THE UTILIZATION OF ABOMASAL SUPPLEMENTS OF PROTEINS AND AMINO ACIDS BY SHEEP WITH SPECIAL REFERENCE TO WOOL GROWTH

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Abstract

Proteins of different amino acid composition (Promine-D, wheat gluten, and zein) were given as abomasal infusions to sheep and effects on wool growth rate, body weight gain, and nitrogen retention were compared with those of casein. These results were considered together with earlier data obtained for whole egg protein, egg albumen, maize gluten, and gelatin. The nutritive value of bloodmeal supplements was also studied. In addition the effects on wool growth of adding lysine and tryptophan to zein, and of adding leucine to casein, were examined.

Abomasal supplements (supplying *c.* 16 g nitrogen per day) of casein, Promine-D, and wheat gluten produced large increases in wool growth rate; zein supplements slightly depressed wool growth. The relative values of the proteins for wool growth were: casein (100), wheat gluten (54), Promine-D (40), and zein (−11). Zein markedly reduced wool fibre diameter and produced an area of weakness in the fibres. Abomasal supplements of casein, Promine-D, and wheat gluten were highly digestible (94–100%); zein was less digestible (80–84%). All these proteins, except zein, consistently supported increases in body weight and gave similar values for nitrogen retention.

Bloodmeal supplements had a low digestibility (62–66%), whether given in the diet or via the abomasum, and were poorly utilized for wool growth and nitrogen retention. There was no advantage of abomasal over dietary supplementation.

When the components of wool growth were measured separately, on average abomasal supplements of zein reduced fibre diameter and wool output, but consistently increased length growth rate of wool. The addition of L-lysine and L-tryptophan to zein did not alter the length growth rate but appreciably increased fibre diameter and wool output above the basal rate. The addition of L-leucine to abomasal supplements of casein, to increase the leucine content to that of zein, had no appreciable effect on fibre diameter or length growth rate of wool.

I. Introduction

The digestive processes in ruminants prevent efficient utilization of the protein in high protein diets. However, when rumen fermentation is avoided by direct administration into the abomasum or duodenum, proteins are much more efficiently utilized (McDonald 1968). Much of this evidence was derived from experiments in which abomasal or duodenal administration of casein was compared with oral administration. Post-ruminal administration increased wool growth rate substantially (Reis and Schinckel 1964; Reis 1969), enhanced nitrogen retention in sheep (Chalmers, Cuthbertson, and Synge 1954; Reis and Schinckel 1961; Little and Mitchell 1967;

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Schelling and Hatfield 1968; Ørskov, Fraser, and Corse 1970), and increased the rate of liveweight gain of sheep (Ørskov, Fraser, and Corse 1970; Black 1970) and of cattle (Swanson *et al.* 1969).

There is less information available about the utilization of other proteins given via the abomasum. Ørskov and Fraser (1969) and Ørskov, Fraser, and Corse (1970) observed increased nitrogen retention and liveweight gain when various protein-rich meals were given in liquid form so as to pass directly to the abomasum. Little and Mitchell (1967) obtained high nitrogen retentions with casein and soyabean proteins given via the abomasum, but obtained low values with zein and gelatin. Colebrook and Reis (1969) compared the value of several proteins, given as abomasal supplements, for wool growth, nitrogen retention, and body weight gain. Casein, whole egg protein, and egg albumen were utilized efficiently, whereas maize gluten supported high nitrogen retention and body weight gain but substantially lower rates of wool growth, and gelatin was of little value. It was suggested that these differences in wool growth rate and nitrogen retention were probably related to the amino acid composition of the proteins and hence to differences in the amounts and proportions of individual amino acids absorbed.

In the present study, some further proteins of different amino acid composition, viz. Promine-D (a protein extracted from soyabeans), wheat gluten, and zein, were given as abomasal infusions, and the effects on wool growth rate, nitrogen retention, and body weight gain were compared with those of casein. In addition, abomasal supplements of bloodmeal were investigated, because of the low value of bloodmeal as a dietary supplement for wool growth reported by Colebrook *et al.* (1968). As zein is lacking in lysine, contains only a trace of tryptophan, and a high proportion of leucine (Block and Mitchell 1946; Block and Bolling 1951; Spector 1956), the effects on wool growth rate of adding lysine and tryptophan to abomasal infusions of zein, and of adding leucine to abomasal infusions of casein, were also investigated.

II. Experimental

(*a*) *Sheep and Diet*

The experimental sheep were mature wethers, fitted with an abomasal cannula near the pylorus; the breeds used are shown in Table 1. The sheep were kept in metabolism cages in a room maintained at $23 \pm 2°C$. A supplement of 1,000,000 i.u. of vitamin D_3 was given to each sheep once every 3 months. The daily ration was a mixture of equal parts of chopped wheaten and lucerne hays containing between 1·5 and 2·0% nitrogen in different batches; the amounts given are shown in Table 1. The mixture was ground and pelleted in experiments 2, 3, and 4. Water was available *ad libitum*. The sheep were fed once daily, between 0900 and 1000 hr, and were weighed once weekly prior to feeding in experiments 1 and 2 except during periods of excreta collection. The body weights were corrected for cumulative fleece growth.

