

Original article

**Fishmeal supplementation of steers fed on grass silage:
effects on rumen function, nutrient flow
to and disappearance from the small intestine**

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Abstract — Four Hereford × Friesian mature steers equipped with rumen, duodenal and ileal cannulas were fed on either grass silage alone (S) or silage supplemented with fishmeal (150 g·kg⁻¹ silage dry matter intake; SFM) to assess effects on rumen fermentation, nutrient flow to and disappearance from the small intestine. The silage was a primary growth of perennial ryegrass with a total-N content of 24.5 g·kg⁻¹ dry matter (DM). Ruminal concentrations of acetate, butyrate and ammonia-N were not different between treatments and averaged 51.7, 8.9 and 8.4 mmol·l⁻¹, respectively. However, molar proportion of propionate was higher on SFM compared to S, 18.7 and 17.6 (SEM 0.18, $P < 0.05$), respectively. The amount and efficiency of microbial protein synthesis were not different between treatments and averaged 71.0 g·d⁻¹ and 35.8 g N·kg⁻¹ organic matter digested in the rumen, respectively. Fishmeal supplementation increased the flow of total-N ($P < 0.05$), non-ammonia-N ($P < 0.05$), and total amino acids ($P < 0.05$) at the duodenum. Amino acid disappearance was increased by fishmeal supplementation, 488 and 717 g·d⁻¹ (SEM 28.6, $P < 0.05$) for S and SFM, respectively. The apparent absorption coefficient for the total amino acids from the small intestine [(expressed as duodenal – ileal)/duodenal] was also increased, 0.57 and 0.72 (SEM 0.028, $P < 0.06$) for S and SFM, respectively.

fishmeal / grass silage / amino acid / absorption

Résumé — Complémentation d'un ensilage d'herbe avec de la farine de poisson : effets sur la fermentation dans le rumen, le flux des nutriments et leur disparition dans l'intestin grêle chez des bœufs matures. Quatre bœufs matures Hereford × Frison munis de canules du rumen, du duodénum et de l'iléon ont été alimentés soit avec de l'ensilage d'herbe seul (S) ou de l'ensilage supplémenté par de la farine de poisson (à raison de 150 g·kg⁻¹ de matière sèche d'ensilage ingéré ;

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SFM) pour évaluer les effets sur la fermentation dans le rumen, le flux des nutriments et leur disparition dans l'intestin grêle. L'ensilage était un ray-grass anglais récolté au 1^{er} cycle de végétation avec une teneur en azote total de 24,5 g·kg⁻¹ de matière sèche. Les concentrations ruminales d'acétate, de butyrate et d'azote ammoniacal n'ont pas été différentes entre les traitements et ont été en moyenne de 51,7, 8,9 et 8,4 mmol·l⁻¹, respectivement. En revanche, la proportion molaire de propionate a été plus élevée avec SFM comparé à S, respectivement, 18,7 et 17,6 (SEM 0,18, $P < 0,05$). Le flux d'azote microbien et l'efficacité de la synthèse protéique microbienne n'ont pas été différents entre les traitements et ont été, respectivement, en moyenne de 71,0 g·j⁻¹ et 35,8 g N·kg⁻¹ de matière organique digérée dans le rumen. La supplémentation en farine de poisson a augmenté le flux d'azote total ($P < 0,05$), d'azote non-ammoniacal ($P < 0,05$) et des acides aminés totaux ($P < 0,05$) au niveau du duodénum. La disparition des acides aminés a été augmentée lors de la supplémentation en farine de poisson, 488 et 717 g·j⁻¹ (SEM 28,6, $P < 0,05$) pour S et SFM, respectivement. Le coefficient de digestibilité apparente des acides aminés totaux au niveau de l'intestin grêle [(exprimé par le rapport duodéno-iléal)/duodéno-iléal] a aussi été augmenté, 0,57 pour S et 0,72 pour SFM (SEM 0,028, $P < 0,06$).

farine de poisson / ensilage d'herbe / acides aminés / absorption

1. INTRODUCTION

A large proportion of European beef is produced in high-forage systems, particularly those based on grass silage. Protein supplements are frequently used [25] to overcome the inadequacies of these diets, which include low intakes, low growth rates and carcasses with a high fat:protein ratio. However, protein supplements are increasingly expensive components of ruminant diets and must be used in situations that will lead to responses.

