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Digestion of concentrates in sheep

5.* The effect of adding fish meal and urea together to cereal diets on protein digestion and utilization by young sheep

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1. In one experiment according to a latin square design five sheep with fistulas in the abomasum and terminal ileum were given diets based on barley. Five diets were compared, one without protein supplementation, two with different levels of fish meal and two with combinations of fish meal and urea.

2. The amount of non-ammonia crude protein passing through the abomasum and removed from the small intestine increased linearly with fish-meal supplementation but the only effect of supplementation with urea was to increase the crude protein removed before the abomasum. It was concluded that urea did not appear to have a sparing effect on the degradation of dietary protein.

3. Seventy-eight lambs were used in a second experiment. They were given thirteen diets made up from a basal barley diet and different combinations of fish meal and urea.

4. Digestibility of organic matter and efficiency of food conversion increased with each level of fish-meal supplementation. The effects of urea supplementation on organic matter digestibility and efficiency of food conversion depended upon the level of fish meal in the diet and at the highest level of fish-meal supplementation there were no effects.

5. The maximum digestibility of organic matter (about 820 g/kg) could be achieved both with urea alone and fish meal alone and was attained at a lower level of nitrogen in the diet with urea. In contrast, the maximum food conversion efficiency that could be achieved with a fish-meal supplement was much greater than could be obtained with a supplement of urea alone.

Previous work (Ørskov, Fraser & McDonald, 1971*b*) on the digestion of concentrates in sheep has shown that in mature sheep there was no increase in the amount of non-ammonia crude protein passing through the abomasum when urea was added to a barley diet containing about 96 g crude protein/kg dry matter. This finding agreed with calculations based on the formation of microbial protein during ruminal fermentation of the diet (Hungate, 1966) and indicated that energy was limiting the amount of microbial protein formed in the rumen. On an unsupplemented barley diet, however, there was a large increase in the amount of nitrogen entering the rumen, resulting from an influx of non-protein N (NPN) from saliva and blood. It was suggested that a young lamb which was actively utilizing most of the amino acids it absorbed for tissue protein synthesis and thus returning less NPN to the rumen would respond to a dietary supplement of NPN. More recently it was found that when urea was added to a barley diet given to young lambs there was increased fermentation of dietary starch in the rumen and also the rates of N retention and live-weight gain increased (Ørskov, Fraser & McDonald, 1972). The optimum level of dietary crude protein for microbial growth in these circumstances was about 130 g/kg dry matter.

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We have studied the response of young lambs to urea supplementation when their barley diet was supplemented with a protein (fish meal). With adult sheep it was found that, although fish-meal supplementation produced quite large increases in the amount of protein passing through the abomasum, more than half the supplement appeared to be degraded to ammonia and volatile fatty acids in the rumen. As this ammonia would serve as a source of N similar to that supplied by urea, there might be little benefit from giving a urea supplement and a protein supplement to a barley diet; unless the urea reduced degradation of the protein supplement it would be unlikely to produce a response. The effects of supplements on digestion in sheep were studied in Expt 1 and the effects on growth rate and N utilization in Expt 2. A brief account of Expt 1 has been published (Ørskov & Fraser, 1972).

EXPERIMENTAL

Expt 1

Animals. Five female Suffolk × North Country Cheviot sheep between 5 and 8 months old were used. They were fitted with cannulas in the abomasum and terminal ileum by the procedure of Ørskov, Fraser & Kay (1969).

Diets. Details of the diets are given in Table 1. Diet 4 was made by replacing 10 g/kg of diet 2 with urea and diet 5 by replacing 10 g/kg of diet 3 with urea. All diets were pelleted through a 7·4 mm die.

Design and treatments. The five sheep were given each diet for a 14 d period according to a latin square design. The level of feeding approached maximum intake on the ration scale previously reported (Ørskov *et al.* 1971*a*, *b*).

