final report

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Optimising the cost-effectiveness of supplementary feeding in sheep meat production systems

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Executive Summary

Introduction

The project described in this report consisted of three major sections and is reported in that fashion. The first part of the project, comprising Section A of this report, consisted of research to develop a new, alkane-based technique which could be used to estimate the supplement intake of grazing animals, since a major impediment to assessing, modelling and thus predicting the response of animals to supplements was the difficulty of estimating the interaction between herbage and supplement intakes. While methods for estimating supplement intake already existed, the development of an alkane-based method would allow it to be combined with the existing alkane method for herbage intake, thus permitting the estimation of both herbage and supplement intake from the same set of analyses.

The second section of the project (Section B of this report) consisted of studies on the response of live weight, body composition and wool growth of large lambs to diets consisting of near- or sub-maintenance intakes of medium-quality roughage with or without protein supplements of differing quality and rumen degradability.

The final section of the project (Section C of this report) involved investigations of the need for and nature of modifications to the decision support tool **GrazFeed**, so that it might better predict the responses to supplements of the kind fed in the second section of the project and thus capture the biological information generated in that section.

Major Findings

Section A: Development of an alkane-based procedure for estimating supplement intake

This section of the project was initially approached by testing the accuracy of C38 alkane as an external supplement intake marker, since even-chain alkanes of this length are not found in pasture plants. In a field study conducted in collaboration with Project UM025, estimates of supplement intake made with C38 alkane were much lower than known intakes, and it was hypothesised that there might be a problem with the faecal recovery of this alkane. A large

indoor feeding trial was then conducted to evaluate C38 alkane further as a supplement intake marker and to compare it with chromic oxide marker and with a new proposed marker, beeswax. Since beeswax has an alkane pattern which reflects its origin as plant material, it cannot be used as an external marker as such. Rather, supplement intake was estimated in this case by regarding the beeswax-labelled supplement as if it were a second 'species' in the diet, and using the patterns of alkane concentrations in labelled supplement, the roughage component of the diet (perennial ryegrass chaff) and faeces to estimate the proportions of supplement and chaff in the diet. The combination of this information with estimates of total intake provided estimates of supplement intake.

Supplement intakes were under-estimated by 15-20% when C38 alkane was used as an intake marker and results confirmed that this under-estimate was due to the poor faecal recovery of this alkane. Similar results were obtained using chromic oxide. By contrast, the use of beeswax accurately estimated supplement proportion in the diet and thus supplement intake.

These results indicate that, due to its poor recovery and high cost, C38 is not a useful supplement intake marker. By contrast, beeswax was cheap, readily applied to supplements and resulted in accurate estimates of intake. Its use in conjunction with orally-dosed, controlled-release devices for delivery of C32 and C36 alkanes provides a means of estimating herbage and supplement intakes rapidly and conveniently, since all results are derived from the same set of chemical analyses.

Section B: Evaluation of the responses in weight, body composition and wool growth of lambs fed protein supplements of a range of rumen degradabilities

Lambs being grown to heavier market weights require supplementation with protein in temperate Australian grazing systems, to overcome the shortage of protein in dry summer pasture. However, the relative responses of live weight, body composition and wool growth to the quality of the protein supplement and to its rumen degradability have not been adequately distinguished and characterised. As a result, decision support tools such as **GrazFeed** do not adequately predict the response of lambs to such supplements.

The first feeding trial in this section compared proteins of different amino acid composition (sunflower meal (SFM), cottonseed meal (CSM), fish meal (FM)) as supplements for lambs fed a maintenance level of medium-quality chaff. Within the SFM, formaldehyde treatment

was used to effect two different degrees of rumen protection, chosen so as to be similar to the CSM and FM, respectively.

Lambs consuming the SFM gained weight at rates comparable with those on the other supplements, but gained significantly less fat. Moreover, reducing the rumen degradability of the SFM increased gains in both live weight and protein, without major increases in fat gain. There were marked wool growth responses to the feeding of protein supplements, especially to the two formaldehyde-treated SFM. The results suggested there was little advantage in feeding expensive supplements such as the FM. An unexpected result of this trial was the very high fat gain of animals fed CSM; a second major indoor trial was therefore conducted to resolve whether the CSM response was as real effect or an artefact of errors in the *in vivo* estimation of body composition.

In this second feeding trial, lambs were fed a similar basal diet at a near-maintenance level, and were supplemented with graded levels of the same CSM as used in the first feeding trial. The first level of CSM was 240 g/d, as in the preceding trial, whilst the next two levels were 360 and 480 g/d, respectively.

There were incremental responses of both live weight and body composition to the increasing intakes of CSM, with the response at the lowest feeding level indicating a loss of body fat, rather than rapid gain as in the earlier trial. The response of empty bodyweight gain to CSM supplementation was 25.9 g/100g CSM fed, whilst those for protein and fat gain were 2.5 and 5.3 g/100g CSM, respectively. Comparison of the results of the two feeding trials indicated that the result in the earlier trial arose from errors in the estimation of final body water content in the lambs on the CSM treatment.

Taken together, the results of the feeding trials indicated that the feeding of protein supplements of reduced rumen protein degradability resulted in increases in wool growth, weight gain, protein gain but, on several treatments, the loss of body fat. These results would not have been predicted by the current version of **GrazFeed**. The final section of the project was thus devoted to an examination of possible modifications to this package so that it might better predict responses such as those observed in the feeding trials.

Section C: Exploration of possible modifications to **GrazFeed**

The decision support tool **GrazFeed** currently models the use of protein and energy by ruminant animals, based on the published Australian Feeding Standards. In the last section of this report, the manner in which **GrazFeed** currently models wool growth and gains in body composition and live weight is described, by way of background to the proposed modifications.

The issue of how better to model the response of wool growth to dietary proteins of lower rumen degradability is addressed by alterations to the functions involving the efficiency of conversion of digestible protein leaving the stomach (DPLS). At present, this has a constant value of 0.116 (ie, 11.6 g clean dry wool per 100g DPLS), up to the point that energy rather than protein limits wool growth. Thereafter, wool growth is calculated as 1.39 g CDW/MJ ME available for wool growth.

Based on the results of the feeding trials in this project, plus published information, the efficency term has been increased for that portion of DPLS which reflects the better sulfur amino acid composition of undegraded protein from the supplement, such that the overall efficiency approaches 0.17. The result of the modification is that predicted wool growth responds not only to DPLS and to ME supply, but also to the proportion of the DPLS derived from undegraded supplement protein with an amino acid composition better suited to wool growth. The final quantification of the modified functions will await collection of digesta flow and wool growth data against which the revised **GrazFeed** predictions can be validated.

Proposed modifications to the body composition and weight gain algorithms are less straightforward. In the present version of **GrazFeed**, the predicted composition of weight gain is a function of the size of the animal relative to its mature size. The consequence of this is that weight gain always contains protein *and* fat, and thus has a relatively high energy content. As a result, **GrazFeed** under-predicts weight gains at feeding levels just above maintenance and does not permit simultaneous gains in protein but losses in fat.

This issue is addressed by making the predicted composition of gain itself a function of feeding level, such that at ME availabilities below about 0.25 of that which will allow maximum potential gain, the protein content of gain rises and fat content falls. This in turn reduces the energy content of gain, so that for a given ME availability for gain, predicted

weight gain will be higher, though the efficiency of use of ME for gain will be lower. Moreover, the weight gain will contain a higher proportion of protein, possibly occurring together with fat loss.

At this stage, the modifications to the body composition and wool growth algorithms must be regarded as conceptual frameworks, but the results presented in the report indicate that the modifications result in much better predictions of body composition and weight changes than those obtained with the unmodified version of **GrazFeed**.

Conclusions

The work conducted within this project has clarified the response of large lambs to diets consisting of maintenance quantities of roughage coupled with protein supplements with reduced rumen degradability. It suggests that protein supplements such as SFM or CSM can be used in this combination to effect a degree of re-partitioning in large lambs, such that they gain weight and protein, but lose fat, while also showing increased rates of wool growth. The development of the new method for estimating supplement intake, based on beeswax, provides a means of closely monitoring intakes in future field trials with such supplements, so that the interactions between supplement, herbage and the animal's body reserves can be evaluated. Finally, the proposed modifications to **GrazFeed** provide a conceptual framework which will predict responses to such proteins more adequately than does the current version of the model. In order for this conceptual framework to evolve into a new release of **GrazFeed**, a phase of validation against experimental results must now commence.

Acknowledgements

The work presented in this Report would have been impossible without the collaboration of many scientific and technical colleagues, and the financial support provided by Meat and Livestock Australia, CSIRO Plant Industry, the Department of Natural Resources and Environment (Victoria), the University of Melbourne and the Ministry of Agriculture, Spain.

The preliminary field evaluation of C38 alkane as a supplement intake marker (Section A2) was conducted with the collaboration of Drs Leo Cummins and Janet Foot of the Pastoral and Veterinary Institute, Hamilton and Dr Brenton Hosking of the University of Melbourne. I also thank Peter Heazlewood and Murray Arnold from PVI for their able technical assistance.

The work reported in Section A3 was conducted in collaboration with Dr Mamen Oliván, a post-doctoral visitor from IEPA, Villaviciosa, Spain; her skilled contributions are gratefully acknowledged. Together, we thank Dr Keith Ellis of CSIRO Animal Production for preparing the alkane CRD; Terry Shepherd, Kim Shelley, Jason Byron and Stephen Speer for their technical assistance and CSIRO Plant Industry and INIA (Ministry of Agriculture), Spain, for the financial support which permitted our collaboration.

Many colleagues assisted with the conduct of the feeding trials reported in Sections B2 and B3. In particular, I thank Jane Pulford for her help with the daily care and feeding of the animals described in Sections B2 and B3; Bruce Reid for his similar assistance during the experiment described in Section B3; Kim Shelley and Jason Byron for their assistance both with the animals and in particular, with the preparation of the experimental diets; Terry Shepherd for help with insertion of dyebands, I/M injections of deuterium oxide and blood sampling; Dr Dennis Poppi of the University of Queensland for the conduct of the urinary purine analyses; and Dr Sam Coleman, visiting McMaster Fellow, for conducting the NIRS assays on the urine samples and the statistical evaluation of the spectral data.

Finally, Section C of this Report, describing proposed modifications to the decision support tool **GrazFeed** was written in collaboration with Dr Mike Freer of CSIRO Plant Industry, the developer of the package. I thank him for his major contributions and for allowing access to and altering the computer code within a version of **GrazFeed**, to generate the prototype modified version.

SECTION A: Development of a new alkane-based technique for the estimation of supplement intake

A1. Introduction

The herbage intake of grazing animals can be estimated accurately using a combination of the alkanes of the herbage cuticular wax and orally-dosed synthetic alkanes (see Dove and Mayes 1996 for details). The method automatically accomodates the differing extent of herbage digestibility in individual animals and its accuracy is unaffected by the consumption of supplement. It is thus an ideal procedure to combine with estimates of supplement intake.

Prior to the commencement of this project, a number of methods existed for estimating the supplement intake of animals. For example, the markers chromic sesquioxide (Cr_2O_3; Lobato *et al.* 1980; Dove and Coombe 1992), tritiated gypsum (Dove 1984; Coombe *et al.* 1987), lithium chloride (Kahn 1994) and ytterbium acetate (Curtis *et al.* 1994) have all been shown to provide accurate estimates of supplement intake. However, each has its disadvantages. Faecal markers such as Cr_2O_3 and ytterbium require either an estimate of faecal output or total faecal collection. Lithium chloride provides an estimate of intake over only a single day and may not be applicable when supplements are fed infrequently. The tritiated gypsum procedure is accurate with very small intakes and with intermittent feeding and also provides an estimate of *in vivo* body composition. However, it is of limited use in practical feeding systems because of the radioactivity of the marker. Finally, all of the above methods require chemical analyses separate from and in addition to the alkane analyses for herbage intake.

The aim of this section (Objective 2) of Project CS232 was to develop an extension of the alkane procedure for herbage intake, which would allow the estimation of herbage and supplement intake from a single set of chemical analyses. When alkanes are used to estimate herbage intake, animals are dosed with C32 alkane which, in combination with either C31 or C33 alkane from the diet, gives an estimate of herbage intake. The alkane dose also contains C36 alkane, the dilution of which in faeces gives an estimate of faecal output and thence digestibility. The proposed extension of this approach employed a further alkane (octatriacontane; C38 alkane) which is not present in pasture species or supplements, as a marker to be applied to the supplement. The product of the C38 concentration in faeces and

the faecal output estimated using the C36 alkane gives an estimate of C38 intake, which is converted to supplement intake using the C38 concentration of the supplement.

The advantages of this approach are first, that any effect of supplement on herbage digestibility is automatically accommodated and second, that only one set of chemical analyses is needed to estimate herbage intake, supplement intake and whole-diet digestibility.

A2. Initial field testing of C38 procedure

Before the commencement of Project CS232 itself, the opportunity arose to evaluate the proposed C38 procedure under field conditions, in a supplementary feeding trial conducted at the Pastoral and Veterinary Institute, Hamilton, Victoria during the late stages of Project UM025. This evaluation comprised Objective 1 of the Project.

Methods

After weaning, 160 cryptorchid cross-bred lambs were allocated to one of four treatments in a 2x2 factorial design. In the late summer-early autumn, animals grazed either annual (volunteer) or perennial (improved) pastures with limited herbage supply (500kg DM/ha or less). All animals received supplements of sunflower meal (SFM; 250g/d per animal); half the animals received SFM which had been treated with formaldehyde to reduce its rumen protein degradability to 25% (protected SFM), while the other half received unprotected SFM.

Six weeks after the introduction of supplements, 10 animals in each group were dosed with intra-ruminal controlled-release devices (CRD) delivering 54 mg/d of each of C32 and C36 alkanes. Over the ensuing 16 days, they also received supplement which had been labelled with 200mg/kg DM of C38 alkane. After an equilibration period of 6 days, samples of faeces (rectal sampling), herbage (hand-harvesting), oesophageal extrusa (adult wethers fistulated at the oesophagus) and labelled supplement were collected and analysed for alkanes by gas chromatography as previously described (Dove 1992). Alkane concentrations were used to estimate herbage intake, supplement intake and whole diet digestibility.

Full details of experimental procedures, the responses of the animals to the supplements and the accuracy of other procedures for assessing supplement intake can be found in the Final Report on Project UM025. The present Report will concentrate on alkane aspects and, in particular, on the evaluation of the proposed C38 procedure.

Results and Discussion

The concentrations of the major alkanes in samples of pasture, oesophageal extrusa and the faeces from the grazing animals are shown in Table A1. In faeces samples, alkanes with odd-numbered carbon chains derive from the consumption of pasture, alkanes C32 and C36 derive from the alkane CRD, while the faecal C38 derives from the consumption of supplement.

Alkane concentrations in oesophageal extrusa were consistent between the animals on a given plot, as evidenced by the small standard errors attendant to mean concentrations. Moreover, the pattern of alkane concentrations in oesophageal extrusa and in pasture was notably similar and indeed, these two data sets could be related by an expression which did not differ from the line of equality (Figure A1). Nevertheless, there were sufficient differences to indicate that inaccurate estimates of herbage intake would have been obtained if they were based on the hand-harvested samples. Estimates of herbage intake were therefore based on the oesophageal extrusa.

