The effect of protein supplementation on digestion and glucose metabolism in young cattle fed on silage

BY MARGARET GILL AND D. E. BEEVER

The Grassland Research Institute, Hurley, Maidenhead, Berks. SL6 5LR

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1. Ryegrass (*Lolium multiflorum* cv. RvP) silage was given to twelve Friesian steer calves (initial live weight (LW) 106 kg) at 2·3 kg dry matter (DM)/calf per d, alone, or with a supplement of fish meal at 50 (FM1) or 100 (FM2) g/kg silage DM per d.

2. The rate of flow of nutrients into the duodenum was measured using a dual-marker technique. Glucose irreversible-loss rates and the contribution of propionate to gluconeogenesis were measured using continuous infusions of [U-^{14}C]glucose and [2-^{14}C]propionate.

3. Amino acid flow into the duodenum was significantly ($P < 0{\cdot}01$) increased from 238 g/d on the control treatment to 319 g/d on FM2, but there was no response to FM1 ($P > 0{\cdot}05$).

4. Glucose irreversible loss rate remained constant with an average value of 1·86 mg/min per kg LW.

5. Neither the percentage of glucose synthesized from propionate (average 30) nor the percentage of propionate converted to glucose (average 45) was significantly altered by treatment ($P > 0{\cdot}05$).

Intake and live weight (LW) gain responses to protein supplementation of silage diets have been reported in cattle (Forbes & Irwin, 1970; Drennan, 1973; Garstang *et al.* 1979) but elucidation of possible mechanisms has not been successful. Digestion studies, carried out mainly on sheep (Beever *et al.* 1977; Gill & Ulyatt, 1977; Siddons *et al.* 1979), have established that protein flow to the duodenum appears to be low on non-additive silages, and recent information for cattle fed on direct-cut ryegrass silage (Thomson *et al.* 1981) confirms these observations. In addition, the molar proportion of propionic acid in the rumen of silage-fed animals appears to be lower than values obtained for other roughage diets fed to both sheep and cattle (Bath & Rook, 1965; Griffiths *et al.* 1973).

Protein and propionic acid are the main glucose precursors available to ruminants (Annison *et al.* 1963; Bergmann *et al.* 1966; Leng *et al.* 1967) and thus play an important role in glucose metabolism, since the amount of glucose absorbed from the alimentary tract of ruminants fed on roughage diets is negligible (Armstrong & Beever, 1969; Beever *et al.* 1972).

The aim of the present experiment was to study the effect of protein supplementation to growing cattle on a silage diet by measuring the daily flow of nutrients into the duodenum, together with glucose irreversible loss rate and the contribution of propionate to gluconeogenesis.

MATERIALS AND METHODS

Diets

Primary growth Italian ryegrass (*Lolium multiflorum* cv. RvP) was cut with a precision-chop forage harvester in mid-June, 1978, and formic acid (Add-F; BP Chemicals Int. Ltd) was applied at the time of harvesting at the rate of 3·0 l/t fresh weight. The grass was ensiled in a clamp silo and sealed. Approximately 4 months later the silo was opened and silage was removed from the clamp daily. The silage was offered either alone (control; C), or with supplements of fish meal (Provimi 66) at inclusion rates of 50 (FM1) and 100 (FM2) g fresh wt/kg silage dry matter (DM). Level of silage feeding was fixed at 10 kg fresh wt/calf per d, equivalent to 2·3 kg DM, and the supplement was mixed by hand with the silage just before feeding.

Animals

Twelve Friesian castrated male calves were fitted with a cannula into the dorsal sac of the rumen and a 'T'-piece cannula into the proximal duodenum at approximately 3 months of age, using previously described techniques (Beever, Kellaway *et al.* 1978). Immediately following surgery, 2 weeks were allowed for post-operative recovery by which time all animals were re-established on a standard silage diet and appeared to be healthy. At this point the animals were transferred to individual metabolism crates, housed in a continuously-illuminated house and allowed free access to water and mineral licks.

