

# Duckweed as a Protein Source for Fine-Wool Merino Sheep: Its Edibility and Effects on Wool Yield and Characteristics

Damry, J. V. Nolan\*, R. E. Bell<sup>1</sup> and E. S. Thomson

School of Rural Science and Natural Resources, University of New England, Armidale, NSW 2351, Australia

**ABSTRACT** : Two experiments were carried out to investigate whether duckweed is useful as a dietary protein source for fine-wool Merino sheep and to evaluate its effects on wool yield and characteristics. In Experiment 1, the sheep were given one of three maintenance diets consisting of oaten chaff (520-700 g/d) supplemented with 16-32 g crude protein/d in the form of fresh (1 kg/d) or sun-dried (50-100 g/d) duckweed. Each ration was estimated to provide 5.4 MJ (1.3 Mcal)/d of metabolisable energy (ME). The sheep readily ingested the fresh or dried duckweed. None of the wool measures (yield, rate of fibre elongation, fibre diameter) differed ( $p>0.05$ ) between dietary treatments. In Experiment 2, oaten-chaff-based diets (800 g/d) supplying 6.5-7.2 MJ (1.6-1.7 Mcal)/d of ME were supplemented with iso-nitrogenous amounts (4-5 g N) either of urea (8 g), cottonseed meal (60 g) or dried duckweed (100 g). In this experiment, the rate of wool fibre elongation, thought to be related to intestinal amino acid absorption, was lower ( $p<0.05$ ) for sheep given the oaten chaff/urea diet than for those given either oaten chaff/cottonseed meal or oaten chaff/duckweed for which the rates did not differ ( $p>0.05$ ). Fibre diameter, which ranged from 16.0-16.7 mm, did not differ ( $p>0.05$ ) between diets, but tended to be lower on the oaten chaff/urea diet so that volume of wool produced was also significantly lower ( $p<0.05$ ) on this diet than on the diets containing duckweed or cottonseed meal. Rumen ammonia concentrations at 4.5 and 7.5 h after feeding were higher ( $p<0.05$ ) for sheep given the oaten chaff/urea diet than for those given the other two diets. A comparison of the rumen ammonia concentrations, wool growth rate and predicted flows of amino acids from the rumen of sheep supplemented with duckweed rather than cottonseed meal suggested that duckweed is a valuable source of 'escape protein' for ruminants. (*Asian-Aust. J. Anim. Sci.* 2001. Vol. 14, No. 4 : 507-514)

**Key Words** : Sheep, Duckweed, Escape Protein, Wool Production, Wool Fibre

## INTRODUCTION

Duckweed is a high-yielding aquatic plant that is a potentially valuable protein-rich feed for farm animals. Its crude protein content is dependent on the nutrient content of the water it grows on, but values ranging from 15 to 43% have been reported (Leng et al., 1994). Its potential value as a feed protein source has been confirmed for species whose amino acids requirements are derived directly from the diet, namely poultry (O'Neill et al., 1996; Haustein et al., 1990; Haustein et al., 1992), pigs (Men et al., 1997) and fish (Van Dyke and Sutton, 1977; Hassan and Edwards, 1992). In contrast, there have been few evaluations of duckweed as a feed for ruminant animals which obtain the majority of their protein requirements from microbial cells produced in the rumen but also derive amino acids from feeds that contain dietary protein in forms that pass from the rumen before being fermented (i.e. 'escape' protein).

Leng et al. (1994) have suggested that the duckweed's main role in ruminant diets would be as a source of ruminally degradable N (RDN) and/or minerals to support rumen microbial growth. In a

study with cattle, Huque et al. (1996) found that duckweed protein was more than 80% degraded in the rumen over 72 h, i.e. only about 20% of the amino acids in duckweed would have 'escaped' to the small intestine. Apart from this study, there is little other research to determine whether duckweed will be ingested by ruminants, or has potential as a source of either rumen degradable or escape protein.