Faecal concentrations of herbage alkanes were also consistent between the 10 animals sampled on each plot, as the small standard errors suggest. Moreover, the concentrations of C32 and especially C36 alkanes were consistent both between and within plots, as might be expected since almost all of the C32 and all of the C36 in faeces is derived from the alkane CRD. Faecal concentrations of C38 alkane were similar in each plot, reflecting the fact that the supplement feeding level was the same in each plot. However, variability in faecal C38 concentration within a plot was greater, presumably because of differences in supplement intake by individual animals.

The 'protected' and 'unprotected' supplements were prepared to contain 200 mg/kg DM of C38 alkane. The actual levels estimated by gas chromatography were 204.5 and 192.1 mg/kg DM respectively. This indicates that the supplement labelling procedure was satisfactory.

Table A1. Alkane concentrations (mg/kg DM) in hand-harvested pasture samples, oesophageal extrusa samples and in faeces samples from grazing sheep. Mean values with their standard errors, except for pasture which represent bulk sample from each paddock.

Paddock:sample	Alkane C25	C27	C29	C31	C32	C33	C36	C38
J1: Pasture	12.3	33.1	128.7	174.6	7.8	60.1	-	-
Oesophageal	17.2±1.0	44.8±1.0	175.0±5.5	187.4±13.2	8.4±0.8	69.5±5.2	-	-
Faeces	23.7±1.9	68.9±2.7	286.3±9.3	386.9±12.9	128.5±6.8	165.2±5.5	99.5±6.4	37.2±5.5
J2: Pasture	12.4	35.0	164.6	251.2	8.4	66.6	-	-
Oesophageal	20.1±1.9	50.7±3.2	182.1±10.6	206.2±12.9	8.8±0.7	73.3±7.7	-	-
Faeces	21.0±1.0	60.3±1.3	253.6±6.6	322.5±8.1	126.5±6.6	125.8±3.7	101.4±6.5	32.6±4.2
50A1:Pasture	19.8	46.1	154.0	158.2	7.4	54.1	-	-
Oesophageal	18.4±0.7	36.0±1.6	119.8±8.2	121.1±14.2	5.8±0.6	42.9±5.5	-	-
Faeces	30.3±1.3	69.6±1.3	236.8±4.1	202.9±2.7	122.8±8.2	61.0±0.9	103.9±5.9	38.8±5.8
50A2: Pasture	17.9	38.4	121.3	119.7	6.4	36.9	-	-
Oesophageal	22.2±1.7	43.6±2.9	158.4±4.4	191.6±10.3	6.6±0.3	55.4±5.4	-	-
Faeces	28.1±1.2	77.9±3.3	362.0±13.6	330.5±13.1	114.8±6.8	85.9±3.5	104.7±7.2	40.5±8.5

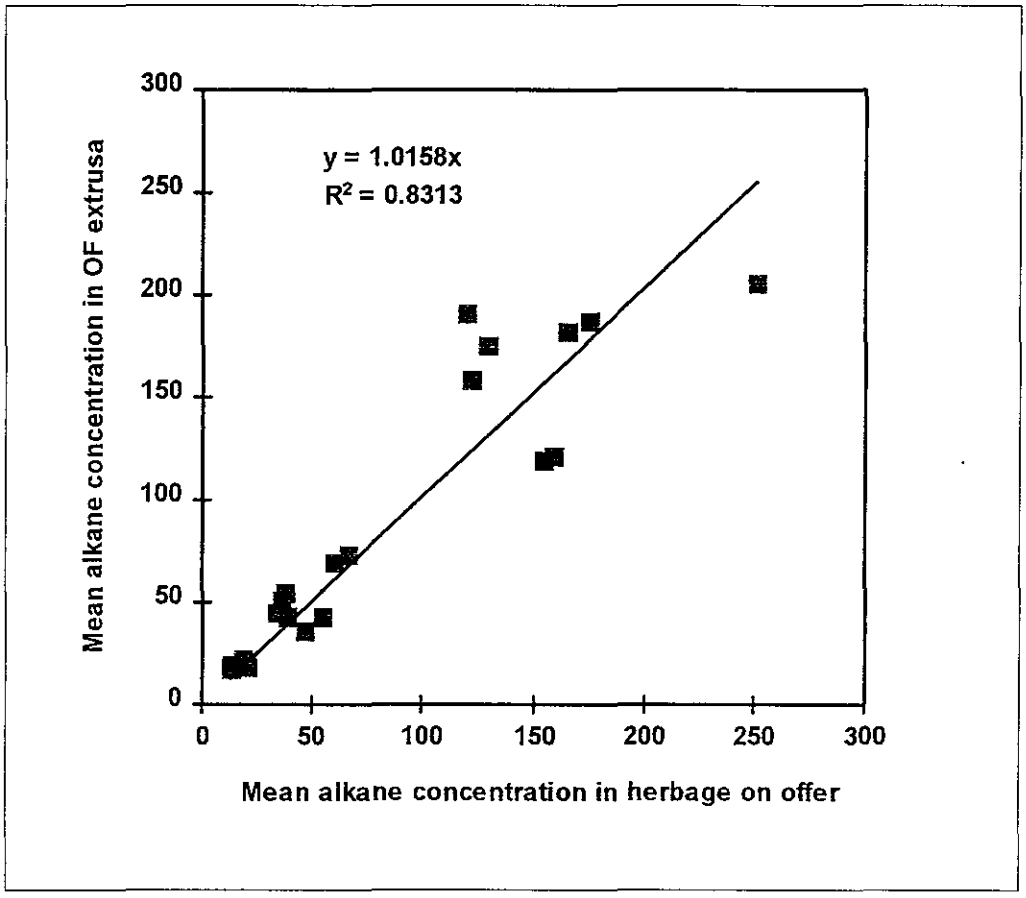


Figure A1. Relationship between the alkane concentrations (mg/kg DM) in oesophageal extrusa and in the herbage on offer. Fitted regression does not differ from the line y = x.

Faecal outputs and estimated pasture and supplement intakes are shown in Table A2. Faecal outputs were computed from the C36 dose rate and C36 concentration in faeces, assuming a faecal C36 recovery rate of 0.95. Pasture intakes estimated using either the C31/C32 or the C33/C32 alkane pair were similar and of the order expected; the values shown in Table A2 are the means of these two estimates.

Table A2. Faecal outputs and pasture and supplement intakes (all g DM/day, mean±se)

Paddock	Faecal output	Supplement intake	Pasture intake
J1	533±3.1	100±1.5	1114±4.9
J2	524±3.2	91±1.4	806±3.5
50A1	509±3.0	103±1.6	767±5.7
50A2	512±3.6	110±2.8	864±4.2

The most notable feature of the results in Table A2 is the low level of the estimated supplement intake, relative to the feeding level of 250 g/d. While there were some refusals of supplement in the period leading up to the estimation of supplement intake, these were not large enough to explain the low estimates. The other major sources of error in the estimation of supplement intake are as follows:

1. The level of C38 applied to the supplement was lower than expected. This has already shown to not be the case.
2. The estimate of faecal output, based on C36 dose and C36 faecal concentration, is too low. However, when alkane concentrations in faeces and oesophageal extrusa were related by regression analysis, as described by Dove *et al.* (1998), the implied whole-diet and pasture digestibilities were 0.52 and 0.47, close to the expected values for animals consuming short summer pasture of low green content. This suggests that the estimates of faecal output and thus C38 faecal excretion are not sufficiently in error to be the cause of errors in estimated supplement intake.
3. The recovery of the synthetic C38 alkane in faeces is less than complete. Based on previous reports of faecal recoveries of alkanes up to C36 (see Dove and Mayes 1996 and below), it was assumed that the faecal recovery of C38 alkane would be complete. The results in Table A2 suggested that this was not the case, and indicated a need for a more detailed indoor assessment of the proposed C38 procedure and in particular, an

examination of the faecal recovery of C38 alkane when used as a supplement intake marker.

A3. Indoor assessment of the C38 procedure and the development of a new procedure using beeswax as a supplement intake marker

Initial attempts to use C38 alkane as an external marker for estimating supplement intake were not successful (see above). It appeared that the most likely reason for the failure of this approach under field conditions was incomplete faecal recovery of C38. The feeding trial described below was conducted to evaluate the procedure under controlled conditions, which permitted a direct estimate of faecal alkane recoveries. The supplement was also labelled with Cr_2O_3, to provide a comparison with previous studies which have used this marker.

Theoretically, supplement intake could also be measured by estimating total intake using the alkane procedure, and then estimating the proportion of supplement in that total, based on the patterns of alkane concentrations in herbage, supplement and faeces. Unfortunately, many supplements, including the sunflower meal (SFM) used in the present study contain virtually no alkanes. We therefore also evaluated a novel approach to estimating supplement intake, in which the supplement is labelled with a non-plant alkane mix in the form of beeswax.

Methods

Twenty-four crossbred young sheep weighing approximately 30 kg were fed, once-daily, one of four dietary treatments consisting of 720g DM/d of mixtures of perennial ryegrass chaff and unpelletted SFM in four different proportions; 7:1, 6:2, 5:3 and 4:4. Eight of the animals were housed in metabolism crates ('crate sheep') to allow total collection of faeces, while the remaining 16 animals ('pen sheep') were housed in individual pens over slatted floors. All animals were fed once daily and had unrestricted access to water.

After a 10-day adjustment period, all animals were dosed with intra-ruminal alkane CRD delivering 54.3 mg/d C32 alkane and 49.4 mg/d C36 alkane, and were switched from unlabelled SFM to SFM labelled with Cr_2O_3 and a beeswax/C28/C38 alkane mixture,

prepared as described by Dove and Oliván (1998). The C28 alkane was added to the beeswax mix in an attempt to ensure that the alkane pattern of the mix was distinctly different from that of the herbage in the diet, but in the event, this proved unnecessary and the C28 alkane concentrations were not included in the calculation of supplement intakes given below.

Six days after dosing and the introduction of labelled SFM, faecal output in the crate sheep was determined by total collection; rectal grab-samples were also taken from these animals at 0900 and 1600h on each day of collection. Pen sheep were only sampled in the mornings. Faecal output in all sheep was also estimated using C36 alkane (from the alkane CRD) as an external marker.

Total intakes (chaff+SFM) were estimated using either the C31/C32 or the C32/C33 alkane pairs, based on the whole-diet and faecal concentrations of these alkanes and the C32 release rate from the alkane-CRD. Supplement intakes were estimated either directly (from the concentrations of Cr_2O_3 or C38 alkane in labelled-SFM and faeces), or indirectly by estimating the proportions of chaff and SFM in the total intake, using the least-squares optimisation package **EatWhat** (Dove and Moore 1995). Further details of chemical analyses, calculation procedures and statistical analyses are given by Dove and Oliván (1998).

Results

Alkane concentrations in perennial ryegrass and SFM supplement

The predominant alkanes in the ryegrass chaff were those with odd-numbered carbon chains, as in previous studies (Table A3; Dove and Mayes 1996). There were only small quantities of the two alkanes with which the animals were dosed (C32 and C36). In the labelled SFM, the labelling procedure was intended to result in concentrations of 50-400 mg/kg DM of the alkanes of beeswax and, respectively, 400 and 200 mg/kg DM of the synthetic C28 and C38 alkanes. It is clear from the data in Table A3 that these desired concentrations were achieved.

Faecal recovery of alkanes

Mean faecal recoveries of alkanes in the crate sheep did not differ significantly between the four dietary treatments and pooled means are shown in Figure A2. Recoveries of the natural alkanes increased with increasing carbon-chain length, in a curvilinear fashion similar to that described previously (see Dove and Mayes 1996). The faecal recovery of C32 and C36

alkanes from the intra-ruminal CRD conformed closely to the pattern noted for the natural alkanes and, for C36 alkane, was essentially complete. By contrast, the recoveries of C28 alkane and especially the C38 alkane which were sprayed onto the SFM with the beeswax, were markedly lower than those found for adjacent natural alkanes.

Table A3. Concentrations of alkanes (mean values ± s.e.) in perennial ryegrass chaff and SFM labelled with C38 alkane and a beeswax/C28 alkane mixture

Alkane (mg/kg DM)	Ryegrass chaff	Labelled-SFM
C25	51.1±0.54	107.3±2.32
C26	3.7±0.20	11.3±0.72
C27	113.9±1.10	432.6±10.90
C28	10.7±0.11	420.1±10.20
C29	270.5±2.87	276.6±6.99
C30	11.9±0.08	7.2±0.24
C31	256.3±2.27	219.0±6.56
C32	5.3±0.07	2.5±0.20
C33	35.8±0.02	33.8±1.51
C35	3.7±0.25	0.8±0.79
C36	5.5±0.13	5.1±0.29
C38	0	206.8±8.18

Estimation of total intake

In the crate sheep, mean DM intake estimated using the C31/C32 alkane pair under-estimated known intake by 2% (706 v. 720 g/day; Table A4). The estimate of DM intake based on the C32/C33 alkane pair was only 0.8% different from the known mean intake (726 v. 720 g/day). In the 16 pen sheep, total DM intake estimated using C31/C32 was very close to known intake, but the mean intake estimated using C32/C33 was 44 g/day (5.8%) higher than known intake (766 v. 722 g/day). The standard errors associated with the estimated intakes in pen sheep were higher than those found with crate sheep.