Management and experimental design

The experiment consisted of two periods, each of 4 weeks duration, with six calves per period. The calves were allocated at random to the two periods and within each period the six calves were again allocated randomly to the three dietary regimens. The second period started 3 weeks after the first period and consequently the mean initial LW of the calves in period no. 2 was 12 kg greater than the mean weight of the calves used in period no. 1 (112 *v.* 100 kg LW).

The experimental diets were introduced over a 7 d period, at the end of which continuous feeding was commenced using automatic belt feeders. A further 7 d was then allowed before the start of sampling for measurement of duodenal flow. Immediately following the duodenal collection, polyvinyl catheters (i.d. 1·00 mm; Portex Ltd, Hythe, Kent) were inserted into each jugular vein at least 48 h prior to the first intravenous infusion. Patency was maintained by regular flushing with heparinized saline (9 g sodium chloride/l containing 250 IU heparin/ml). A total of three separate tracer infusions was carried out, comprising an intravenous infusion of D-[U-^{14}C]glucose to determine glucose turnover followed at 4 d intervals by intrarumen infusions of sodium [2-^{14}C]propionate and of sodium [1-^{14}C]propionate to measure rumen propionate production and the contribution of propionate to gluconeogenesis.

Measurement of duodenal flow

The rate of flow of digesta at the duodenum was estimated using the dual-phase marker system described by Faichney (1975). A solution of ruthenium phenanthroline (80 μg/ml) and chromium ethylene diamine tetra acetic acid (CrEDTA; 650 μg/ml) was continuously infused into the reticulo-rumen by means of a peristaltic pump (Quickfit, Stone, Staffs.) at a constant rate of 20 ml/h. The infusion was continued for 7 d and on the last day of the infusion, collection of duodenal digesta was undertaken. This comprised manual spot sampling from the 'T'-piece cannula at hourly intervals between 09.00 and 20.00 hours inclusive, with 100 ml digesta being collected on each occasion. The twelve samples obtained from each calf were bulked and mixed using a Silverson homogenizer (Model L2R, Silverson Machines Ltd., Chesham, Bucks.). Approximately 400 ml of the sample was removed and freeze-dried to represent whole digesta, whilst the remainder was centrifuged at 20000 ***g*** for 30 min to obtain a residue which was freeze-dried and used as the alternative digesta phase as outlined by Faichney (1975). All samples were subsequently ground through a small laboratory mill and stored at $-5°$ until required for analysis.

Measurement of IRL of glucose

A solution of [U-^{14}C]glucose (0·75 μCi/ml) was prepared in autoclaved physiological saline containing 1 mg carrier glucose/ml, and infused for 9 h using a Gilson peristaltic pump (Anachem Ltd, Luton, Beds) at a rate of 13 μCi/h into the right jugular vein. From 5th to 9th hour of infusion, samples of venous blood were withdrawn at 30 min intervals from the non-infused jugular vein by means of a syringe attached to the catheter and placed

immediately into heparinized tubes. The tubes were stoppered, gently inverted and approximately 18 ml heparinized blood were weighed into a beaker and deproteinized using zinc sulphate and sodium hydroxide (Somogyi, 1945). After allowing to stand for 5 min the solution was centrifuged at 2500 *g* for 15 min and the resulting supernatant fraction filtered through Whatman No. 1 filter paper before storage at −5° until required for analysis of glucose specific activity. In addition, in the first period, a further 12 ml heparinized blood was centrifuged immediately and 6 ml of the resulting plasma was processed as described previously.

Measurement of propionate metabolism

Rumen production of propionate was estimated by means of a continuous intrarumen infusion of sodium [2-^{14}C]propionate solution (11·5 μCi/h, 1 mg Na propionate/ml) with samples of rumen fluid (approximately 10 ml) being obtained by means of a syringe (at 30 min intervals) during the last 3 h of infusion. The samples were acidified immediately using a small quantity of 5 M-sulphuric acid and stored at −5° until required for analysis. The contribution of propionate to gluconeogenesis was derived from this infusion of sodium [2-^{14}C]propionate and a subsequent intrarumen infusion of sodium [1-^{14}C]propionate (14·9 μCi/h, 1 mg Na propionate/ml) (Judson & Leng, 1973*a*; Thompson, 1971). Prior to the start of each infusion a blood sample was taken from each animal to determine background ^{14}C levels. Each infusion lasted 9 h and blood samples were taken from the left jugular catheter at 30 min intervals over the last 4 h of the infusion. Samples of blood and plasma from period no. 1 and blood only from period no. 2 were processed as described earlier.