Management and sampling procedure. The sheep were kept on slats in individual pens. Half their daily food allowance was given at 08.00 hours and half at 20.00 hours. The amount of uneaten food was recorded daily and dried to constant weight at 100°. Fresh water was always available. During the last 24 h of each treatment period, samples of abomasal, ileal and rectal contents were obtained at 2 h intervals (Ørskov *et al.* 1971*a*). It was assumed that the compositions of the pooled samples were representative of the compositions of the total 24 h flow at each sampling point. The disappearances of digesta between the mouth and the abomasum, between the abomasum and the terminal ileum and between the terminal ileum and rectum are referred to as disappearances in the rumen, the small intestine and the large intestine, respectively. The disappearance of food components in each organ was assessed from changes in the concentration of Cr_2O_3 relative to that which was incorporated into the diets as a mixture baked with a wheat-flour paste to ensure homogenous mixing.

During the last three periods and in a subsequent period when the five sheep received a similar diet supplemented with urea alone, samples of rumen liquor were obtained by stomach tube on the last day of each period at 11.00 hours.

Analytical procedure. The procedures of Ørskov *et al.* (1971*b*) were used. N content was determined by the Kjeldahl method of Davidson, Mathieson & Boyne (1970), ammonia content was estimated by the method of Conway (1957) and the chromium

Table 1. *Ingredients (g/kg fresh weight) and crude protein content (g/kg dry matter) of the barley and barley–fish-meal diets used in Expts 1 and 2*

Expt no.	Diet*	Barley	Fish meal	Dicalcium-phosphate	Limestone	Crude protein
1	1	960	0	15	15	95
1	2	912	48	15	15	121
1	3	868	94	15	15	156
2	A_1	967	0	18	15	98
2	B_1	943	30	12	15	115
2	C_1	919	60	6	15	131
2	D_1	895	90	0	15	155

* All diets contained (per kg): 1·5 mg retinyl palmitate, 0·025 mg cholecalciferol, 20 mg α-tocopheryl acetate, 200 mg MgO, 150 mg $ZnSO_4.7H_2O$, 80 mg $MgSO_4.4H_2O$, 1·0 mg KI and 0·43 mg $CoSO_4.7H_2O$. Diets 1, 2 and 3 also contained 10 g Cr_2O_3 mix (consisting of 1 part by weight Cr_2O_3 baked with 4 parts of wheat flour).

content of ashed samples was estimated by the method of Stevenson & Clare (1963) as modified by Mathieson (1970). Diamino-pimelic acid (DAPA) content was determined by the method of Mason (1969). The amounts of volatile fatty acids were estimated by gas–liquid chromatography with a flame-ionization detector (Model no. 104; Pye Unicam Ltd, Cambridge, England) as described by Fell, Kay, Whitelaw & Boyne (1968).

Expt 2

Animals. Thirty-nine male and thirty-nine female Suffolk × (Finnish Landrace × Dorset Horn) sheep were used. They were weaned at 4 weeks of age and were allocated to the experimental treatments at an average weight of 14 kg, when they were about 6 weeks of age.

Diets. The ingredients of the barley diet (A_1) and of the barley–fish-meal diets (B_1, C_1 and D_1) are given in Table 1 (on a fresh-weight basis). Urea was substituted for barley in diets A_1–D_1 as follows: 7 g/kg for diets A_2, B_2, C_2 and D_2; 14 g/kg for diets A_3, B_3 and C_3; and 21 g/kg for diets B_4 and C_4.

Design and treatments. The lambs were divided into six blocks of thirteen according to live weight and sex, and one lamb from each block was allocated at random to each of the thirteen diets. Feeding levels were adjusted each week for each animal according to its live weight, by means of a feeding scale which was estimated to approximate to maximum intake.

Management. The animals were given their daily allocated rations in two equal amounts at 08.00 and 20.00 hours. The amount of uneaten food was recorded at weekly intervals and dried to constant weight at 100°. Live weights were recorded weekly, fresh water was always available, and the animals were bedded with sawdust.

At about 25 kg live weight the male lambs were placed in metabolism cages for a period of 14 d, during the last 8 d of which the faeces and urine were collected.

The lambs were slaughtered when about 35 kg live weight and the weights of liver at slaughter and of cold carcass 24 h after slaughter were recorded. N contents of the diets, and of faeces and urine, were determined by methods used in Expt 1.

