The effect of fishmeal on the digestion of grass silage by growing cattle

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The effect of two levels of fishmeal substitution (50 (FM1) and 150 (FM2) g/kg) of a grass silage control diet (C) on the rumen digestion of organic matter and nitrogen, and the small intestinal disappearance of amino acids was examined in young growing cattle each equipped with simple PVC cannulas in the dorsal sac of the reticulo-rumen, the proximal duodenum and the terminal ileum. The silage was a primary growth of perennial ryegrass (*Lolium perenne*) (+formic acid) with a total N content of 22 g/kg dry matter (DM) (diet C). Fishmeal substitution increased this to 26 (diet FM1) and 34 (diet FM2) g/kg DM. On diets C and FM1, approximately 0.71 of digestible organic matter intake was apparently digested in the rumen, but this was significantly ($P < 0.05$) reduced on diet FM2 (0.60). Whilst duodenal flows of non-ammonia N and total amino acids were significantly ($P < 0.01$) increased at the highest level of fishmeal inclusion only, the synthesis of microbial N was significantly ($P < 0.001$) reduced by fishmeal inclusion, and feed N degradability declined progressively in response to increased fishmeal. Both levels of fishmeal addition caused a significant ($P < 0.05$) reduction in the fractional outflow rate of water from the rumen, and on the highest level of fishmeal significant ($P < 0.05$) increases in rumen ammonia concentration and rumen propionate molar proportions were observed. The net effect of the highest level of fishmeal substitution was to increase amino acid absorption from the small intestine by 0.47 compared with the control diet ($P < 0.05$), but due to an elevated ileal flow of amino acid no such effect was detected at the lowest level of fishmeal substitution. Composition of the absorbed amino acid fraction was relatively unaffected by the treatments imposed, despite large changes in the composition of the duodenal protein. The apparent non-linearity of response to fishmeal substitution is discussed and the amino acid supply findings are compared with the protein retention findings obtained in an earlier study by Gill *et al.* (1987). By two methods of calculation it was estimated that the amino acid N fraction disappearing from the small intestine was utilized with an efficiency of between 0.51 and 0.53 and no apparent effects due to diet or level of amino acid supply were detected.

Fishmeal: Grass-silage digestion: Cattle

The rate of live-weight gain in young growing cattle offered grass-silage diets can be quite variable and often below theoretical expectations. Poor levels of voluntary feed consumption and an impairment in protein supply have been suggested as possible explanations, supported by the studies of Lonsdale (1976) and Gill *et al.* (1987) which demonstrated high fat:protein ratios in cattle receiving grass silage only as feed. When Thomson *et al.* (1981) in conjunction with C. R. Lonsdale and D. J. Thomson (unpublished results) and Gill *et al.* (1987) attempted to enhance protein supply either by the use of a

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formaldehyde-containing silage additive or fishmeal supplementation respectively, significant improvements in protein retention were observed whilst levels of fat accretion declined or remained unchanged.

The objective of the present experiment was to quantify the effect of two levels of fishmeal substitution on nutrient digestion and supply using similar animals and the same grass silage as described by Gill *et al.* (1987). The diets were offered in two separate feeds each day and the estimates of amino acid disappearance from the small intestine were related to the protein anabolic effects observed by Gill *et al.* (1987). In a parallel study, Dawson *et al.* (1988) used the same silage to examine protein metabolism in the rumen, but owing to the experimental procedures used, the diets were offered hourly by means of automatic feeders.

A preliminary report on part of the experiment presented here has already been published (Beever *et al.* 1987).

MATERIALS AND METHODS

Preparation of diets

A primary growth of perennial ryegrass (*Lolium perenne* cv. Cropper) was cut on 24 and 25 May 1984 and harvested, after a short wilt (to 220 g dry matter (DM)/kg), with a precision-chop forage-harvester. Formic acid (3.3 litres/tonne fresh grass) was applied at the time of harvesting and the grass was ensiled in a concrete-walled clamp silo. The silage for the production study (Gill *et al.* 1987) was removed from the silo from 13 to 23 weeks after ensiling. During this time, sufficient silage for the present experiment was removed from the clamp and placed into 25-kg sacks before storing at $-20°$. The silage was held frozen for approximately 2 months before the start of the present experiment, at which stage sufficient feed was removed on a daily basis and gently thawed for approximately 48 h before feeding. The three experimental diets comprised silage alone (C) or with inclusion of fishmeal (Provimi 66; British White Fish Meals Ltd) equivalent to 50 (FM1) and 150 (FM2) g fresh weight/kg total diet DM with all diets fed at 24 g DM/kg live weight. The fishmeal and silage were thoroughly mixed just before feeding.

Animals

Six Friesian steers (initially 120 kg, 5–6 months old) were used. Before the experiment, each animal was prepared, in two stages, with a PVC cannula (i.d. 38 mm) into the dorsal sac of the reticulo-rumen and PVC 'T'-piece cannulas (i.d. 20 mm) into the proximal duodenum anterior to the bile duct, and in the terminal ileum, using techniques previously described by Beever *et al.* (1978).

During the post-operative recovery period and between measurement periods when diet change-overs were effected, all animals were housed in individual pens, and transferred to metabolism crates (Cammell, 1977) for each measurement period. At all times, the animals were held in a well-ventilated, continuously illuminated environment with free access to fresh water and mineral blocks. The animals were weighed before and at the end of each measurement period to allow intake levels to be adjusted according to individual animal live weights.