Chemical analyses

Feeds

Oven-DM content of the silage was determined every 7 d by drying in a forced-draught oven at 103° for 24 h, and the values so obtained were used to adjust levels of fish meal supplementation. At the end of the experiment fresh samples of silage taken daily over the duration of the two measurement periods were bulked and analysed for toluene-DM (Dewar & McDonald, 1961), total nitrogen, hot-water-insoluble N (Lonsdale *et al.* 1981), ammonia-N (Siddons *et al.* 1979), lactic acid (Elsden & Gibson, 1954) and individual volatile fatty acid contents (Beever, Terry *et al.* 1978).

Gross energy content of the silage was determined by means of an adiabatic bomb calorimeter using freeze-dried material, with appropriate corrections for the content of volatile components (Terry & Osbourn, 1980). In addition, a freeze-dried sample of the silage was used to determine organic matter (OM, ashing at 550° overnight) and individual amino acid contents (Beever, Terry *et al.* 1978).

The DM content of the fish meal was determined on representative samples taken at regular intervals throughout the experiment according to the procedure described previously, whilst OM, total N and individual amino acid contents were determined on a bulk sample.

Digesta

All analyses of whole and centrifuged duodenal digesta were conducted using freeze-dried material. Contents of OM, total N and individual amino acids were determined using techniques previously referred to, whilst chromium content was determined using the techniques described by Beever, Kellaway *et al.* (1978). Ruthenium content of duodenal digesta was determined by X-ray fluorescence spectrometry (Evans *et al.* 1977). Samples of the marker infusates were analysed for chromium and ruthenium contents using the previously-mentioned procedures.

Rumen fluid

After thawing, all rumen fluid samples were centrifuged at 2500 *g* for 10 min and the supernatant fractions were removed. The quantities and molar proportions of the individual volatile fatty acids were determined by using a Hewlett Packard 5570 gas–liquid chromatograph packed with Chromosorb 101 and operating at a temperature of 160°, whilst total ^{14}C contents were determined by liquid scintillation (Beever *et al.* 1976).

Blood and plasma

Glucose concentrations of all deproteinized blood and plasma samples were determined using a u.v. hexokinase (*EC* 2.7.1.1) method (Boehringer Mannheim test combination; Boehringer Corp. Ltd, Lewes, Sussex). Assay of levels of radioactivity of blood and plasma glucose was performed by preparing the glucose pentaacetate derivative (Jones, 1965) and final counting by liquid scintillation, using Toluene, with 2,5-diphenyloxazole (PPO, 4 g/l) and 1,4-di-2-(5-phenyloxazolyl)-benzene (POPOP, 2 g/l) as the scintillant.

Calculation of results

The quantity of DM flowing into the small intestine was estimated according to the procedures outlined by Faichney (1975) and nutrient flows were calculated from a knowledge of the latter and the concentration of individual nutrients in the whole and centrifuged digesta.

The net production of propionate in the reticulo-rumen was determined by standard isotope techniques (Leng *et al.* 1967; Weller *et al.* 1967) with no allowance for possible inter-conversion which, in the case of propionate, was considered to have minimal effect.

