based on the assumption that rumen production rate = whole body entry rate; ** Not corrected for hepatic utilisation.

glucose infusion and were significantly lower in the LF than in the HF group.

The percentage of $CO_2$ derived from acetate oxidation was similar between groups (*ca.* 16.5%) and accounted for a lower portion of the $CO_2$ pool than propionate. The FFA entry rate was significantly higher ($p < 0.05$) in the HF than the LF group. This value has not been corrected for portal appearance and hepatic tracer utilisation.

The HF group converted 23.63 ± 2.3 mmol more acetate per hour into FFA than did the LF group. Conversion into FFA accounted for 14.6 and 4.0 ± 0.781% ($p < 0.05$) of ruminal acetate production rates for the HF and LF groups, respectively. This data suggests that considerably more of the acetate production in the HF fed group is converted to FFA than in the LF group despite almost identical rates of acetate turnover and oxidation.

## Blood hormone concentrations

The concentrations of insulin, glucagon, thyroid hormones, carbon dioxide ($CO_2$) and oxygen ($O_2$) as well as their ratios to each other, are shown in Table 8. The plasma concentrations of insulin, glucagon and thyroxin ($T_4$) were significantly higher in the HF than in the LF group, while triiodothyronine ($T_3$) did not differ between groups. This resulted in a lower ratio ($p < 0.05$) of insulin to glucagon and $T_3$ to $T_4$ in the HF than in the LF group. Oxygen concentrations in the HF group were significantly higher than in the LF group, however the ratio of $CO_2$ to $O_2$ did not differ between groups since the $CO_2$ concentrations were correspondingly higher in the HF than in the LF group. These values are similar to a wide range of insulin concentrations reported by others: 0.2–0.8 ng.ml$^{-1}$ (Bloch *et al.*, 1986) 25 ng.ml$^{-1}$ (Waghorn *et al.*, 1987) and 824 ng.ml$^{-1}$ (Brockman, 1990). These results support the findings of the growth study (Linington *et al.*, 1996) where the insulin concentrations were lower in animals fed concentrates than in those fed roughage.

**Table 8** Arterial plasma concentrations of hormones and oxygen as well as the ratios between hormones or metabolites. ($n$ = 4 per group; mean ± s.e.m.)

| | HF | LF | s.e.m. | $p$ |
|---|---|---|---|---|
| **Concentrations** | | | | |
| Insulin ($ng.ml^{-1}$) | 1.38 | 1.14 | ±0.046 | 0.05 |
| Glucagon ($pg.ml^{-1}$) | 98.0 | 43.5 | ±1.73 | 0.05 |
| $T_4$ ($pmol.l^{-1}$) | 1.93 | 1.65 | ±0.043 | 0.05 |
| $T_3$ ($pmol.l^{-1}$) | 20.7 | 21.1 | ±0.828 | NS |
| $CO_2$ (mM) | 18.9 | 15.6 | ±0.875 | 0.05 |
| Oxygen (mM) | 5.07 | 4.58 | ±0.220 | 0.05 |
| **Ratios** | | | | |
| Insulin : Glucagon | 14.1 | 26.2 | ±0.326 | 0.05 |
| $T_3 : T_4$ | 10.7 | 12.8 | ±0.481 | 0.05 |
| $CO_2 : O_2$ | 3.7 | 3.4 | ±0.190 | NS |

s.e.m. = standard error of the mean; NS = not significant

## Discussion

### Ruminal VFA production

The total VFA concentrations and molar proportions of the individual acids given in Table 1 were similar to those found in a previous study using the same diets but different sheep (Linington *et al.*, 1997) and are within the range (60–150 mM) reported in the literature (Sutton *et al.*, 1986; Sutton *et al.*, 1988). This would suggest that the digestion and fermentation kinetics of the diets in the two groups of sheep were essentially similar.

Total VFA concentrations did not differ significantly between diets (91.5 & 91.3 mM) . The molar proportions of acetate : propionate : butyrate were 70:21:7 and 60:25:12 for the HF and LF diets, respectively. These results are similar to those reported by Sutton *et al.* (1986), who compared a roughage to a low-fibre diet and found the VFA concentrations in the rumen to be 110 and 120 $mmol.l^{-1}$, respectively, and the acetate : propionate : butyrate ratio to be 62:25:13 and 54:31:9 for their roughage and low-fibre diets, respectively. Although it has been suggested that frequent feeding may alter the ruminal fermentation pattern in the rumen (MacLeod *et al.*, 1984) the present study found no effect of feeding frequency on the VFA pattern and neither did Sutton *et al.* (1986).

The production rates of propionate on the two diets are similar to those reported in the literature, and are dependent on the type of diet, the DOM and the ME intake. The reported increase in propionate concentration appears to result from an increase in production rate (Table2). Bauman *et al.* (1971) reported that the propionate production rate increased from 13 to 31 moles per day with increasing concentrate intake, while the acetate production rates remained almost the same. This would clearly lead to a decrease in the acetate : propionate ratio. In this study the difference in production rates was constant (16%) between diets and was much less than that reported by Bauman *et al.* (1971), but similar to that of Seal *et al.* (1989), who reported propionate production rates of 3 and 3.3 $mmol.min^{-1}$ for cattle fed either a forage (grass) or a forage-concentrate (50% grass–50% maize) diet, respectively.