Experimental design

The experiment comprised two 3×3 Latin squares. The animals were allocated at random to each square and, subsequently, to the three dietary treatments within each square. Each experimental period lasted approximately 5 weeks, i.e. a 2 week adaptation period to the

diets, when the animals were offered equal feeds at 09.00 and 17.00 hours each day, followed by a 3 week measurement period when equal feeds were offered at 12 h intervals (09.00 and 21.00 hours). Refusals, if any, were removed daily at 08.30 hours and dry weights were recorded.

Experimental procedures

During each experimental measurement period, total faecal output was collected and weighed daily for 7 d and a subsample, bulked over the whole period was obtained for each animal. Subsequently each animal received an intrarumen dose of CrEDTA (100 ml; 3300 μg Cr/g) at 09.00 hours and strained rumen samples (15 ml) were taken hourly over the next 12 h to estimate rumen fractional outflow rate of water. The following day Dacron bags were placed in the rumen with each bag containing fresh experimental silage equivalent to approximately 5 g DM. Rates and extent of silage organic matter (OM) and nitrogen digestion were estimated by sequential removal of bags over the next 48 h (Siddons *et al.* 1982*a*). On the following day strained rumen contents (15 ml) were taken from each animal at 30-min intervals between 09.00 and 21.00 hours using automatic sampling apparatus (R. T. Evans, unpublished), and acidified with 2·5 M-sulphuric acid. Subsequently, ytterbium acetate (YbAc; 50 mg Yb/kg DM intake) and CrEDTA (120 mg Cr/kg DM intake) were infused (20 ml infusate/h) continuously into the rumen using separate infusion lines. After maintenance of the infusions for 7 d, samples of ileal contents were collected hourly from each animal between 09.00 and 21.00 hours on two successive days (Beever *et al.* 1971). ^{15}N-labelled ammonium sulphate (95% enriched; 1 g $(^{15}NH_4)_2SO_4$/d) was then added to each Yb-containing infusate and the infusions were maintained for 2 d before collection of duodenal digesta and for a further 2 d until digesta collection was completed. Duodenal digesta were collected using the automatic sampling apparatus described by Evans *et al.* (1981), with 2×24 h collections being attempted for each animal. Following completion of duodenal sampling, diet change-overs were commenced and feed levels were adjusted according to live weight.

Sample preparation and analysis

Samples of the silage and fishmeal were taken daily and DM determined weekly by oven drying to allow offered feed levels to be adjusted. During the faecal and digesta collection periods fresh samples of both silage and fishmeal were frozen, subsequently freeze-dried, and ground before chemical analysis. After thorough mixing, representative subsamples of the accumulated faecal samples were oven-dried to determine DM content, whilst a further portion was freeze-dried, ground and retained for analysis.

Accumulated samples of daily ileal and duodenal digesta were thoroughly mixed and centrifuged at 2000 rev/min for 10 min to provide samples of centrifuged digesta (residue), in addition to representative samples of whole digesta. These were subsequently freeze-dried, ground and retained for analysis. Finally, the supernatant fraction obtained from duodenal digesta after removal of the residue was centrifuged at 20000 ***g*** for 30 min and after two washes, with glass-distilled water, the resultant microbial fraction was freeze-dried before analysis.

Following removal from the rumen, the Dacron bags were washed thoroughly in cold water, oven dried for 24 h and the dry weight of the contents recorded. Subsequently these were ground and retained for analysis.

All rumen samples were stored at $-20°$ until required for analysis of Cr, volatile fatty acid or ammonia concentrations (Beever *et al.* 1985; Siddons *et al.* 1985*a*).

Analytical procedures for estimation of silage constituents and fishmeal composition were as described by Gill *et al.* (1987). OM, total N, ammonia N, Yb, Cr and ^{15}N contents of

faecal, ileal, duodenal and microbial samples were analysed (as appropriate) using previously described techniques (Beever *et al.* 1978, 1985; Siddons *et al.* 1982*a*, *b*, 1985*b*). Contents of individual amino acids in whole and centrifuged duodenal and ileal samples were determined by cation exchange chromatography using an LKB 4400 amino acid analyzer operating with sodium buffers, after hydrolysis under N_2 with 7·5 ml 6 M-hydrochloric acid using between 100 and 200 mg sample. Cystine and methionine contents were determined after oxidation with formic acid (10 vol. hydrogen peroxide, 80% formic acid, 5 mg phenol/ml) without N_2, before the hydrolysis step.

Calculation of results and statistical analysis

Estimates of nutrient flow to the duodenum and the terminal ileum were obtained with Yb–Cr as dual-phase markers as proposed by Faichney (1975), using whole digesta and centrifuged digesta as the two distinct digesta phases. Estimates of microbial N synthesis were obtained by comparison of the ^{15}N enrichments of microbial N and total non-ammonia N in duodenal digesta according to the procedures of Siddons *et al.* (1982*a*). The estimate of duodenal non-microbial N was further fractionated into endogenous and undegraded dietary N by application of a standard endogenous N flow of 2·98 g/kg OM intake (Bartram, 1987). Fractional outflow rate of water from the rumen was estimated from a log plot of Cr concentration against time according to Warner & Stacy (1972), whilst the rates and extents of OM and N digestion in the rumen were estimated by fitting the values obtained for nutrient disappearance from the Dacron bags *v.* time to the Mitshcherlich equation, according to the procedures of Ørskov & McDonald (1979).