Many studies have shown feed intake and liveweight gain responses when grass silage was supplemented with fishmeal [10, 12, 34]. Gill et al. [14] reported that fishmeal supplementation improved protein retention while levels of fat accretion declined or remained unchanged. Similar findings were observed in lambs [22]. Contrary to this, some studies have found little response when supplementing with fishmeal [30].

The differences in response to fishmeal supplementation are likely to be associated with differences in the quality of the basal diet [25, 26]. The greatest responses to fishmeal have generally been obtained with poor-quality silages, although again the

responses are not always clear. This may in part relate to the changes in rumen metabolism or the changes in body tissue metabolism, or a combination of both [8].

The studies conducted by Dawson et al. [8] and Beever et al. [4] suggested that the responses from steers fed on grass silage with fishmeal may be associated with an increase in the efficiency of utilisation of dietary nitrogen (amino acids).

The objectives of this study were to investigate the nature of the response in (1) aspects of rumen function and (2) nutrient flow to and absorption from the small intestine when additional protein in the form of fishmeal was supplemented in the diet of steers fed on grass silage.

2. MATERIALS AND METHODS

2.1. Animals and diets

Four Hereford × Friesian steers (520 ± 20 kg, 20 months old) prepared with a PVC cannula into the dorsal sac of reticulo-rumen (38 mm internal diameter) and PVC 'T'-piece cannula into the proximal duodenum and in the terminal ileum (19 mm internal diameter) were used. Animals were maintained in

individual pens and housed in a unit that was continuously illuminated.

First cut perennial ryegrass (*Lolium perenne*) was prepared in 15th May 1996. After a minimum wilt (1–3 h), formic acid (3 litres per tonne fresh grass) was applied at the time of harvesting and the grass was ensiled in a concrete-walled clamp silo until it was opened on 2nd November 1996. The two experimental diets consisted of grass silage alone, or supplemented with 150 g fishmeal (United Fish Products Ltd, Aberdeen) per kg silage dry matter (DM) intake. Diets were fed at 90% of ad libitum intake of each animal and daily feed allocation was offered on an hourly basis using automatic feeders. Animals were allowed free access to water and were offered a vitamin/premix (100 g·d⁻¹; Rumens Cattle Regular, Rumenco, Burton, Staffordshire, UK), mixed in on top of the silage.

2.2. Experimental design

The experiment was a balanced two period changeover design, with animals allocated at random to each treatment. Each experimental period lasted 28 days with 14 d for diet adaptation, assessment of ad libitum intake and adjustment of feed intake to the experimental level (90% ad libitum). During this time animals were kept in individual pens and feed was offered in two equal portions at 09:00 and 16:00 hours. On d 14 animals were moved into individual stalls and faecal collection equipment was fitted. Faecal measurements were made between d 15–20, followed by rumen, duodenal and ileal digesta collection from d 22–28.

2.3. Experimental procedures

The experiment ran between November and December 1996. Total faecal output for each animal was collected and weighed daily for 6 d and a sub-sample (5%), bulked over

the whole period. The faeces was stored frozen (–20 °C) in sealed containers for later analysis. Strained rumen contents (10 ml) were taken hourly from each animal between 09:00 and 21:00 hours, and rumen pH was measured immediately and the samples were then acidified with 2.5 M sulphuric acid. Digesta flow at the duodenum and the ileum were estimated using a dual-phase marker system with ytterbium acetate (YbAc) and chromium ethylene diamine tetra-acetic acid (CrEDTA) as the particulate and liquid markers, respectively [11]. Samples of duodenal and ileal digesta were taken prior to infusion to assess background levels of digesta markers. On d 20, YbAc (50 mg Yb·kg⁻¹ DM intake) and CrEDTA (1 750 mg·d⁻¹) were then infused intraruminally via separate lines at a rate of 20 ml·h⁻¹. On d 26, ileal samples (500 ml) were collected manually using a 500 ml plastic bottle every 4 h over a 24 h period followed by duodenal samples (500 ml) on d 28 as described for the ileal samples.