IRL of glucose was calculated by comparing the rate of labelled glucose infusion with the plateau specific radioactivity (SR) of glucose in whole blood (both periods) or plasma (period no. 1 only). The percentage of glucose synthesized from propionate and the percentage of propionate converted to glucose were determined as follows (Leng *et al.* 1967);

$$\% \text{ glucose from propionate} = \frac{\text{SR glucose from propionate infusion } (\mu\text{Ci/mg C})}{\text{SR propionate from propionate infusion } (\mu\text{Ci/mg C})}.$$

$$\text{Amount of glucose synthesized from propionate} = \text{glucose IRL} \times \% \text{ from propionate}$$

$$\% \text{ propionate converted to glucose} = \frac{2 \times \text{amount glucose from propionate (mol/d)}}{\text{propionate production rate (mol/d)}}.$$

An alternative calculation of the contribution of propionate to gluconeogenesis, which takes account of the crossover of label within the Krebs cycle, as proposed by Thompson (1971), was also considered, details of which are given below.

The first step requires calculation of the glucose ratio (GR);

$$\text{GR} = \frac{\text{SR glucose from [2-}^{14}\text{C]propionate infusion}}{\text{SR glucose from [1-}^{14}\text{C]propionate infusion}}.$$

Values for GR were calculated from infusions into the six animals in period no. 1 and the mean value was used to estimate the proportion (X) of oxaloacetate which leaves the Krebs cycle to form phosphoenolpyruvate using the following equation;

$$X = \frac{5 - \text{GR}}{1 + \text{GR}}.$$

Table 1. *Chemical composition of the dietary ingredients (g/kg dry matter (DM) unless stated)*

	Grass silage	Fish meal
Toluene DM	230	ND
pH	3·9	ND
Organic matter	901	878
Total nitrogen	19·1	112·9
Insoluble N	2·10	22·0
Amino acid-N	13·6	100·2
Gross energy (MJ/kg DM)	19·0	21·1
Lactic acid	129·0	ND
Acetic acid	19·2	ND
Butyric acid	0·6	ND

ND, not determined.

Values for X were then used to calculate the underestimation of propionate contribution to glucose metabolism which was incurred by using [2-^{14}C]propionate, i.e.;

$$\text{percentage underestimation} = 100 - \frac{(5X - X^2)}{2 + 2X}\,100.$$

Statistical analysis

The results were assessed by analysis of variance of the 3×2 factorial design and the treatment means were compared using Student-Newman-Keul's test.

RESULTS

The chemical composition of the silage and the fish meal is shown in Table 1. The existence of a low pH and relatively high lactic acid content indicates a well-fermented silage. The total N content of the silage (19 g/kg) was low, with 70% being present in the form of amino acid-N, while hot-water-insoluble N comprized 11% of total silage N and almost 20% of total fish meal N. The effect of the fish meal was to increase total N intake from 19·1 on the control silage to 26·8 g/kg diet DM at the higher level of supplementation and insoluble N from 2·1 to 4·0 g/kg diet DM.

Nutrient intake and digestion

The mean quantities of OM, total N and total amino acids consumed and entering the small intestine are shown in Table 2. The low level of fish meal inclusion had no significant effect on the quantities of OM, total N or total amino acids flowing to the small intestine relative to the values observed on diet C. Both diets showed approximately 51% of ingested OM to be lost before the small intestine, while the 15 g difference in N intake on the two diets was reduced to a non-significant difference in the amounts entering the duodenum. Likewise, total amino acid intake was 85 g/d greater on diet FM1 than on diet C, but the overall effects of rumen digestion reduced this difference and similar amounts of amino acids were estimated to enter the duodenum.

The increased flow of OM, total N and total amino acids into the small intestine on diet FM2 compared with the other two diets ($P < 0·05$) was related to a reduced extent of digestion in the rumen. N flow at the duodenum was 7·3 g/d less than the amount consumed but was significantly higher than the values recorded for diets C and FM1 ($P < 0·05$), whilst

Table 2. *The mean quantities (g/d) of organic matter, total nitrogen and total amino acids consumed and entering the small intestine of calves fed on grass silage with and without fish meal supplementation at two levels (50 (1) or 100 (2) g/kg silage dry matter per d)*

Dietary regimen*	Silage	Silage + fish meal 1	Silage + fish meal 2	SE of mean
Organic matter:				
Intake	2050	2193	2218	13·0
Entering small intestine	1033[a]	1041[a]	1204[b]	18·9
Total N:				
Intake	43·4	58·2	66·2	0·27
Entering small intestine	44·9[a]	47·1[a]	58·9[b]	1·59
Total amino acids:				
Intake	192·4	276·4	314·4	1·23
Entering small intestine	238·4[a]	239·9[a]	319·2[b]	10·1

[a, b], values that do not share a common superscript letter are significantly different ($P < 0·01$).
* For details, see p. 37.