(*b*) *Protein and Amino Acid Supplements*

During a pretreatment period of at least 8 weeks, the sheep received the roughage diet only. There were four experiments in which protein and amino acid supplements were given in two consecutive periods, usually as abomasal infusions, as shown in Table 1.

The protein supplements were casein (Casinal; Glaxo–Allenburys, Sydney), Promine-D (supplied by Food Additives of Australia, Rydalmere, N.S.W.), wheat gluten (Andrew Baker Pty. Ltd. Sydney), zein (Zein G200, supplied by Corn Products Co., New York), and bloodmeal (New South Wales State abattoirs). The following amino acids were added to proteins in some

experiments: L-lysine (L-lysine hydrochloride, feed grade, 98% purity; Feed Chemicals Pty. Ltd., Artarmon, N.S.W.), L-leucine (laboratory grade; Koch–Light Laboratories, U.K.), and L-tryptophan (laboratory grade; A.E.C., France).

Proteins and amino acids were infused into the abomasum as aqueous solutions or suspensions, volume 1500–2000 ml, for a period of 6–8 hr each day, commencing when the sheep were fed. A peristaltic pump was used to maintain a steady rate of infusion. When amino acids were given, aqueous solutions of the amino acids were added to the protein supplements before commencing the daily infusion. Solutions of casein were administered as described by Colebrook and Reis (1969). Bloodmeal, wheat gluten, and Promine-D were suspended in water (Promine-D was largely dissolved), and were stirred continuously during infusion to prevent sedimentation and to allow a uniform rate of infusion. Methylpolysiloxane was added to the

TABLE 1

EXPERIMENTAL PLANS

All supplements were given as abomasal infusions except as indicated in experiment 2. The values quoted after each supplement are g/day of dry matter. In experiment 1 all proteins supplied *c.* 16 g nitrogen per day. Lysine was given as L-lysine hydrochloride but the amount quoted is free lysine. The breed symbols are M (Merino), EL × M (English Leicester × Merino), and C (Corriedale)

Expt. No.	Sheep	Breed	Intake of basal diet (g/day)	Protein and amino acid supplements	
				Period 1	Period 2
				7 weeks	7 weeks
1	8002	M	800	Promine-D, 104	Casein, 99
	8005	M	800	Casein, 99	Promine-D, 104
	1024	EL × M	800	Wheat gluten, 128	Casein, 99
	1038	EL × M	800	Casein, 99	Wheat gluten, 128
	4489	C	600	Zein, 100	Casein, 99
	4494	C	600	Casein, 99	Zein, 100
				12 weeks	8 weeks
2	1095	EL × M	400	Bloodmeal, 68*	Bloodmeal, 68
	2627	EL × M	400		
				2 weeks	2 weeks
3	5482	M	800	Casein, 40	Casein, 33·5
	6003	M	800		L-Leucine, 6·5
				2 weeks	2 weeks
4	1095	EL × M	800	Zein, 76	Zein, 69
	8002	M	800		L-Lysine, 6
	8004	M	800		L-Tryptophan, 1
	9062	M	800		

* Included in the diet.

latter three proteins to minimize foam formation (Colebrook and Reis 1969); in addition 6–8 drops of Tween 80 (Atlas Chemical Industries, Wilmington, California) were added to the daily infusion of bloodmeal to facilitate wetting. A suspension of zein in water could not be pumped because of swelling of the particles which resulted in blocking of the infusion line. Consequently, zein was dissolved in water by adjusting the final pH to 11 with NaOH. The pH was gradually reduced to 6·5 by addition of 0·1N HCl while stirring with a high-speed mixer. The very fine precipitate of zein obtained was infused in the same manner as described for wheat gluten, with continuous stirring and the addition of methylpolysiloxane.

(c) *Collection of Excreta and Nitrogen Analysis*

Excreta were collected during the last 10 days of each experimental period, in experiments 1 and 2, as described by Reis and Tunks (1969).

Representative samples of excreta, the roughage diet, and the protein supplements were taken for analysis. Nitrogen was determined by a Kjeldahl method, and dry matter content by drying at 104°C for 24 hr.

The digestibilities of the protein supplements in experiments 1 and 2 were calculated from the increase in faecal nitrogen output during protein supplementation. Tissue nitrogen retention was calculated in experiment 1 as: nitrogen intake − (faecal nitrogen + urinary nitrogen + wool nitrogen); clean dry wool was assumed to contain 16% nitrogen.

(d) *Wool Growth*

In experiments 1 and 2 the supplementation periods were of 7–12 weeks duration, and measurements of wool growth rate were made by the tattooed patch method (Reis 1967). Wool from areas (*c.* 10 by 10 cm) defined by tattooed lines on the left and right shoulders, was removed with Oster small animal clippers (size 40) at intervals of 2 weeks, except for the first clipping in each supplementation period of experiment 1, which was after 1 week. Wool samples were cleaned by the method of Reis (1967), and total growth of clean dry wool per sheep (grams per day) was calculated as described by Reis and Schinckel (1964). The wool growth rates given in Tables 2 and 4 are those for the last 6 weeks of the pretreatment period and for the last 4 weeks of each treatment period. Yield of wool from two sheep in experiment 1 was calculated by expressing clean dry wool as a percentage of greasy wool. Wool fibre diameter was measured on samples from the same sheep by the "snippet method" of Chapman (1960).