2.4. Sample preparation and chemical analysis

Separate samples of fresh silage and fishmeal were taken daily during the digestion periods. Sub-samples were either stored frozen or freeze-dried and ground for chemical analysis. After thorough mixing, representative sub-samples of the bulked faecal samples were oven-dried to determine DM content, whilst a further portion was freeze-dried and ground for subsequent analysis. Accumulated samples of daily duodenal and ileal digesta were thoroughly mixed, and sub-samples were centrifuged at 15 000 rpm for 20 min to provide samples of centrifuged digesta with residue, in addition to representative samples of whole digesta. These were subsequently freeze-dried and ground. The supernatant fraction obtained from duodenal digesta by centrifugation at a low speed (2 000 rpm for 5 min) before further centrifugation at 15 000 rpm for 20 min and

after two washes with 0.9% saline (w/v) plus distilled water in a final wash, the resultant microbial fraction was freeze-dried before analysis. Hourly collected rumen samples were stored at -20°C until required for analysis of ammonia-N and volatile fatty acid (VFA) concentrations.

The DM, OM, total-N, neutral and acid detergent fibre (NDF and ADF), volatile components of silage, and chemical components of the rumen fluid were determined using the techniques described by Scollan et al. [24]. YbAc and CrEDTA concentrations in ileal, and duodenal samples were measured by atomic absorption spectrophotometry [29]. The contents of purine bases were determined according to Cozzi et al. [7] using HPLC (LDC/Milton Roy, USA) and used to estimate microbial protein synthesis in the rumen [7, 35]. Amino acid analysis of feed, duodenal and ileal samples were determined by HPLC, Pico-Tag system (Waters chromatography division, Millipore, Milford, MA, USA) through the hydrolysis steps with 6 M HCl, using DL-Norleucine as an internal standard.

2.5. Calculations and statistical analysis

Estimates of nutrient flow to the duodenum and the terminal ileum were calculated after mathematical reconstruction of true digesta as proposed by Faichney [11]. Whole digesta and centrifuged digesta were used as the two distinct digesta phases. Estimates of microbial N synthesis were obtained by comparison of the purine bases [7, 35]. An endogenous N flow of $2.98 \text{ g}\cdot\text{kg}^{-1}$ OM to duodenum [3] was applied for the fractionation of the estimated duodenal non-microbial-N into endogenous and undegraded dietary-N [4]. Feed N degradability in the two diets was calculated by difference from the quantity of undegraded dietary N flowing to the duodenum after allowing for microbial and endogenous-N flow [8]. Hence the equations used were:

$$\begin{aligned} \text{Undegraded N flow} &= (\text{total N flow} - \text{ammonia-N flow} - \text{endogenous-N} - \text{microbial-N flow}) \\ \text{N degradability} &= 1 - \frac{(\text{non ammonia N flow} - \text{microbial N flow} - \text{endogenous N flow})}{\text{N intake}} \end{aligned}$$

The hourly estimates of rumen pH, ammonia and VFA concentrations were averaged over the 12 h period and analysed by analysis of variance.

The response to fishmeal supplementation of silage was examined by analysis of variance using Genstat 5 [19]. The model fitted was:

$$Y_{ijk} = \mu + A_j + P_i + D_k + e_{ijk}$$

where μ is the overall mean, A_j is the effect of animal, P_i is the effect of period, D_k is the effect of diet and e_{ijk} is the residual error term. The level treated as not significant was $P > 0.05$ but trends were also expressed ($P < 0.1$).

3. RESULTS

The chemical composition of the experimental diets is presented in Table I. The low pH and relatively high lactic acid content indicates that the silage was well fermented but the ammonia-N content was relatively high (12% of total-N). Effect of diet on rumen fermentation characteristics is given in Table II. Fishmeal supplementation had no effect on rumen pH and total VFA concentrations (mean value over the 12 h sampling period). Fishmeal resulted in a numerically substantial increase in rumen ammonia concentration but this was not significant. However, the molar proportion of propionate on fishmeal-supplemented silage was higher ($P < 0.05$) than on silage alone. Apparent N digestibility was higher ($P < 0.05$) on fishmeal-supplemented silage.

Table I. Chemical composition of the experimental diets (g·kg⁻¹ DM unless otherwise stated).

	Silage	Fishmeal
Toluene DM (g·kg ⁻¹ fresh)	195.7	ND
Organic matter	916.7	793.5
Total-N	24.5	110.8
Ammonia-N	2.94	ND
Soluble-N	16.9	ND
Neutral-detergent fibre	565.6	ND
pH	3.95	ND
Buffering capacity (meq·kg ⁻¹ DM)	861.4	ND
Water soluble carbohydrate	6.6	ND
Lactic acid	101.2	ND
Acetic acid	23.0	ND
Gross energy (MJ·kg ⁻¹ DM)	19.3	ND

ND, not determined.