In the present study, Merino sheep were given a basal diet of oaten chaff that was supplemented with duckweed (*Spirodela punctata*, harvested from a sewage treatment works in northern NSW) to provide additional protein for wool growth. In Experiment 1, the aim was to determine whether sheep would readily learn to ingest fresh or dried duckweed, and to investigate its post-ingestive effects on live-weight gain and wool production. In Experiment 2, rate of wool fibre elongation and fibre diameter were compared in sheep given a basal diet of oaten chaff supplemented with iso-nitrogenous amounts of either urea (a source of non-protein N that is completely degraded in the rumen), cottonseed meal (approximately 40 % 'escape' or 'by-pass' protein; McDonald et al., 1992) or duckweed, so that the 'escape' protein value of duckweed could be evaluated.

## MATERIALS AND METHODS

### Animals and their management

Thirty-six Merino sheep (castrated males, aged

\* Corresponding Author: J. V. Nolan. Tel: +61-2-6773-3275, Fax: +61-2- 6773-3275, E-mail: jnolan@metz.une.edu.au.

<sup>1</sup> BioTech Waste Management, P.O Box 870, Armidale, NSW 2350, Australia.

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about 6 months, mean live weight of  $39.2 \pm 0.57$  kg and in good body condition) were obtained from a fine-wool flock at the University of New England, and used in Experiment 1 (21 November 1997-13 March 1998). A smaller group ( $n=21$ ) drawn from the same 36 sheep was used in Experiment 2 (30 July-22 November 1998). During both experiments, the animals were confined indoors in individual pens ( $0.9 \times 1.2$  m). The pens had a feed trough at the front and a water trough at the back from which fresh water was always freely available. New feed was given each day at 09.00 h. The sheep were weighed (at 14.00 h) at strategic times throughout the experiments.

### Experiment 1

*Pre-experimental phase:* From 21 November to 3 December 1997, the sheep were housed indoors and offered a mixed diet of oaten chaff (600 g/d) and lucerne chaff (50 g/d), and allowed to become accustomed to their new situation. On 3 December 1997, by which time all the animals were eating the oaten chaff/lucerne chaff mixture, the diet was changed to a mixture of sun-dried duckweed (50 g/d) and oaten chaff (700 g/d). On the same day, wool was removed from a mid-side patch on the left side of each animal and retained for detailed description, and a dye-band was placed in the wool next to the skin surface about 5 cm posterior to the mid-side patch.

During the next 7 weeks (3 December 1997-27 January 1998), the sheep were offered the same ration of 700 g oaten chaff and 50 g sun-dried duckweed so that all animals could experience the taste and metabolic consequences of ingesting duckweed. On 27 January, the wool that had grown on the mid-side patch was removed by clipping close to the skin surface, and retained for detailed description, and a second dye-band was placed in the wool at the same site as the original dye-band. The characteristics of wool produced by individual sheep in the period 4 December 1997-27 January 1998 were used later in an analysis of covariance when comparing the effect of diet on the same characteristics during the experimental period (27 January-13 March 1998).

*Experimental phase:* On 27 January, the animals were allocated to 4 groups of 9 sheep, each group being assigned to one of 4 experimental diets. The allocation was made on the basis of the animals' live weight and the mean fibre diameter of their wool obtained from the mid-side patch. The diets, live weights and diameter of wool fibre from the sheep in each of the treatment groups on the first day of the experimental period are given in table 1. The diets consisted of oaten chaff supplemented with different amounts of fresh or sun-dried duckweed. They were designed to be iso-energetic and to provide a metabolisable energy intake (MEI) of 5.4 MJ (1.3

Mcal)/d. This MEI was expected to be just adequate to enable the sheep to maintain live weight (SCA, 1990). Diet 1 (OC) provided 700 g oaten chaff /d, and was estimated to provide about 9 g N/d. Diet 2 (630 g OC+ 50 g sun-dried duckweed/d; OC+50 DW) and Diet 4 (630 g OC+ 1000 g fresh duckweed/d; OC+1000 DW) were expected to provide the same amount of N and dry matter (DM), with sun-dried or fresh duckweed (4.9% DM; 33% crude protein) providing an additional 2.6 g N/d, respectively, whereas the duckweed included in Diet 3 (540 g OC +100 g sun-dried duckweed; OC+100 DW) would have provided an additional 5.2 g N/d. When predicting MEI, it was assumed that duckweed ME content was 9.7 MJ (2.3 Mcal)/kg DM.