All results were subjected to analysis of variance of a two 3×3 Latin square design with one animal per sequence. There were 8 df associated with error term but due to missing values, error df were reduced to either 5 or 6. In Latin square designs it is a common problem that pairwise interactions among columns, rows and treatments enter the error term and, thus, can make error variance too large for variance-ratio test of treatment effects. However, if the row and column effects are large then this overestimation of the error terms is unimportant (Wilk & Kempthorne, 1957). We found this to be the case in the analysis of data reported here. Whilst the use of additive analysis of variance model appears to be justified, a further test of additivity (Tukey, 1955) was undertaken to establish the validity of an additive model:

$$Y_{ijk} = u + a_i + p_j + t_k + e_{ijk},$$

where u is the overall mean and a_i, p_j and t_k are the animal, period and treatment effects respectively and e_{ijk} are normally and independently distributed with zero mean and constant variance. We used the procedure outlined by Rojas (1973) which is equivalent to the Tukey (1955) and Snedecor & Cochran (1969) methods but computationally easier to use. Rojas (1973) used the model:

$$y = u + a_i + p_j + t_k + ex_{ijk} + e_{ijk},$$

where x_{ijk} is a covariate constructed from the estimates of a, p and t from the analysis of variance based on the additive model, i.e. ${}_ix_{jk} = (\hat{a}\hat{p} + \hat{a}\hat{t} + \hat{p}\hat{t})$. The test of additivity then reduces to the test of significance of regression coefficient $\hat{\theta}$ in an analysis of covariance.

It was found that the analyses based on the additive model were satisfactory and even when $\hat{\theta}$ approached significance ($P < 0{\cdot}01$) the conclusions reached were unaltered. Treatment contrasts were used to test the effect of fishmeal compared with the control and also among fishmeal levels. The difference among the fishmeal levels was small, thus indicating non-linear response to fishmeal substitution.

Table 1. *Chemical composition of the silage (g/kg dry matter (DM))*

Toluene DM* (g/kg fresh wt)	205
Organic matter	927
Neutral-detergent fibre	546
Total nitrogen	22·1
Ammonia N (g/kg total N)	96·0
Gross energy (MJ/kg DM)	19·3
pH	3·7
Lactic acid	86·3
Acetic acid	22·9
Ethanol	40·5
In vivo DOMD (g/g)	0·74

DOMD, digestible organic matter digested.
* Corrected for ethanol content.

RESULTS

The composition of the silage is presented in Table 1. From an assessment of the contents of fermentation acids, pH and ammonia N content (as a proportion of total N) it appeared that the silage was well-preserved, whilst estimates of in vivo digestible OM and neutral-detergent fibre content indicated that the nutritive value of the silage was high, although total N content was quite low.

The fishmeal had an OM content of 837 g/kg DM and an N content of 105 g/kg DM, with the overall effect of fishmeal substitution being to increase total dietary N to 26·2 and 34·5 g/kg DM (diets FM1 and FM2 respectively).

Values in Table 2 refer to the rumen fermentation characteristics with the three diets. The first level of fishmeal inclusion (diet FM1) had no effect on rumen ammonia concentration, but at the higher level (diet FM2) a significant ($P < 0·05$) increase was observed, whilst both levels of fishmeal caused a significant ($P < 0·05$) decline in the fractional outflow rate of water from the rumen. *In situ* degradation studies indicated that silage OM and total N potential degradability were high (mean values 0·89 and 0·92 respectively) and not significantly affected by fishmeal inclusion. Similarly the rates of OM and N degradation were not significantly affected by fishmeal inclusion, although it should be noted that both rates were lower with diet FM1 compared with diets C and FM2. Total volatile fatty acid concentrations, representing a mean value over the 12 h sampling period were unaffected by the treatments, and equally diet FM1 had no effect on volatile fatty acid molar proportions compared with diet C. In contrast, with diet FM2, molar proportion of propionate increased whilst acetate decreased compared with the other two diets. Although this difference was not significant, the overall effect was a reduction in the ratio of acetate + butyrate : propionate from 3·81 (diets C and FM1) to 3·27 (diet FM2).

This observation is further illustrated in Fig. 1 where the effect of the two levels of fishmeal inclusion on rumen molar proportions of propionate during one feeding cycle is presented. In response to feeding (09.00 hours) all diets showed a pronounced increase in propionate molar proportion, but the values appeared to be higher at all times with diet FM2 compared with diets C and FM1, with the magnitude of the difference increasing at 3–4 h after feeding.