Table II. Rumen fermentation characteristics in steers fed on grass silage alone or silage supplemented with fishmeal (150 g·kg⁻¹ silage DM intake) (*n* = 4).

	Silage	Silage + Fishmeal	SEM	<i>P</i> -value
pH	6.82	6.86	0.013	NS
Ammonia-N (mmol·l ⁻¹)	6.42	10.46	1.064	0.12
Total VFA (mmol·l ⁻¹)	81.20	78.40	1.02	NS
Apparent N digestibility	0.71	0.79	0.008	0.05
Molar proportion of VFA				
Acetate	65.1	64.40	0.51	NS
Propionate	17.6	18.7	0.18	0.05
Butyrate	11.1	11.2	0.18	NS

NS, not significant.

The effects of fishmeal supplementation on OM and N digestion in the rumen are presented in Table III. Intake and duodenal flow of OM were higher ($P < 0.05$) on fishmeal-supplemented silage but the proportion of OM apparently digested in the rumen (OMADR) was significantly higher ($P < 0.05$) on silage alone. Approximately, 0.32 and 0.53 of the duodenal OM flow was digested in the small intestine on the silage and fishmeal supplemented silage, respectively. This increase was reflected in the estimates of duodenal non-ammonia-N

supply and was entirely disappeared from small intestine, and consequently N flow at ileum was similar on both diets. Both microbial-N flow and efficiency of microbial protein synthesis in terms of OMADR did not differ between the two diets. The supply of undegraded dietary-N to the duodenum was estimated assuming an endogenous-N flow to the duodenum of 2.98 g N·kg⁻¹ OM intake [3]. This suggested that fishmeal-supplemented diet supplied more undegraded-N than silage alone ($P < 0.05$). The degradability of total dietary-N was marginally but

Table III. The mean quantities of organic matter, total-N, microbial-N and total amino acids flowing into the small intestine of steers fed on grass silage alone or silage supplemented with fishmeal (150 g·kg⁻¹ DM intake) (*n* = 4).

	Silage	Silage + Fishmeal	SEM	<i>P</i> -value
Organic matter (kg·d ⁻¹)				
Consumed	4.71	5.25	0.077	0.05
Duodenal flow	2.54	3.11	0.067	0.05
Ileal flow	1.75	1.46	0.097	NS
OMADR**(g·g ⁻¹ OM intake)	0.46	0.41	0.003	0.05
Total-N (g·d ⁻¹)				
Consumed	132.3	209.0	4.42	0.01
Duodenal flow	135.0	182.8	4.38	0.05
Ileal flow	59.8	58.1	2.03	NS
Total non-ammonia-N (g·d ⁻¹)				
Duodenal flow	124.0	166.5	4.53	0.05
Ileal flow	57.4	54.9	2.12	NS
Total amino acids (g·d ⁻¹)				
Consumed	531	798	15.5	0.01
Duodenal flow	488	717	28.6	0.05
Ileal flow	210	197	8.32	NS
Absorbed	278	521	35.6	0.05
Undegraded dietary N at duodenum (g·d ⁻¹)	45.5	73.5	2.06	0.01
Microbial-N at duodenum (g·d ⁻¹)	64.6	77.5	4.08	NS
EMPS* (g N·kg ⁻¹ OMADR**)	29.9	36.1	1.86	NS
Endogenous-N at duodenum (g·d ⁻¹)	14.0	15.6	0.23	0.05
Feed N degradability	0.652	0.646	0.0009	0.05

NS, not significant; *EMPS, efficiency of microbial protein synthesis; **OMADR, organic matter apparently digested in the rumen.

significantly decreased ($P < 0.05$) on fishmeal-supplemented silage compared to silage alone. The responses in N flow to and disappearance from the small intestine were reflected in the total amino acids results. The quantities of total amino acids consumed and flow at the duodenum were 50 ($P < 0.01$) and 47% ($P < 0.05$) higher on fishmeal-supplemented silage, respectively. The increased flow of amino acids at the duodenum ($P < 0.05$) on fishmeal diet was completely absorbed from the small intestine, with the result that amino acid flow to the ileum was similar on both diets.