At the end of the experimental period, on 11 March 1998, the sheep were weighed and wool was again clipped from the mid-side patches, and a third dye-band was placed in the wool, close to the skin surface and below the previous two dye-bands. The sheep were then put out onto pasture to allow time for the third dye-band to grow away from the skin surface. Dye-banded wool staples were removed from the animals on 27 April 1998. The sheep were returned to pasture and allowed to graze as one group until the end of July 1998 when they were returned to the animal house in preparation for Experiment 2.

### Experiment 2

*Pre-experimental phase:* The sheep were housed as in Experiment 1 and, from 30 July given a daily ration of 800 g oaten chaff and lucerne chaff (50 g) mixed with 8 g urea until 18 October 1998. A dye-band was placed next to the skin in a wool staple on the right side of the sheep on 12 August 1998.

*Experimental phase:* On 19 October 1998, the animals were allocated to 3 groups of 7 sheep based on their mean wool fibre diameter and live weight at the end of Experiment 1 (table 2). The sheep were assigned to receive, from 19 Oct to 22 Nov 1998, one of three diets, viz. 800 g oaten chaff (OC) plus 8 g urea (OC+U), 800 g oaten chaff plus 60 g cottonseed meal (OC+CSM) or 800 g oaten chaff plus 100 g

**Table 1.** Dietary treatments, mean live weight and wool fibre diameter of sheep in the treatment groups at the start of the experiment on 26 January 1998 (experiment 1, group means  $\pm$  SEM,  $n=9$ )

Diet	Diets	Live weight (kg)	Mean fibre diameter ( $\mu$ m)
1	OC	$35.7 \pm 1.1$	$17.8 \pm 0.63$
2	OC+50DW	$37.5 \pm 0.9$	$17.9 \pm 0.59$
3	OC+100DW	$39.2 \pm 1.3$	$17.7 \pm 0.76$
4	OC+1000DW	$38.9 \pm 1.3$	$18.1 \pm 0.83$

\* OC=oaten chaff; DW=duckweed

sun-dried duckweed (OC+DW). The diets were intended to provide approximately similar amounts of total N (17-18 g N/day) and of supplementary N (4-5 g N/day) in the form of urea, duckweed or cottonseed meal. Analyses of the ingredients in these diets are given in table 3.

A second dye-band was placed next to the skin below the first dye-band on the 21 October 1998. Rumen fluid was collected using a stomach tube on 20 November 1998 at 0.5, 4.5 and 7.5 h after feeding. Rumen fluid pH was determined immediately after the samples were collected, and a sub-sample (16 ml) was transferred into a wide-neck McCartney bottle containing 0.3 ml of 18 M H<sub>2</sub>SO<sub>4</sub> and stored at -18°C pending analysis to determine ammonia concentration.

A third dye-band was inserted close to the skin surface below the second one at the end of the experimental period (22 Nov. 1998) after which animals were again put out on pasture to allow time for the dye-band to grow away from the skin. Dye-banded wool samples were removed from the sheep on 29 Jan 1999; at the time of this sampling, one sheep from the (OC+U) group and two sheep from the (OC+DW) treatment groups could not be found and associated data were treated as missing values for statistical purposes.

#### Laboratory analyses

The distance from the base of the first dye-band to the base of the second band, and then from the base of the second band to the base of the third band indicated the length of the wool staple grown during the pre-experimental and experimental periods, respectively. Measurements were made using 5 randomly chosen wool staples from each sheep. To estimate the mean diameter of the wool fibre grown in the pre-experimental and experimental phases, the staples were guillotined at the base of the second and the third dye-bands to give a 2 mm sample immediately above the cut. This sample was washed twice with n-hexane that was then removed by double washing with hot water (60°C). The resulting clean wool sample was dried in a forced-draught oven at 75°C for 1.5 h, and left overnight in a controlled environment. The following morning, it was mini-cored to provide 2000 snippets of fibre that were subjected

**Table 2.** Dietary treatments, mean ( $\pm$ SEM; n=7) live weight and wool fibre diameter in treatment groups at the start of the experimental period (experiment 2, 19 October 1998)

Diet	Diets	Live weight (kg)	Mean fibre diameter ( $\mu$ m)
1	OC+U	41.3 $\pm$ 1.72	16.2 $\pm$ 0.88
2	OC+CSM	38.7 $\pm$ 1.30	16.3 $\pm$ 0.65
3	OC+DW	38.6 $\pm$ 0.65	16.5 $\pm$ 0.94

to optical fibre diameter analysis (IWTO, 1995).