Table 2. *Expt* 1. *Intake and disappearance of organic matter (g/d) in rumen, small and large intestines in sheep given rolled-barley diets supplemented with either fish meal or combinations of fish meal and urea*

(Mean values for groups of five sheep adjusted to a dry-matter intake of 1025 g/d)

Diet	Supplement (per kg diet)	Organic matter intake	Organic-matter disappearance in: Rumen Apparent	Rumen Dietary	Small intestine	Large intestine	Organic matter in faeces
1	None	975	452	609	237	83	203
2	Fish meal 48 g	969	466	560	248	63	193
3	Fish meal 94 g	950	438	558	282	51	180
4	Diet 2 + urea 10 g	959	467	557	268	51	173
5	Diet 3 + urea 10 g	959	486	597	239	59	174
SE of treatment means		—	30	22	31	12	10

Table 3. *Expt* 1. *Composition of abomasal (ab) and ileal (il) contents of sheep given rolled-barley diets supplemented with either fish meal or combinations of fish meal and urea*

(Mean values for groups of five sheep)

Diet	Supplement (per kg diet)	Non-ammonia crude protein (g/kg dry matter) ab	Diamino-pimelic acid (g/kg protein*) ab	Ammonia (mg N/l) ab	Non-ammonia crude protein (g/kg dry matter) il	Ammonia (mg N/l) il
1	None	222	6·33	64	148	189
2	Fish meal 48 g	251	4·88	72	151	212
3	Fish meal 94 g	261	3·62	99	173	322
4	Diet 2 + urea 10 g	254	4·32	114	165	271
5	Diet 3 + urea 10 g	262	4·31	167	176	308
SE of treatment means		9	0·40	7	5	52

*(N × 6·25).

RESULTS

Expt 1

The mean dry-matter intakes varied between treatments, being 891, 970, 1044, 1060 and 1070 g/d for treatments 1–5 respectively. Estimates of quantities of digesta were all adjusted to a dry-matter intake of 1025 g/d, direct proportionality being assured.

Mean values for intake of organic matter and its apparent disappearance in different segments of the gut for each treatment are given in Table 2. An estimate of the true disappearance of dietary organic matter in the rumen (Table 2) was obtained by adjusting for bacterial organic matter on the assumptions that bacteria contain 105 g N/kg organic matter (Hungate, 1966) and 1 g DAPA/19·1 g N (Hutton, Bailey & Annison, 1971). There were no significant differences between treatment means. The mean apparent disappearance of digestible organic matter was about 60% in the rumen, 32% in the small intestine and 8% in the large intestine, but estimates

Table 4. *Expt* 1. *Intake and disappearance of crude protein (g/d) in different parts of the digestive tract in sheep given rolled-barley diets supplemented with either fish meal or combinations of fish meal and urea*

(Mean values for groups of five sheep adjusted to a dry-matter intake of 1025 g/d)

		Crude protein		Non-ammonia crude protein		Crude protein	
Diet	Supplement (per/kg diet)	Intake	Dis-appearance from rumen	Passing through abomasum	Dis-appearance from small intestine	Dis-appearance from large intestine	Excreted in faeces
1	None	97	−33	131	80	10	44
2	Fish meal 48 g	124	−20	144	97	7	42
3	Fish meal 94 g	160	6	154	104	15	39
4	Diet 2+urea 10 g	163	21	142	96	12	37
5	Diet 3+urea 10 g	213	73	140	90	14	40
SE of treatment means		—	8	7	7	3	2

of bacterial protein content showed that 74 % of the digestible dietary organic matter disappeared in the rumen.

The amount of N in abomasal and ileal contents is given in Table 3. The percentage of non-ammonia crude protein (NACP) in the abomasal dry matter increased linearly with fish-meal supplementation ($P < 0.05$). The proportion of DAPA in abomasal protein decreased linearly with fish-meal supplementation ($P < 0.001$) but was not significantly changed by urea supplementation. Ammonia concentration in abomasal fluid also increased linearly with fish-meal supplementation ($P < 0.01$) and was further increased by urea supplementation ($P < 0.001$). The concentration of NACP in ileal fluid increased linearly with fish-meal supplementation ($P < 0.01$), but was not significantly affected by urea supplementation. The concentration of ammonia in the ileal contents did not differ between treatments. The treatment mean values for intake and disappearance of crude protein in various segments of the gut are given in Table 4. There was a linear increase in the amount of crude protein disappearing in the rumen with increasing amounts of fish meal in the diet ($P < 0.01$). Also the amounts of NACP passing through the abomasum and disappearing in the small intestine increased linearly with fish-meal supplementation ($P < 0.05$), by 3·8 g and 3·9 g respectively for each additional 10 g of protein from fish meal. The only significant effect of urea was to increase the disappearance of N in the rumen ($P < 0.001$). There were no significant effects of protein supplementation on the crude protein disappearing in the large intestine or excreted in the faeces.