Individual flows of amino acids to the duodenum and ileum, and the disappearance from the small intestine (duodenum–ileum) are presented in Table IV. With fishmeal supplementation there were marked increases in the supply of most amino acids except for alanine and lysine, and tendency for increases in glutamic acid, glycine, leucine and phenylalanine ($P < 0.1$). At the duodenum, the flow of essential amino acids (EAA) and non-essential amino acids (NEAA) on the fishmeal-supplemented diet were greater (51 and 42%, respectively) than on silage alone ($P < 0.05$). The effect of these

Table IV. Mean quantities of individual amino acids ($\text{g}\cdot\text{d}^{-1}$) flow to duodenum and ileum in steers fed on grass silage alone or silage supplemented with fishmeal ($150 \text{ g}\cdot\text{kg}^{-1}$ DM intake), and the individual amino acid composition ($\text{g}\cdot\text{kg}^{-1}$) of the total amino acid fraction apparently absorbed from the small intestine ($n = 4$).

	Duodenal flow				Ileal flow				Composition of apparently absorbed amino acid			
	Silage	Silage + Fishmeal	SEM	<i>P</i> -value	Silage	Silage + Fishmeal	SEM	<i>P</i> -value	Silage	Silage + Fishmeal	SEM	<i>P</i> -value
Aspartic acid	58.7	79.1	2.97	0.05	14.0	10.8	1.08	NS	161.8	130.8	6.54	0.08
Glutamic acid	72.0	104.6	6.01	0.06	30.9	25.2	1.91	NS	148.0	150.5	6.66	NS
Serine	28.1	40.5	1.93	0.05	17.7	15.3	0.94	NS	37.2	48.0	1.63	0.05
Glycine	34.6	47.3	2.31	0.06	15.9	15.0	0.62	NS	67.2	62.5	3.01	NS
Histidine	13.1	19.6	1.02	0.05	8.0	7.0	0.39	NS	17.5	24.3	1.98	NS
Arginine	21.6	35.9	1.28	0.05	7.2	7.6	0.11	NS	51.7	54.7	1.41	NS
Threonine	26.6	40.1	1.93	0.05	12.0	11.7	0.42	NS	52.0	54.5	1.82	NS
Alanine	35.1	44.7	2.56	NS	16.7	16.3	0.67	NS	66.5	55.7	7.60	NS
Proline	24.7	37.0	1.17	0.05	13.6	12.9	0.65	NS	39.7	46.2	1.68	NS
Tyrosine	14.4	21.4	0.45	0.01	5.5	5.6	0.17	NS	32.2	30.8	2.02	NS
Valine	30.6	46.9	2.73	0.05	14.9	14.4	0.45	NS	55.7	62.2	4.37	NS
Methionine	9.4	16.9	0.59	0.01	4.0	4.3	0.22	NS	19.3	24.8	3.25	NS
Isoleucine	24.8	40.8	2.63	0.05	9.4	9.9	0.18	NS	54.7	59.5	3.38	NS
Leucine	43.2	62.2	3.85	0.07	16.9	17.0	0.49	NS	93.7	88.0	6.41	NS
Phenylalanine	27.7	43.2	2.73	0.06	10.3	10.3	0.30	NS	62.0	63.0	3.47	NS
Lysine	22.1	34.7	3.04	NS	11.7	12.3	0.50	NS	39.0	41.7	5.58	NS
EAA	268	406	15.5	0.05	117	116	3.1	NS	544.7	559.0	6.77	NS
NEAA	219	311	13.8	0.05	94	80	5.3	NS	455.3	441.0	6.77	NS

NS, not significant; EAA, essential amino acid; NEAA, non-essential amino acid.

Table V. Apparent absorption¹ of essential and total amino acids from the small intestine in steers fed on grass silage alone or silage supplemented with fishmeal (150 g·kg⁻¹ DM intake) (*n* = 4).

	Silage	Silage + Fishmeal	SEM	<i>P</i> -value
Histidine	0.38	0.64	0.051	0.069
Arginine	0.66	0.79	0.017	0.036
Valine	0.51	0.69	0.047	0.108
Methionine	0.57	0.75	0.024	0.034
Isoleucine	0.61	0.76	0.043	NS
Leucine	0.60	0.73	0.035	NS
Phenylalanine	0.62	0.76	0.040	NS
Lysine	0.46	0.63	0.056	NS
Total amino acids	0.57	0.72	0.028	0.059

NS, not significant.