Samples of feed used in Experiment 2 were dried in a forced-draught oven at 105°C for 24 h and then burnt in an ashing oven at 600°C for 6 h to enable DM and organic matter (OM) content to be estimated. Nitrogen content of finely ground feed samples was determined using a Nitrogen Analyser (Leco FP 2000, St Joseph, MI USA). The results are given in table 3.

Ammonia-N in rumen fluid was analysed colorimetrically using a Technicon autoanalyser (Biertz, 1974).

#### Statistical analysis

Statistical analysis was performed on a personal computer using S-plus 2000 (MathSoft Inc.). Differences in the rates of wool fibre elongation and in fibre diameter during the experimental phases of both experiments were evaluated by analysis of covariance with adjustments for these same parameters determined in each of the pre-experimental phases. Yields of greasy wool obtained in Experiment 1 were also subjected to analysis of variance. In Experiment 2, means for dietary intake during the experimental phase and rate of fibre elongation were evaluated by analysis of covariance, while treatment means for rumen fluid ammonia-N concentration and pH were evaluated by analysis of variance.

## RESULTS

#### The edibility of duckweed

When the sheep were first brought into the animal house in both experiments, they were allowed to become accustomed to their new surroundings and to learn to eat oaten chaff and lucerne chaff as well as duckweed. The sheep were offered a basal diet of oaten chaff, but were encouraged to investigate their feed bins and their contents by placing some lucerne and common salt in the bins. The animals took about 4-5 days to become accustomed to eating all of these feed ingredients.

During the pre-experimental phase of Experiment 1 when duckweed was first introduced to the animals in

**Table 3.** Dry matter (% air dry), organic matter and crude protein contents (% dry matter) of dietary ingredients (experiment 2)

Feed component	Chemical composition		
	Dry matter	Organic matter	Crude protein*
Oaten chaff	91.6	91.3	10.4
Lucerne chaff	92.5	94.1	19.6
Duckweed (sun-dried)	88.8	84.5	35.5
Cottonseed meal	92.0	92.9	46.5

\* Total N (% dry matter)  $\times$  6.25.

either dried or fresh forms, there were occasional feed refusals when fresh duckweed was mixed in the diet, but the amounts refused were small and the duckweed containing diets were readily accepted within a few days.

During the experimental phase of Experiment 1, duckweed was completely ingested by the sheep each day. When the sheep were re-introduced to the duckweed in the experimental phase of Experiment 2, they recognised and immediately accepted it. No clinical ill-effects associated with the ingestion of duckweed by the sheep were observed in either experiment.

#### Feed intakes

The MEI and crude protein (Experiment 1) and DM, OM, crude protein and MEI (Experiment 2) are given in tables 4 and 5, respectively. In Experiment 2, intakes of DM, OM and ME (adjusted for intakes in the pre-experimental phase) by sheep offered Diet OC+U were lower ( $p < 0.05$ ) than for those given Diets (OC+CSM) or (OC+DW) (table 5). Crude protein intake of sheep on Diet (OC+DW) was less ( $p < 0.05$ ) than that of animals given Diet (OC+U) or Diet (OC+CSM).

#### Live-weight change

During the pre-experimental phase of Experiment 1 the sheep lost live weight from a mean of 39.1 kg on 1 Dec 97 to be 1.4 kg live weight lighter on 26 Jan 98. During the experimental phase (26 Jan-11 March 98), there was a slight tendency for animals to regain weight slightly (mean 0.8 kg;  $p = 0.08$  for Diet OC+50DW).