MacLeod *et al.* (1984) suggested that the correlation between production rate and molar proportions is poor when sheep are fed concentrate diets. In an *in vitro* study, these authors found that the

low pH found in the rumen of sheep fed concentrate diets, decreases acetate absorption and the production rate is no longer proportional to the concentration. In this study the sheep were fed half-hourly, to avoid any possible decline in pH. No studies which employed a frequent-feeding regime could be found. As a result, the VFA production rates were found to be similar regardless of which VFA was used to determine the production rate, i.e. acetate or propionate.

The effective production rates in Table 2 were derived by correcting the gross rates for VFA interconversion. The extent of conversion found in the present study is lower than those of Bergman *et al.* (1965) and Van der Walt & Briel (1976) but higher or similar to those reported by Leng & Leonard (1965). The major inter-acid conversion in the rumen was found to be butyrate from acetate. Butyrate may be formed in the rumen from both acetate and compounds which form acetyl-CoA. The interconversion of acetate and butyrate in the rumen is energetically interesting. Conversion of butyrate to acetate is advantageous in that there is a net synthesis of ATP, whereas the reverse reaction consumes net amounts of ATP (Dijkstra, 1994). This latter reaction, however, may be advantageous in that it supplies reducing factors ($NADPH_2$) for anabolic reactions (Martin *et al.*, 1994). Excessive reducing power generated by the formation of acetate and butyrate, or the conversion of acetate to butyrate, is used in the formation of propionate, or converted to methane (Martin *et al.*, 1994). Little interconversion of propionate with acetate or butyrate has been reported and this is confirmed by the results of this study.

Extrapolating an hourly production rate to a daily production rate is subject to many potential errors such as a possible fluctuation in VFA concentrations and/or production rate. However, the frequent feeding regime appeared to reduce the fluctuations, thereby making extrapolation of the data to daily production rates more acceptable. In the present study, the VFA supplied 60–72% of the digestible energy intake. This contribution is higher than that reported by Faichney (1968) but is close to the range (35–70%) reported by other authors (Bines *et al.*, 1988; Reynolds & Huntington, 1988). In none of these studies, however, were diets high in concentrates fed. It thus appears that the contribution of VFA produced in the rumen to DE intake increases as the starch content of the diet increases.

Although the LF diet supplied higher quantities of starch than did the HF diet, the VFA accounted for more of the DE intake than the HF diet. Since the VFA production rates were similar when expressed as a percentage of ME intake, this is possibly due to a lower DE intake in the LF group. Acetate appeared to supply one-third of the DE intake on either of the diets. There was a significant increase in the proportion of DE intake from propionate which was not only as a result of increased production, but also the higher energy contribution of propionate than that of acetate. The contribution of propionate to the DE intake is similar to that reported by other authors for concentrate diets (Herbein *et al.*,1978; Seal *et al.*, 1989).

## Whole body metabolism

Glucose entry rates in ruminants appear to be only slightly lower than those found in non-ruminants despite little glucose being absorbed from the gastro-intestinal tract on most diets. Many glucose entry and irreversible loss rates have been reported in the literature from 7.9 $mmol.h^{-1}$ in sheep starved for 6 days (Steel & Leng, 1973a) to 38.92 $mmol.h^{-1}$ in sheep fed maize-based diets (Janes *et al.*, 1985a,b). It appears that glucose entry and irreversible loss rates are dependent on the quality and quantity of the diet (Steel & Leng, 1973b). GIL rate is dependent on digestible organic matter intake (Steel & Leng, 1973a,b; McNiven, 1984), protein intake (König *et al.*, 1984) and ME intake and body weight (Oddy *et al.*, 1984), propionate production in the rumen (Judson & Leng, 1973a,b), the proportion of grain in the diet (Annison *et al.*, 1974), digestible energy intake (Herbein *et al.*, 1978; Harmon *et al.*, 1983), DE intake and the energy balance of the animal.

The GIL rate was significantly higher in the LF compared to the HF group. Our results are similar to those of Janes *et al.* (1985a,b) who fed grass- or maize-based diets to sheep. Increased glucose flux has also been reported when starch was fed (Judson *et al.*, 1968). The increase in glucose flux is usually associated with an improved nutritional state and many authors have reported no difference in GIL rate when isoenergetic, but not isonitrogenous, diets were fed (Herbein *et al.*, 1978; Harmon *et al.*, 1983). The results of the present study, where ME and nitrogen intakes were similar, are supported by the results of Lee *et al.*, (1987) who also found an increase in glucose entry rate when isonitrogenous and isoenergetic diets were fed. It would appear therefore that glucose irreversible loss rate is not only dependant on ME intake on but the quantity of starch and nitrogen in the diet as well.