¹ Expressed as (duodenal flow – ileal flow) / duodenal flow.

changes on the composition of the apparently absorbed amino acid fraction was small. Supplementation of fishmeal increased the flow of serine ($P < 0.05$) but the remainder of the amino acids were not different. Within each dietary treatment the apparent absorbability in the small intestine was expressed by the equation:

$$\text{Apparent absorbability} = \frac{(\text{duodenal flow} - \text{ileal flow})}{\text{duodenal flow}}$$

For total amino acids, apparent absorption was 0.72 vs. 0.57 ($P = 0.059$) for the fishmeal diet compared to silage alone (Tab. V). The disappearance coefficients for individual amino acids ranged from 0.37 to 0.65 and 0.64 to 0.79 for the silage alone and fishmeal supplemented silage, respectively.

4. DISCUSSION

4.1. Rumen fermentation and OM digestion

In general, fishmeal is considered to be a good source of undegraded protein. However, ruminal-N degradation of fishmeal ranges between 30–70% [16, 20] depending on the manufacturing process [17]. Thus,

the degradable proportion of fishmeal may alter rumen metabolism, resulting in an increase in ammonia-N concentration in the rumen if the degradable proportion is increased. Dawson et al. [8] reported an increase in the concentration of ammonia-N in the rumen of the steers fed on 150 g fishmeal·kg⁻¹ silage DM intake with values of 7.8 and 10.7 mmol·l⁻¹ for silage alone and fishmeal supplemented silage, respectively. These values are in close agreement with the results found in this study (6.4 and 10.5, respectively). In both of these studies, animals were maintained under steady-state conditions. However, it is not immediately apparent why the molar proportion of propionate was higher on fishmeal supplemented silage although the results are in agreement with Beaver et al. [4].

The digestibility of silage is often improved by fishmeal inclusion [15, 21] and this effect may, in part, be explained by effect on an increase in fibre digestibility in the rumen [21]. However, it is likely that the apparent proportional depression of OM digestion in the rumen when fishmeal is added to silage alone probably occurs because fishmeal is, as stated previously, relatively resistant to rumen degradation [2]. The crude protein content of fishmeal in the present study was

693 g·kg⁻¹ DM, representing approximately 0.87 of the OM. Assuming that only 30% of the crude protein fraction is capable of being degraded in the rumen, the rest of the fraction would flow to the duodenum undegraded. This would lead to significant differences in OMADR as well as duodenal flow of OM ($P < 0.05$) between the two diets. On the other hand, OM digestibility in the whole tract was unaffected by fishmeal supplementation, 0.78 and 0.79 ($P > 0.05$, data not shown in Tab. IV) for silage alone and fishmeal supplemented silage, respectively, which supports the results of Gill et al. [14].

4.2. Flow and digestion of nitrogenous constituents

4.2.1. Flow of N to the duodenum

Fishmeal supplemented silage increased the flow of both non-ammonia-N ($P < 0.05$) and total amino acids ($P < 0.05$) to the duodenum. Similar responses to fishmeal supplementation were reported by Dawson et al. [8] and Gill and Beever [13]. However, the response appears to depend on the level of fishmeal supplementation. For example, 50 g·kg⁻¹ silage DM intake compared to 150 g of fishmeal inclusion did not increase the flow of N to the duodenum [4, 13] and the explanation for this remains unclear. It is likely that incremental responses of both non-ammonia-N and amino acids were in general attributable to an increased flow of rumen undegraded protein at the level of 150 g fishmeal·kg⁻¹ silage DM intake (Tab. IV).

Undegraded dietary-N flow at duodenum on silage alone (45.5 g·d⁻¹) was relatively high compared to literature values (14.9; [4], 19.3; [8]). This was reflected in the low estimation for feed-N degradability in the rumen (0.65). Agricultural Research Council [ARC, 2] reported a range of 0.71–0.90 for feed-N degradability of grass silage (wilted and unwilted), but suggested

caution in the interpretation since the measurements of microbial-N and endogenous-N are uncertain and may vary with the methodology employed.

There was no loss of N across the forestomachs with silage alone but a net loss of 26.2 g N·d⁻¹ (12.5% of N intake) with the fishmeal supplemented diet. N losses of between 5 to 35% of N intake have been reported for cattle [6] and sheep [28] offered silage diets, reflecting the inefficient utilisation of silage-N attributed to the rapid and extensive degradation of silage non-protein-N [31]. In the present study, such an effect may be reduced by the increased frequency of feeding, resulting in a relatively constant supply of N to the rumen as observed by Dawson et al. [8]. The reasons for the net loss of N on the fishmeal-supplemented diet are not clear but are in agreement with Gill and Beever [13]. Supplementing silage with fishmeal induced an elevation in the rumen ammonia concentration (although non-significant) possibly due to the rapidly degradable-N fraction in the rumen. However, if this degradable fraction did not contribute much to microbial protein synthesis, then it may have been absorbed from the rumen, resulting in net loss of N across this tissue.