Table 3. *Amino acid composition (g/kg total amino acid) of the grass silage and fish meal and the duodenal contents of animals receiving the three diets*
(Fish meal was fed at 50 (1) and 100 (2) g/kg silage dry matter per d)

			Duodenal digesta			
Dietary regimen*	Grass silage	Fish meal	Grass silage	Grass silage + fish meal 1	Grass silage + fish meal 2	SE of mean
Histidine	17·7	30·6	19·0	20·9	19·6	0·90
Isoleucine	70·9	47·6	61·4	65·3	63·0	1·74
Leucine	87·2	78·3	78·4	78·5	78·7	1·05
Lysine	70·0	81·8	71·1	74·9	73·2	2·42
Methionine	22·9	32·2	23·6	25·2	29·3	1·57
Phenylalanine	52·7	42·2	54·2	54·3	53·5	1·09
Threonine	49·9	47·6	57·4	55·9	56·2	0·59
Tyrosine	26·8	37·7	44·2	44·4	44·3	0·76
Valine	68·8	53·9	65·5	65·2	64·1	0·92
Alanine	89·2	63·3	71·1	68·9	68·5	0·95
Arginine	36·8	64·1	48·6	47·5	51·1	1·37
Aspartic acid	112·8	91·6	104·2	99·9	101·3	1·55
Glutamic acid	118·3	149·0	127·3	125·6	131·1	1·42
Glycine	64·4	77·4	73·1	70·9	67·9	3·24
Proline	60·2	51·8	48·9	53·1	48·2	2·27
Serine	51·4	50·6	52·1	49·5	50·6	1·27

* For details, see p. 37.

total amino acid flow to the small intestine was virtually identical to the amount consumed and approximately 33% greater than the values recorded on diets C and FM1.

Values for individual amino acid contents in the diets and in the digesta entering the duodenum are presented in Table 3. There were no statistically significant ($P > 0·05$) differences but there were marked increases in the contents of methionine in particular, and also lysine, arginine and glutamate. Thus, whilst diet FM2 increased total amino acid flow

Table 4. *Mean rates of propionate production and molar percentage of the volatile fatty acids in the rumen of calves fed on grass silage with or without the addition of fish meal at two levels (50 (1) or 100 (2) g/kg silage DM)*

Dietary regimen	Silage	Silage + fish meal 1	Silage + fish meal 2	SE of mean
Propionate production (mmol/min)	1·83	1·85	1·11	0·314
Molar percentage:				
Acetate	69·1	68·6	70·8	0·82
Propionate	23·9	23·7	22·0	1·08
Butyrate	7·0	7·7	7·2	0·49

Table 5. *Glucose concentration and specific activity of blood and plasma samples taken from six calves in period no. 1 and the irreversible loss rates of glucose calculated from these values*

	Blood	Plasma	SE of mean	Statistical significance of difference: *P*
Glucose concentration (mg/l)	560	764	11·2	< 0·001
Glucose specific activity (μCi/mg × 10^{-3})	1·238	1·235	0·0165	NS
Irreversible loss of glucose (mg/min)	202·0	201·5	2·85	NS

NS, not significant.

by 34% compared with diet C, methionine flow was increased from 5·6 to 9·4 g/d, an increase of 68% ($P < 0·01$). The increases in lysine, arginine and glutamate flow were 35, 41 and 38% respectively compared with diet C.

The mean rumen propionate production rate (see Table 4) fell by approximately 39% in response to the high level of fish meal inclusion but this did not achieve statistical significance. Molar proportion of propionate on diet FM2 was slightly lower than on diets C and FM1, but the difference was not statistically significant ($P > 0·05$).