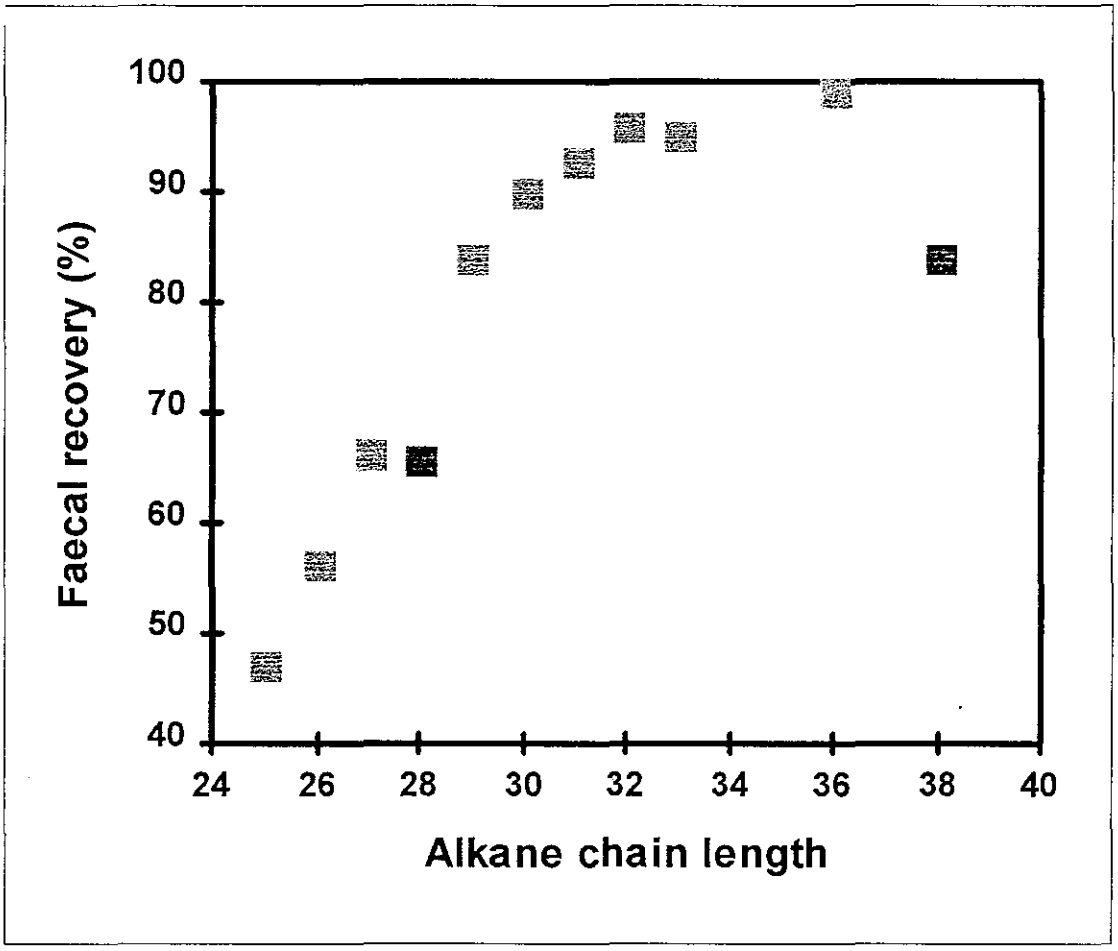


Figure A2. Faecal recoveries of alkanes in sheep fed mixtures of perennial ryegrass:labelled SFM and dosed with intra-ruminal alkane controlled-release devices.

Table A4. Comparison of known total intakes (g DM/day), faecal outputs (g DM/day; crate sheep only) and DM digestibilities (%; crate sheep only) with estimates based on the C31/C32 or the C32/C33 alkane pairs (intake) or C36 alkane (faecal output). Mean values±s.e.

	Crate sheep			Pen sheep		
	Known	C31/C32	C32/C33	Known	C31/C32	C32/C33
Intake	720±1.9	706±11.7	726±5.6	722±0.9	724±22.8	766±22.4
Faec. output	282±2.5	269±7.8	269±7.8	-	260±6.7	260±6.7
Digestibility	60.8±0.35	61.9±1.01	63.0±0.87	-	63.8±1.03	65.9±0.94

Faecal DM output estimated using C36 alkane was 5% less than known faecal output (269 v. 282 g/day) despite the almost complete faecal recovery of this alkane. As a result, whole-diet

digestibility was slightly over-estimated (63.0 and 61.9% v. 60.8%). In pen sheep, true faecal outputs and digestibilities were by definition unknown, but estimates of faecal output based on faecal grab samples taken only in the morning, were similar to those found with the crate sheep.

Estimation of supplement intake

Mean supplement intakes in crate and pen sheep were 229±38.7 and 230±26.6 g DM/day respectively. These were significantly under-estimated ($P<0.05$) when C38 was used as the supplement marker (crate sheep 185±32.7; pen sheep 205±23.6 g DM/d).

In the crate sheep, the relationships between known supplement intakes and the various estimates are shown in more detail in Figure A3. When C38 alkane was used, the mean estimated supplement intake (185±32.7 g DM/day) was significantly less than the known intake ($P<0.05$) and was related to it by an expression in which was significantly different from the line of equality (Fig. A3(a), slope = 0.84±0.036; $P<0.05$). The under-estimate was due entirely to the poor faecal recovery of C38 alkane and the adjustment of the estimates for this faecal recovery resulted in a relationship between estimated and known intakes which did not differ from the line of equality (Fig. A3(b)). Similar relationships existed for the C38-based estimates in the pen sheep, and the Cr_2O_3-based estimates (Fig. A3, (c) and (d)).

Table A5. Comparison of the known proportions of supplement (SFM) in the diet and those estimated from the pattern of alkane concentrations in faeces and dietary components (using the least-squares package *EatWhat*), and comparison of resultant estimated supplement intakes with known intakes (means ± s.e.)

	Crate sheep		Pen sheep	
Estimated SFM:	Known	Estimated	Known	Estimated
Proportion	0.32±0.053	0.34±0.056	0.32±0.036	0.33±0.039
Intake (g DM/day)	229±38.7	244±40.5	230±26.6	238±26.4

By contrast, the dietary proportions of chaff and SFM estimated from the pattern of alkane concentrations in each and in faeces (using **EatWhat**), were not significantly different from known proportions (Table A5). The resultant estimates of supplement intake (Table A5) did not differ significantly from known intakes and in both the pen sheep and the crate sheep (Fig.

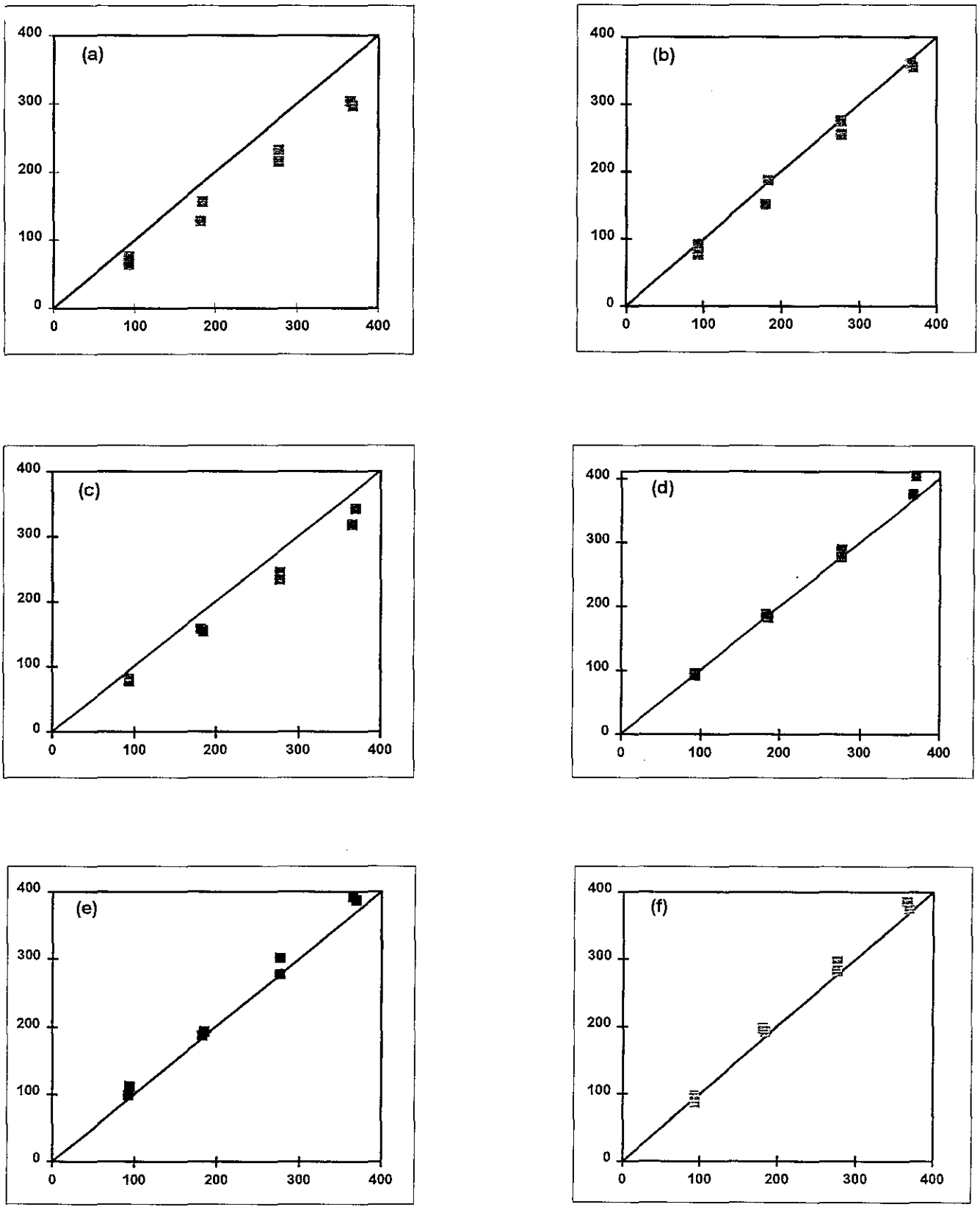


Figure A3. Relationships between known supplement intake (x, g DM/d) and estimates (y, g DM/d) obtained using the following procedures:
(a) C38 alkane as external marker,
(b) C38 alkane as external marker, corrected for faecal recovery of C38
(c) Cr_2O_3 as external marker,
(d) Cr_2O_3 as external marker, corrected for faecal recovery of Cr_2O_3,
(e) pattern of alkane concentrations in herbage and in beeswax applied as supplement marker,
(f) pattern of alkene concentrations in herbage and in beeswax applied as supplement marker.
(Solid lines are lines of equality, y = x)

A3 (e)) were related to these known intakes by equations which did not differ from the line of equality.

In addition to the saturated hydrocarbons (alk*a*nes), beeswax also contains hydrocarbons with one or more double bonds (alk*e*nes). Estimates of supplement proportion and intake based on alkene concentrations in the diet components and the faeces were also very close to known intakes (eg, Fig. A3 (f)), despite the fact that the recovery of alkenes in faeces was only of the order of 35-40%. This emphasises the point made by Dove and Mayes (1996) that it is the *relative*, rather than the *absolute* recoveries which are important in obtaining an accurate estimate of diet composition. Despite their low absolute faecal recoveries, the alkenes still functioned well as supplement intake markers because their recoveries relative to each other were so similar.

Discussion and Conclusions

The results of this feeding trial confirm the usefulness of the alkane procedure for estimating total intake in animals consuming a mixture of forage and supplement. With the exception of C28 and particularly C38 alkane, faecal alkane recoveries (Fig. A2) were similar to published values (see Dove and Mayes 1996) and increased with carbon-chain length.

The faecal recoveries for C32 and C36 alkanes (Fig. A2) can be considered to relate to the pure alkanes derived from the intra-ruminal CRD, since over 95% of the input of these alkanes came from this source. The lower recoveries of pure C28 and C38 are thus difficult to explain but may suggest that, in contrast to the alkanes of beeswax or the C32 and C36 released gradually from the CRD, pure alkanes sprayed onto dietary components may behave differently during passage through the gut. As the data in Fig. A3 (a) and (b) indicate, the low recovery of C38 alkane was the major cause of the significant under-estimates of supplement intake found with this alkane; when allowance was made for the incomplete faecal recovery of C38, estimated supplement intakes in the crate and pen sheep were 221 and 244 g DM/day respectively, close to the known intakes of 229 and 230 g DM/day respectively.

The use of beeswax as an alkane-bearing marker allowed the alkane procedure to be used for the simultaneous estimation of total and supplement intake, in a situation where the supplement itself did not contain alkanes. The proportion of supplement in the diet was

accurately estimated, using the non-negative least-squares procedure in the **EatWhat** programme. Since total intakes were accurately estimated using either the C31/C32 or C32/C33 alkane pairs, the product of these and the supplement proportion in the diet of individual animals resulted in accurate estimates of supplement intake, over a four-fold range of supplement intakes. The absence of any benefit from adding C28 alkane to the beeswax suggests that beeswax on its own would be a useful marker, and it has the added advantages of being cheap (approximately $5/kg) and easily applied to supplements. By contrast, the high cost and poor performance of C38 alkane suggest it offers no advantage over beeswax.

SECTION B: Evaluation of the responses in live weight, body composition and wool growth of lambs when fed protein supplements of a range of rumen degradabilities

B1. Introduction

If either carry-over lambs or lambs being grown to larger market weights, are to reach target weights by late summer or early autumn, some supplementation with protein sources seems to be necessary, partly because of the relatively high protein requirements of these animals, and partly because protein supplements help to alleviate the shortage of rumen-degradable nitrogen which can occur in young animals grazing dry summer pasture. However, responses to supplements in grazing lambs (eg, Freer *et al.* 1988) may differ from those observed when similar supplements are offered to animals in pens (Doyle *et al.* 1988) or small yards (Freer *et al.* 1985). The pattern of response in the carcass may also differ from the response in live weight (Dove *et al.* 1991). All these studies were based on relatively degradable supplements (2:1 oat grain:sunflower meal (SFM)).

The work conducted in Project UM025 demonstrated rapid weight gains and unusually low substitution rates between supplement and herbage when young sheep were given supplements of slowly-degrading protein sources such as cottonseed meal (CSM) and fishmeal (FM). These responses would not have been predicted by the current version of **GrazFeed,** and they suggest that the cost-effectiveness of supplements for lambs might be increased by using these less-degradable protein sources. Research in both Britain (Vipond *et al.* 1989) and Australia (Bell and Bower 1990) indicates that it is also likely that supplements based on slowly-degrading protein sources may offer a means of reducing the carcass fat content of large lambs, without reducing the yield of saleable lean. In order to achieve this, it seems necessary to feed the protein supplements together with maintenance or sub-maintenance amounts of low-quality roughage. Some reports have indicated that when this is done, fat content of the animal is reduced and the lean content and carcass weight either stay the same or increase (Vipond *et al.* 1989; Bell and Bower 1990). By contrast, the results obtained by Drs Oddy and Hegarty of NSW Agriculture led them to suggest that rumen-

escape protein (in their case, formaldehyde-treated casein) had "...only a small effect on growth and carcass composition...", but that it could "...significantly improve feed conversion efficiency of finishing lambs" (see p.ii, Final Report, Project DAN056). The improved feed conversion efficiency confirms results reported by Beermann *et al.* (1990) and by Sainz *et al.* (1994), except that these authors also observed effects on carcass components.

Hence, despite the promise offered by using slowly-degrading protein sources for larger lambs, the approach has yet to be evaluated under practical feeding systems for the production of leaner lambs in Australia. Before this could be done, the relative effects of protein source and of degree of rumen degradability need to be examined further. In particular, there is a need to include in such comparisons, both protein sources of different degradability and the same protein source treated to achieve different degrees of rumen degradability. The aim of this section of Project CS232 (Objective 3 of the Project) was to examine whether the differences in response in the above suite of studies could be explained in terms of differences in the degree of rumen degradability versus the amino acid composition of the protein sources. Since treated casein cannot be regarded as a 'practical' protein supplement, SFM, CSM and FM were used as the benchmark protein sources. This permitted reference back to our earlier indoor and field trials (Freer *et al.* 1988; Doyle *et al.* 1988) and to the work conducted in Projects DAN056 and UM025.

Objective 4 of the Project required the selection, in consultation with the then Corporation, of a 'best-bet' supplement for obtaining the desired gains in carcass composition, when offered to heavier lambs (>35 kg) grazing low-quality pasture over the summer-autumn period. However, as is discussed in Section B2 below, responses in body fat content of housed lambs to CSM were unusually large and required resolution before any attempt could be made to modify the way in which **GrazFeed** deals with the effects of supplements on changes in live weight, body composition and wool growth.