In experiments 3 and 4 the supplementation periods were of 2 weeks duration, and the wool growth responses were assessed during the last 4 days of each period by the autoradiographic technique of Downes, Clarke, and Dagg (1967). Both length growth rate and diameter of fibres were measured and fibre volume was calculated from these parameters assuming that the fibres were cylindrical. As the fibres were not medullated, these volumes can be regarded as proportional to the mass of wool. Intravenous injections of L-[^{35}S]cystine were given 4 days apart at the end of each period and fibre diameter was measured at the front of each radioactive zone. Fibres were taken from 4 sites on each sheep; 25 fibres per site were measured in experiment 3 and 20 fibres per site were measured in experiment 4. A supplementation period of 2 weeks was considered to be adequate to assess wool growth responses by this technique, as valid estimates of the responses to supplements of casein and sulphur-containing amino acids (*S*-amino acids) (Downes *et al.* 1970; Reis and Downes 1971) and to changes in nutrition (Downes and Sharry 1971) have been obtained over this period.

III. Results

(a) *Comparative Value of Various Proteins for Wool Growth*

Abomasal supplements of casein, Promine-D, and wheat gluten all produced large increases in the rate of wool growth in experiment 1 (Table 2). However, both Promine-D and wheat gluten had a considerably lower value than casein for wool growth; the relative values were: casein (100), wheat gluten (54), and Promine-D (40). In contrast, zein supplements infused into the abomasum decreased the wool output of one sheep and had no effect on the other sheep (Table 2); the relative values of casein and zein for wool growth were 100 and −11 respectively. It should be noted that the 7-week supplementation periods would be adequate to remove any carry-over effects between periods 1 and 2 (Reis 1969; Colebrook and Reis 1969).

The wool grown during the periods of zein infusion could be detected subsequently as a band of abnormal wool (Fig. 1). This band represented a "break" or area of weakness in the wool fibres, and was also characterized by having a low yield of clean, dry wool

TABLE 2

EFFECT OF VARIOUS PROTEIN SUPPLEMENTS ON WOOL GROWTH AND NITROGEN RETENTION

These data relate to experiment 1, Table 1. Tissue nitrogen retention = nitrogen retention—nitrogen stored in wool; the values were calculated from the original data

Sheep	Abomasal supplement	Supplementation period	Growth of clean dry wool (g/day)	Increase in wool growth (%)	Wool growth response relative to casein (%)	Digestibility of protein supplement (%)	Nitrogen retention (g/day)	Tissue nitrogen retention (g/day)
8002	Nil	—	4·2	—		—	1·4	0·8
	Casein	2	14·1	236		100	3·6	1·4
	Promine-D	1	6·9	64	27	97	3·3	2·2
8005	Nil	—	4·0	—	40	—	1·4	0·8
	Casein	1	14·1	252		98	3·9	1·6
	Promine-D	2	9·4	135	53	95	1·5	0·0
1024	Nil	—	5·4	—		—	2·0	1·1
	Casein	2	14·9	176		97	2·8	0·4
	Wheat gluten	1	9·0	67	38	94	3·6	2·2
1038	Nil	—	4·2	—	54	—	1·3	0·6
	Casein	1	9·1	117		99	3·2	1·8
	Wheat gluten	2	7·6	81	69	100	2·7	1·5
4489	Nil	—	2·6	—		—	0·8	0·3
	Casein	2	7·7	196		98	2·6	1·4
	Zein	1	1·6	−38	−20	80	1·5	1·2
4494	Nil	—	2·8	—	−11	—	1·3	0·8
	Casein	1	8·7	211		97	4·1	2·7
	Zein	2	2·7	−4	−2	84	−0·5	−0·9

(Table 3). Wool fibre diameter was significantly reduced by zein infusion and increased by casein infusion in each sheep ($P < 0{\cdot}001$) (Table 3). The changes in fibre cross-sectional area due to casein (Table 3) accounted for only a proportion of the increased wool output (Table 2), as would be expected from previous work (Reis and Schinckel 1964; Reis and Downes 1971). However, with zein infusions the decrease in cross-sectional area (Table 3) for each sheep was greater than the decrease in wool output (Table 2), implying that length growth rate must have increased.