Values in Table 3 indicate the effects of fishmeal inclusion on OM and N digestion and total amino acid supply. OM intakes were similar for all three diets, but whilst OM digestibility was high on the control silage, fishmeal supplementation caused small but significant ($P < 0·05$) increases (diet C 0·794, diets FM1 and FM2 0·818). The quantity of OM entering the small intestine was similar with diets C and FM1 (mean 1·61 kg/d), but

Table 2. *The effect of fishmeal on rumen fermentation characteristics in young cattle offered a basal diet of grass silage*

Diet*...	Control	FM1	FM2	SE of mean
Rumen ammonia nitrogen concentration (mg/l)	113	109	149	5·2
Fractional outflow rate of water from the rumen (h)	0·19	0·14	0·12	0·011
Silage digestion characteristics:				
Potential degradability (g/g)				
OM	0·88	0·90	0·90	0·012
N	0·91	0·93	0.93	0·008
Rate of degradation (/h)				
OM	0·072	0·061	0·077	0·0054
N	0·16	0·11	0·15	0·0015
Rumen VFA concentration (mmol/l)	80·4	81·3	81·0	3·10
Molar proportions of rumen VFA:				
Acetate	0·68	0·69	0·67	0.016
Propionate	0·21	0·21	0·23	0·011
Butyrate	0·11	0·11	0·10	0·006

FM1, 50 g fishmeal/kg total dry matter; FM2, 150 g fishmeal/kg total dry matter; OM, organic matter; VFA, volatile fatty acids.

* For details, see p. 490 and Table 1.

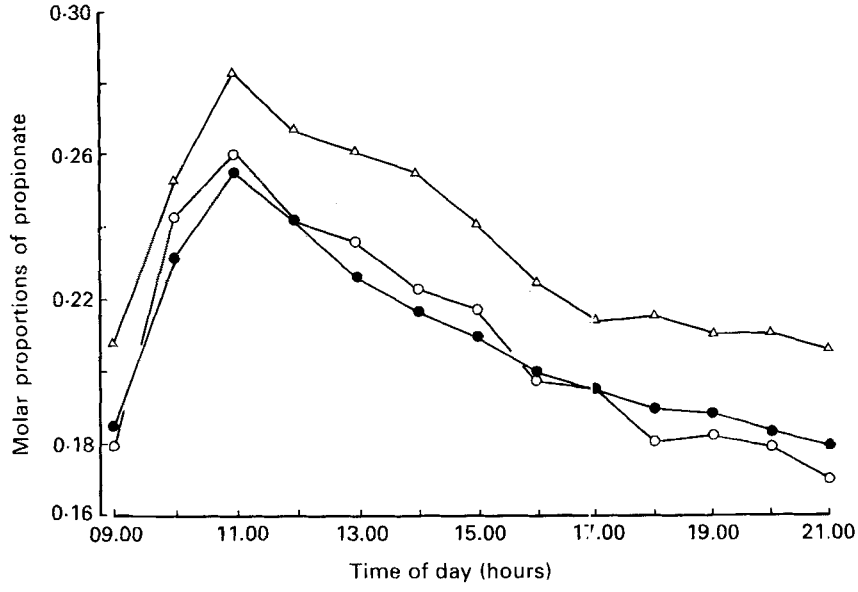

Fig. 1. The effect of fishmeal on the molar proportion of propionic acid in the rumen of cattle consuming a grass silage diet. (○), Control diet; (●), diet FM1 (50 g fishmeal/kg total dry matter; (△), diet FM2 (150 g fishmeal/kg total dry matter). Each data point is the mean observation for six animals. For details of diets see p. 490 and Table 1.

approximately 20% higher with diet FM2. Consequently only 0·6 of the digestible OM consumed on this diet was apparently digested in the rumen compared with 0·71 and 0·72 for the other two diets ($P < 0·05$). With diet C, approximately 0·50 of duodenal OM was digested in the small intestine, but the extent of digestion with diet FM1 was reduced (0·33)

Table 3. *The mean quantities of organic matter (OM; kg/d) and total nitrogen (or non-ammonia N; g/d) consumed, entering and leaving the small intestine and in the faeces of young cattle consuming silage (control) and fishmeal-containing silage diets. Values for total quantities of amino acids (g/d) entering and leaving the small intestine, and absorbed from the small intestine are also presented*

Diet*...	Control	FM1	FM2	SE of mean
Organic matter:				
Consumed	3·79	3·78	3·79	0·034
Entering small intestine	1·62	1·59	1·93	0·167
Leaving small intestine	0·82	1·07	0·80	0·070
In faeces	0·78	0·71	0·67	0·019
Apparent digestibility (g/kg DM intake)	0·794	0·812	0.823	0.0036
Proportion of digestible OM digested in rumen	0·721	0·713	0·596	0·0496
Total N:				
Consumed	90	108	143	1·9
Entering small intestine†	106	111	140	5·2
Leaving small intestine	36	48	43	2·4
In faeces	26	27	28	0·63
Total amino acids:				
Entering small intestine	462	497	656	37·5
Leaving small intestine	137	191	177	13·7
Absorbed	326	306	479	25·9

DM, dry matter; FMI, 50 g fishmeal/kg total DM; FM2, 150 g fishmeal/kg total DM.
* For details, see p. 490 and Table 1.
† Non-ammonia N.

despite a similar duodenal OM supply. In contrast, apparent digestibility of duodenal OM in the small intestine for diet FM2 was almost 0·60, and despite the higher duodenal OM supply on this diet, ileal OM flow was identical to that of diet C, and both were significantly ($P < 0·05$) lower than that of diet FM1.