Therefore after consultation with Drs I. Johnsson and L.P. Thatcher (May 8, 1998), Objective 4 was altered so that it addressed directly the question of body fat responses to CSM supplement. This was satisfactorily resolved in the final experiment in the Project, discussed in Section B3 below.

B2. Comparative effects of amino acid composition and degree of rumen degradability of supplement protein on live weight, body composition and wool growth of lambs

This animal house study consisted of two parts, a feeding trial in which the live weight, body composition and wool growth responses of 56 lambs were evaluated, and a digestibility trial in which both digestibilities and daily nitrogen (N) balances were measured in 14 lambs housed in metabolism crates.

Methods

Feeding trial

Animals and feeding: Fifty-six close-shorn crossbred lambs were housed in individual pens in the Ginninderra Animal House. Half the lambs were ewes (liveweight 33.8±0.4kg) and the other half cryptorchid males (liveweight 36.7±0.3), in order to examine the response of the two sexes to the dietary regimens. Animals received one of 7 diets (8 lambs/diet):

1. Medium-quality chaff (DM digestibility 60%; 8.4% CP), fed at a level calculated to maintain liveweight (600 g DM/d; Control),
2. Chaff + 230g solvent-extracted, decorticated SFM (37.6% CP), in which (based on previous studies in this laboratory) rumen protein degradability was approximately 75% (SFM75),
3. Chaff + 230g SFM, treated with formaldehyde solution at the rate of approximately 6g formaldehyde/kg protein (SFM50),
4. Chaff + 230g SFM, treated at the rate of 12g formaldehyde/kg protein (SFM25),
5. Chaff + 240g CSM (42.5% CP),
6. Chaff + 125g FM (62.8% CP),
7. Chaff + 125g FM + 70g wheat starch (<0.5% CP).

Previous work in this group has indicated that the rate of formaldehyde used to treat diet 4 should have resulted in the reduction of the rumen degradability of SFM protein to approximately 25%. The treatment level used for diet 3 was an attempt to achieve a degradability of approximately 50%, comparable with that found commonly for CSM and FM. The quantities of CSM and FM fed in diets 5-7 were intended to be isonitrogenous with

supplements 2-4, but as is discussed below (see Table B2), variations in the N content of these supplements from the expected resulted in some treatment variability in N intake. Due to the higher protein content of the FM compared with SFM and CSM, the attempt to make diet 6 isonitrogenous with the other supplemented diets resulted in the feeding of less total energy. Starch was therefore added to the FM in diet 7 to raise the total energy content of the mixture to that of supplements 2-5. Animals were fed once daily (0900h). Food refusals were collected daily and water was freely available at all times. Anthelminthics were administered to keep animals free of internal parasites.

Measurements: After an adjustment period of 21d on the experimental diets, and again 42d later, all animals were denied food and water for 15h and then given deuterium oxide (0.2 g/kg live weight) by deep I/M injection. Six hours later, animals were bled by jugular puncture in order to obtain samples of body water for the measurement of the dilution of deuterium oxide. Feed and water were then returned to the animals.

Blood samples were allowed to clot over 24h; body water was removed from the resultant blood serum by lyophilisation and the concentrations of deuterium oxide determined as decribed elsewhere (Dove 1988). Body composition *in vivo* was then estimated using the prediction equations of Donnelly and Freer (1974).

On the same occasions as the *in vivo* body composition measurements, dyebands were inserted in the fleece (right midsides) for the measurement of wool growth, using commercial hair dye (Schwarzkopf Igora Royal N1 Black) as described by McCloghry (1997).

Digestibility trial

Commencing on day 42 of the feeding trial, a further 7 lambs were housed in individual metabolism crates and offered the same diets as the 56 lambs in the feeding trial (1 lamb/diet). After lambs were accustomed to the crates and diets, total collections of faeces and urine were made by standard procedures, for the determination of diet digestibility and N balance. These procedures were repeated immediately after the feeding trial with another 7 lambs drawn from that trial, such that estimates of digestibility and N balance were ultimately obtained for 14 animals (one ewe and one cryptorchid lamb per diet).

In addition to analysis of total N content, the concentrations of urea and total purine derivatives in urine were estimated by Dr D. Poppi and colleagues, University of Queensland,

to establish whether the differences in rumen degradability of the supplement proteins brought about differences in the extent of rumen microbial protein synthesis, and consequent urinary purine derivative excretion. As an adjunct to this section of the work, Dr S.W. Coleman, a visiting McMaster Fellow in this laboratory, scanned all urine samples by near-infrared reflectance spectroscopy (NIRS), in the hope of establishing useful calibration equations for total purine derivatives and/or their component compounds. The availability of such a set of calibration equations would greatly assist the application of the urinary purine approach to estimating rumen microbial protein synthesis, since it would obviate the need for all samples to be anaysed by high-performance liquid chromatography (HPLC), as is currently the case.

Urine samples (0.1 ml) from each sheep were scanned in duplicate to determine if spectral data associated with the nitrogenous compounds reported by HPLC could be detected by NIRS. Since water is a strong absorber in the near infra-red region, spectral information in liquid or very wet samples is difficult to obtain unless the water is removed. In order to eliminate this problem, the samples were aspirated onto glass fiber filters and allowed to air dry. The residue left on the filter consisted of the solids and non-volatile solutes from the urine. Each filter was scanned in normal reflectance mode on an NIRsystems model 9500 spectrophotometer (Foss International, Silver Spring, Maryland, USA), in a spinning sample cup. Reflected energy was captured by silicon (400-1100 nm) and lead sulfide (1100-2500 nm) detectors at 2 nm intervals and the 1000 data points were collected by a microcomputer and stored for later analysis. Calibration equations were generated using partial least-squares regression which combines principal components and reference chemistry to arrive at the final loadings.

Results

Digestibility trial

The *in vivo* digestibilities of the diets are shown in Table B1, together with the digestibilities calculated for the supplements, assuming no associative effects.

The calculated digestibilities of the SFM supplements were somewhat lower than is usual for this supplement (~65%) but errors attendant to the assumption of 'no associative effects' must be emphasised. The digestibility of SFM25 supplement appeared lower than the more degradable sunflower meals but whether or not this is a real effect must await measurements

of rumen degradability, which are yet to be done. The digestibilities of the other supplements were as expected, though that for FM was lower than usual. As might be expected, the digestibility of the wheat starch was essentially 100%.

Table B1. Estimated *in vivo* digestibilities of the diets and calculated digestibilities of the supplements

Diet	*In vivo* digestibility	Supplement	Calculated digestibility
Control	60.3±1.35		
SFM75	61.1±1.25	SFM75	63.1±0.99
SFM50	61.7±1.76	SFM50	65.1±2.86
SFM25	60.5±2.13	SFM25	60.8±4.19
CSM	63.6±1.89	CSM	71.6±3.25
FM	62.5±2.63	FM	72.4±8.61
FM + starch	64.8±2.67	(FM+starch) mix, starch	79.4±5.77, 99.5±6.69

The components of daily N balance, measured during the digestibility trial, are shown in Table B2. The effect of curvilinearity in response to treatment was examined by fitting both linear and quadratic terms in the analyses of variance. In addition, the response to treatment was partitioned into single degree of freedom comparisons (shaded area) as follows:

1. Control v. supplemented lambs,
2. Plant protein sources (SFM+CSM) v. animal protein source (FM),
3. FM v. (FM + starch),
4. Sunflower meal treatments v. CSM,
5. Effect of formaldehyde treatment of sunflower meal.

The N intake of control lambs (7.6 gN/d) was close to the expected 7.2 gN/d, equivalent to 45 gCP/d. The major treatment effect on N intake was, as expected, the difference between control and supplemented lambs. The supplemented diets were formulated to provide a total of 130 gCP/d (chaff 45 gCP/d; supplement 85 gCP/d) or 20.8 gN/d; on the SFM diets, N intakes were close to this (mean 20.6±0.31 gN/d). Lambs offered CSM or FM diets had significantly higher or lower N intakes respectively (Table B2), because the protein contents

Table B2. Components of nitrogen balance in digestibility trial

Variate	Nitrogen intake (g/d)	Faecal nitrogen (g/d)	Digestibility of N (%)	Supplement N dig. (%)	Urinary nitrogen (g/d)	Nitrogen balance (g/d)
Control	7.61	4.38	42.4	n/a	4.19	-0.96
SFM75	20.07	6.48	67.7	83.1	13.60	-0.01
SFM50	20.71	5.83	71.9	88.9	12.62	2.25
SFM25	21.12	6.82	67.7	82.0	11.35	2.96
CSM	22.42	7.24	67.7	80.7	12.91	2.27
FM	19.18	5.25	72.6	92.5	11.76	2.17
FM+starch	18.96	5.72	69.8	88.1	10.96	2.28
Treatment: linear	***	**	**	n/a	**	*
Treatment: quadratic	***	**	**	n/a	*	*
LSD (P<0.05)	0.43	0.85	3.7	6.4	2.30	1.8
Supp v. none	***	***	***	*n/a*	***	**
(SFM+CSM) v. FM	***	**	*	**	*0.10>P>0.05*	*NS*
FM v. FM+starch	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
SFMs v. CSM	***	*	*NS*	*NS*	*NS*	*NS*
Formaldehyde-treat	**	*0.10>p>0.05*	*	*0.10>p>0.05*	*NS*	*

found for these supplements differed from those used in the ration formulation. The differences in N intake, taken with those in faecal N excretion, resulted in significant treatment differences in N digestibility, with the digestibility of N in supplemented treatments being 25-30 percentage units higher than that of the control chaff. The FM treatments had slightly though significantly higher N digestibilities than the other supplemented diets, whilst the first level of formaldehyde treatment significantly increased the digestibility of N in diet SFM50. These effects are more clear in the N digestibilities calculated for the supplements themselves (assuming no associative effects) and provide strong evidence that the formaldehyde treatment did not 'over-protect' the SFM protein and thereby reduce the whole-tract digestibility of N.

The major treatment difference in urinary N excretion was between control and supplemented treatments, though there was a tendency for animals fed FM to have lower urinary N outputs. Animals fed the control diet were in negative N balance, and the provision of protein supplements effected a significant increase in N balance. The provision of untreated SFM brought animals essentially into daily N balance, but the provision of a like amount of N as SFM50 brought these animals significantly into positive N balance. The SFM25 supplement increased daily N balance slightly further; other supplemented groups were in similar N balance to those fed SFM50.

Feeding trial

The effects of supplement and type of animal (cryptorchid, ewe) on fasted live weights and *in vivo* body composition are shown in Table B3, for each of the two measurements (21d, 63d after the commencement of experiment). Within supplemented groups, the use of actual N intake as a covariate did not influence responses.

Cryptorchid lambs were significantly heavier when allocated into treatments ($P<0.001$) and as can be seen in Table B3, they maintained this advantage in fasted live weight throughout the trial. The use of allocation weight as a covariate removed the sex difference in fasted live weight and therefore was used as a covariate in all analyses of body composition. Nevertheless, by the end of the trial, cryptorchid lambs still had significantly higher body water contents in terms of both amount ($P<0.01$) and percentage of fasted live weight ($P<0.05$). The percentage fat in cryptorchids was also significantly lower ($P<0.05$) while the difference in the amount of fat approached significance ($0.10>P>0.05$). After adjusting for differences in allocation weight, there were no sex differences in body protein content.

Table B3. Responses of live weight and body composition to supplement and type of animal

Variate	Fasted wt after 21d	Fasted wt after 21d	Body water (kg) 21d	%Body water 21d	Fat (kg) 21d	%Fat 21d	Protein (kg) 21d	%Protein 21d
Covariate	-	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt
Control	31.9	31.6	18.9	59.9	6.9	21.7	4.2	13.2
SFM75	32.2	31.9	19.2	60.3	6.8	21.2	4.2	13.2
SFM50	31.7	32.1	19.6	61.0	6.5	20.3	4.2	13.1
SFM25	33.0	32.8	20.3	62.2	6.3	19.1	4.3	13.1
CSM	32.1	32.3	19.8	61.4	6.5	19.9	4.2	13.1
FM	30.8	31.2	20.1	64.4	5.1	16.3	4.1	13.3
FM+starch	32.1	31.9	19.9	62.4	6.0	18.7	4.2	13.2
Treatment	NS	NS	**	*	*	*	NS	NS
Cryptorchids	33.1	32.1	19.9	62.1	6.1	19.0	4.2	13.2
Ewes	30.8	31.8	19.4	61.1	6.5	20.2	4.2	13.2
Type	***	NS	*	NS	NS	NS	NS	NS
Variate	Fasted wt after 63d	Fasted wt after 63d	Body water (kg) 63d	%Body water 63d	Fat (kg) 63d	%Fat 63d	Protein (kg) 63d	%Protein 63d
Covariate	-	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt
Control	31.4	31.1	18.5	59.4	6.7	21.5	4.2	13.5
SFM75	33.8	33.5	20.3	60.5	7.0	20.7	4.4	13.2
SFM50	34.4	34.8	21.4	61.5	7.0	19.9	4.6	13.1
SFM25	35.4	35.2	21.7	61.7	6.9	19.6	4.6	13.1
CSM	34.9	35.0	20.4	58.3	8.3	23.8	4.6	13.1
FM	32.9	33.2	20.1	60.5	6.9	20.6	4.4	13.3
FM+starch	34.7	34.5	20.9	60.5	7.3	21.0	4.6	13.1
Treatment	***	***	***	NS	NS	NS	***	***
Cryptorchids	35.0	34.0	20.9	61.6	6.7	19.6	4.5	13.2
Ewes	32.9	33.9	20.0	59.1	7.6	22.5	4.5	13.2
Type	***	NS	**	*	0.10>P>0.05	*	NS	NS

After only 21d, there were already treatment differences in body composition, which related principally to the amount and proportion of fat in lambs consuming the FM diets. As can be seen in Table B3, by the end of the trial there were treatment differences in the amount of body water ($P<0.001$) and both the amount ($P<0.001$) and the proportion ($P<0.001$) of body protein. Most of these differences seemed related to the difference between unsupplemented and supplemented lambs.

The responses to supplements are seen more readily in terms of the daily gains of fasted live weight and its components, shown in Table B4. There were no differences between cryptorchid and ewe lambs in these gains so that the effect of sex of lamb is not considered further. Unsupplemented lambs lost fasted live weight over the 63d of the trial, though most of this loss was as body water. There was a pronounced effect of supplementation on all gains ($P<0.001$), as might be expected. Protein source had no significant effect on fasted liveweight gain but within the SFM treatments, lambs consuming SFM50 and SFM25 grew faster ($P<0.05$) than those given untreated SFM. They also gained significantly more protein ($P<0.05$) and ash ($P<0.05$).