In Experiment 2, mean live weight at the start of the pre-experimental period was 39.5 kg (table 2), but live weight change was not recorded thereafter.

#### Wool yield, fibre elongation and fibre diameter

In Experiment 1, yield of greasy wool on mid-side patches (62-80 g/m<sup>2</sup>/d) and rate of wool fibre elongation (0.171-0.192 mm/d) were higher, before covariate adjustment, in the pre-experimental phase than in the experimental phase (46-60 g/m<sup>2</sup>/d and

**Table 5.** Least-square means for intakes (g/d) of organic matter and crude protein based on analysis of ingredients, and estimated metabolisable energy intake (MEI; MJ (Mcal)/d) during the experimental phase (experiment 2)

Nutrients	Chemical composition		
	OC+U	OC+CSM	OC+DW
Organic matter (g/d)	659 <sup>a</sup>	721 <sup>b</sup>	722 <sup>b</sup>
Estimated MEI (MJ (Mcal)/d)*	6.5 (1.6) <sup>a</sup>	7.2 (1.7) <sup>b</sup>	7.2 (1.7) <sup>b</sup>
Crude protein (g/d)	102 <sup>a</sup>	102 <sup>a</sup>	93.0 <sup>b</sup>
DPLS (microbial +escape protein) <sup>1</sup> (g/d)	42	50	50

<sup>a,b</sup> Means within a row without a common superscript differ ( $p < 0.05$ ).

\* Estimated using published (SCA, 1990).

<sup>1</sup> Digestible protein leaving stomach (estimated using GrazFeed; Freer et al., 1997).

0.157-0.177 mm/d, respectively). During the experimental phase, neither the adjusted yield of greasy wool nor the adjusted rate of wool fibre elongation differed significantly between diets (table 6).

In Experiment 2, the adjusted rate of wool fibre elongation in the experimental phase was lower ( $p < 0.05$ ) for the sheep supplemented with urea than for those offered duckweed or cottonseed meal (table 7).

There were no between-diet differences in the mean fibre diameter of wool grown during Experiment 1 (table 6). Fibre diameter declined from a mean of 18.8  $\mu$ m to 17.9  $\mu$ m during the pre-experimental period and declined further during the experimental period when, even though the animals maintained weight, the mean fibre diameter was lower (15.9  $\mu$ m) than in the pre-experimental phase. In the last 10 d before the insertion of the third dye-band, fibre diameter of the wool grown (the mean diameter of a 2 mm snippet below this band) was even lower (15.3  $\mu$ m) than the mean for the whole experimental phase.

In Experiment 2, the adjusted rate of wool fibre elongation was lower ( $p < 0.05$ ) for the diet containing urea (0.137  $\pm$  0.006 mm/d) than for the diets containing

**Table 4.** Least-square means for organic matter, metabolisable energy (MEI) and crude protein intakes, and the amounts of digestible protein leaving the stomach (experiment 1)

Nutrients	Diets			
	OC	OC+50DW	OC+100DW	OC+1000DW
Organic matter (g/d)	644	624	611	630
Estimated MEI (MJ (Mcal)/d)*	5.5 (1.3)	5.4 (1.3)	5.3 (1.3)	5.3 (1.3)
Crude protein (g/d)	42.5	51.9	61.9	49.4
DPLS (microbial+escape protein) (g/d)	27	29	32	28

\* Estimated using published (SCA, 1990).

Digestible protein leaving stomach (estimated using GrazFeed; Freer et al., 1997).

**Table 6.** Least-square means ( $\pm$ SEM) for yield of greasy wool (mid-side patch), rate of wool fibre elongation (dye-band) and wool fibre diameter in the experimental phase (experiment 1)

Items	Diets			
	OC	OC+50DW	OC+100DW	OC+1000DW
Yield of greasy wool (g/m <sup>2</sup> /d)	53 $\pm$ 2.86	53 $\pm$ 2.95	57 $\pm$ 2.86	53 $\pm$ 2.95
Rate of fibre elongation (mm/d)	0.159 $\pm$ 0.007	0.175 $\pm$ 0.007	0.154 $\pm$ 0.007	0.168 $\pm$ 0.007
Adjusted fibre diameter ( $\mu$ m)	15.9 $\pm$ 0.24	15.8 $\pm$ 0.24	16.0 $\pm$ 0.24	15.7 $\pm$ 0.24
Adjusted 2 mm snippet ( $\mu$ m)*	15.4 $\pm$ 0.42	15.0 $\pm$ 0.29	15.4 $\pm$ 0.75	15.5 $\pm$ 0.62