4.2.2. Microbial protein synthesis

The amount and efficiency of microbial protein synthesis is, in general, low in cattle fed on fresh, conserved or low quality forages [1, 9]. The main objective of protein supplementation is to improve flow of N (protein) to the small intestine by (1) improving microbial protein synthesis and/or (2) increased supply undegraded dietary protein. The effect of fishmeal supplementation of silage on microbial protein synthetic activity has often lacked consistency [4, 8]. Fishmeal supplementation of silage did not significantly improve microbial-N flow or the efficiency of rumen microbial protein synthesis in the present study, which is in agreement with the observations of Beever

et al. [4] but in contrast with those of Dawson et al. [8]. Statistically, the low number of observations in the present study may in part, have masked the substantial effect between treatments since the numerical difference in the means was approximately 17%. The value obtained for the efficiency of microbial protein synthesis on the silage diet (29.9 g N·kg⁻¹ OMADR) is relatively high compared with other literature values. For example, values of 26.7, 21.6 and 22.7 g N·kg⁻¹ OMADR were reported for similar diets by Beever et al. [5], Siddons et al. [27] and Thomas et al. [32], respectively. However, an average value of 30 g microbial N·kg⁻¹ OMADR has been adopted for all diets by the ARC [2] and this was confirmed elsewhere [4, 8, 9]. Dawson et al. [8] suggested that higher results (30.8 g N·kg⁻¹ OMADR) may be achieved if the silage is of good quality and is offered using a continuous feeding regimen, perhaps reflecting an increased 'balance' in the supply of N and energy to the rumen microbes.

4.2.3. Amino acid flow to and absorption from small intestine

Amino acid flow to and disappearance from small intestine in response to fishmeal supplementation was similar to that for N. However, as a proportion of N intake, amino acid-N flow at the duodenum was relatively low, approximately 0.49 g amino acid-N·g⁻¹ N intake. For steers fed on grass silage based diets, Thompson et al. [33] and Beever et al. [4] reported a value of 0.8 whereas Rooke et al. [23] reported 0.62 g amino acid-N·g⁻¹ N intake. Reasons for such differences are most likely related to daily DM intake.

ARC [2] has defined the apparent absorbability of amino acids in the small intestine as the net disappearance of amino acids between the duodenum and the terminal ileum as a fraction of the amino acids entering the duodenum. A value of 0.70 was considered appropriate [2]. However, Agricultural and Food Research Council [AFRC, 1] suggested that 0.85 was more appropriate when

taking into account true endogenous-N losses from the body including urine and faeces. Based on the early equation of ARC [2], values of 0.57 and 0.72 for silage alone and fishmeal-supplemented diet, respectively were obtained in the present study. The value from the silage diet was lower compared with those of other workers [2] using forage diets. However, forage variety, species and stage of growth are likely to be important factors contributing to the difference. For example, the values were lower in artificially dried legume and higher in fresh or frozen grasses [2]. In addition, most data were obtained from sheep with little data available for the direct comparison with growing cattle even though ARC [2] stated that there appeared to be no observable difference between values for sheep and for cattle. In studies with steers fed on silage and supplemented with fishmeal, the apparent absorption of total amino acids was in the range of 0.61–0.73 [4] and 0.56–0.69 [18]. Nevertheless, the increase in apparent amino acid absorption from the small intestine when supplementing silage with fishmeal is interesting.

In conclusion, fishmeal supplementation had little effect on rumen fermentation characteristics. However, there was a significant increase in molar proportion of propionate ($P < 0.05$) and a tendency for ammonia-N to increase. The duodenal flows of total-N, non-ammonia-N and total amino acids in animals consuming silage supplemented with fishmeal were higher ($P < 0.05$) than those consuming silage alone. Fishmeal supplementation had little effect on the individual amino acid composition of the total amino acid fraction apparently absorbed from the small intestine. However, the efficiency of apparent absorption of total amino acids was lower on the silage compared to fishmeal-supplemented silage ($P = 0.059$).

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