As would be expected, supplemented animals gained more fat than the control lambs ($P<0.01$) which lost a small amount of fat per day. Lambs consuming the plant protein sources (SFM, CSM) gained less fat than those on the two FM treatments ($P<0.01$) and within the plant protein sources, lambs consuming CSM gained much more fat than those on any of the SFM treatments ($P<0.001$). When the data presented in Table B4 were examined further by partitioning the treatment effect into the same single degree of freedom comparisons, lambs consuming CSM were found at the end of the study to contain significantly more fat ($P<0.05$) and a higher proportion of fat ($P<0.01$) than those consuming the SFM supplements. Similarly, those consuming (FM+starch) had significantly more body protein than those given FM alone ($P<0.05$) though the absolute difference was small (4.6 v. 4.4 kg). Moreover, the higher protein content of those given (FM+starch) actually formed a significantly lower proportion of the final fasted live weight than in those consuming FM (13.1% v. 13.3%; $P<0.05$). Effects on protein content were therefore small relative to effects on fat content.

Trends in the gains in ash content were similar to those in fasted gains, as were those in the sum of body components. The latter data are presented principally as a check on the accuracy of estimation of body components by deuterium oxide dilution and indicate that on average,

Table B4. Responses of liveweight and body composition gains to supplement and type of animal

Variate	Fasted gain g/d (fleece-free)	TBWgain g/day	Fat gain g/day	% Fat in gain/loss	Protein gain g/day	% Protein in gain/loss	Ash gain g/day	Total dry gain g/day	Dry+TBW gains g/day	FCE (g feed/g LWG)
Control	-17.8	-11.4	-8.0	25.2	0.2	3.6	1.2	-6.6	-18.0	-22.8
SFM75	30.4	24.3	-2.5	negative	4.8	16.5	2.0	4.3	28.5	27.7
SFM50	54.9	44.7	-0.4	negative	7.1	13.5	2.4	9.0	53.7	16.0
SFM25	47.0	31.5	5.8	13.3	6.4	14.6	2.3	14.4	45.9	19.2
CSM	55.9	15.1	32.8	53.7	7.2	13.3	2.4	42.4	57.6	15.0
FM	40.3	2.0[A]	31.5	43.0	5.7	15.3	2.1	39.4	41.3	20.1
FM+starch	54.0	23.3	22.3	31.1	7.0	13.4	2.3	31.6	54.9	14.1
Treatment	***	***	***	*	***	*	***	***	***	***
LSD (P<0.05)	18.1	21.0	22.0	48.4	1.7	3.3	0.3	22.7	18.1	12.6
Supp v. none	***	***	**	*	***	*	***	***	***	***
(SFM+CSM) v. FM	*NS*	**	**	*	*NS*	*NS*	*NS*	**	*NS*	*NS*
FM v. FM+starch	*NS*	*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
SFMs v. CSM	*NS*	*	***	**	*NS*	*NS*	*0.10>P>0.05*	***	*	*NS*
Formaldehyde-treat	*	*NS*	*NS*	*NS*	*	*NS*	*	*NS*	*	*NS*
Cryptorchids	34.4	19.0	8.1	12.8	5.2	13.7	2.1	15.3	34.3	16.3
Ewes	41.2	17.9	15.2	17.7	5.8	12.0	2.1	23.1	41.1	9.3
Type	NS	NS	NS	NS	NS	0.10>P>0.05	NS	NS	NS	*

[A]On this treatment, cryptorchids lost 4.8 g/d TBW, ewes gained 8.8 g/d

99.6% of fasted weight gain could be accounted for in the sum of the estimated gains in body components.

Treatment effects on feed conversion efficiency (FCE) are also shown in Table B4; in assessing treatment responses it should be remembered that FCE calculated for small gains or losses either side of weight maintenance are very sensitive to small changes in weight. Nevertheless, there was a large effect of supplementation on FCE ($P<0.001$) as might be expected. Surprisingly, ewes had a significantly better FCE than cryptorchids, despite their greater fat gains. This may in part be an artefact of the greater variability in FCE which was found in the cryptorchids. In the ewes, the effect of formaldehyde-treatment of SFM on FCE was more consistent and marked than in the cryptorchids (32.6, 16.9, 13.6 in SFM75, SFM50, SFM25 respectively) and also approached significance ($0.10>P>0.05$). Within the cryptorchids, there were no significant differences in FCE between the supplemented treatments.

The effects of the various protein supplements on wool growth were more marked than on liveweight or body composition changes, with significant treatment effects observed for greasy fleece weights, wool length growth and fibre diameter, washing yield and daily greasy and clean dry wool (CDW) growth rates (Table B5).

As with changes in live weight and its components, the effect of supplementation itself was marked but significant differences were also observed within the supplements. Lambs fed plant protein supplements had heavier fleeces than those fed the FM treatments ($P<0.01$), principally because of the significantly heavier fleeces found in the SFM50, SFM25 and CSM treatments. Although similar trends are obvious for wool length growth, fibre diameter and daily wool growth, these were not statistically significant.

By contrast, formaldehyde treatment of the SFM supplement consistently influenced all the measured parameters of wool growth. Fleece weights of lambs fed SFM50 and SFM25 were about 25% heavier than those of lambs fed untreated SFM (SFM75), while wool length growth rate was increased about 10-20% ($0.10>P>0.05$) and fibre diameter 10-15% ($P<0.01$). These resulted in a significant increase of about 15-30% in daily greasy wool growth. The response in daily CDW was 22-28%, despite the slight reduction in washing yield effected by the formaldehyde-treated SFM.

Table B5. Responses in wool growth to supplement and type of animal (all values adjusted by covariance for amount, yield or fibre diameter of wool grown before experiment commenced)

Variate	Greasy fleece weight (g)	Wool length growth(µm/d)	Yield of wool grown (%)	Fibre diam (micron)	Wool growth g/d greasy	Wool growth g CDW/d
Control	1213	237	79.7	21.5	11.2	8.9
SFM75	1375	278	75.7	24.1	13.0	9.8
SFM50	1718	308	71.1	26.8	15.2	11.0
SFM25	1721	326	73.0	27.6	17.1	12.5
CSM	1570	299	73.0	25.5	15.3	11.2
FM	1434	310	74.6	25.6	12.6	9.4
FM+starch	1440	303	75.6	24.5	15.6	10.8
Treatment	***	***	**	***	**	*
LSD (P<0.05)	174	43	4.2	1.8	2.9	2.3
Supp v. none	***	***	***	***	**	*
(SFM+CSM) v. FM	**	*NS*	*NS*	*NS*	*NS*	*NS*
FM v. FM+starch	*NS*	*NS*	*NS*	*NS*	*0.10>P>0.05*	*NS*
SFMs v. CSM	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*
Formaldehyde-treat	***	*0.10>p>0.05*	*0.10>P>0.05*	**	*	*
Cryptorchids	1529	280	74.5	24.5	14.7	10.8
Ewes	1462	309	74.8	25.7	13.9	10.2
Type	NS	*	NS	*	NS	NS

Responses in urinary metabolites and possible development of NIRS calibration for urinary purines

As discussed above, samples obtained from the digestibility trail component of this experiment allowed an assessment of possible effects of the dietary treaments on urinary urea, creatine/creatinine and purine derivatives. Moreover, collaboration with Dr S.W. Coleman allowed an attempt to derive an NIRS calibration set for urinary purines, a set which would obviate the need for complex wet chemistry (HPLC) of all samples.

Responses of the urinary metabolites to treatment are shown in Table B6. There were marked curvilinear effects of supplementation on the urinary excretion of both urea and creatine. Within the supplemented animals, those consuming FM plus starch excreted significantly more urinary urea and creatine, while there was a significant trend ($P<0.05$) for formaldehyde treatment of SFM to reduce the excretion of urea and creatine. Creatinine excretion was unaffected by diet. Perhaps surprisingly, there were no marked effects of treatment on the excretion of individual or total purine derivatives in urine, though responses in the proportion of of the major derivative allantoin, were similar to those observed for urea and creatine.

By contrast, attempts to establish NIRS calibration equations for purine derivatives, creatine and creatinine were very encouraging (Table B7). In the tabulated data, 'SE calibration' is estimated from the sum of squares of residuals of the regression relationship between spectral and analytical data; the coefficient of determination in column 3 refers to these relationships. The 'SE validation' (strictly 'cross-validation') is an indicator of the extent to which each value in the data set can be individually predicted when the calibration is established using all remaining values; r_v^2 refers to the goodness of fit of these relationships.

Uric acid levels in urine varied little between treatments (Table B6) and were not well predicted by NIRS (Table B7, column 4). To a lesser extent, the same was true of hypoxanthine. However, it is clear from the results in Table B7 that for most of the other metabolites, the attempts to establish NIRS calibration equations were extremely encouraging.

Table B6. Concentrations of urinary metabolites in sheep from digestibility trial

Treatment	Urinary concentration (mmol/L) of: Urea	Creatine	Creatinine	Allantoin	Uric acid	Hypoxanthine	Xyanthine	Proportion allantoin
Control	2.94	7.39	8.08	9.45	4.77	2.89	2.07	0.519
SFM75	6.05	5.02	8.49	9.42	3.87	1.34	1.57	0.581
SFM50	5.80	4.77	7.30	11.58	4.99	1.50	1.21	0.602
SFM25	5.37	2.79	8.09	10.40	3.30	1.08	0.91	0.669
CSM	5.41	4.04	8.16	9.48	2.87	1.24	1.13	0.632
FM	4.67	4.06	7.22	7.30	2.98	0.83	0.77	0.619
FM+starch	6.87	5.95	10.77	9.91	3.54	1.18	1.24	0.603
Treatment: linear	***	***	NS	NS	NS	NS	NS	***
Treatment: quadratic	***	***	NS	NS	NS	NS	NS	**
LSD (P<0.05)	0.57	1.77	4.24	5.13	1.78	2.03	1.41	0.076
Supp v. none	***	***	NS	NS	0.10>P>0.05	*	0.10>P>0.05	**
(SFM+CSM) v. FM	NS	NS	NS	NS	NS	NS	NS	NS
FM v. FM+starch	***	*	NS	NS	NS	NS	NS	NS
SFMs v. CSM	NS	NS	NS	NS	NS	NS	NS	NS
Formaldehyde-treat	*	*	NS	NS	NS	NS	NS	0.10>P>0.05

Table B7. Standard errors and corresponding coefficients of determination of partial-least squares relationships between NIRS spectral data and nitrogen metabolite concentrations in urine of unsupplemented lambs or lambs consuming a range of protein supplements

Constituent	SE calibration	r^2	SE validation	r_v^2
Creatine	0.776	0.92	1.487	0.72
Creatinine	0.496	0.93	0.755	0.83
Allantoin	0.667	0.95	1.194	0.86
Uric acid	0.864	0.61	1.135	0.34
Hypoxanthine	0.254	0.60	0.283	0.49
Xyanthine	0.344	0.86	0.464	0.74
Total purine derivatives (PD)	2.239	0.93	3.696	0.80
Total PD + creatine/creatinine	1.723	0.88	3.027	0.65

Discussion

The dietary regimens in this study were established on the basis that lambs would maintain weight when fed 600g/d of the control diet. It is clear from the data presented above (Tables B3, B4) that the change in fasted live weight over the trial was close to maintenance. By contrast, supplemented lambs gained up to 5kg weight over the course of the trial, with daily liveweight gains (fleece-free) ranging from 30-55 g/d (Table B4).

From the body protein gains (Table B4) and wool growths (Table B5) observed in this study, it can be calculated that expected daily N balances should have been in the range 1.3-2.8 gN/d, with supplemented animals in the range 2.2-2.8 gN/d. As the data in Table B2 indicate, the observed levels of N balance were in this range for all but the control treatment (expected 1.3 gN/d; observed -1.0 gN/d) and SFM75 treatment (expected 2.2 gN/d; observed 0 gN/d). Hence for the formaldehyde-treated SFM supplements, there was conformity between observed wool growths and body protein gains and the observed N balances, suggesting that the formaldehyde treatment had not reduced the whole-tract availability of supplement protein.

The use of FM, with or without starch did not result in increased protein gains, but rather resulted in increased rates of gain in body fat and higher proportions of fat in the daily gain

(Table B4). Moreover, the rate of gain of fasted live weight and final fasted live weights were no higher than the equivalent responses of lambs fed plant protein sources. At least in terms of the basal ration and level of feeding employed in the present study, there seemed no advantage to using this expensive animal protein source.

Within the plant protein supplements, the highest absolute rate of gain was in animals fed CSM rather than the SFM treatments, but in contrast to a range of previous studies (see **Introduction**) the use of CSM resulted in much higher rates of fat gain (Table B4) and animals of higher fat content (Table B3). This result was unexpected, but may be a consequence of problems with the estimate of final body water content in this treatment (see below).

Lambs consuming SFM had, on average, rates of gain comparable with those consuming the other supplements, and gained significantly less fat. Moreover, reducing the degradability of SFM protein increased both liveweight gain and body protein gain. Lambs fed SFM25 had over twice the rate of fat gain than those on the other SFM treatments, though this difference was not statistically significant. In terms of desired changes in body composition, lambs fed SFM50 had among the fastest rates of liveweight gain and protein gain, but did not gain body fat. By contrast with some previous studies (eg, MRC-Project DAN056), the feeding of supplements with decreased rumen protein degradability did not result in significant inprovements in FCE, though this result should be interpreted with caution because of the relatively low rates of weight gain involved in the present study,

Responses in wool growth were not of primary concern within this study, though they are still of interest in terms of helping to explain treatment responses and thus to provide the information required to modify supplement response mechanisms in **GrazFeed**. Moreover, the improved wool growth is important in practical terms, by increasing the value of skins from meat-type lambs. The efficiency of wool growth, defined as the daily growth of CDW/kg DM intake, was of the same order as those reported by Masters *et al.* (1998b). For treatments SFM75, SFM50 and SFM25 the efficiency increased progressively (13.1, 14.8, 16.7 gCDW/kg DMI), though only the difference between SFM75 and SFM25 was significant ($P<0.05$). Efficiencies calculated for other supplement treatments did not differ significantly from these or within themselves. The data in Table B5 again suggest there is little advantage in feeding expensive FM supplements, and indicate significant responses of

wool growth to both SFM and to the formaldehyde-treatment of that supplement. Responses to CSM were intermediate between those of FM and the SFM50/SFM25.

The preliminary analyses presented in this report suggest that, on balance, SFM treated to reduce its protein degradability to about 50% would be the preferred supplement. The most unexpected response in the present study was the very high level of fat gain found with the CSM supplement. This was the consequence of the low percentage final body water and thus small increase in body water content found on this treatment (see Tables B3, B4 and the equations of Donnelly and Freer (1974)) and suggests the possibility of error in the estimation of the final body water content in animals on this treatment. In turn, this suggests a need to re-examine the response to graded levels of CSM in a similar group of lambs. After discussion with Drs Johnsson and Thatcher of MRC, this became the objective of the final experiment in the project, discussed in the following section.

B3. Evaluation of the live weight and body composition responses of lambs consuming graded levels of cottonseed meal (CSM) supplement

This study addressed Objective 4 of the Project, modified on the basis of the previous experiment, as described above. At the time of writing, the lambs involved in this final experiment have yet to be shorn, so wool growth data are not available. Moreover, this necessitates the presentation of live weight and body composition data without correction for fleece gains.