Treatment mean values for the apparent digestibilities of dry matter, organic matter, crude protein and ash are given in Table 5. The only significant effects of treatments were linear increases in the apparent digestibility of protein with increasing fish-meal supplementation ($P < 0.001$) and further increases when urea was included ($P < 0.01$), and a linear increase in the apparent digestibility of ash with increasing fish-meal supplementation ($P < 0.05$). There were no apparent differences between the treatments in proportions of volatile fatty acids in the samples of rumen contents

Table 5. *Expt* 1. *Dry-matter, organic-matter, crude protein and ash digestibilities in sheep given rolled-barley diets supplemented with either fish meal or combinations of fish meal and urea*

(Mean value for groups of five sheep)

Diet	Supplement (per kg diet)	Dry matter	Organic matter	Crude protein	Ash
1	None	0·762	0·792	0·556	0·178
2	Fish meal 48 g	0·773	0·801	0·663	0·269
3	Fish meal 94 g	0·775	0·811	0·757	0·323
4	Diet 2 + urea 10 g	0·790	0·820	0·775	0·358
5	Diet 3 + urea 10 g	0·787	0·818	0·813	0·336
SE of treatment means		0·012	0·010	0·020	0·048

Table 6. *Expt* 2. *Effect of urea and fish-meal supplementation of rolled-barley diets on digestibility of dry matter and nitrogen and on N balance in lambs*

(Mean values for three male lambs in each group)

	Supplement (g/kg)		Digestibility ratio						
Diet	Urea	Fish meal	Dry matter	N	N intake (g/d)	Faecal N (g/d)	Urinary N (g/d)	N retained (g/d)	Ratio, retained N:digestible organic-matter intake
A_1	0	0	0·715	0·493	10·0	5·0	2·5	2·5	0·510
A_2	7	0	0·757	0·594	12·0	4·9	3·5	3·6	0·732
A_3	14	0	0·793	0·727	19·0	5·2	6·5	7·3	1·114
B_1	0	30	0·741	0·649	14·4	5·0	2·8	6·5	1·141
B_2	7	30	0·796	0·720	18·5	5·2	5·3	8·0	1·198
B_3	14	30	0·805	0·782	20·8	4·5	9·2	7·1	1·073
B_4	21	30	0·800	0·796	24·4	5·0	10·2	9·2	1·391
C_1	0	60	0·761	0·649	18·3	6·4	4·4	7·5	1·155
C_2	7	60	0·788	0·761	22·9	5·4	9·7	7·7	1·097
C_3	14	60	0·803	0·786	26·8	5·7	12·5	8·5	1·163
C_4	21	60	0·803	0·826	31·2	5·4	17·0	8·8	1·165
D_1	0	90	0·805	0·770	21·7	4·9	8·7	8·1	1·168
D_2	7	90	0·800	0·780	25·1	5·5	12·0	7·6	1·076
SE of means			0·013	0·020	1·5	0·5	0·9	1·2	0·145

taken in the last three periods and the one additional period. The twenty observations gave a range in composition from 15 to 40 molar % of propionic acid and from 33 to 55 molar % of acetic acid. There was no indication of any relationship between these proportions and the amount of protein passing through the abomasum.

Expt 2

The results of the balance trials are given in Table 6. There were similar and statistically significant differences in apparent digestibility of dry matter ($P < 0·001$) and of organic matter ($P < 0·001$). The effects of urea supplementation on apparent digestibility of organic matter are shown in Fig. 1. The response to urea supplementation was dependent on the level of fish meal in the basal diet. Only in the absence of fish meal was there a significant increase in digestibility between the first and second levels of urea supplementation, and at the highest level of fish meal there was no response to urea at all.