Fishmeal inclusion increased total N intake by 18 (diet FM1) and 53 (diet FM2) g/d but this increase was not fully reflected in the estimates of duodenal non-ammonia N supply and only on diet FM2 was a significant increase observed (140 *v.* 109 g/d (diets C and FM1), $P < 0·01$). With diets C and FM2, small-intestinal availabilities of duodenal non-ammonia N (measured by reference to ileal total N) were 0·66 and 0.68 respectively, compared with 0·57 for diet FM1, with ileal N flows being significantly ($P < 0·05$) higher for diet FM1 compared with diets C or FM2. Hind-gut digestion of N was greatest with diet FM1, and consequently faecal N outputs were similar for all diets. In response to fishmeal inclusion, total amino acid flows into the small intestine increased by 8 ($P > 0·05$) and 42% ($P < 0·05$) with diets FM1 and FM2 respectively compared with diet C. However, both fishmeal-containing diets showed increased quantities of total amino acids leaving the small intestine, and small-intestinal loss of total amino acids was only increased on diet FM2 ($P < 0·05$). Mean apparent digestibility of duodenal amino acids in the small intestine were 0·71, 0·62 and 0·73 for diets C, FM1 and FM2 respectively.

Both fishmeal treatments significantly ($P < 0·05$) reduced microbial N synthesis by an average of almost 12% (Table 4), whilst the estimated quantities of undegraded dietary N flowing to the small intestine increased significantly at both levels of inclusion ($P < 0·001$). Consequently, degradability of total dietary N which was high with diet C (0·83 g/g) declined progressively in response to the lower ($P < 0·001$) and higher ($P < 0·001$) levels of

Table 4. *The mean quantities (g/d) of total, microbial, undegraded, and ammonia nitrogen flowing into the small intestine of young cattle consuming silage (control) or fishmeal-containing silage diets, the apparent degradabilities of feed N (g/g) and the efficiencies of rumen microbial N synthesis (g/kg organic matter (OM) apparently (OMADR) or truly (OMTDR) digested in the rumen)*

Diet*...	Control	FM1	FM2	SE of mean
Total N	113·0	118·8	148·9	5·17
Ammonia N	6·9	7·5	8·8	0·21
Microbial N	80.6	69·9	73·2	2·41
Undegraded dietary N	14·9	30·2	57·1	2·46
Endogenous N†	11·3	11·3	11·3	—
Feed N degradability				
Total dietary N	0·83	0·72	0·60	0·0175
Fishmeal supplement		0·28	0·35	
Efficiency of microbial N synthesis:				
OMADR‡	36·5	31·9	39·4	4·21
OMTDR	26·6	24·5	28·7	4·43

FM1, 50 g fishmeal/kg total dry matter; FM2, 150 g fishmeal/kg total dry matter

* For details, see p. 490 and Table 1

† Endogenous N assumed from Bartram (1987).

‡ Assuming a microbial N content of 101·8 (control) and 94·9 (FM1 and FM2), g/kg OM (Dawson *et al.* 1988).

fishmeal supplementation, and a significant ($P < 0·001$) difference between the two fishmeal diets was also observed. If it is assumed that the degradability of the silage was unaffected by the inclusion of fishmeal (i.e. remained at 0·83 g/g) then estimates of fishmeal degradability of 0·28 and 0·35 g/g (diets FM1 and FM2 respectively) can be obtained. It may be concluded that on diet FM2, the increase in duodenal non-ammonia N supply was wholly attributable to an increased passage of undegraded dietary N.

Efficiency of microbial N synthesis in relation to the quantity of OM apparently digested in the rumen (OMADR) ranged from 32 to 39 g/kg with no significant or consistent effects due to diet. When related to OM truly digested in the rumen, values were lower (24–29 g/kg) and again not apparently related to dietary treatments.

Values presented in Table 5 relate to the duodenal and ileal supply of individual amino acids, and the composition of the amino acids which disappeared from the small intestine for the three diets.

Fishmeal at the lowest level of inclusion marginally increased the duodenal supply of all amino acids compared with the control diet whereas with diet FM2, all amino acid flows were substantially increased compared with the other two diets. Comparison of the control diet against the two fishmeal diets indicated significant ($P < 0·05$) increases in duodenal flow of all amino acids except valine and phenylalanine, whilst comparison of diet FM2 with diet FM1 revealed significant ($P < 0·05$) increases in all amino acids except methionine and arginine.

Despite the differences observed in the duodenal flow of amino acids, amino acid flows at the ileum were, in most instances, only marginally higher on the fishmeal-containing diets compared with the control, and no statistically significant effects were detected apart from lysine (diet C *v.* fishmeal-containing diets (FM1 and FM2); $P < 0·05$).