Methods

Animals and feeding

Thirty close-shorn crossbred lambs of the same provenance of those used in the previous experiment were housed indoors in the Ginninderra Animal House. Half the lambs were ewes (live weight at allocation 37.1±0.44 kg) and, since cryptorchid lambs were unavailable, the other half were wether lambs (allocation live weight 38.5±0.35 kg).

In order to clarify the response to CSM as observed in the previous experiment, 28 of the lambs were divided into four groups which received one of the following diets (7 lambs/diet):

1. Medium quality chaff similar to that used in Section B2 above, fed at a level calculated to maintain live weight (600 g DM/d; Control),
2. Chaff + 240 g CSM (42.5% CP) from the same source as in Section B2 above,
3. Chaff + 360 g CSM,
4. Chaff + 480 g CSM.

The remaining two spare lambs received the control diet, but since they were not required to replace any of the original 28 animals selected, these two animals were ultimately included in the statistical analyses of the experiment. Hence, the control diet comprised 9 animals and all other diets 7 animals. Moreover, dietary treatment and lamb gender were not balanced. All aspects of daily management of the lambs were as described in Section B2 above

Measurements

After an adjustment period of 18 days on the experimental diets (cf 21d in the previous experiment), and again 50d later, estimates of deuterium oxide dilution space were made using the techniques described above. Dyebands to estimate daily wool growth were inserted on each occasion, but these data are not yet available since lambs have yet to be shorn.

Statistical analyses

Treatment effects were initially assessed and least significant differences (LSD, $P<0.05$) calculated from single classification analyses of variance. Possible differences in response of ewes and wethers were examined using multiple regression, which also provided an estimate of the response of live weight and body composition per 100 g of CSM consumed.

Results

The effects of level of CSM supplement and of sex (wether, ewe) on fasted weights and *in vivo* body composition are shown in Table B8, for each of the two measurements (18d, 68d after the start of the experiment). To allow comparison with the previous experiment, results for the equivalent treatment (600 g/d chaff DM + 240 g/d CSM) are also included. Allocation weight was used as a covariate in these analyses, since wethers were heavier than ewes at allocation. Nevertheless, there were significant effects of gender, especially on final live weights and body compositions. Wethers had heavier final live weights than ewes and contained more body water and protein, but the difference in fat content

Table B8. Responses of live weight and body composition to level of cottonseed meal (CSM; 240, 360 or 480 g/d)

Variate	Fasted wt[C] after 18d	Fasted wt after 18d	Body water 18d	%Body water 18d	Fat 18d	%Fat 18d	Protein 18d	%Protein 18d
Covariate	-	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt
Control	31.1	31.2	19.2	61.7	5.7	18.0	4.4	14.1
CSM240	32.5	33.0	19.9	60.2	6.6	19.8	4.6	13.8
CSM360	34.1	33.5	19.8	59.1	7.2	21.2	4.6	13.8
CSM480	33.5	33.5	19.9	59.5	7.0	20.7	4.6	13.8
CSM240 Previous (21d)	*32.1*	*32.3*	*19.8*	*61.4*	*6.5*	*19.9*	*4.2*	*13.1*
Treatment	*	**	NS	*	**	*	**	**
LSD (P<0.05)[B]	2.1/2.2	1.5/1.6	0.9/1.0	1.9/2.0	0.8/0.9	2.1/2.2	0.1/0.2	0.2/0.2
Response/100g CSM[A]		0.49**	NS		0.30***		0.05**	
Type (weth. v. ewe)[A]		*	*	NS	NS	NS	*	*

Variate	Fasted wt after 68d	Fasted wt after 68d	Body water 68d	%Body water 68d	Fat 68d	%Fat 68d	Protein 68d	%Protein 68d
Covariate	-	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt	Alloc. wt
Control	29.8	29.9	18.8	63.0	4.9	16.3	4.3	14.4
CSM240	34.4	34.8	21.8	62.8	6.0	17.2	4.8	13.8
CSM360	37.6	37.1	22.6	60.9	7.4	19.8	5.0	13.5
CSM480	38.2	38.1	23.2	60.8	7.6	19.9	5.1	13.4
CSM240 Previous (63d)	*34.9*	*35.0*	*20.4*	*58.3*	*8.3*	*23.8*	*4.6*	*13.1*
Treatment	***	***	***	NS	***	*	***	***
LSD (P<0.05)[B]	1.8/2.0	1.4/1.5	1.2/1.2	2.7/2.9	1.2/1.2	2.9/3.1	0.14/0.15	0.2/0.2
Response/100g CSM[A]		1.79***	0.97***		0.57***		0.18***	
Type (weth. v. ewe)[A]		*	***	NS	NS	NS	*	*

[A]Based on results of multiple regression analysis
[B]First value for comparison of control with other treatments; second value for comparisons within CSM treatments
[C]All weights in kilograms and uncorrected for fleece growth

was not significant. However, none of these effects was large and for the remainder of this report, discussion will focus on treatment responses.

As in the previous experiment, effects of treatment on live weight and body composition were apparent by the end of the adjustment period (18d). Lambs fed CSM supplement were already larger ($P<0.01$) and contained more protein ($P<0.01$) and fat ($P<0.01$). At this stage, the fasted live weight, body water and body fat contents of lambs fed CSM240 were very similar to those of lambs fed the equivalent treatment in the previous experiment, while their protein content was slightly larger.

By the end of the experiment (68d), there were pronounced effects of supplementation on all body components except body water content, which was also the response which distinguished CSM240 lambs from the equivalent treatment in the previous experiment. As a result, lambs fed CSM240 were estimated to contain much less fat than those on the equivalent earlier treatment, though their fasted weights and protein contents were similar.

Significant differences between the levels of CSM offered and consumed were also observed. These are probably best summarised by the responses per 100g CSM, derived by multiple regression and also shown in Table B8. In these terms, the response to CSM by 68d was 1.8kg live weight, 1.0kg body water, 570g fat and 180g protein, per 100g CSM.

Responses in daily gains of fasted live weight and its components are summarised in Table B9. Lambs given no supplement lost slightly more weight than those on the equivalent treatment in the previous experiment; this effect will be more marked when wool growth is taken into account. Lambs on treatment CSM240 grew less fast than on the equivalent treatment in the previous experiment, though a highly significant 'dose response' to CSM is obvious, amounting to 26g/d fasted liveweight gain per 100g CSM consumed ($P<0.001$). Responses in the daily gain in total body water (Table B9) show similar pattern ($P<0.001$), with the value for CSM240 lambs being more than twice that of the equivalent lambs in the previous experiment. As a result, daily gains in body fat show a consistent and significant response of just over 5g body fat gain/100g CSM ($P<0.05$), with lambs fed CSM240 showing a loss of about 8g/d body fat in this experiment, compared with a gain of 33g/d in the previous experiment. By contrast, gains in other body components are more similar between experiments.

Table B9. Responses of liveweight and body composition gains to level of cottonseed meal (CSM; 240, 360 or 480g/d)

Variate	Fasted gain g/day	TBWgain g/day	Fat gain g/day	Protein gain g/day	% Protein in gain/loss	Ash gain g/day	Total dry gain g/day	Sum dry+TBW gains g/day
Control	-27.1	-9.5	-15.0	-1.8	7.0	0.4	-16.4	-25.9
CSM240	38.3	38.5	-7.7	4.8	14.1	1.6	-1.4	37.1
CSM360	69.7	57.3	0.4	7.8	11.3	2.1	10.3	67.7
CSM480	93.7	66.5	12.2	10.1	10.8	2.5	24.8	91.3
CSM240 Previous (63d)	*55.9*	*15.1*	*32.8*	*7.2*	*13.3*	*2.4*	*42.4*	*57.6*
Treatment	***	***	NS	***	***	***	*	***
LSD (P<0.05)[B]	24.4/25.9	21.5/22.9	22.9/24.3	2.4/2.6	2.7/2.9	0.4/0.5	24.4/25.9	23.7/25.2
Response/100g CSM[A]	*25.9****	*16.8****	*5.3**	*2.5****	*0.9**	*0.5****	*8.3****	*25.0****
Wethers v. Ewes[A]	NS	NS	*	NS	NS	NS	*	NS

[A]Based on results of multiple regression analyses
[B]First value for comparison of control with other treatments; second value for comparisons within CSM treatments

Discussion

The results presented in Tables B8 and B9 show an incremental response of live weight and body composition to increasing consumption of CSM supplement, with the response to 240g/d CSM indicating fat loss rather than the rapid fat deposition noted in the previous study. Moreover, the changes in body fat noted over the range of intakes of CSM are similar in magnitude to those found with the SFM supplements in the previous study, after allowance is made for the larger losses of weight and body fat of lambs fed the control ration. Taken together, these data strongly suggest that, as suggested earlier, the apparent gains in body fat seen with CSM in the previous study represent errors in the estimation of final total body water content of the lambs on that treatment.

In addition, the data from the present experiment suggest that if grazing lambs were to consume approximately the same amount of herbage OM as the control lambs, the provision of about 400g/d CSM would result in liveweight and protein gains of about 80 and 9g/d respectively, with the daily gain of only about 2.5g fat. Maintained over 75d of supplementary feeding in the field, these responses would amount to gains of 6kg in live weight, 0.7kg in protein content and less than 0.2kg in fat content. Body composition could be manipulated by feeding either lower of higher levels of CSM. For example, the data in feeding half the amount of CSM (200g/d) for 100d would result in gains of 2.8kg in live weight and 380g in body protein, but a loss of almost 1kg in body fat content. In larger lambs such as those used in the present study, such an effect could have economic consequences.

However, the relativities between the treatments responses shown in Tables B8 and B9 will alter when fleece growth is taken into account, though the general response will stay the same. Moreover, the calculations in the previous paragraph are all approximations based on intakes and responses staying the same over the course of the projected feeding period. A better prediction of the likely response to supplements can be gained through the use of decision-support packages such as **GrazFeed**, but only if this package adequately represents the response of lambs to the improved amino acid supply effected by the slowly-degrading protein sources such as CSM or the formaldehyde-treated SFM used in the previous experiment. The current version of **GrazFeed** would not have predicted the responses described in Section B of this report. The next major section of the report is devoted to a description of proposed changes to this package, so that it can more adequately predict responses such as those observed in the current section of the report.

SECTION C: Implications of the observed effects of the rumen degradability of protein supplements for the modelling of live weight, body composition and wool growth in *GrazFeed*

The final objective of the Project, as modified during discussion with Drs Johnsson and Thatcher (May 1998), was either to modify or to propose modifications to the decision support system **GrazFeed**, to allow it better to model the response to slowly-degrading protein supplements. In order to approach this and to see how the results in Section B of this report could be used to modify **GrazFeed**, it is first necessary to examine how the package currently models responses in live weight, body composition and wool growth.

C1. *GrazFeed* - the current situation

GrazFeed models the use of energy and protein for animal production based on the Australian Feeding Standards as encapsulated in SCA (1990). The animal biology model within **GrazFeed** is described in detail by Freer *et al.* (1997) and only a summary can be presented here.

Responses of animals to consumed nutrients are modelled in terms of metabolisable energy (ME) intake and the supply of truly digestible protein leaving the stomach (DPLS). The latter is in turn computed as a function of microbial CP (MCP) supply plus the truly digestible portion of the undegraded dietary protein (UDP; see Freer *et al.* 1997). Two points should be noted here. First, although the treatment of a dietary protein to reduce its rumen degradability will increase the supply of UDP, it also has the potential to reduce the supply of MCP, if the treatment results in there being insufficient rumen degradable protein in the consumed diet. The overall effect on the supply of DPLS is thus a balance between these two effects. Second, a distinction needs to be drawn, especially for the modelling of wool growth, between the effect of reduced rumen protein degradability on DPLS supply and its effect on the amino acid composition of that part of the DPLS which comes from UDP. **GrazFeed** currently accommodates the former effect, but not the latter.

In predicting the response to ME and DPLS supply, **GrazFeed** first estimates the requirements for maintenance, then the additional requirements for pregnancy and lactation should these states prevail. Responses in wool growth and live weight and its components are then predicted. In considering weight gain in lambs, the requirements for pregnancy/lactation are clearly not relevant. Despite the fact that the lamb production system is aimed at optimising the response in terms of lean meat (body protein), in considering possible ways in which **GrazFeed** might be modified it is instructive first to examine how the response in wool growth is estimated.

Modelling wool growth in GrazFeed

In a sense, the modelling of wool growth is simpler because the product is essentially protein and the energy cost of wool growth is not great. Leaving aside the effects of potential fleece weight and of the relative size (maturity) of the animal (as it increases surface area but reduces wool follicle density), daily wool growth is predicted as the minimum of that estimated from the amount of DPLS available for wool production, or that estimated from the ME available for wool production. In the current version of **GrazFeed**, the 'switch' from DPLS-driven to ME-driven wool growth occurs when the ratio of DPLS/ME (g/MJ) exceeds 12.0 (see Freer *et al.* 1997 for supporting discussion and references).

If the above ratio is <12, then the predicted efficiency of conversion of DPLS to clean wool is 11.6% for average Merino sheep (Hogan *et al.* 1979). Greasy wool production is then calculated from CDW production and the percentage yield. It must be stressed that this efficiency of conversion from DPLS to CDW is only appropriate for the situation in which most of the DPLS is of microbial origin, albeit that this is the usual situation. If supplements are modified in a manner which reduces their rumen protein degradability and results in increases in the post-ruminal supply of UDP and especially the limiting sulfur-containing amino acids (SAA), then an increase in the efficiency would be required if the response were to modelled adequately (see Stuth *et al.* 1999). The present results with SFM, which contains substantial quantities of SAA, plus those in recent publications using canola meal, which has a substantial degree of rumen-protection, (Mata *et al.* 1995, Masters *et al.* 1998a, Masters *et al.* 1998b) indicate that within **GrazFeed**, there is now a need to allow for the effect of the amino acid compositionn of UDP on the efficiency of conversion of DPLS to wool growth. The data in these studies also provide the information with which to make the necessary modifications to **GrazFeed**.

In the current **GrazFeed**, if the supply of DPLS is such that DPLS/ME exceeds 12, then the wool growth calculated from the ME supply will prevail, rather than that calculated from the protein supply. The rate of wool growth per MJ ME will be 12.0 * 0.116 or 1.392 g/MJ. Hence a hypothetical situation can arise in which a protein supplement is treated so as to increase UDP and thus DPLS supply, with the result that DPLS/ME exceeds 12.0 and wool growth becomes ME-driven. It is for this reason that in order to allow **GrazFeed** to respond to the provision of the same amount of protein supplement but supplying different amounts of SAA beyond the rumen, the alteration of efficiencies of conversion of DPLS to wool is probably the more useful approach.

Modelling gains in live weight and body composition in GrazFeed

In the current version of **GrazFeed**, the weight change is predicted as a function of the ME supply after allowing for the energy requirements for maintenance and wool growth (see Freer *et al.* 1997). Hence the first step within the prediction of weight change is the estimation of the energy and, secondarily, the protein available for that weight change. Based on the degree of maturity of the animal, the model estimates the energy content of empty weight gain; the potential gain is then the energy supply divided by the energy content of gain.