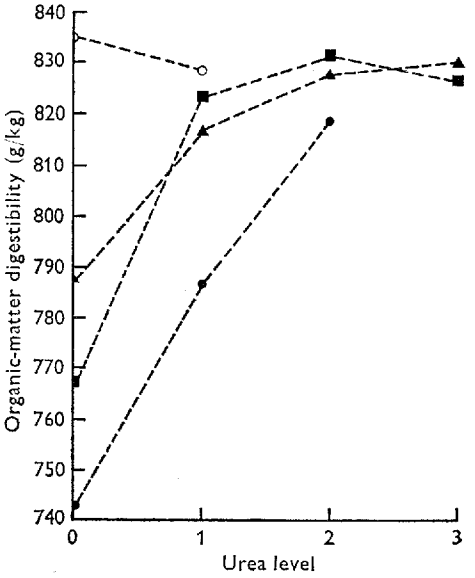

Fig. 1. Effect on organic-matter digestibility of giving lambs barley diets supplemented with urea and containing four graded levels of fish meal (g/kg): o (●); 30 (■), 60 (▲), 90 (○).

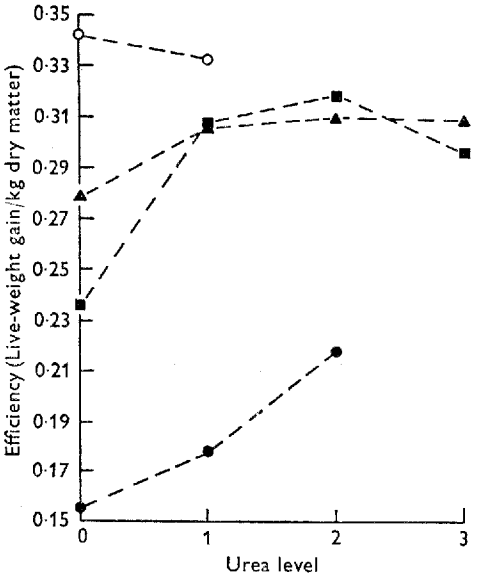

Fig. 2. Effect on food conversion efficiency of giving male lambs barley diets supplemented with urea and containing four graded levels of fish meal (g/kg): o (●); 30 (■); 60 (▲) and 90 (○).

Table 7. *Expt 2. Effect of urea and fish-meal supplementation of rolled-barley diets on rate of live-weight gain and food conversion ratio in lambs*

(Mean values for three male and three female lambs in each group)

Diet	Supplement (g/kg) Urea	Fish meal	Initial wt (kg)	Final wt (kg)	Carcass wt (kg)	Rate of gain (g/d)	Food conversion ratio (dry matter intake:live-wt gain) ♂	♀
A_1	0	0	15·2	34·1	16·4	102	6·39	5·43
A_2	7	0	15·1	34·0	17·2	141	5·69	4·44
A_3	14	0	14·7	34·0	17·9	158	4·58	4·51
B_1	0	30	14·5	34·7	17·1	176	4·24	4·20
B_2	7	30	14·2	34·9	17·6	219	3·23	3·71
B_3	14	30	14·8	35·1	17·9	239	3·13	3·42
B_4	21	30	14·2	34·8	18·0	220	3·39	3·68
C_1	0	30	15·3	35·1	17·9	210	3·63	3·85
C_2	7	30	14·8	34·9	17·7	237	3·28	3·35
C_3	14	60	15·6	34·7	17·5	228	3·24	3·61
C_4	21	30	15·8	35·0	17·7	235	3·26	3·52
D_1	0	90	15·0	36·0	17·7	269	2·91	2·94
D_2	7	30	14·6	33·9	17·5	249	3·02	3·34
SE of means			—	—	—	0·30	8·6	0·19	0·19

There were also treatment differences in urinary N and digestibility of N ($P < 0·001$), retained N ($P < 0·05$), and the ratio of retained N to digestible organic matter intake ($P < 0·05$). The digestibility of N increased as N supplementation increased. The N retention was increased by urea supplementation, particularly at the low level of fish meal (30 g/kg) in the diet, and was increased by fish-meal supplementation.

In Table 7, initial weight, final weight, carcass weight, rate of live-weight gain and food conversion ratios have been given. There were significant treatment effects on growth rate and on food conversion ratio ($P < 0·001$). Results for the latter could not be combined for males and females; therefore separate mean values have been shown. The results for male lambs are shown in Fig. 2 by plotting food conversion efficiency (the reciprocal of food conversion ratio) against level of urea supplementation. The reciprocal was used to facilitate comparison with Fig. 1. The response to urea supplementation, as in Fig. 1, was apparently dependent on the protein level in the basal diet. Only in the absence of fish meal was there a significant increase in food conversion efficiency as a result of the change from the first to the second level of urea supplementation and at the higher protein levels there was no response to urea.