The effect of these changes on the composition of the apparently absorbed amino acid fraction was remarkably small. Both levels of fishmeal significantly ($P < 0·05$) reduced the content of isoleucine (diet C 57, diets FM1 and FM2 53), tyrosine (diet C 53, diets FM1

Table 5. *Mean quantities of individual amino acids (g/d) entering and leaving the small intestine of young cattle consuming silage (control) or fishmeal-containing silage diets, and the individual amino acid composition (g/kg) of the total amino acid fraction apparently absorbed from the small intestine*

	Entering small intestine				Leaving small intestine				Apparently absorbed			
Diet*...	Control	FM1	FM2	SE of mean	Control	FM1	FM2	SE pf mean	Control	FM1	FM2	SE of mean
Aspartic acid	50·6	54·6	71·2	4·43	14·2	20·7	19·7	1·39	113	113	106	3·0
Threonine	26·0	28·2	35·9	2·20	9·6	12·5	11·8	0·69	48	51	49	2·6
Serine	22·3	22·5	29·3	1·80	7·7	10·9	10·0	0·91	44	38	40	1·6
Glutamic acid	60·2	66·7	88·0	5·12	18·6	26·6	23·2	1·66	129	130	136	2·0
Proline	21·0	23·8	30·3	1·46	10·2	12·6	12·1	1·45	31	37	38	3·5
Glycine	30·0	32·6	44·2	2·94	9·3	13·0	12·1	0·91	64	63	68	3·4
Alanine	32·6	33·5	46·7	3·03	10·6	14·5	12·9	0·98	68	62	70	2·2
Valine	29·2	29·3	38·2	2·23	8·8	10·7	10·7	1·16	61	61	59	3·1
Methionine	9·8	12·1	13·7	0·92	2·4	2·8	2·9	0·65	23	30	23	2·0
Isoleucine	25·8	27·0	34·8	1·95	7·2	10·4	9·3	0·77	57	54	53	0·7
Leucine	38·9	43·6	61·3	3·95	12·0	17·1	15·4	1·20	85	87	95	4·6
Tyrosine	21·3	21·5	27·1	1·38	4·3	6·7	6·4	0·74	53	48	44	1·5
Phenylalanine	27·5	27·7	35·6	2·08	7·5	10·7	9·7	0·87	61	55	54	1·0
Lysine	33·7	37·5	49·2	2·67	6·5	10·1	9·8	0·63	85	89	82	1·2
Histidine	10·4	10·9	15·5	0·84	2·9	4·4	4·2	0·31	23	21	24	0·4
Arginine	23·1	25·9	34·5	1·90	4·7	6·9	6·8	0·55	57	61	58	0·8

FM1, 50 g fishmeal/kg total dry matter; FM2, 150 g fishmeal/kg total dry matter.
* For details see p. 490 and Table 1.

and FM2 46) and phenylalanine (diet C 61, diets FM1 and FM2 55) compared with diet C, whilst alanine and histidine were significantly ($P < 0·05$) higher with diet FM2 compared with diet FM1.

DISCUSSION

Nutrient supply on silages

There is considerable agreement between the findings obtained in the present study and the work reported by Thomson *et al.* (1981). The silage used by Thomson *et al.* (1981) had slightly lower N and higher neutral-detergent fibre contents than the silage used in the present experiment but the proportion of digestible OM which was apparently digested in the rumen was similar for both diets. Equally the two silages gave estimated small-intestinal flows of approximately 0·80 g amino acid N/g N intake. Rooke *et al.* (1987) on the other hand reported a lower value of 0·65 g amino acid N/g N intake for a silage with higher neutral-detergent fibre and lower N contents. With respect to the efficiency of microbial protein synthesis the comparison is less clear. Rooke *et al.* (1987) reported a value of 22 g/kg OMADR and this agrees with other values obtained on silage diets, albeit mainly with sheep (Thomas, 1982), and supports the general view that overall microbial synthetic efficiency is low on such diets. In contrast, this present study gave a value of 37 g/kg OMADR, whilst in the parallel study where Dawson *et al.* (1988) gave the same silage in equal hourly meals to cattle, a value of 31 g/kg OMADR was obtained.

Reasons for such differences in the estimates of the efficiency of microbial protein synthesis are not immediately apparent. Both the results of the present study and those reported by Dawson *et al.* (1988) are considerably higher than general expectations, albeit much of the earlier information was obtained using sheep at maintenance levels of feed intake. In this

regard, both animal species and level of intake may be influencing the efficiency of microbial protein synthesis. In a recent review, McAllan *et al.* (1987) concluded, from a detailed analysis of available data, that values for the efficiency of microbial protein synthesis tended to be higher with cattle than sheep for both silage alone (cattle 27 g microbial N/kg OM digested in the rumen, sheep 20 g/kg), and silage plus concentrate diets (cattle 33 g/kg, sheep 25 g/kg). Equally, in the study of Rooke *et al.* (1987) where a value of 22 g/kg was reported, the cattle used were both non-pregnant and non-lactating and received only 12·5 g DM/kg body-weight, a level of intake approximating to 0·50 of that achieved in the present study. It is difficult to speculate further on the mechanisms involved, but such variation, which has been recognized but then often ignored, should provide a stimulus for future studies designed to understand, rather than simply quantify, N kinetics in the rumen of forage-fed cattle.