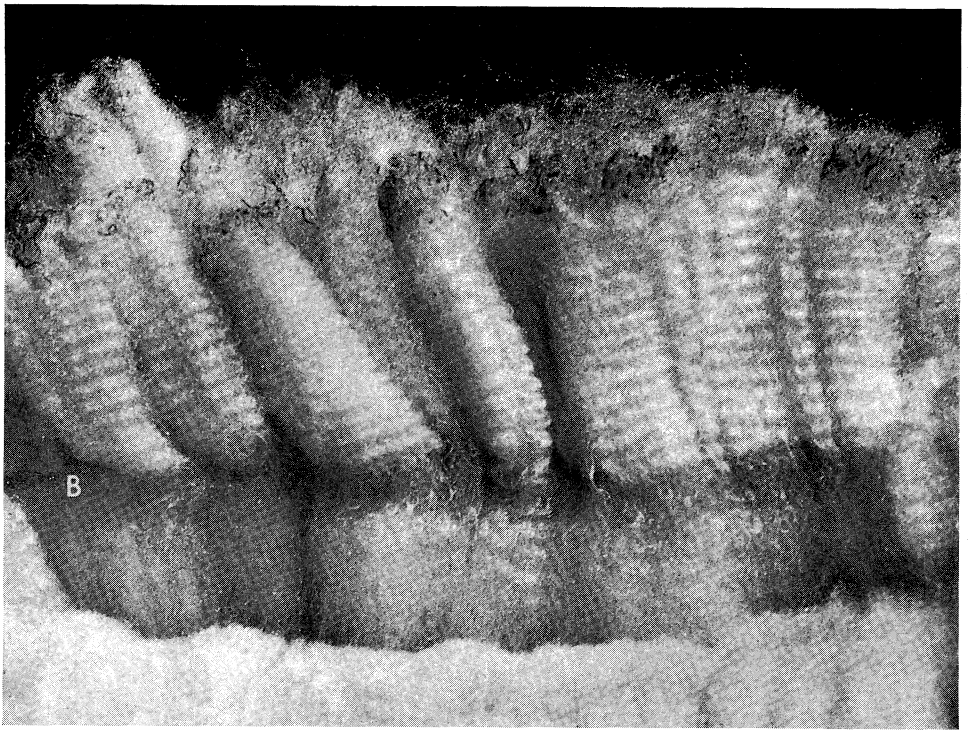

Fig. 1.—Wool staple from sheep 4489, experiment 1 (see Table 1 for details). The band B of abnormal wool was grown during supplementation with zein via the abomasum. Wool immediately above this band is control wool, and wool immediately below the band was grown during casein supplementation.

Addition of bloodmeal supplements to the diet or directly into the abomasum in experiment 2 increased the very low rates of wool growth obtained on the basal diet alone (Table 4). However, the wool growth rates obtained with bloodmeal supplements were also low and there was no advantage of abomasal over dietary supplementation. Factors were not available to calculate wool grown per sheep in this experiment, but the values quoted for bloodmeal supplements (Table 4) would be approximately 4–5 g of clean dry wool per day.

(*b*) *Digestibility of Protein Supplements, Nitrogen Retention, and Body Weight Changes*

Abomasal supplements of casein, Promine-D, and wheat gluten were highly digestible (94–100%), whereas zein was less digestible (80–84%) (Table 2). The

digestibility of the bloodmeal supplements was low (62–66%) whether given in the diet or via the abomasum (Table 4).

TABLE 3

EFFECT OF SUPPLEMENTS OF ZEIN AND CASEIN ON FIBRE DIAMETER AND YIELD OF WOOL

These data are for two sheep from experiment 1, Table 1. Each value for fibre diameter is the mean for 100 fibres. Fibre cross-sectional area was calculated assuming fibres were circular in cross-section

Sheep	Abomasal supplement	Supplementation period	Wool fibre diameter +S.E. (μm)	Change in fibre cross-sectional area (%)	Yield of clean dry wool (%)
4489	Nil	—	20·4±0·39	—	64
	Casein	2	27·1±0·50[a]	76	71
	Zein	1	15·0±0·35[a]	−46	42
4494	Nil	—	18·9±0·36	—	58
	Casein	1	24·9±0·46[a]	74	66
	Zein	2	17·2±0·32[a]	−18	45

[a] Significantly different from fibre diameter with no protein supplement ($P < 0·001$).

Values for nitrogen retention and tissue nitrogen retention obtained with the various proteins in experiment 1 are given in Table 2; the values for each protein tended to be higher in period 1 than in period 2. Zein appeared to be of less value than the other proteins for nitrogen retention and tissue nitrogen retention. The nitrogen retention values for bloodmeal in experiment 2 (Table 4) confirm the poor utilization of bloodmeal, especially as an abomasal supplement. Tissue nitrogen retention could not be calculated in this experiment.

TABLE 4

EFFECT OF BLOODMEAL SUPPLEMENTS ON WOOL GROWTH AND NITROGEN RETENTION

These data relate to experiment 2, Table 1

Sheep	Bloodmeal supplement	Supplementation period	Growth of clean dry wool (mg/cm²/day)	Digestibility of bloodmeal nitrogen (%)	Nitrogen retention (g/day)
1095	Nil	—	0·24	—	0·4
	68 g/day in diet	1	0·58	66	1·7
	68 g/day via the abomasum	2	0·44	62	0·8
2627	Nil	—	0·29	—	0·4
	68 g/day in diet	1	0·65	66	1·9
	68 g/day via the abomasum	2	0·61	64	1·4

Figure 2 shows the changes in fleece-free body weight in experiment 1 in response to abomasal supplements of proteins. Increases in body weight were consistently

obtained with all proteins except zein, which failed to increase body weight in sheep 4494. Casein and wheat gluten appeared to have similar effects, whereas Promine-D appeared to support slightly lower rates of body weight increase.