DISCUSSION

Effect of urea on digestion of fish meal

The effects of increasing the amount of fish meal in the diet are similar to those reported previously (Ørskov *et al.* 1971*b*); an increase of 10 g fish protein in the diet increased the protein passing the abomasum by 3·5 to 4 g and this protein was completely digested in the small intestine. The amount of NACP disappearing from

the small intestine increased by 3·9 g/10 g increase in dietary protein. The increased protein passing the abomasum may have been dietary protein, since the concentration of DAPA in digesta decreased.

When urea was added to the diets supplemented with fish meal there was no increase in NACP passing the abomasum. This may be compared with the earlier observation (Ørskov *et al.* 1971*b*) that the addition of urea to a barley diet did not increase the NACP passing the abomasum. It may be concluded that urea does not appear to spare dietary protein from degradation.

Effect of protein and NPN on apparent digestibility of dry and organic matter

The highly significant response of digestibility to N supplementation found in Expt 2 was almost certainly the result of an increase in the amount of rumen fermentation (Ørskov *et al.* 1972). The plateau in digestibility shown in Fig. 1 therefore indicates when there was adequate N for microbial fermentation. Since fish meal is only partly degraded in the rumen, the concentration of dietary N necessary to give an adequate N supply for the microbes would be expected to be higher with fish-meal than with urea supplementation. The results shown in Fig. 1 are consistent with this idea; the plateau of digestibility was reached at a lower concentration of N when urea formed all or part of the supplement. Therefore there may be a nutritional situation in which urea would be superior to fish meal as a supplement of N. This would occur with animals whose protein requirements could be completely satisfied by microbial protein and undegraded protein from the basal diet, e.g. non-lactating sheep in early pregnancy.

Effect of urea and fish meal on food conversion

It can be seen from Fig. 2 that the sheep could not attain their optimum feed conversion efficiency when the basal diet was supplemented with urea alone. This pattern differs from that of the digestibilities shown in Fig. 1, and the comparison provides an interesting illustration of the difference between the N requirement of the rumen microbes and that of the lamb. With each addition of fish meal the lambs responded by increasing their efficiency of food conversion. This was expected since even the highest level was slightly below the optimum concentration of protein (Andrews & Ørskov, 1970). When urea was added to diets containing the lower levels of fish meal, food conversion efficiency increased with the first increment but there was no further increase. At the highest level of fish meal, urea supplementation had no effect; there was a slight but non-significant decrease in food conversion efficiency. This result is consistent with the conclusion from Expt 1 that urea does not spare dietary protein from degradation. When the N generated as a result of protein degradation together with recycled N was sufficient to provide N for maximum microbial growth, no response to urea would be expected.

Choice of type of supplement for cereal diets for ruminants

Our results indicate that when a choice has to be made between supplementation with protein, with urea, or with a mixture of the two, the following principles may provide some guidance: (1) Urea can be used as the only source of supplementary N whenever the animal's protein requirement can be satisfied by undegraded basal protein together with the microbial protein produced when carbohydrates are fermented; it is possible that in these circumstances urea N might sometimes be even better than a protein supplement. (2) Supplementation will have to be with protein when, as with the young animals used here, requirements exceed those which can be satisfied by microbial protein. Whether it will be advantageous to provide urea additionally will depend on whether microbial requirements can be satisfied by degradation of protein from the basal diet and from the protein supplement. (3) Urea will be useful in combination with a protein supplement if the latter is protected from microbial degradation, for instance with formaldehyde (Ferguson, Hemsley & Reis, 1967) or with tannin (Leroy & Zelter, 1970), or is given so that it bypasses the rumen (Ørskov, Fraser & Corse, 1970).

Urea may be useful when given with a low-protein supplement, which does not satisfy microbial requirements from the degraded fraction. This combination would be appropriate when the animal's requirement was only slightly higher than that which could be met by microbial and undegraded basal protein alone. The level of supplementary protein above which urea supplementation would cease to be beneficial would vary with the susceptibility of that particular protein to degradation (See Schoeman, de Wet & Burger, 1972).

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