Again, based on the relative maturity of the animal, **GrazFeed** then estimates the protein content of the potential weight gain and checks whether the supply of DPLS available for gain is sufficient to allow the potential to be reached. If it is not, weight gain is reduced accordingly.

It should be stressed that the above calculations in **GrazFeed** are based on concepts derived from the analysis of body composition changes by Searle and Griffiths (1976). This has several consequences. First, it is the supply of energy compared with the energy content of gain which determines body composition. Although the calculation allows DPLS supply to influence liveweight gain, it does so indirectly. Moreover, should DPLS supply already be 'adequate' in these terms, then the provision of extra DPLS by a reduction in rumen degradability will not result in any further increase in weight gain. Second, there is no mechanism whereby an improvement in the amino acid pattern of UDP can influence the body composition. This is probably an inadequate approach, since recent data indicate that such improvements can influence liveweight change and body composition, as well as wool

growth (eg, Masters *et al.* 1998b). Finally, the current approach does not permit gains in protein and body weight but losses of fat, such as those observed by Vipond *et al.* (1989) and those in Tables B4 and B9.

A potential mechanism whereby **GrazFeed** could allow such a response to reduced degradability of dietary protein might be as follows:

1. A potential empty liveweight gain is estimated from the ME supply above maintenance and the energy content of empty bodyweight gain for the maturity of animal being considered.
2. A second potential empty liveweight gain is estimated as some function of the DPLS supply, the protein content of empty gain and the efficiency of use of DPLS for gain.
3. If the second estimate of potential gain is higher, then it predominates and the extra ME required is obtained via the mobilisation of existing body fat.

However, such a 'comparative supply/demand' approach will in most situations result in a predicted DPLS-driven weight gain which is much greater than that driven by energy supply, and which could not be accommodated by the combination of dietary energy supply and fat mobilisation.

A more potent approach is probably to alter the premise of the calculations within **GrazFeed** such that below a certain (low) level of intake, the predicted protein content of gain increases markedly with a corresponding decrease in the fat content of gain, such that the energy content of gain falls markedly. For a given ME supply available for gain, this will have several consequences.

1. The predicted bodyweight gain at low levels of intake will be higher than that predicted by the current **GrazFeed**.
2. The protein content of that gain will be higher.
3. It would be possible to have protein gains but losses of fat.

This proposed alternative approach is discussed in more detail below.

C2. *GrazFeed* - possible modifications

In this section, possible modifications to the **GrazFeed** decision support sytem are proposed. Two points should be stressed.

1. Although the modifications can be proposed here and shown to be biologically sensible, they have to be regarded as conceptual and indicative of the final changes, rather than quantitatively definitive. Their incorporation into a released version of **GrazFeed** must await validation against experimental data.
2. **GrazFeed** predictions of the gains in empty body weight and its components are based on the ME and protein available after the demands for wool growth have been met. Hence, proposed modifications to the wool growth algorithms in **GrazFeed** have to be considered first, followed by proposed modifications to the weight gain algorithms.

Modifications to the GrazFeed *wool growth algorithms*

The issue of how to modify **GrazFeed** so that it better responds to dietary proteins which provide increased amounts of SAA post-ruminally has been approached by using the current version to estimate the supply of ME and DPLS in a 35 kg crossbred wether weaner consuming a diet of similar composition to the SFM-based diets described in Section B2 above. Moreover, wool growths were predicted for ME intakes of 6.6 MJ/d, which is close to that prevailing in the experiment described in Section B2, as well as intakes of 7.6, 8.6 and 9.6 MJ/d. In order to simplify the number of variables being considered, constraints related to the potential wool growth of the animals or to their size relative to mature size were ignored. The predicted wool growths therefore represent only the interaction between the DPLS and the ME supplied by the hypothetical diets. In using **GrazFeed** under practical circumstances, the effects of potential wool growth and of relative size would have to be considered.

The results of the **GrazFeed** predictions, with either constant or progressively changing efficiencies of use of DPLS for wool growth, are shown in Figure C1, in which daily clean wool growth rates are related to the supply of DPLS and ME. The basic question addressed was whether the prediction algorithms could be altered to accommodate the increased supply of DPLS of better amino acid composition, which might arise from decreased rumen degradability of supplement protein?

The results in Fig. C1(a), estimated using the current version of **GrazFeed**, can be likened to the familiar N intake:N balance relationships at a range of energy intakes. At each level of ME intake, wool growth increases with increasing DPLS supply at a rate of 0.116 g wool/g DPLS, up to the point that DPLS no longer limits wool growth. The daily wool growth is then a function of the ME supply, at the rate of 1.392 g wool/MJ ME as described above. At each level of ME, the slope of the lines relating DPLS to wool growth is the same, so that the slope of the overall line is also 0.116.

The results shown in Fig. C1(b) were obtained with an amendment to the efficiency of conversion of DPLS to wool growth so that, unlike the current version of **GrazFeed**, the efficiency of conversion of DPLS to wool itself increases as the proportion of DPLS derived from undegraded supplement protein increases. The actual steps in this revised algorithm were as follows:

1. The lowest level of DPLS for a given ME intake occurred when the degradability of the hypothetical supplement was 0.80. This was taken as the 'baseline' wool growth for the given ME intake and DPLS supply converted to wool with an efficiency of 0.116 as in the current algorithm, regardless of whether the DPLS was derived from microbial protein ($DPLS_{MCP}$) or from undegraded dietary protein ($DPLS_{UDP}$). Hence,

$$\text{Wool (g/d)} = 0.116*(DPLS_{MCP}) + 0.116*(DPLS_{UDP})$$

2. As the degradability of supplement protein was progressively reduced at a given ME intake, DPLS supply increased and a larger proportion was derived from the supplement. $DPLS_{MCP}$ was converted to wool with an efficiency of 0.116, as above. The remaining DPLS, derived from UDP was regarded as 2 pools. The first pool, equal in size to the original $DPLS_{UDP}$ in 1. above, was also converted to wool with an efficency of 0.116. The second pool, representing the increase in the $DPLS_{UDP}$ attributable to the decreased supplement degradability, was converted to wool with an efficiency of 0.25. Hence,

$$\text{Wool (g/d)} = 0.116*(DPLS_{MCP}) + 0.116*(\text{original } DPLS_{UDP}) + 0.25*(\text{increase in } DPLS_{UDP})$$

The choice of 0.25 as the incremental efficiency was based on recent estimates of the efficiency of wool growth (g/g DPLS) obtained in studies in which the sulfur amino acid content of post-ruminal protein was made non-limiting by sulfur amino acid infusion

(Mata *et al.* 1995; Masters *et al.* 1998a). At any given ME intake and supplement protein degradability, the overall efficiency of conversion of DPLS to wool is then the weighted average of efficiencies of 0.116 and 0.25, applied to the MCP and the two UDP pools.

A point to note is that as DPLS increases due to decreased degradability of supplement protein, a slight fall in $DPLS_{MCP}$ supply is also predicted. Though relatively small (4-6 g DPLS/d), this is sufficient to introduce the slight curvilinearity in response which can be seen in Fig. C1(b). Once DPLS no longer limits wool growth, the levels of wool growth predicted from ME supply in Fig. C1(b) are the same as in Fig. C1(a). However, the DPLS supply at which this changeover occurs is lower and represents a DPLS/MEI ratio not of 12 (1.392/0.116), but of 1.392/(weighted average efficency). For example at the lowest ME intake, the change from DPLS-driven to ME-driven wool growth occurs at at a DPLS/MEI ratio of 9.25 (1.392/0.1505).

In the current version of **GrazFeed**, there can be one of two predicted wool growths at a given DPLS supply, depending on whether DPLS is limiting or non-limiting for wool growth. The proposed modification to **GrazFeed** now permits a third possible outcome, related to what increase in the proportion of $DPLS_{UDP}$ has resulted from decreased degradability of supplement protein. For example, at a DPLS supply of about 84 g/d, predicted wool growth can be:

1. 9.2 g/d (blue symbols); wool growth constrained by low MEI (6.6 MJ/d),
2. 9.7 g/d (red symbols); MEI (9.6 MJ/d) non-limiting but wool growth constrained by DPLS supply,
3. 10.6 g/d (cyan symbols); wool growth constrained by MEI but at higher level (7.6 MJ/d),
4. 11.3 g/d (green symbols); MEI non-limiting, wool growth higher than (2) since more of the total DPLS supply comes from undegraded supplement protein.

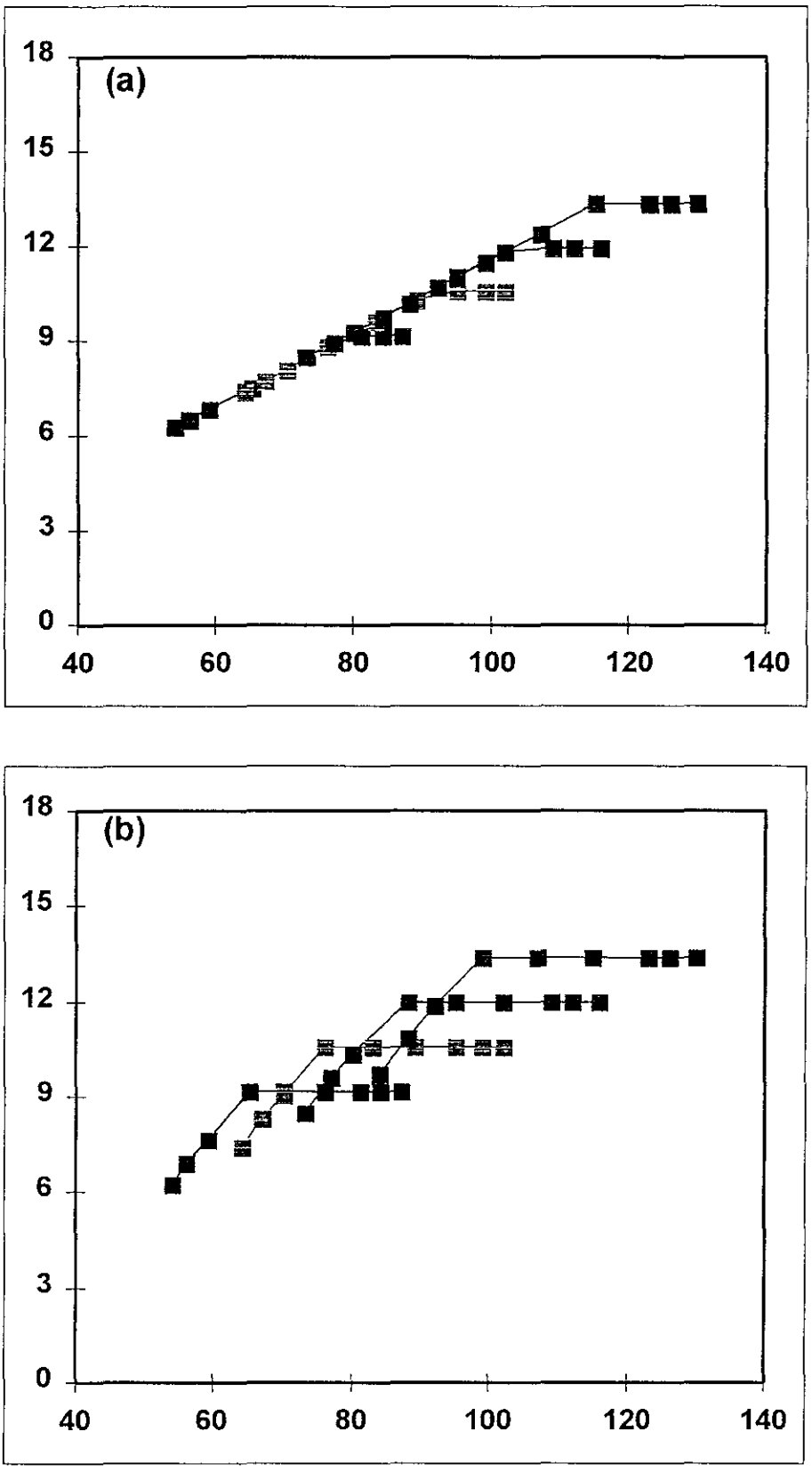


Figure C1. Predicted relationships between clean dry wool growth (y, g/d) and the amount of digestible protein leaving the stomach (x, g/d) in 35 kg crossbred lambs at four different levels of ME intake: 6.6 (blue), 7.6 (cyan), 8.6 (green) or 9.6 (red) MJ/d.
(a) Current version of *GrazFeed*
(b) Version with amendment to efficiency of conversion of DPLS to wool

In summary, the proposed modification to **GrazFeed** permits wool growth responses to total DPLS or total MEI and, for the first time, a response which reflects explicitly, the proportion of the DPLS supply derived from a protein supplement rich in SAA which escape degradation in the rumen. The change in the efficiency of wool growth in this situation must itself be function of supplement type, since it relates to the amino acid and especially SAA composition of the supplement protein. The incorporation of this proposed modification into a **GrazFeed** release will now await collection of digesta and wool growth data against which the revised **GrazFeed** predictions can be validated.

Modifications to the GrazFeed *algorithms for estimating fat, protein and bodyweight gains*

In the current version of **GrazFeed**, the proportional contributions of fat and protein to weight gain relate primarily to the size of the animal relative to its expected mature size (Freer *et al.* 1977); the energy available for gain then sets the amount of gain which will occur. Hence, the proportions of protein and fat in the gain are constant for an animal of a given relative size, and there is no effect of energy supply as such on the expected contributions of protein and fat to the predicted weight gain. Since the gain predicted by the current **GrazFeed** always contains fat, the energy content of gain is high and predicted weight gains will be low when the ME available for gain is low. For example, in animals consuming a sub-maintenance diet of medium-quality chaff supplemented with 200-250 g/d of protein supplement (eg, SFM75, Table B4; CSM240, Table B9), gains of less than 5 g/d would be predicted by the current version of **GrazFeed** (see also Table C1 below). Moreover, as discussed above, the current version of **GrazFeed** would not predict simultaneous protein gain and fat loss (Tables B4, B9) nor a response to formaldehyde-treatment of supplement protein such as that reported for SFM in Table B4. In an attempt to address these issues, the following modifications to **GrazFeed** are proposed.

When the availability of ME for gain (ME_g) falls below about 0.25 of that which will support the maximum potential gain, the proportion of protein in gain progressively increases above that which would prevail at higher relative ME supplies (ascribed the relative value of 1.0) while the corresponding proportion of fat in the gain declines. The alteration in the proportion of protein, relative to the value of 1.0 at higher relative ME availability, is effected by a function of the form:

$$RP = a/(RME + b)$$

where RP is the proportion of protein predicted in the gain, relative to that at higher RME, RME is the ME_g relative to that required for maximum potential gain and 'a' and 'b' are constants. In the prototype revised version of **GrazFeed**, these constants have values of 0.44 and 0.178 respectively. The relative proportion of fat in the gain is estimated as the complementary function

$$RF = 2.0 - RP$$

The nature of the responses generated by these functions is shown in Figure C2(a). When the ME available for gain is high relative to that which would support maximum potential gain, the relative values for the proportions of protein and fat in the gain are unity and the actual protein and fat contents of gain are calculated from the relative size of the animal, as in the current version of **GrazFeed**. This results in a relatively constant energy content of gain.