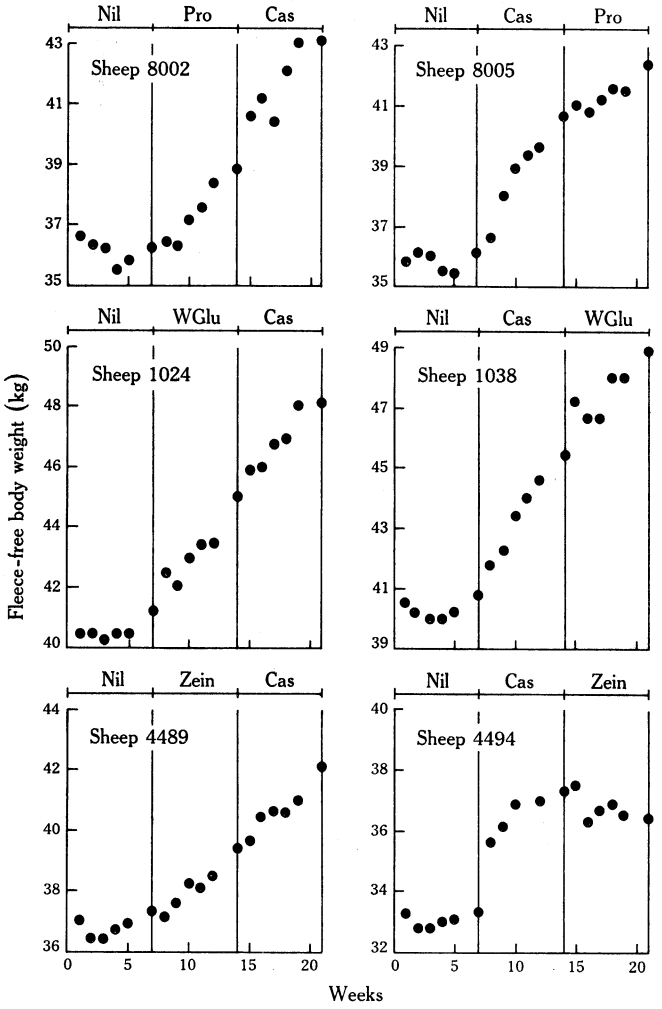

Fig. 2.—Effect of abomasal protein supplements on fleece-free body weights (see experiment 1, Table 1 for details). The protein supplements were casein (Cas), wheat gluten (WGlu), Promine-D (Pro), and zein.

(c) *Amino Acid Supplementation of Zein and Casein*

The low value of zein for wool growth may be related to its unusual amino acid composition, namely a high proportion of leucine, a trace of tryptophan, and no lysine (Block and Mitchell 1946; Block and Bolling 1951; Spector 1956). Consequently, two experiments were carried out to investigate these possibilities. Wool growth was measured by the autoradiographic technique (Downes, Clarke, and Dagg 1967) to enable

short infusion periods to be used and to obtain separate estimates of length growth rate and fibre diameter.

In experiment 3 possible adverse effects of excess leucine were investigated; casein was compared with a leucine-enriched casein, the total leucine content being *c.* 24%, as is present in zein. Casein supplementation increased length growth rate, fibre diameter, and hence fibre volume; the addition of leucine to the casein had no appreciable effect on fibre diameter or length growth rate of wool (Fig. 3). The effects were similar in each of the two sheep (Table 5).

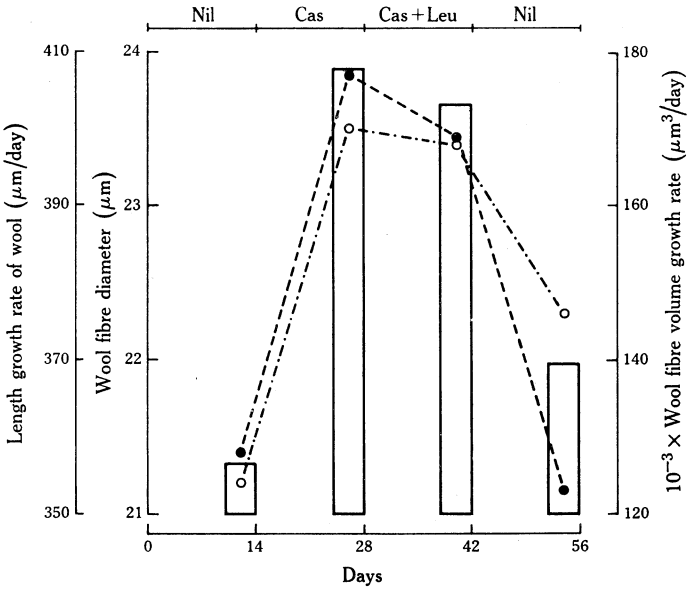

Fig. 3.—Effect of abomasal supplements of casein and leucine-enriched casein on the components of wool growth (see experiment 3, Table 1 for details). The components of wool growth are indicated as follows: fibre volume (vertical columns), fibre diameter (○), and length growth rate (●). Values are means from two sheep.