Glucose metabolism

In the first period, glucose IRL was calculated using the SR of both whole blood and plasma glucose. The values presented in Table 5 show that glucose concentration was, as expected, approximately 200 mg/l higher in plasma than in blood. However, blood and plasma glucose SR values and thus the calculated IRL values were remarkably similar. Consequently, only whole blood samples were assayed for glucose SR in period no. 2, and all glucose metabolism results presented and discussed subsequently were based on blood assays only.

The mean values of glucose IRL for the three diets are given in Table 6 and ranged from 190 to 210 mg/min or 1·75 to 1·92 mg/min per kg LW. Although there appeared to be a slight positive trend with fish meal inclusion, none of the differences obtained was statistically significant. The contribution of propionate to gluconeogenesis was calculated

Table 6. *Criteria of glucose metabolism in calves fed on grass silage with or without fish meal at two levels*

Treatments	Live wt (kg)	Blood glucose concentration (mg/l)	Irreversible loss of glucose (mg/min)	Percentage propionate converted to glucose	Percentage glucose synthesized from propionate
Silage alone	108·3	522	189·9	43·1	38·0
Silage + fish meal 1	104·8	548	198·9	45·2	34·3
Silage + fish meal 2	109·5	580	209·7	47·1	19·5
SE of mean	3·06	18·8	11·84	2·09	5·17
Statistical significance of difference	NS	NS	NS	NS	NS

NS, not significant.

from the [2-^{14}C]propionate infusion (Table 6) and showed no apparent treatment differences (mean 45%). The percentage of total glucose production which was synthesized from propionate is also shown in Table 6, with animals on diets C and FM1 obtaining 36% of total glucose production from rumen propionate, whilst for diet FM2 the value was only 20% but this difference was not statistically significant ($P > 0{\cdot}05$).

DISCUSSION

The studies of Garstang *et al.* (1979) have demonstrated that protein supplementation of grass silage fed to young cattle can improve animal performance. Inclusion of fish meal at 50, 75 and 100 g/kg silage DM, gave rise to increases in voluntary food intake of 7, 13 and 12% respectively, and daily LW gains which were 40, 72 and 116% greater than the values observed on unsupplemented silage with associated improvement in food conversion efficiencies. Thomas *et al.* (1980) showed fish meal inclusion (50 g/kg silage DM) to significantly increase silage intake by 8%, but when expressed on an LW basis, no intake differences were observed. In this study, LW change was not recorded.

The results of the present study failed to detect any significant change in digestion with the low level of fish meal inclusion, whereas at the higher level there was a substantial increase in duodenal amino acid flow. The significance of this increase in terms of LW gain can be estimated using the recently published system for calculating nutrient requirements for ruminants (Agricultural Research Council, 1980). Such calculations give an expected LW gain of 850 g/d on the high fish meal supplemented silage diet compared with a mean estimate of 600 g/d on the control and low fish meal treatments. This calculation emphasizes the need for protein supplementation of silage diets to obtain high rates of LW gain.

A further advantage of fish meal supplementation was observed in an improved balance of amino acids entering the duodenum. Total amino acid flow was increased 34% on diet FM2 compared with diet C, while methionine flow on diet FM2 was 68% greater than on the control diet. The effect of low methionine availability on silage diets in limiting production in sheep and dairy cattle has been reported previously (Barry *et al.* 1973; Gill & Ulyatt, 1977; Thomas *et al.* 1980) and it is possible that similar limitations might exist in growing animals.

These responses in duodenal protein supply were only observed at the higher fish meal level and this non linearity of response is somewhat surprising. Partition of duodenal protein into microbial and undegraded feed protein was not attempted in this experiment, but it

would appear that fish meal degradability was different under the two feeding regimens, which suggests that the adoption of a constant degradability value of 0·30 for fish meal is not a valid assumption (Mercer & Annison, 1976; Osbourn *et al.* 1977).