\* Wool grown in the last 10 days of the period of supplementation.

cottonseed meal and duckweed which did not differ significantly ( $0.157 \pm 0.005$  mm/d): the volume of wool produced per day was also lower ( $p < 0.05$ ) on the diet supplemented with urea. As with Experiment 1, the adjusted mean fibre diameter of the wool grown in the experimental phase, which did not differ ( $p > 0.05$ ) between dietary treatments, was  $16.4 \mu\text{m}$  (cf.  $15.9 \mu\text{m}$  in Experiment 1).

#### Rumen fluid ammonia concentration and pH (Experiment 2)

Ammonia concentration in rumen fluid in Experiment 2 increased from 96.3 to 176 mg N/l between 0.5 and 4.5 h after feeding and then decreased to 84.4 mg N/l at 7.5 h after feeding (figure 1). The rumen fluid ammonia concentrations did not differ ( $p > 0.05$ ) between diets in samples collected at 0.5 h after feeding, but were higher ( $p < 0.05$ ) for Diet (OC+U) at 4.5 and 7.5 h than for the other two diets which did not differ ( $p > 0.05$ ). Rumen fluid pH decreased significantly ( $p < 0.001$ ) from  $7.0 \pm 0.03$  h after feeding time, to  $6.5 \pm 0.05$  at 4.5 h and  $6.4 \pm 0.04$  at 7.5 h after feeding, but did not differ significantly between diets.

## DISCUSSION

These studies demonstrated that duckweed given as

**Table 7.** Least-square means ( $\pm$ SEM) for rate of wool fibre elongation (mm/d) and fibre diameter in the experimental phase (experiment 2)

Items	Diets		
	OC+U	OC+CSM	OC+DW
Rate of Fibre elongation (mm/d)	0.137 <sup>a</sup> $\pm$ 0.006	0.15 <sup>b</sup> $\pm$ 0.005	0.159 <sup>b</sup> $\pm$ 0.006
Adjusted fibre diameter ( $\mu$ m)	16.2 <sup>a</sup> $\pm$ 0.30	16.0 <sup>a</sup> $\pm$ 0.28	16.7 <sup>a</sup> $\pm$ 0.33
Fibre volume production (mm <sup>3</sup> /d $\times 10^5$ )	2.89 <sup>a</sup> $\pm$ 0.16	3.12 <sup>a</sup> $\pm$ 0.15	3.51 <sup>b</sup> $\pm$ 0.18

<sup>a,b</sup> Means within a row without a common superscript differ ( $p < 0.05$ ).

a supplement to a basal diet of oaten chaff, in both fresh and sun-dried forms, was well accepted by Merino sheep and generated responses in wool production similar to those obtained when cottonseed meal was provided as a dietary supplement. Clinical ill-effects associated with the ingestion of duckweed were not apparent in either study.

It is expected that animals will exhibit neophobia (fear of novelty) when first offered a food they have not previously experienced; however, after they ingest small amounts of the food and detect the metabolic consequences, they may form conditioned aversions or preferences for the food depending on whether the post-ingestive metabolic effects of the food ingredients are detrimental or beneficial (Tien et al., 1999). The sheep formed a strong preference for duckweed after a short period of exposure, and we therefore conclude that the beneficial nutritional properties of duckweed outweighed any toxic or other detrimental effects that may have been present.

Diets in Experiment 1 were formulated to provide a maintenance level of energy intake, and this was successfully achieved, as indicated by the relatively constant live weight of animals during the experiment. Under these conditions, it seemed likely that wool growth might be stimulated by an increase in the intestinal supply of amino acids when duckweed was included in the basal diet (Black et al., 1973). However, despite duckweed-supplemented animals ingesting more crude protein those receiving oaten chaff only, there were no significant effects of including duckweed in the diet on wool yield or characteristics.