In experiment 4 the effect of supplementing zein with lysine and tryptophan was investigated by adding sufficient of each amino acid to give the approximate amount present in casein, viz. 8% lysine and 1·4% tryptophan (Block and Mitchell 1946; Block and Bolling 1951; Spector 1956). Results for individual sheep are given in Table 5 and the average effects are shown in Figure 4. Abomasal supplementation with zein caused a significant reduction in fibre diameter ($P < 0{\cdot}001$). However, as inferred from the data in experiment 1 (Tables 2 and 3), the length growth rate of wool was consistently increased by zein supplementation ($P < 0{\cdot}001$). The combination of these two effects produced little change in fibre volume, except for a decrease with sheep 1095 (Table 5). This result was similar to that found in experiment 1 (Table 2), when a larger amount of zein was given. The addition of lysine and tryptophan to zein did not alter the length growth rate, but appreciably increased fibre diameter ($P < 0{\cdot}001$) and

fibre volume ($P < 0{\cdot}001$) in all sheep (Table 5 and Fig. 4). The values for fibre volume, fibre diameter, and length growth rate, obtained during amino acid supplementation of zein (Fig. 4), were all significantly greater than the pretreatment values ($P < 0{\cdot}001$).

TABLE 5

EFFECT OF CASEIN, ZEIN, AND AMINO ACID SUPPLEMENTS ON WOOL GROWTH

These data relate to experiments 3 and 4, Table 1. The nil supplementation values are for the pretreatment period

Expt. No.	Sheep	Abomasal supplement	Length growth rate of wool (μm/day)	Wool fibre diameter (μm)	$10^{-3} \times$ Wool fibre volume growth rate (μm^3/day)
3	5842	Nil	363	22·4	143
		Casein	416	24·8	202
		Casein + leucine	405	24·9	197
	6003	Nil	353	20·0	111
		Casein	398	22·2	154
		Casein + leucine	392	22·0	149
4	1095	Nil	416	29·0	275
		Zein	442	24·8	214
		Zein + (lysine, tryptophan)	455	31·2	349
	8002	Nil	364	21·7	135
		Zein	422	20·4	138
		Zein + (lysine, tryptophan)	396	23·1	166
	8004	Nil	457	26·4	250
		Zein	484	24·6	231
		Zein + (lysine, tryptophan)	482	27·5	286
	9062	Nil	417	22·9	172
		Zein	434	22·7	175
		Zein + (lysine, tryptophan)	441	24·1	201

IV. DISCUSSION

It is apparent from the present data and from earlier published work (Reis and Schinckel 1964; Colebrook and Reis 1969) that the mixture of amino acids available for absorption from the small intestines has a substantial effect on the rate of synthesis of wool proteins. The differences in wool growth rate observed with various protein supplements given via the abomasum are presumably brought about by alterations in the amounts and proportions of amino acids in plasma (Reis and Tunks 1970; Reis and Colebrook, unpublished data), and hence in the assemblage of amino acids available for protein synthesis in the wool follicles. However, apart from specific effects of the absorbed amino acids on the wool follicles, the possibility of more general effects on amino acid metabolism in the animal, especially in relation to hormones such as insulin, growth hormone, and cortisol, must be considered.

In experiment 1, wool growth rates obtained in response to the various protein supplements should not be influenced by the sequence of supplementation, as the periods were sufficiently long to remove any carry-over effects from the previous treatment (Reis 1969; Colebrook and Reis 1969). The effects on wool growth observed in experiment 1 can be discussed in conjunction with the results of Colebrook and Reis (1969). The relative values for wool growth of the various proteins given as abomasal supplements in these two studies are shown in Figure 5. It is apparent that the proteins studied fall into three groups: casein, whole egg protein, and egg albumen, which have a

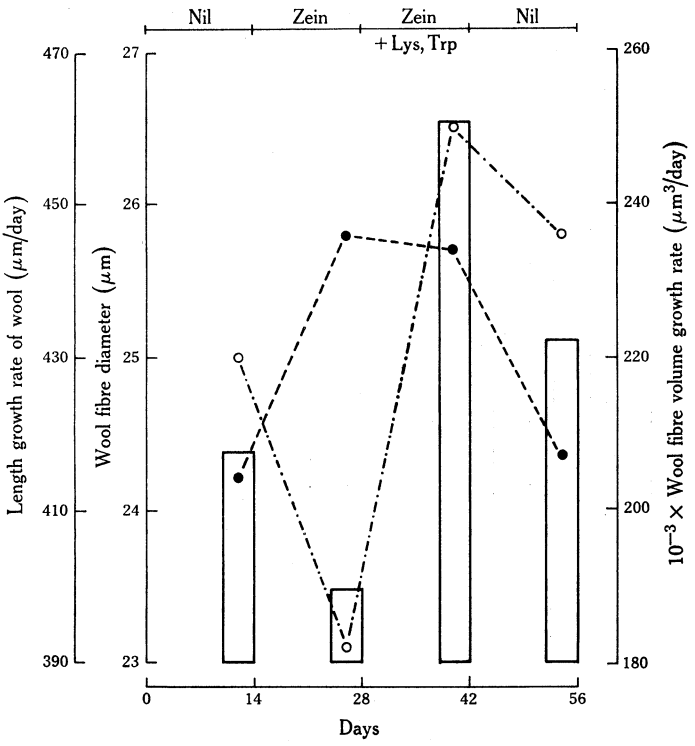

Fig. 4.—Effect of abomasal supplements of zein and amino acid-supplemented zein on the components of wool growth (see experiment 4, Table 1 for details). The components of wool growth are indicated as follows: fibre volume (vertical columns), fibre diameter (○), and length growth rate (●). Values are means from four sheep.