Below an available ME supply of about 0.25 of that for maximum potential gain, the relative value of protein in the gain increases sharply. For example, when ME supply is 0.1 of that which would support maximum potential gain, the relative value for protein content is 1.58, that is the protein content of gain is 58% higher than that which would be estimated in the current version of **GrazFeed**. Similarly, the estimated fat content of gain is (2.0-1.58) = 0.42 of that which would be estimated in the current **GrazFeed**.

The effects of these proposed modifications on the energy (NE) content of gain, and the amounts of this derived from protein and fat, are shown in Figure C2(b). Below a relative ME availability of 0.25, the NE content of gain which derives from protein approximately doubles, while that derived from fat falls sharply and is negative when ME available for gain is below about 0.05 of that needed for maximum potential gain. As a result, the estimated NE content of gain also falls sharply. With these proposed modifications, the estimation of changes in body weight and its components will proceed as follows:

1. The availability of ME for gain will be calculated as in the current version of **GrazFeed**.
2. This will be compared with the energy content of gain, which will be the same as in the current version of **GrazFeed** when the relative ME availability is above about 0.25, but much lower at lower relative ME availabilities.

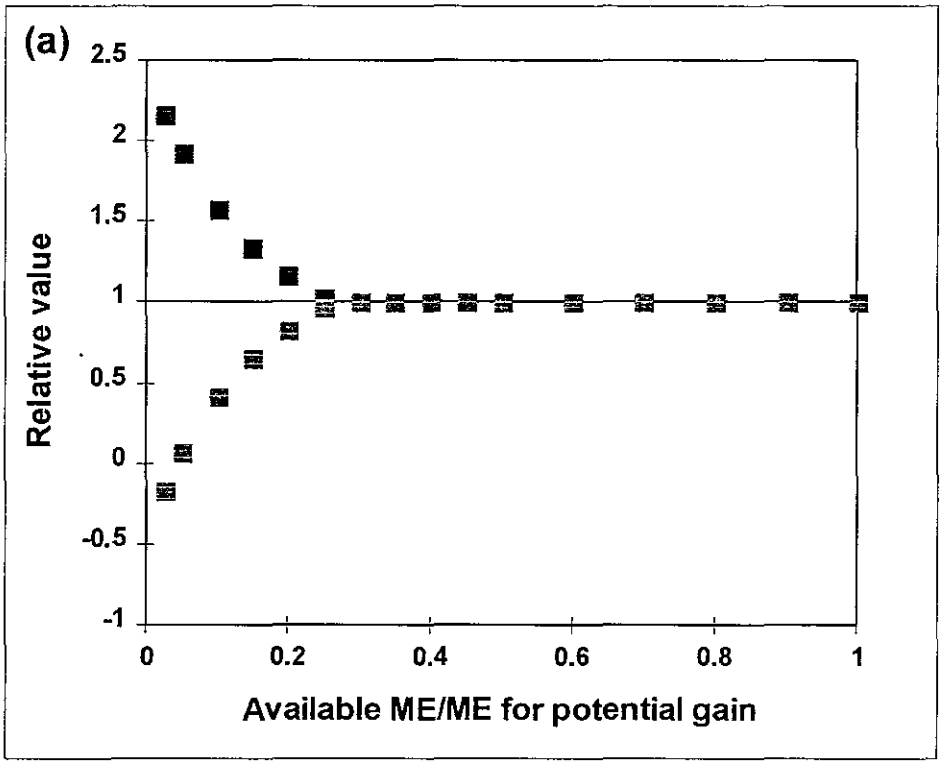
3. A provisional gain will be computed from 1. and 2. and the supply of protein then checked to establish that there is sufficent to support the predicted gain, as in the current **GrazFeed**.

The first major consequence of the proposed modifications to this section of **GrazFeed** is that at ME availabilities which are low relative to those which will support maximum potential gain, the weight gain predicted by the modified **GrazFeed** will be considerably higher than is predicted by the current version. The extent to which it is higher will relate directly to the energy content of gain calculated either with or without the modification. As can be seen in Fig. C2(b), this falls by a factor of approximately 2 as the ME available for gain falls from 0.3 to 0.1 of that required for maximum potential gain, and by a factor of about 4 at a relative ME availability of 0.05. Note that these effects in turn imply a reduction in the efficiency of use of ME for gain. The effect of the decreases in the energy content of gain on predicted weight gains is shown in Figure C3. The values in Fig. C3(a) represent the relative increase in gain effected by the proposed modification. As can be seen, these correspond to the decreases in the energy value of gain shown in Fig. C2(b).

A comparison is shown in Fig. C3(b) for a **GrazFeed** example involving crossbred 35 kg lambs grazing high-quality pasture (digestibility 0.8) of progressively declining availability. The proposed modification to **GrazFeed** begins to exert an effect at a liveweight gain below about 70 g/d, with predicted liveweight gains being higher with the modified version.

The second major consequence of the proposed modification is that at low relative availabilities of ME for gain, the energy content of gain is less than 5 MJ NE/kg gain as a result of their being a gain in protein but a loss of fat. The modification thus permits gains of protein and body weight, but a loss of fat, in animals such as those in Section B which were receiving high-protein diets at feeding levels not far above maintenance.

The daily gains in empty body weight, protein and fat for treatments SFM75 (see Section B2) and CSM240 (see Section B3) are compared in Table C1 with those predicted by the current **GrazFeed**. As indicated above, observed bodyweight gains were consistently under-estimated by the current version, due to the high value for the energy content of weight gain calculated in this version. The proposed modifications to **GrazFeed** result in lower values for the energy content of weight gain and accordingly, result in much better correspondence between predicted protein and bodyweight gains and those observed in the feeding trials.



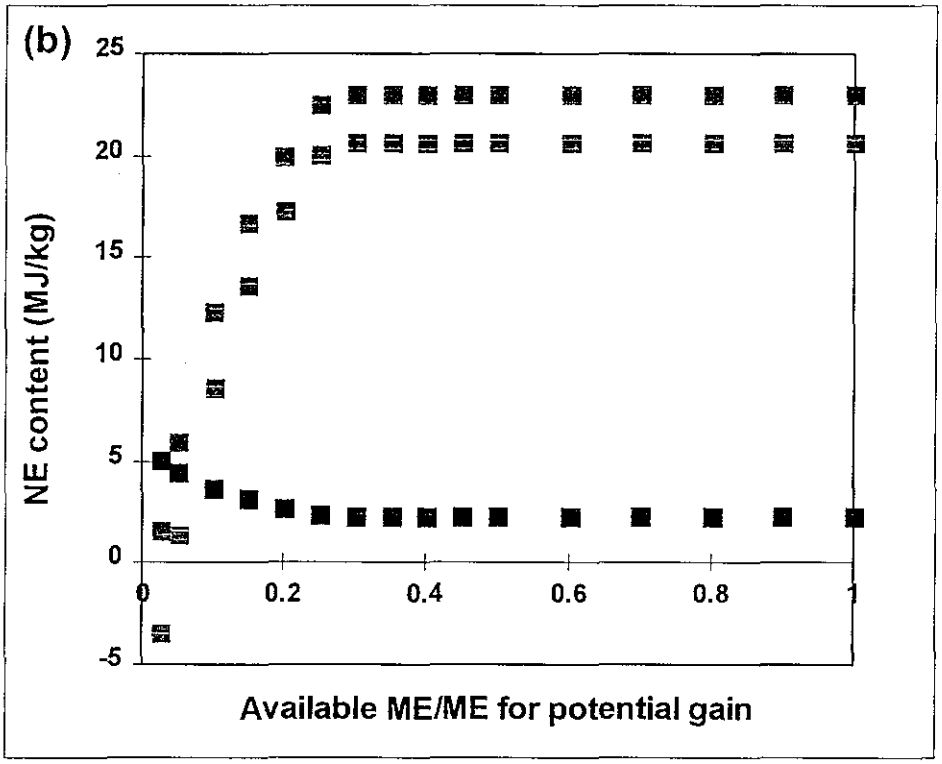
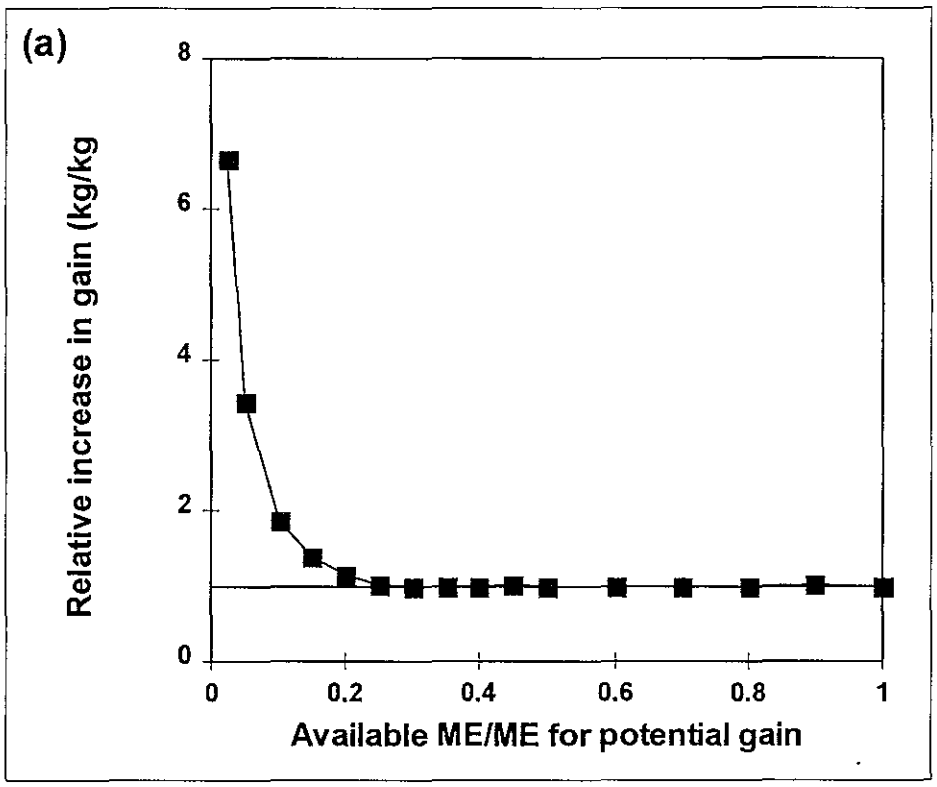


Figure C2. (a) Proposed adjustments within *GrazFeed* to the relative contributions of protein (blue) and fat (cyan) to the energy content of empty weight gain, considered in relation to the relative ME avilability for gain.

(b) Effect of imposition of adjustments on the contribution of NE from protein (blue) and from fat (cyan) to the total NE content of empty weight gain (green)



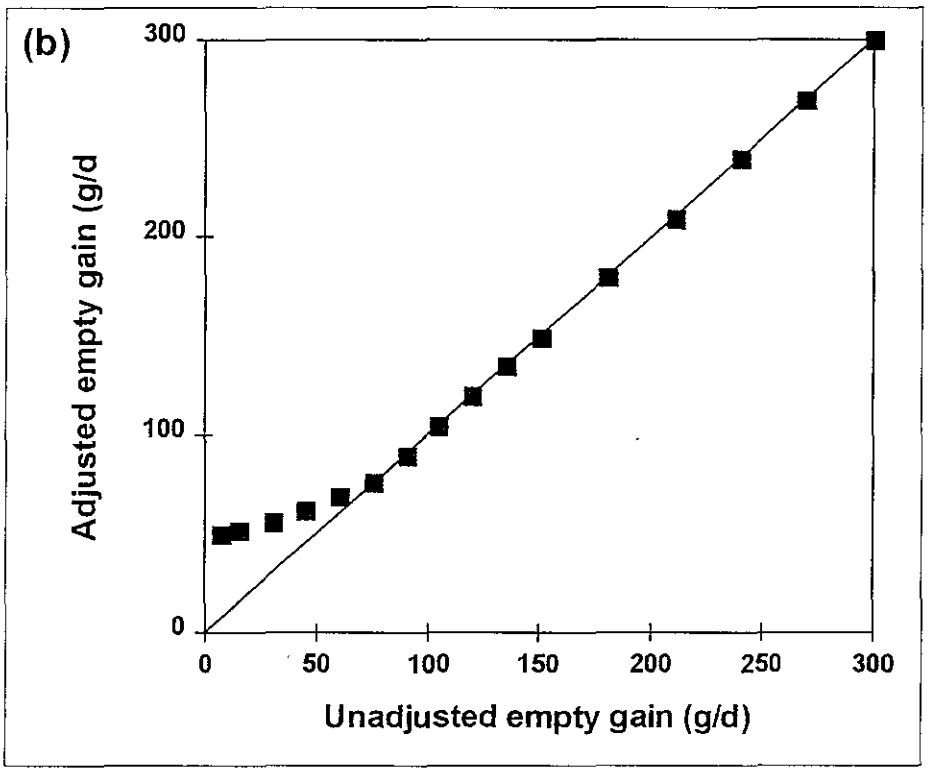


Figure C3. Effect of proposed adjustments to GrazFeed on

(a) the relative increase in daily gain at low ME availability,

(b) the predictions of empty weight gains in grazing 35 kg crossbred lambs, compared with those obtained without adjustment to the body composition algorithms.

The modified version of **GrazFeed** predicted small daily losses of fat, rather than small daily gains as in the current version. However, the predicted losses are still less than those observed, especially with the CSM treatment.

Table C1. Comparison of the observed daily gains (g/d) in empty body weight, protein and fat in feeding trials (Tables B4, B9 above) with those predicted by the current version of *GrazFeed* (GFD) and the version with proposed modifications to the body composition algorithms.

Treatment	Result	Bodyweight	Protein	Fat
SFM75, Table B4	Observed	30.4	4.8	-2.5
	Predicted (unmodified GFD)	*2*	*0.2*	*1*
	Predicted (modified GFD)	35	5	-1
CSM240, Table B9	Observed	38.3	4.8	-7.7
	Predicted (unmodified GFD)	*3*	*0.3*	*1.5*
	Predicted (modified GFD)	37	4	-2

In summary, the second proposed modification to **GrazFeed** results in better predictions of daily gains in empty body weight and protein, by making the energy value of gain a function of the relative availability of ME for gain. Moreover, the approach taken permits **GrazFeed** to predict positive changes in body protein and empty body weight combined with negative changes (ie, losses) of body fat. At this stage, the functions employed may be under-estimating the extent of such fat loss (see Table C1 above), but it must be emphasised that the modification is presented as a concept, rather than the 'quantitatively final' version.

It must also be emphasised that in contrast with that suggested for the wool growth algorithm, the modification proposed for the body composition algorithm does not, at this stage, permit a response reflecting a possibly improved amino acid composition of the total DPLS supply, arising from a protein supplement of low rumen degradability. Future work with **GrazFeed** will concentrate on appropriate quantification of the proposed modifications, on the validation of predicted responses against experimental data, and on the incorporation of responses of body composition to the quality of post-ruminal protein supply.

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