The total amounts of amino acids available for intestinal absorption in Experiment 1 were predicted using the software model GrazFeed (Freer et al., 1997) assuming that duckweed would be 80% degradable in the rumen (Huque et al., 1996) and oaten chaff 70% degradable. GrazFeed predicted that, for sheep given oaten chaff only, 27.0 g/d total digestible protein would have passed out of the stomach, whereas in those offered oaten chaff supplemented with 50 g, 100 g or 1 kg duckweed, the values would have increased only slightly to 29, 32 and 28 g/d, respectively. An increase of 1-5 g/d in the amounts of total digestible

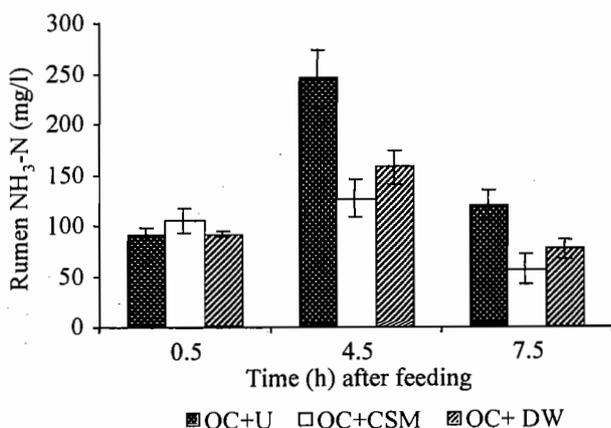
protein leaving the stomach may have been too small to generate any detectable increase in wool growth in this experiment, especially as the additional protein may have been used in part to prevent tissue protein loss rather than to supply the wool follicle.

The suggestion that the amounts of digestible crude protein leaving the stomach and potentially available for intestinal absorption in animals in Experiment 1 (27-32 g/d) were insufficient to enhance wool growth is consistent with the continued decline of wool yield and fibre diameter observed throughout the experiment. Black et al. (1973) found that, under nutritional conditions that were closely similar to those in Experiment 1, wool production of fine-woolled Merino sheep (33 kg) declined from about 4 g/d to 2 g/d over a period of 8 weeks during which they were given a complete diet by abomasal infusion that provided 5 MJ (1.2 Mcal)/d of gross energy and 20 g/d of protein. At the same rate of energy infusion but higher rates of protein infusion (40-80 g protein/sheep/d) and in conditions rather similar to our Experiment 2, these authors found that the higher initial rates of wool growth (5-9 g wool/sheep/d) were just maintained or slightly increased over an 8-week period. In our Experiment 2, the rate of wool fibre elongation was similar to that during Experiment 1. However, elongation rate was higher when animals were given oat chaff supplemented with cottonseed meal or duckweed, estimated to provide 50 g/sheep/d of digestible protein to the small intestine, than when they were supplemented only with urea (estimated to provide 40 g/sheep/d of digestible protein). Results of the current study and those of Black et al. (1973) therefore suggest that wool growth is less responsive to an increase in intestinal protein supply if this

supply is below that required to support live weight maintenance.