high value for wool growth; the glutens and Promine-D, which have about half the value of the first group; gelatin and zein, which have no value for, or are slightly inhibitory to, wool growth. These differential responses in wool growth rate appear to be related to differences in amino acid composition of the various proteins. Thus, proteins with an adequate content of all essential amino acids and a high biological value for monogastric animals (whole egg protein, egg albumen, and casein) produce substantial increases in wool growth rate. The glutens, while supplying adequate *S*-amino acids compared with the previous group of proteins, are unbalanced with respect to some other essential amino acids. They are deficient in lysine and tryptophan and contain a high proportion of leucine; this pattern is more pronounced with maize gluten. Promine-D contains relatively small amounts of *S*-amino acids (*c.* 2%); total essential amino acids appear to be adequate. The relatively low value of the glutens

and Promine-D for wool growth may thus be related to differences in the assemblage of amino acids absorbed from the intestines and hence available to the wool follicles. It should be pointed out that the precise mixture of amino acids required for optimal wool growth is not known, but the amino acid mixture supplied by proteins of the first group is obviously superior to the mixture supplied by the other proteins studied. The negligible effects of gelatin supplements on wool growth have been discussed previously (Reis and Schinckel 1964; Colebrook and Reis 1969); the results are explicable in terms of a deficiency of most essential amino acids, especially *S*-amino acids and tryptophan. The low value of gelatin does not appear to be related to its high content of some non-essential amino acids, as a large excess of glycine added to casein did not reduce its value for wool growth (Reis 1970). Though zein contains adequate amounts of

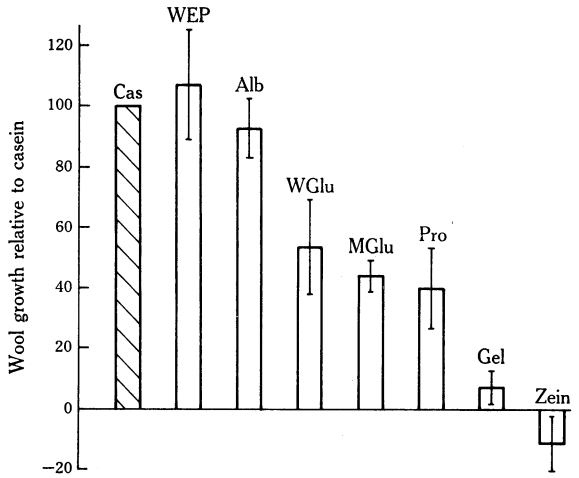

Fig. 5.—Relative value of various abomasal protein supplements for wool growth. The data are derived from experiment 1 (Table 1), and from the experiment of Colebrook and Reis (1969). Each unhatched column represents the average increase in wool growth rate obtained with a protein supplement relative to the increase obtained with the same sheep receiving casein supplements. The vertical bars represent the range of individual values for each protein. The proteins were casein (Cas), whole egg protein (WEP), egg albumen (Alb), wheat gluten (WGlu), maize gluten (MGlu), Promine-D (Pro), gelatin (Gel), and zein.

S-amino acids, it is devoid of lysine, contains only a trace of tryptophan, and a considerable excess of leucine, and thus supplies a very unbalanced mixture of amino acids. Whether the adverse effects of zein on wool growth are due to its unusual amino acid composition must still be regarded as uncertain; the possibility of some "toxic" substance in the zein could also be considered. However, the effect of the addition of lysine plus tryptophan to zein renders this unlikely. The results obtained with gelatin (Reis and Schinckel 1964; Colebrook and Reis 1969) and with zein show that amino acids other than the *S*-amino acids are important for wool growth.

In contrast to the effects on wool growth rate in experiment 1, it was difficult to assess the comparative value of the protein supplements for promoting tissue nitrogen

retention or body weight gain, as these parameters may be influenced by the cumulative effects of the previous supplements. However, no consistent differences were apparent between casein, wheat gluten, and Promine-D in their value for promoting nitrogen retention or body weight gain in experiment 1. The observation that nitrogen retention tended to be higher in period 1 than in period 2 (experiment 1) is in accord with earlier observations that nitrogen retention decreases with time (Reis and Schinckel 1961; Colebrook and Reis 1969). The low values for nitrogen retention obtained with abomasal infusions of bloodmeal in experiment 2 may be explained on the basis of a time effect. In agreement with the observations of Little and Mitchell (1967), zein was inferior to casein for promoting nitrogen retention. Zein supplementation in one sheep (4489) supported body weight gain and nitrogen retention but wool growth was depressed below the basal rate. This result indicates that the amino acid requirements for the synthesis of wool proteins and tissue proteins may be different.