The proportion of ruminal propionate being converted to glucose varies from 16 to 60% (Bergman *et al.*, 1966; Amaral *et al.*, 1990), and is dependent on the GIL rate, the glucogenic potential of the diet and the amount of glucose absorbed from the small intestine. In the present study the amount of propionate converted to glucose was not affected by the type of diet, similar to the results of Teleni *et al.*, (1989) but unlike others (König *et al.*, 1984; Waghorn *et al.*, 1987). The proportions reported here are within the range found in the literature i.e. approximately 30–53% of the glucose entry rate can be derived from ruminal propionate (Teleni *et al.*, 1989).

The FFA entry rate was lower in the LF than in the HF group. A lower FFA entry rate is usually indicative of a lower rate of lipolysis, and coupled to the lower FFA concentrations it would appear as if FFA synthesis and esterification was higher in the LF group than in the HF group (Bauchart, 1993; Faulconnier *et al.*, 1994). Although the acetate concentrations were higher in the HF than in the LF group, the percentage oxidised to $CO_2$ was similar for both diets, i.e. *ca.* 16% of the $CO_2$ was derived from acetate, supporting the role of acetate as a major energy source in the ruminant (Prior *et al.*, 1980). It would appear that the *de novo* synthesis of fatty acids from fatty acids was higher in the HF than the LF group. This estimate is however, very indirect since acetate is converted into fatty acids which then join the triglyceride pool. Since the rate of lipolysis appears to be higher in the HF than in the LF group, the rate at which acetate would appear in FFA and the percentage FFA from acetate would also be greater. Furthermore, the actual amount used in fatty acid synthesis is probably far higher than the amount appearing in the blood.

Although most of the consequences of altering the nutritional status on metabolism are direct, nutritional status also affects the endocrine balance of the animal. The partitioning of nutrients between tissues appears to be regulated mainly by glucagon, insulin, growth hormone (GH) and thyroid hormones (Sartin *et al.*, 1985; Coxam *et al.*, 1989; Eisemann, 1994).

Glucose normally causes increased insulin concentrations although the VFA, specifically propionate and butyrate, also stimulate insulin secretion (Johnsson *et al.*, 1985; Zainir *et al.*, 1989; Sano *et al.*, 1993). Basset (1972) suggested that the role of VFA in eliciting an insulin response was minor since the concentrations required to produce an increase in insulin concentrations were pharmacological. In the present study, the plasma insulin concentrations were lower in the LF than in the HF sheep even though both plasma glucose concentrations and ruminal VFA concentrations were higher. Despite the lower insulin concentrations, sheep fed the LF diet used more glucose. Livingston *et al.* (1984) suggested that insulin sensitivity in rats might be due to the synergistic action of hormones other than insulin, e.g. growth hormone, catecholamines or glucocorticoids, although species differences may dilute this observation (Broad *et al.*, 1983).

De Boer *et al.* (1985) suggested that the balance or ratio of hormones to each other is more important than their absolute concentrations. The insulin – glucagon axis is important in ruminant glucose homeostasis since glucagon acts primarily on the liver, and insulin exerts its effects on peripheral tissue. The normal insulin glucagon molar ratio ranges from 0.9–2.3, a low ratio repre-

senting a dominance of glycogenolytic and gluconeogenic actions, while a high ratio represents glycogenesis and peripheral glucose use. Thus it appears that the insulin : glucagon ratio was one of the determining factors in glucose utilisation in the present study.

Thyroid hormones exert many effects on glucose metabolism but its role in glucose homeostasis remains unclear (Trenkle, 1978; Barry *et al.*, 1985; Müller *et al.*, 1989). In humans $T_3$ and $T_4$ can act both synergistically and antagonistically with glucagon and insulin and may act as either anabolic or catabolic agents depending on the hormonal and metabolic milieu, thereby having an ambiguous role in metabolism (Müller *et al.*, 1989). The data on thyroid hormone function in sheep is limited and conflicting. In the present study there was no change in $T_3$ concentrations but plasma concentrations of $T_4$ were higher in HF sheep than LF sheep. Barry *et al.*, (1985) found elevated plasma $T_4$ and GH concentrations were associated with increased protein, while Rosemberg *et al.* (1989) found no effect after injecting and implanting sheep with $T_4$ and GH, respectively. $T_4$ increased carcass mass and dressing percentage but there was no effect on composition or feed conversion ratios.

It is clear from the preceding that the effect of endocrine status and metabolic milieu on metabolite partitioning is not well understood. Data from this study and others indicate that glucose and lipid metabolism may be linked through antagonistic hormones, i.e. insulin and glucagon. Furthermore, the effect of the hormones on nutrient partitioning is not absolute but dependent upon the concentrations of other hormones, such as growth hormone and thyroid hormones. A 30% difference in the efficiency in ME utilisation was observed by Linington *et al.* (1996) in karakul lambs fed the same diets. The lambs fed the LF diet were more efficient compared to lambs fed the HF diet even though ME and CP intakes were similar. This improvement could have resulted from a better utilisation of absorbed nutrients reflected in a lower FFA entry rate i.e. lower lipolytic rate.

The appearance of nutrients in portal blood after absorption and the effect of the liver on circulating metabolites are to be reported later.

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