The highest level of fishmeal inclusion gave responses in nutrient supply which were more or less in line with expectations. Increased N intake gave an incremental response in duodenal non-ammonia N flow of 0·64 g/g, which was wholly accounted for by an increased flow of undegraded dietary (presumably fishmeal) protein. At the same time fishmeal inclusion at the highest level caused significant increases in rumen ammonia concentrations. A similar response was reported by Dawson *et al.* (1988) for the same grass silage and the result has recently been confirmed by Ortigues *et al.* (1989) with fishmeal supplementation of straw-based diets. Both the present study and Dawson *et al.* (1988) found the highest level of fishmeal to increase duodenal amino acid supply by approximately 200 g/d, but unlike the present study, Dawson *et al.* (1988) found increases in both microbial protein synthesis and undegraded dietary N flow to the intestines. This led Dawson *et al.* (1988) to suggest that the efficiency of capture of rumen-degradable N (RDN) by the microbes had been increased due to an improvement in the quality of the RDN fraction available in the rumen. The *in situ* digestion studies undertaken in the present experiment suggested that the digestion rate of silage N was almost twice that of silage OM, from which the possibility of an imbalance between degraded N and OM for microbial synthesis with diet C can be inferred. However, in the present study diet C gave a value of 0·81 for the apparent efficiency of microbial capture of RDN, and values on the fishmeal diets (0·79 and 0·78, diets FM1 and FM2 respectively) suggest that there were no apparent improvements due to a possible improvement in the composition of the RDN fraction. In contrast, Dawson *et al.* (1988) reported a lower value of 0·72 for the unsupplemented silage, and this increased to 0·84 on the fishmeal-containing diet. It may be that this response was related in part to the increased frequency of feeding used in the present study.

The response to fishmeal inclusion at the lower level in the present experiment (diet FM1) was below expectations and the non-linearity of response to fishmeal increments was similar to the result of Gill & Beever (1982). Whilst undegraded dietary N flow to the small intestine increased in response to the extra dietary N supplied, microbial N synthesis fell more on this diet than it did on diet FM2. In attempting to reconcile this effect it is necessary to realise that in the study by Dawson *et al.* (1988) fishmeal was given as a supplement to grass silage, whereas in the present study fishmeal was substituted for grass silage in the total diet. Thus, Dawson *et al.* (1988) had an increased RDN supply (11 g/d) and this appeared to stimulate net microbial synthesis and efficiency of RDN capture. In contrast, in the present study, RDN supplies were increased by only 3 g/d on the two fishmeal treatments and these were accompanied by reductions in microbial protein synthesis of between 7 and 10 g/d.

Examination of these findings adds considerable support to the view that fishmeal inclusion in the diet may be exerting effects on both the rumen and post-rumen processes

of digestion. The marked reduction in fractional outflow rate of water from the rumen with both fishmeal-containing diets supports this contention. The origin of this effect is not clear, although it may be related to the extra mineral intake associated with fishmeal feeding. It is interesting to note that recent experiments by Ortigues *et al.* (1989) and A. B. McAllan (personal communication) which examined fishmeal supplementation of different forage types have established similar reductions in rumen fractional outflow rates. The values for volatile fatty acid molar proportions adds further support to the suggestion of a rumen effect of fishmeal-containing diets, and whilst the overall effect on acetate + butyrate: propionate molar ratio was not large (diets C and FMI 3·8 *v.* diet FM2 3·3), the sustained effect as illustrated in Fig. 1 appeared to be a real one.

Estimates of feed N degradability by both in vivo and in vitro methods are fraught with problems as no direct methods exist. However, the values in Table 4 do appear to be realistic with respect to the control silage diet, the fishmeal-containing diets and the partial degradabilities calculated for fishmeal, although it has to be recognized that the estimate of endogenous N flow used in the in vivo calculations could have a major effect on these calculations.

Nutrient utilization

The silage used in the present experiment was identical to that offered to young growing cattle in the production–slaughter study reported by Gill *et al.* (1987). Without fishmeal supplementation high levels of *ad lib.* intake (24 g DM/kg body-weight) and satisfactory live weight (0·7 kg/d) and empty-body-weight gains (0·57 kg/d) were obtained. The silage used by Thomson *et al.* (1981) was also offered to growing cattle of similar initial weights at similar intake levels (C. R. Lonsdale and D. J. Thomson, unpublished results) and comparable empty-body-weight gains were observed. However, despite apparent similarities in nutrient digestion and supply, Gill *et al.* (1987) reported a much higher protein: fat ratio in the gain (1·03 *v.* 0·55). Reasons for this difference are not immediately apparent.

In the study of Gill *et al.* (1987), fishmeal supplementation similar to the levels of substitution used in the present study gave live-weight gain responses of 0·17 and 0·26 kg/d when anabolic growth promoters were not used. Values for the composition of empty-body-weight gain were obtained only for the control diet and at the highest level of fishmeal substitution, and the results indicated that virtually all the extra gain could be accounted for by increased protein retention. The actual levels of carcass N accretion are given in Table 6. Assuming forage-based diets have an average metabolizable energy content of 15·83 MJ/kg digestible OM intake (Ulyatt *et al.* 1981; Cammell *et al.* 1966) it may be calculated that fishmeal substitution in the present study increased total amino acid disappearance from the small intestine per unit metabolizable energy supply from 6.8 g/MJ (diet C) to 9·7 g/MJ (diet FM2). Clearly the cattle responded to this increased concentration of protein in the metabolizable energy, but the responses in live weight, empty body-weight and carcass composition are in disagreement with proposals of the Agricultural Research Council (1980), which suggested that optimal absorbed protein per unit metabolizable energy requirement of a 200 kg steer gaining 0·75 to 1·0 kg/d on a diet of metabolizability of 0·6 was 4·8 g/MJ. Equally Thomson *et al.* (1981) found similar responses in animal performance when absorbed protein supply was estimated to increase from 7·8 to 10·8 g/MJ metabolizable energy on formalin-compared with formic acid-treated silages offered to growing cattle. In the absence of more information on the mechanisms of nutrient utilization, the discrepancy between the requirement proposed by the Agricultural Research Council (1980) and the observation that animals receiving a higher supply of protein in the basal diet were able to respond to further protein cannot be resolved.