In Experiment 2, the rate of wool fibre elongation was higher in animals supplemented with cottonseed meal or duckweed than in those supplemented with urea, even though crude protein intake was lower in animals supplemented with duckweed than for those offered the other two diets. The faster rate of fibre elongation for animals supplemented with cottonseed meal and duckweed was probably because the protein in these supplements escaped intact from the rumen, increasing the intestinal availability of digestible crude protein above that by microbial cells passing from the rumen of sheep supplemented with urea. The predicted total digestible crude protein (of microbial and dietary origin) leaving the stomach predicted using GrazFeed (Freer et al., 1997) in animals supplemented with cottonseed meal would be 50 g/d, assuming 60 % rumen degradability of cottonseed meal, and 50 g/d for sheep supplemented with duckweed, assuming 80% rumen degradability, whereas for those supplemented with urea it would be 42 g/d. Based on these predictions, it is possible that the rumen degradability of the duckweed protein used in our experiments was lower than the assumed 80%, and perhaps closer to the 60% assumed for cottonseed meal, i.e. its 'escape protein' value was higher than that assumed. At these levels of intestinal availability of amino acids, it is unlikely, on the basis of the findings of Black et al. (1973) and Reis et al. (1992) that the MEI, which was also higher for animals supplemented with cottonseed meal and duckweed than for those receiving urea, was solely responsible for the enhanced wool growth observed. Those studies showed that increasing gross energy intakes from 3.01 to 10.04 MJ /sheep/d (0.7 - 2.4 Mcal/sheep/d) in fine-woolled Merino sheep stimulated wool growth only when the amount of protein infused into the abomasum was 100 g/d, but not when it was 20 or 60 g/d, i.e. there was no effect of energy when conditions were similar to those in our experiments. We conclude that is likely that the response in wool production in Experiment 2 was primarily due to the enhanced supply of amino acids of dietary origin when duckweed or cottonseed meal rather than urea was used to supplement a basal diet of oat chaff.

In Experiment 2, the lower concentrations of rumen ammonia found 4.5 or 7.5 h after feeding in sheep supplemented with iso-nitrogenous amounts of duckweed or cottonseed meal in place of urea was probably a result of a slower rate of ammonia production from ruminal degradation of duckweed and cottonseed meal proteins relative to that from degradation of urea. Effects of pH, which can alter the rate of absorption of ammonia across the rumen wall (Siddons et al., 1985), were unlikely to have



**Figure 1.** Rumen fluid ammonia concentration (means  $\pm$  SEM; mgN/l) of animals given oat chaff (OC) plus one of three iso-nitrogenous supplements (urea, U; cottonseed meal, CSM or sun-dried duckweed, DW) at 0.5, 4.5 and 7.5 h after feeding (experiment 2)

generated differences in rumen ammonia concentration between diets in this experiment because there were no significant between-diet differences in pH. Degradation of dietary protein, intra-ruminal recycling of microbial N and influx of endogenous N (including urea) all contribute to the ammonia present in rumen fluid, whereas microbial assimilation of ammonia for protein synthesis, outflow to the lower tract and absorption through the rumen wall account for ammonia disappearance (Nolan, 1993). Of these, however, degradation of dietary protein (influx) and microbial assimilation (removal) are often the most significant ammonia transactions in the rumen. Even though rumen ammonia concentrations were apparently affected by diet in Experiment 2, the concentrations of rumen ammonia in all animals at 7.5 h after feeding (84.4-176mg N/l) were above the minimum of 50 mg N/l thought to be required for optimum microbial protein synthesis (Satter and Slyter, 1974) or 80 mg/l needed for optimum feed digestion (Leng et al., 1993). It therefore seems that the rumen ammonia concentration in the present study was not a major constraint to achieving optimum feed digestion or microbial protein synthesis.

Huque et al. (1996) reported that sun-dried duckweed proteins were extensively degraded in dacron bags placed in the rumen of bulls for 72 h (80% for Spirodela; 87% for Lemna and 94% for Wolfia). If this had also been the case in the sheep used in the present study, most of the protein present in duckweed would have been available to rumen microbes and only small amounts of amino acids would have been available from duckweed for absorption in the small intestine. Results of Experiment 2, however, showed that the concentrations of ammonia in the rumen of sheep supplemented with duckweed were similar to those supplemented with cottonseed meal. It therefore seems probable that, in our study, the resistance of proteins in the duckweed to degradation in the rumen was rather similar to that in cottonseed meal, which is considered to be a good source of undegradable protein for ruminants (Krysl et al., 1987). The higher resistance of duckweed to ruminal degradation in our study compared with that of Huque et al. (1996) may be due to differences in (a) the residence time of duckweed in the rumen (perhaps considerably less than 72 h), (b) duckweed-drying conditions or (c) ruminal conditions between our sheep and their cattle.

We conclude that the duckweed used in our experiments was readily eaten by sheep and that it was more effective than urea, and as effective, as a protein source for wool growth, as cottonseed meal - and cottonseed meal is considered to be an excellent source of 'escape' protein for ruminants (Leng et al., 1983).

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