Despite the variable response in terms of duodenal protein supply to the two levels of fish meal supplementation, the IRL of glucose was similar on all three diets inferring that, despite the low supply of glucose precursors on the silage-alone diet, the animal managed to meet its glucose requirements, and when supplied with more glucose precursor in the form of protein (diet FM2) there was only a small increase in total glucose IRL. This is contrary to the findings of Lindsay & Williams (1971), Ford & Reilly (1969) and Judson & Leng (1968) who showed glucose IRL to increase as the level of crude protein (N × 6·25) in the diet was increased, but agrees with the values of Ulyatt *et al.* (1970) and Herbein *et al.* (1978) who showed no increases in glucose turnover in response to diet when digestible energy intakes were equated. Lindsay (1970) has drawn attention to the relationship between digestible energy intake and glucose turnover and it is pertinent to note that in studies where glucose turnover rates increased in response to dietary protein changes, digestible energy intakes were not held constant.

In the present experiment there were no statistically significant differences in either percentage conversion of rumen-derived propionate to glucose or in percentage of glucose IRL synthesized from propionate (Table 6). However, the latter value is dependent on estimation of propionate production rate and the high error associated with this technique (Table 4) may preclude the detection of any true difference. The mean conversion of propionate to glucose, estimated by [2–^{14}C]propionate infusion, was 45·1% which agrees closely with values of 43 and 50% recorded by Judson & Leng (1973*b*) and Bergmann *et al.* (1966) respectively.

On diets C and FM1 36·2% of total glucose was derived from rumen absorbed propionate. Judson & Leng (1973*b*) recorded values of 25–55% in short-term propionate infusion experiments, up to a maximum value of 83% at an infusion rate of 1·12 mmol propionate/min, whilst Bergmann *et al.* (1966) recorded a value of 27% in sheep. On diet FM2 a value of 19·5% was obtained, which is a direct consequence of the reduced rate of propionate production noted on this diet.

Thompson (1971) raised doubts as to the authenticity of the techniques for measuring the contribution of propionate to gluconeogenesis which were proposed by Leng *et al.* (1967) and used in the present experiment. Thompson suggested the use of a correction factor involving infusions of both [1-^{14}C]- and [2-^{14}C]propionate to account for crossover of label within the Krebs cycle. Calculation of this correction factor is described in the section on MATERIALS AND METHODS and in the present experiment a mean correction factor of $0{\cdot}43 \pm 0{\cdot}100$ was obtained. This increased the mean percentage of propionate converted to glucose from 45 to 63% and the percentage of glucose synthesized from propionate from 36 to 51% on diets C and FM1 and from 19 to 33% on diet FM2.

Wiltrout & Satter (1972) also took account of a possible crossover of label within the Krebs cycle and for dairy cows concluded that 61% of glucose IRL was derived from rumen propionate. This is somewhat higher than the corrected value obtained in the present study, a difference which could easily be a reflection of higher rumen production of propionate on the concentrate-based diet used by Wiltrout & Satter (1972).

From a review of the literature it would appear that in considering the contribution of propionate to glucose IRL, few workers have taken into account the possibility of label crossover as suggested by Thompson (1971). However, it can clearly be seen from the results of this study that applying the correction may have a dramatic effect on the final outcome in terms of glucose precursors. The current view of the authors is that the hypothesis of Thompson (1971) is worthy of further consideration and there is a real need for critical

research to validate or reject his proposals. Until such time as this issue is satisfactorily resolved, we conclude from our data that 31% of glucose IRL was derived from rumen propionate but the distinct possibility exists that this value may be an underestimate.

In conclusion, this direct comparison of nutrients available for digestion in the intestines with metabolism of glucose in the blood, has shown that an increase of 80 g amino acids/d entering the duodenum produced no significant change in glucose IRL, measured in venous blood. Neither was there any significant change in the proportion of propionate converted to glucose. The proportion of glucose synthesized from propionate appeared to be lower on diet FM2 but this difference did not reach statistical significance. In general, the metabolism results reported for a grass-silage diet are in close agreement with those reported by other workers using more conventional diets.

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