The low value of bloodmeal for wool growth and nitrogen retention when given as an abomasal supplement probably explains the poor response of wool growth to the inclusion of bloodmeal in the diet, in the experiments of Colebrook *et al.* (1968). In the present study abomasal supplements of bloodmeal supported low rates of wool growth by comparison with abomasal supplements of casein given to sheep receiving the same dietary regime (Reis 1969). In marked contrast to casein (Reis 1969) dietary supplements of bloodmeal were perhaps superior to abomasal supplements. The low nutritional value of bloodmeal cannot be explained on the basis of amino acid composition, and it is probable that the protein had been damaged during processing. The low digestibility of the bloodmeal supports this suggestion, and evidence of heat damage during processing is also provided by measurements of degradation in an *in vitro* rumen system carried out by Dr. J. A. Hemsley (personal communication). Thus, the bloodmeal used in experiment 2 and oven-dried whole blood were very resistant to degradation to ammonia, whereas freeze-dried whole blood was readily degraded.

The effects of zein on wool growth are particularly interesting. A differential effect of a nutritional treatment on length growth rate and fibre diameter has not previously been observed. Variable effects on length growth rate and fibre diameter have been observed with other treatments, such as large amounts of some hormones (Downes and Wallace 1965) and exposure of the skin to low temperatures (Downes and Hutchinson 1969; Lyne, Jolly, and Hollis 1970). Nutritional changes usually produce changes in both length growth rate and diameter in about the same proportions, which maintain a fairly constant ratio of length : diameter for each sheep (Downes and Sharry 1971). The differential effects of zein on length and diameter were consistent for the four sheep in experiment 4, and resulted in the mean length : diameter ratio before treatment of 16·7 being increased to 19·3; the pretreatment ratios were restored (mean 16·9) by the addition of lysine and tryptophan to the zein. It is not possible with present knowledge to suggest whether these responses in length growth rate and fibre diameter represent specific effects on mitotic activity or protein synthesis in the follicles.

The considerable decrease in fibre diameter and the small decrease in wool output during zein supplementation in experiment 1 were associated with a marked weakening of the fibre. The stress imposed on fibres at 15% extension in water has been shown to be a convenient reference value for comparison of the mechanical properties of single wool

fibres, being largely independent of the variance of fibre cross-sectional area (Collins and Chaikin 1965). Such measurements were carried out by Mr. B. J. Rigby, Division of Textile Physics, CSIRO, Ryde, N.S.W., on lengths of wool fibres grown by sheep 4489 prior to treatment and during zein infusion. After allowance was made for the reduction in diameter, the "zein fibres" were still less than half as strong as the control fibres. Complete fibres consistently broke in the "zein" section when extended, before the "control" section had reached the yield region of the stress–extension curves. Thus, the physical structure of the wool fibres appears to have been markedly affected by altering the mixture of amino acids available to the wool follicles. These results with zein indicate the possibility of a new approach to chemical shearing. Further work would be needed to determine whether the strength of wool fibres can, in fact, be substantially reduced merely by altering the mixture of amino acids available to the wool follicles over a short period of time. This approach would have advantages over the use of antimitotic agents (Dolnick *et al.* 1969), which produce toxic side-effects.

The addition of lysine and tryptophan to zein in experiment 4 removed the adverse effects of zein and actually increased wool output above the basal rate. This is the first demonstration of an effect of specific amino acids, apart from *S*-amino acids, on wool growth. However, even though the gross amino acid deficiencies of zein were rectified, the increase in wool growth above the basal rate was still much less than would be expected with casein. Because lysine and tryptophan were added together, the relative importance of each amino acid to the response observed is not known. Tryptophan is the least abundant amino acid in the free amino acid pools of various tissues in the rat and in most proteins synthesized from these pools, and appears to be the amino acid limiting the rate of translation of messenger RNA and hence the rate of protein synthesis (Munro 1970). Limited information for cattle suggests that tryptophan is the least abundant essential amino acid in plasma (Oltjen and Putnam 1966; Love and Wiygul 1969). No data are available for free tryptophan in sheep tissues, but the unique importance of tryptophan may well apply to ruminants and explain the poor performance of proteins such as zein and gelatin. The adverse effects of zein may also be related to its unusual amino acid composition, namely an excess of leucine (*c.* 24%) coupled with a deficiency of lysine and tryptophan. Plasma amino acids measured during zein infusion (Reis and Colebrook, unpublished data) showed a substantial increase in leucine and very low levels of lysine (tryptophan was not measured) and indicated that the mixture of amino acids available to the follicles may be unbalanced. The addition of leucine to casein, to increase the leucine content to that of zein, had no appreciable effect on either fibre diameter or length growth rate of wool. This result does not support the hypothesis that high levels of leucine alone have an adverse effect on wool growth. However, further investigations are needed on this point.

The large range of responses in wool growth to various proteins administered into the abomasum, observed by Colebrook and Reis (1969) and in the present study, demonstrates the need to establish the nutritional value of any protein source which it is desired to protect from ruminal degradation (Ferguson, Hemsley, and Reis 1967). Damage to protein-rich meals during processing, as well as the amino acid composition of the protein, must be considered. Wool growth appears to be particularly sensitive to the balance of amino acids supplied to the wool follicles, but the actual requirements for, and interactions between, the various amino acids are still poorly understood.

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