Table 6. *The effect of fishmeal substitution (150 g/kg total diet dry matter) on body nitrogen retention and the efficiency of utilization of absorbed amino acid N*

Diet*...	Control	FM2
N intake (g/d)*	76·5	115·2
Amino acid N absorption (g/d)†	37·8	53·0
N retention (g/d)*	15·1	23·2
Increment responses:		
N retention	—	8·1
Amino acid N absorption	—	15·2
Partial efficiency of amino acid N utilization (g retained/g supplied)	—	0·53
Incremental responses above maintenance:		
Amino acid N available for production‡	29·8	45·0
Efficiency of utilization of absorbed amino acid N (g retained/g supplied)	0·51	0·51

* As reported by Gill *et al.* (1987).
† Calculated from data presented in Table 5, adjusted to N intake reported by Gill *et al.* (1987).
‡ After deduction of maintenance N requirement calculated from Agricultural Research Council (1980).

Findings relating to total amino acid N supply, as measured in the present experiment but corrected for the differences in silage and fishmeal intakes recorded in the two studies, are also presented in Table 6. While calculated amino acid N supply increased by 15.2 g/d, carcass N retention increased by only 8.1 g/d, suggesting a partial efficiency of utilization of the extra amino acid N of 0·53. On the other hand, if body protein N maintenance costs are estimated on the basis of the Agricultural Research Council (1980) proposals, the utilization of amino acid N available for production on the two diets (C and FM2) was 0·51. These values are surprising in two respects. First the values are all considerably less than the value of 0·75 currently adopted by the Agricultural Research Council (1980), but are in agreement with values of between 0·4 and 0·5 calculated from the studies of C. R. Lonsdale and D. J. Thomson (unpublished results) and Thomson *et al.* (1981) referred to earlier. Furthermore, it is surprising that the cattle on the control diet, which on the basis of the observed production responses could be considered to be on a protein-limiting diet, did not show a higher efficiency of utilization of amino acids than the supplemented diets. On the control diet, protein deposition (169 g/kg empty-body-weight gain) was similar to the value of 165 g/kg suggested by the Agricultural Research Council (1980), whilst the value for the diet with the highest level of fishmeal approached 190 g/kg, and Sanderson & Thomas (1987) have recently reported a similar value for cattle fed on grass silage and fishmeal. These results suggest that where both total gain and protein content of that gain change in response to dietary perturbations, with a reduction in the proportional diversion of available energy into adipose tissue, empirical calculations of the efficiency of protein utilization without recognition of the underlying mechanisms may be inappropriate.

The overall effect of fishmeal substitution on the composition of the amino acid fraction absorbed from the small intestine was small despite the contribution of undegraded dietary N to total duodenal non-ammonia N increasing from 14% for diet C to 41% for diet FM2. This may in part have contributed to the lack of any apparent increase in the efficiency of utilization of the absorbed amino acid fraction. Clearly the results do not suggest any sustained increases in the proportional contributions from methionine, which was suggested by Thomas (1982) as possibly the first limiting amino acid for growing cattle on grass-silage diets. Equally the findings for other potentially limiting amino acids, e.g. lysine, histidine and threonine, do not suggest any major improvement in potential biological value of the absorbed amino acid fraction. It is unlikely, however that specific amino acid

deficiencies could account for the large discrepancy between the efficiency values of 0·51–0·53 noted in the present study and 0·75 proposed by the Agricultural Research Council (1980).

In this respect, recent studies with contrasting forage:concentrate diets (Beever *et al.* 1988; Thomas *et al.* 1988) given to cattle and a predominantly barley diet fed to growing lambs (Pell *et al.* 1989) have indicated that the form of the energy (i.e. cereal *v.* forage) may have a major effect on the metabolic efficiency of utilization of absorbed amino acids in favour of the concentrate-enriched diets. However, this hypothesis needs to be tested before real reasons for the apparently low efficiency of absorbed amino acid utilization on forage-based diets can be elucidated.

In conclusion, the results of the present study have confirmed previous reports (Beever *et al.* 1977; Siddons *et al.* 1979; Rooke *et al.* 1987) that silage N is extensively degraded in the rumen. Consequently resultant amino acid supply is likely to limit growth in young cattle offered rations based on grass silage. Fishmeal will, at reasonably high levels of inclusion, give desirable increases in protein supply to the animal, but at lower levels of inclusion this response may be variable. The present study demonstrates that fishmeal substitution can affect rumen metabolism, although the increased molar proportion of propionate appeared to contrast with the decreased propionate production rate reported by Gill & Beever (1982). It is clear that the animal response noted by Gill *et al.* (1987) was largely attributable to the increased protein supply which occurred as a result of fishmeal inclusion in the diet, but the relatively low estimates of efficiency of utilization of the absorbed amino acids on both the control and fishmeal diets is of great concern.

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